### Infectious Disease

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Abstract

Anti-malarial drug resistance is a major public health problem which hinders the control of malaria. In India, resistance of *Plasmodium falciparum* (Pf) to chloroquine, the cheapest and the most used drug was first reported in the year 1973. Resistance to commonly used antimalarial drugs like chloroquine (CQ), mefloquine (MQ), sulfadoxine/pyrimethamine (SP) have been reported across the country. Emergence of artemisinin resistance in *P. falciparum* strains from Cambodia and neighboring countries is a major setback to the antimalarial program of these countries and an ominous sign.

Various drug-resistant genetic markers identified for *P. falciparum* and *P. vivax* antimalarial drugs, like *Plasmodium falciparum* Multidrug Resistance Protein 1 (PfMDR1), *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT), The K76T mutation in PfCRT protein is a potent molecular marker of CQ resistance and susceptibility. Antimalarial drug susceptibility and resistance to quinine, amodiaquine (AQ), piperazine, and lumefantrine is affected by variation in the PfCRT protein mutation. Mutation in C-terminal region of K13 protein is associated with the artemisinin resistance. Detection of drug-resistant parasites in *P. vivax* malaria is also prevalent worldwide. Prolonged courses of artemisinin-based combination therapies for 6 days are currently efficacious in areas where standard 3-day treatments are failing.

Introduction

Malaria is a major cause of morbidity and mortality in the tropical countries. Malaria cases in India have reduced from 2 million annually in the 1990s to 1 million in 2012, with a declining trend since 2002. However, the *Plasmodium falciparum* (Pf) cases reported has increased to 50.01% in 2012 from 39% in 1995. Notably, antimalarial drug resistance is becoming a major public health problem throughout the globe. In India, chloroquine-resistance in *P. falciparum* was first reported in 1973 from Assam. The National Vector Borne Disease Control Programme (NVBDCP) has been monitoring the response of antimalarial drugs in *P. falciparum* malaria since 1978 throughout India. This helps in formulation of National Drug Policy and recommends necessary changes in the control strategy including treatment policy to contain resistant *P. falciparum* foci. National Drug Policy recommends the use of ACT as first-line of treatment for *P. falciparum* since 2010 as resistance to commonly used antimalarial drugs like chloroquine (CQ), Mefloquine (MQ), sulfadoxine/pyrimethamine (SP) has been reported across the country.

Tools for Monitoring

Drug sensitivity status in India was being assessed following conventional WHO in-vivo protocol till 2002. This is changed in 2003 since when WHO protocol on “Therapeutic efficacy of antimalarial drugs in uncomplicated *P. falciparum* malaria” is being used. The criteria classify response in three categories:

- Adequate Clinical and Parasitological Response (ACPR),
- Early Treatment Failure (ETF), and
- Late Treatment Failure (LTF).
Criteria for Change of Drug Policy

The treatment drug policy is changed for the area/Block PHC, which reports ≥10% total treatment failure (ETF+LTF) to the tested drug, that is, the currently used antimalarials in a sample of minimum 30 *P. falciparum* test cases. To reduce emergence of drug resistance, the National Drug Policy on Malaria recommends use of combination therapy in chloroquine-resistant areas, that is, Artesunate plus Sulfadoxine-Pyrimethamine (AS+SP) for treatment of *P. falciparum* cases.1

Genetic Markers of Drug Resistance

The various drug-resistant markers identified for *P. falciparum* and *P. vivax* antimalarial drugs are discussed here.

*Plasmodium falciparum* Multidrug Resistance Protein 1 (PfMDR1)

The PfMDR1 gene located on chromosome 5, encodes for P-glycoprotein homolog 1 protein,4,5 which belongs to the ATP-binding cassette (ABC) superfamily. Resistance to antimalarials is believed to be due to polymorphism, amplification, or variation in mRNA expression level of the PfMDR1 gene.5

Drug susceptibility to chloroquine, quinine, mefloquine, halofantrine, lumefantrine, and artemisinin are affected by mutations in Pfmdr1 gene at the following positions (N86Y, Y184F, S1034C, N1042D, and D1246Y). Amodiaquin resistance is associated with PfMDR1 mutations at N86Y and N1042D position. Chloroquine resistance is associated with K76T and A220S mutation in the Pf chloroquine resistance transporter (PfCRT) gene and N86Y mutation in the PfMDR1 gene.6-10

*Plasmodium falciparum* Chloroquine Resistance Transporter

As discussed, PfCRT gene mutation has potential role in chloroquine resistance. This gene is localized to chromosome 7 and PfCRT protein belongs to the drug/metabolite transporter superfamily and chloroquine resistance transporter-like transporter family with ten putative transmembrane domains spanning the digestive vacuole membrane of the parasite.

The K76T mutation in PfCRT protein is a potent molecular marker of CQ resistance and susceptibility.11 K76T mutation located in the first transmembrane domain of PfCRT protein, allows the efflux of diprotonated chloroquine out of the digestive vacuole by active transport.12 Other mutations cause resistance only in association with K76T mutation and include C72S, M74I, N75E, A220S, Q271E, N326S, I356T, and R371I.12

Antimalarial drug susceptibility and resistance to quinine, amodiaquine (AQ), piperaquine, and lumefantrine is affected by variation in the PfCRT protein mutation.13-16 There is cross-resistance of chloroquine with AQ and quinine mainly mediated by 76T, and lumefantrine has an inverse cross-resistance having reduced susceptibility in association with wild-type K76.17

*Plasmodium falciparum* Multidrug Resistance-Associated Protein (PfMRP)

PfMRP is a transmembrane protein produced by PfMRP gene located on chromosome 1. This multidrug resistance-associated protein aids the parasite in transporting out of drug and organic anionic substrates like oxidized glucuronate, glutathione, etc. from its inside, thus effecting resistance.18,19

Chloroquine and quinine resistance are associated with two mutations in PfMRP at position Y191H and A437S. High sensitivity to various antimalarial drugs such as chloroquine, quinine, primaquine, piperaquine, and artemisinin can be demonstrated by genetic knocking out of PfMRP gene in the resistant parasite, and more accumulation of chloroquine and quinine is observed in the sensitive parasite. Thus, PfMRP is not only involved in determining the drug resistance but has role in varying the antimalarial response to resistance.19

*Plasmodium falciparum* Sodium Hydrogen Exchanger (PINHE)

This gene on chromosome 13 codes for a protein associated with quinine resistance in Pf, and is involved in active efflux of protons in the parasite to maintain pH at 7.4, in response to acidification by anaerobic glycolysis, the primary energy source for the parasite.20 Polymorphism in the microsatellite ms470 region exhibited a decrease in quinine susceptibility with an increase in DNNND repeat motif, whereas increase in quinine susceptibility is observed with a rise in NHNDNHNDDD motif. Three mutations at 790, 894, and 950 codons and polymorphism in the microsatellite region (msR1 and ms3580) showed
no association with quinine resistance. Thus, repeat polymorphism in PfNHE gene may be used as a valid genetic marker to determine quinine resistance in some regions.

**Plasmodium falciparum** Bi-functional Dihydrofolate Reductase-Thymidylate Synthase (PfDHFR-TS)

The PfDHFR-TS gene has one exon located on chromosome 4 encoding for PfDHFR protein and is a bi-functional enzyme involved in two main folate metabolic activities—biosynthesis of dTMP by thymidylate synthase activity and the reduction of dihydrofolate to tetrahydrofolate by dihydrofolate reductase (DHFR) activity. The folate mechanism of PfDHFR-TS enzyme is inhibited by the action of antifolate drugs such as pyrimethamine and cycloguanil, thus reducing the production of pyrimidine for DNA replication.

Pyrimethamine resistance is mainly associated with point mutation in the PfDHFR protein at S108D codon, and further mutation at N51I, C59N, and I164L positions strengthens the resistance besides amplification of gene.

**Plasmodium falciparum** Dihydropteroate Synthase (PfDHPS)

The PfDHPS gene is located on chromosome 8 and encodes for PfDHPS protein. PfDHPS synthesizes dihydrofolate, a folate precursor that is essential for the synthesis of pyrimidine in the parasite by catalyzing the reaction of pterin derivative and p-aminobenzoic acid (PABA). This catalytic enzyme action to synthesize dihydrofolate is inhibited by sulfa drugs (sulfadoxine and dapsone), which act as an analog to PABA.

Resistance to sulfadoxine in *P. falciparum* is due to S436A/F, A437G, L540E, A581G, and A613T/S mutations in the PfDHPS protein. Sulfadoxine is always provided in combination with pyrimethamine (SP) as monotherapy is associated with antimalarial drug resistance. Point mutation in both PfDHFR and PfDHPS gene is associated with resistance to SP.

**Cytochrome B**

Cytochrome b (Cyth) gene is a subunit of cytochrome bc1 complex present in mitochondrial inner membrane of the parasite. It catalyses the transfer of electrons across the inner mitochondrial membrane to maintain the electrochemical potential of the membrane. The antimalarial drug atovaquone disrupts the electrochemical potential by binding to the ubiquinol binding site of cytb, and hence is lethal for the parasite. Mutation at the ubiquinol binding site confers atovaquone resistance.

Single mutation at Y268N/S/C codon in the cytb gene leads to resistance to atovaquone in *P. falciparum* field isolates.

**Kelch**

Kelch-13 (K13) protein has one exon located on chromosome 13. Mutation in C-terminal region of K13 protein in this region is associated with artemisinin resistance in both clinical and field isolates by disrupting the domain scaffold. However, the exact function of K13 protein, and the effect of various mutations on the protein will only be known after several studies are conducted to ascertain its nature completely.

Nonsynonymous polymorphism at Y493H, R539T, I543T, and C580Y positions observed in the kelch repeat region of K13 propeller domain is associated with higher artemisinin resistance. Mutations at codons F446I, Y493H, P574L, R539T, and C580Y confer a higher degree of resistance to artemisinin, and the frequency of C580Y allele mutation is higher and takes longer time for parasite clearance when compared to variation in other sites. Therefore, K13 propeller protein polymorphism can serve as a potent molecular marker indicating emergence and spread of artemisinin-resistant *P. falciparum*.

**Drug Resistance in Vivax Malaria**

Drug-resistant *Plasmodium vivax* malaria is also prevalent worldwide. Its treatment primarily consists of two drugs: Chloroquine (blood schizonticide) targeting the asexual blood stages of the parasite, and Primaquine (tissue schizonticide), which targets the dormant live stage (hypnozoites) of the parasite, responsible for relapse.

*P. vivax* is also associated with molecular markers of drug-resistance, like *P. falciparum*. Increased susceptibility to chloroquine in *P. vivax* is strongly associated with the Y976F mutation in PvMDR1 gene, which is a homolog of PfMDR1. However, the PICRT gene homolog in *P. vivax*, that is, the PvCRT gene, is not associated with chloroquine-resistance (unlike in *P. falciparum*). Mefloquine resistance
in *P. vivax* is associated with amplification of *PvMDR1* gene. Mutation at Y976F position of *PvMDR1* in *vitro* has been shown to be associated with resistance to mefloquine and artesunate, in addition to chloroquine.\(^{28,30}\)

Point mutations at F57L/I, S58R, T61M, and S117T/N codons of *PvDHFR* gene are linked to pyrimethamine resistance and consequently treatment failure with pyrimethamine in *P. vivax*. The wild type residue V585 in *PvDHPS* gene has shown innate resistance to sulfadoxine and is enhanced by the mutation in A383G and A553G codons in *PvDHPS*, which is similar to *PfDHPS* mutation at codons A347G and A581G.\(^{31,32}\)

### Conclusion

Antimalarial drug resistance poses a major hurdle to our victory over the malarial parasite and contributes to failure in treatment of malaria. Combination therapies targeting different mechanisms of action can delay the emergence and spread of drug-resistant parasites. Molecular markers of drug resistance play a vital role in the detection of resistance in clinical and field isolates when compared to the *in vivo* efficacy studies and *in vitro* tests. Thus, earlier clinical isolates will aid in employing immediate and appropriate treatment that in turn reduces treatment failure and thereby mortality, and also prevents the spread of resistance. Hence, continuous monitoring and surveillance of drug-resistant molecular markers in malaria endemic regions is important in determining and assisting an effective national drug policy for malaria treatment. Therefore, more research is necessary to find new antimalarial drugs/vaccines for multidrug resistance parasites.

### References


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**Infectious Disease**


Abstract

Ever since the advent of vaccination against communicable diseases there has been concern in the mind of certain people about its adverse outcome. While there is no doubt about efficacy of the time-tested vaccines which have been able to either eradicate or minimize many dangerous communicable diseases but the apprehension remains there, which were not unfounded. There was apprehension of increased incidences of Autism (MMR Vaccine), GBS (DPT, Influenza vaccine) which later on proved wrong. Dengvaxia saga (2015 with Dengue vaccination), Cutter polio incident (1955), and currently Covid vaccine related adverse effects are few examples which were highlighted out of proportion despite their numbers were few and statistically insignificant. In this article we highlight the various aspects of various controversies revolving around the vaccinations and their scientific explanations which would help us to overcome these apprehensions for future vaccination strategies.

Introduction

Ever since Edward Jenner had introduced the first vaccine in 1796, the global use of vaccines has been detrimental in reducing or eradicated the incidence and spread of childhood diseases.\(^1,2\) Despite the fact that there are a lot of evidences behind the benefits of vaccines, to provide immunity against diseases, there has been opposition in certain quarter against the use of vaccines.\(^3\) Some of these biased views are based on perception of personal, religious, or cultural beliefs that vaccines can be more harmful than good in people who receive them. The Controversy of vaccination is about:

- Safety
- Efficacy
- Dosing
- Availability of different vaccines for different region.

This can be attributed to lack of awareness, political and vested interests. However, this fear was not without reasons. Following is the historical facts of various concerns against the vaccinations.

The Cutter Incident:\(^4\) In 1955 Cutter Laboratories produced 120,000 polio vaccines (in which the process of inactivating the live virus proved to be defective), which caused 40,000 cases of polio, 53 cases of paralysis, and 5 deaths. This in turn also infected the family members of the recipients leading to additional 113 cases of paralytic polio and another 5 deaths. It has been rightly described as the worst Pharmaceutical incident.

Other historical facts are given below which created doubts and suspicion in the mind of people regarding efficacy and side effects of vaccinations (Table 1).

Despite all the rebuffs to the Antivax (Anti-vaccination) lobby the concerns raised were not without reasons due to the emergence of certain adverse effects in the ensuing
### TABLE 1

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<th>Allegation</th>
<th>Evidence</th>
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<td>MMR vaccine and autism&lt;sup&gt;1&lt;/sup&gt;</td>
<td>MMR vaccine can cause autism</td>
<td>• Initial report suggesting an association between vaccination and autism was drawn back&lt;br&gt;• Autism usually occurs in early age group than before recommended age of MMR vaccination (12 months) and is genetically determined&lt;br&gt;• In studies conducted by the epidemiologists there is no increased risk of autism associated with MMR vaccine</td>
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<td>DPT</td>
<td>Neurological conditions following immunization&lt;sup&gt;7,8&lt;/sup&gt;</td>
<td>• Commission on Vaccination and Immunization (JCVI) confirmed that the risks or neurological problems due to DPT were quite low&lt;br&gt;• With universal childhood immunization, the number of reported cases fell by &gt;95%, and mortality rates decreased even more dramatically&lt;sup&gt;9&lt;/sup&gt;</td>
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<td>Thimerosal&lt;sup&gt;10&lt;/sup&gt;</td>
<td>Increases risk of autism and other neurodevelopmental disabilities may be increased by Thimerosal</td>
<td>• Ethyl mercury (an active ingredient of thimerosal) does not accumulate in the body to harmful levels with consecutive vaccinations&lt;br&gt;• Even after removal of thimerosal from childhood vaccines, incidence of autism continued to increase&lt;br&gt;• Studies have not found an increased risk of autism or other neurodevelopmental disabilities associated with thimerosal-containing vaccines in epidemiological studies&lt;sup&gt;11&lt;/sup&gt;</td>
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<td>Influenza vaccines may cause GBS&lt;sup&gt;12&lt;/sup&gt;</td>
<td>GBS (1978)</td>
<td>It is observed that risk of GBS is greater following natural influenza infection than possible vaccination</td>
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<td>Meningococcal vaccine and can cause GBS&lt;sup&gt;12&lt;/sup&gt;</td>
<td>GBS (2005–2008)</td>
<td>A large study proved there was no link between Menactra and GBS</td>
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<td>Autoimmunity&lt;sup&gt;13&lt;/sup&gt;</td>
<td>Vaccines may be responsible for chronic diseases of autoimmune etiology</td>
<td>There is no established mechanism to explain how vaccinations cause autoimmune disease and epidemiologic studies have failed to support the hypothesis that vaccines cause autoimmune diseases</td>
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<td>HPV vaccine: Safety</td>
<td>Risk of autoimmune and other disorders may be increased with HPV vaccination</td>
<td>• Several large studies have not established increased risks of autoimmune or neurologic diseases&lt;br&gt;• Other studies failed to establish increased risks of POI, POTS, or CRPS</td>
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<td>Aluminum</td>
<td>Autoimmune diseases and a variety of other disorders, including Macrophagic Myofasciitis can be attributed to aluminum in vaccines&lt;sup&gt;14&lt;/sup&gt;</td>
<td>• The serum levels of aluminum are well below the toxic range due to aluminum containing vaccines&lt;br&gt;• There is hardly any correlation between infant blood or hair aluminum concentrations and vaccine history&lt;br&gt;• There were lower incidence of autoimmune disease with higher quantities of injected aluminum adjuvants&lt;br&gt;• Systemic symptoms of MMF secondary to aluminum salts at injection sites have never been established&lt;sup&gt;15&lt;/sup&gt;</td>
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<td>Too many too soon</td>
<td>Too many vaccines given early in life might interfere with the immune system</td>
<td>• Infants can handle as many as 10,000 vaccines at one time&lt;br&gt;• Long-lasting, gross alterations of the immune system has been found with childhood vaccinations&lt;br&gt;• The number of vaccines or vaccine antigens received in early childhood did not increase risk of disease or developmental delay as per many epidemiological studies</td>
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<td>Hepatitis vaccine 1998&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Multiple sclerosis</td>
<td>The IOM committee concluded that there is no link between MS and hepatitis B vaccination</td>
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CRPS, complex regional pain syndrome; GBS, Guillain-Barré syndrome; HPV, human papillomavirus; IOM, Institute of Medicine; MMR, measles, mumps, and rubella; POI, primary ovarian insufficiency; POTS, postural orthostatic tachycardia syndrome
years. To highlight these concerns, few following events throw insight into the safety and concerns about the vaccination.

**Dengue Vaccine Controversy**\(^{16,17}\)

About 400 million people are infected with Dengue every year, out of which almost 25,000 people die of dengue hemorrhagic fever (DHF). Due to lack of cure for Dengue and high mortality rate in the countries with less advanced medical system, scientists have since long been on a quest to develop a vaccine. Recently, Dengvaxia (Vaccine against Dengue) was approved by Food and Drug Administration (FDA) to prevent dengue. Despite the fact that this vaccine has the capability to save millions of lives, its approval was not without controversy.

Philippines in 2015 launched a massive campaign to immunize children against the dreaded disease. After about 1 million children were vaccinated, the controversy was raised as the vaccine was associated with the deaths of three children in the Philippines. However, before addressing this issue it is imperative, we understand how the immune system reacts to dengue virus. There are two types of antibodies generated against any pathogen—neutralizing and non-neutralizing. The dengue virus has four serotypes and the antibodies generated against one serotype while it may react with another serotype as well, it does not always neutralize the antigen. This may be acceptable for other pathogens since the antibody-pathogen complex reach the macrophage and is eventually destroyed. However, dengue virus is unique as it primarily infects the macrophage. Thus, antibodies that have developed against one variant of dengue can make subsequent infections far more lethal. The preexisting non-neutralizing antibodies bind to dengue virus antigen and attract macrophages facilitating spread. In this manner the non-neutralizing antibodies allow the virus to infect multiple cells with ease, thus causing the severity of the second infection with respect to the first one. This phenomenon is referred as antibody enhancement.\(^{18}\)

Now, though dengvaxia was indeed engineered to protect against all four serotypes of dengue, many people did not develop neutralizing antibodies against all the serotypes. In this unfortunate people, the vaccine acted as a primary infection thus making the second infection far more lethal (Fig. 1).

**Current recommendations of dengue vaccine:** Despite these shortcomings, dengvaxia has been approved by FDA for use in the following conditions—
- Children 9–16 years old in endemic areas
- Laboratory confirmed previous infection

**Controversies Surrounding HPV Vaccination**

The controversy surrounding HPV Vaccine is about its—
- Safety concerns
- Dose scheduling
- Two strains/four strains vaccine
- Whether males should be vaccinated
- Till what age one should be vaccinated

**Safety concerns:** The main adverse reactions reported with HPV vaccination were: headaches (70%), general fatigue (53%), coldness of the legs (53%), limb pain (50%), limb weakness (48%), difficulty in getting up (48%), fainting (43%), decreased capability to learn (43%), arthralgia (43%), tremulousness (40%), gait disturbances (40%), disturbed menstruation (35%), and dizziness (30%). Besides this, it was associated with enhance autoimmune response and CNS side effects including syncope. Possibility of presence of the active aluminum adjuvant might be contributing to these side effects.\(^{19}\)

In India, with regards to HPV vaccine, Program for Appropriate Technology in Health (PATH),\(^{20}\) a nonprofit based in Seattle, in 2009 launched a $3.6 million HPV trial, funded by the Bill & Melinda Gates Foundation, in 24,777 adolescent girls in Andhra Pradesh and Gujarat states; however, it was prematurely terminated by the government when news outlets reported the death of seven girls. However, on further analysis it was found that five of those deaths were evidently unrelated to the vaccine itself. The causes attributed to their death were drowning (1 girl), snake bite (1 girl), suicide (2 girls), malaria (1 girl). The other two deaths were still uncertain.

**Dosing of vaccine and cost:** Another dilemma with respect to the HPV vaccine is the dosing schedule.\(^{21}\) While one dose by itself is quite immunogenic so as to provide lasting protection from HPV 16 and 18, data regarding long-term protection after 7 years and protection from HPV and cervical precancerous lesions is lacking. Conversely the immunogenicity produced by two-dose and three-dose HPV vaccine schedules, measured using antibody responses in young females, is comparable.\(^{21}\)
Current Recommendations

**Children and adults ages 9 through 26 years:** HPV vaccination is routinely recommended at age 11 or 12 years; although it can be started as early as age 9 years. HPV vaccination is recommended for all women before attaining 26 years of age who were not adequately vaccinated earlier. Children below 15 years require only two doses to be fully protected. People who start the series at age 15 or older and people with weakened immune system need three doses to be fully protected.

**Adults ages 27 through 45 years:** Despite the facts that HPV vaccine is approved by FDA to be given through age 45 years, HPV vaccination is not recommended for all adults ages 27 through 45 years as effect of vaccination starts to diminish by age 18. Therefore, its benefit for cancer prevention is doubtful as people get older. The ACS (American Cancer society) does not recommend HPV vaccination for persons older than age 26 years. Instead, ACIP recommends that because more people have already been exposed to the virus. HPV vaccination
in this age range provides less benefit; clinicians consider discussing with their patients in this age group about the benefits of the vaccination.

Male HPV vaccination: The US Advisory Committee on Immunization Practices (ACIP) licensed HPV4 for boys and young men in 2009. HPV vaccine in males can help prevent genital warts as well as a variety of cancers associated with HPV.

Influenza Vaccination

Influenza (flu), which caused a pandemic in 2009 accounting for 100,000–400,000 deaths, still remains a cause of global concern. According to the World Health Organization (WHO), 1 billion cases surface every year globally, causing about 200,000–600,000 deaths. According to Flu Net, the WHO Global Influenza Surveillance and Response System (GISRS) laboratories; out of total cases 98.6% were influenza A and 1.4% were influenza B cases. Out of these influenza A viruses, 65.2% were influenza A H1N1 and 34.8% were influenza A H3N2. Influenza A H3N2 infection was more virulent than influenza A H1N1. In India also the strain influenza A (H1N1) has accounted for most cases—which peaked in the third week of February 2019. Although the report said that vaccination was the most effective tool to create immunity against the disease, but certain doubts remain there about vaccination.

- Which type of vaccination in India should be used? Northern hemisphere/Southern hemisphere.
- High dose versus routine dose in 65 year plus people (High dose vaccination not available in India).
- Serial vaccination and do annual vaccination cause less immunization in subsequent vaccination? Antigenic distancing hypothesis.
- Universal vaccination: Will single vaccination for lifetime (like other vaccines) see some light in time to come?

Seasonal Influenza Vaccine Recommended for the Season of 2019-2020 in India

In India, ICMR and Ministry of Health and Family Welfare, Government of India, have recommended Northern hemisphere quadrivalent/tetravalent vaccine.

Due to the constant changing of antigenicity, the efficacy of influenza vaccination is potentiated when circulating viruses and vaccine viruses correspond to each other. Even when they do correspond, efficacy of vaccine is not beyond 40–60%. But, if the vaccine virus as recommended by WHO is different from the locally circulating virus, it may be partially effective or not effective at all. Hence, vaccines are not foolproof method of protection against influenza. Instead due attention must be given to personal hygiene, frequent washing of hands, social distancing, use of proper masks, etc. should not give a false sense of security.

In various studies it was found that high-dose vaccine (fluzone) was 24.2% more effective (CI 9.7–36.5%) in preventing flu in adults 65 years of age and older relative to a standard-dose vaccine and high-dose influenza vaccine can reduce risk of respiratory-related hospital admissions from nursing home residents aged 65 years and older.

In people who received vaccination in two consecutive seasons, protection against H3N2 was lesser than those who had only been vaccinated in current season suggesting that repeated vaccination against influenza may weaken the immune system. However, as per CDC recommendations, annual flu vaccination remains the first and most important step in protecting against flu and its complications. One of the studies postulated that twice annual influenza vaccinations in elderly population of tropical and subtropical areas has improved efficacy. However, the observations were in contradiction to the above said postulation.

Future Vaccine Considerations

An ideal universal vaccine should be:
- Effective for longer duration of time and 75% effective against all strains of Influenza A virus in all age groups.
- A cell based or a recombinant formulation should be favored over an egg-based vaccine.
- The hemagglutination inhibition response for a nanoparticle vaccine formulated with a saponin-based adjuvant was greater than a high dose vaccine.
- Traditional influenza vaccine target IgG portion. However, since different parts of IgA trap many parts of influenza virus simultaneously, IgA may be a more effective target.
- Manufacturing time should be shortened.
Universal Influenza Vaccine

A ball and spike model depicts well of a structure of influenza virus where hemagglutinin protein on the surface represent as spikes. Each spike having a stalk and a cap.

While all current influenza vaccines target at the cap portion of the hemagglutinin proteins which change its antigenicity frequently requiring annual vaccination. Instead the stalk portion of the hemagglutinin protein is stable among different influenza viruses and does not alter antigenicity annually. Therefore, a vaccine targeted at the stalk portion of the hemagglutinin protein can turn out to be a universal vaccine.

Measles Cases in 2019\(^{2,33}\)

It is worth understanding the mechanism of transmission of measles as it is highly contagious like COVID-19 virus. It has been noted that the viability of the virus lasts for about 2 hours and individuals who have not been vaccinated against measles have a 90% chance of contracting measles. These people now have the potential to spread the disease to 9–18 other people.\(^2\) However, in the global scenario, measles is the leading cause of vaccine-preventable disease and deaths despite the widespread use of vaccines and in fact every year 100,000 people die from measles.\(^2\) World Health Organization reports that there were 1,234 cases of measles in the US and 91 cases in Canada from January 2019 to September 2019. On the other hand, in 2018, 372 cases were reported in the US and 28 in Canada. The recent spike in measles outbreak is due to vaccine deterrence (out of fear of Autism and ignorance and inertia). This along with increase in international travel has facilitated the disease to enter into areas where it was once considered eliminated.\(^4\)

Current Recommendations for Adults

It is advisable for the adults including students at post high school educational institutions, health-care personnel, international travelers who do not have significant evidence of immunity to get a single dose of MMR vaccine. However, certain adults especially students at post high school educational institutions may need two doses separated by at least 28 days. Also, the killed measles vaccine available in 1963–1967 was not very effective. So, they should be vaccinated again.

Tdap/DTaP Vaccination\(^{35,36}\)

*Bordetella pertussis* may be the etiologic agent in 12–30% of adults with cough that does not improve within 2 weeks. This is a highly contagious disease with attack rates of 80–100% among unimmunized household contacts and 20% within well immunized household contacts. Despite their efficacy whole-cell pertussis vaccines have been associated with adverse events both common (fever, pain at injection site, erythema and swelling; irritability) and uncommon (febrile seizures, hypotonic hyperresponsive episodes). There have also been alleged associations of whole-cell pertussis vaccine with encephalopathy, sudden infant death syndrome, and autism, which although not substantiated, have succeeded in initiating an active anti-immunization lobby.

Low dose diphtheria toxin along with acellular pertussis vaccine in otherwise previous DPT vaccine has reduced its dreadful side effects. Currently, diphtheria toxoid vaccine is coadministered with tetanus vaccine (with or without acellular pertussis). DTaP (full-level diphtheria toxoid, tetanus toxoid, and acellular pertussis vaccine) is currently recommended for children up to the age of 6 years. DTaP replaced the earlier whole-cell pertussis vaccine DTP in 1997. Tdap is formulated for adolescents and adults and consists of acellular pertussis, tetanus toxoid and reduced diphtheria toxoid, and acellular pert Tdap was licensed for use in the US in 2005 and is recommended for children ≥7 years old and for adults. It is recommended that all adults who have never received Tdap before; a single dose of Tdap should be given regardless of the fact that they got Td vaccine before. This should be followed by either a Td or Tdap booster every 10 years.

Efficacy of vaccine can be ascertained by certain historical events. During the 1990s, an epidemic affecting 150,000 people occurred in the former states of the Soviet Union out of which 5000 had died. This was associated with a clonally related strain of ET8 complex. This outbreak was attributed to the failure of the public health infrastructure to vaccinate the people.

Controversy: In the United States, routine vaccination that started in 1940s led to a 100-fold reduction in the incidence of pertussis, and the disease appeared to be on the road to elimination.\(^2\) However, since the mid-1970s, the disease has once again resurfaced,\(^3\) steadily increasing in incidence to 15.1 cases per 100,000 in 2012.
The recent surge of pertussis in developed countries led to a controversy as to its cause. Domenech de Cellès et al. modeled the transmission of pertussis using incidence data from Massachusetts, US. They found that it was not the switch to acellular pertussis vaccine but rather the waning vaccine conferred immunity that contributed to the Massachusetts outbreak. This could be explained by the local increased incidences in pertussis cases in the subpopulation who were not vaccinated. Simulations suggested that administration of existing boosters to children may be an effective strategy to halt the transmission of pertussis.

Current recommendation: Every Adult >10 years of age should be given Tdap followed by booster dose of Td or Tdap every 10 years including pregnant women in early pregnancy.

COVID-19 Vaccination

Three types of mechanisms are involved for manufacturing of Covid SARS 2 vaccines (as depicted in Figure 2) which are being developed by more than 300 drug companies.

As of now following vaccines (Table 2) are at various stage of development:

<table>
<thead>
<tr>
<th>Vaccine candidates, developers, and sponsors</th>
<th>Mechanism</th>
<th>Sponsor</th>
<th>Approved/phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad5-nCoV</td>
<td>Recombinant vaccine (adenovirus type 5 vector)</td>
<td>CanSino Biologics</td>
<td>Approved China</td>
</tr>
<tr>
<td>AZD1222</td>
<td>Replication-deficient viral vector vaccine (adenovirus from chimpanzees)</td>
<td>The University of Oxford; AstraZeneca; IQVIA; Serum Institute of India</td>
<td>Phase 3</td>
</tr>
<tr>
<td>Covaxin</td>
<td>Inactivated vaccine</td>
<td>Bharat Biotech; National Institute of Virology</td>
<td>Phase 3</td>
</tr>
<tr>
<td>JNJ-78436735 (formerly Ad26.COV2.S)</td>
<td>Non-replicating viral vector</td>
<td>Johnson &amp; Johnson</td>
<td>Phase 3</td>
</tr>
<tr>
<td>NVX-CoV2373</td>
<td>Nanoparticle vaccine</td>
<td>Novavax</td>
<td>Phase 3</td>
</tr>
<tr>
<td>Gam-COVID-Vac (Sputnik V)</td>
<td>Non-replicating viral vector</td>
<td>Gamaleya Research Institute of Epidemiology and Microbiology</td>
<td>Russia: Ministry of Health</td>
</tr>
</tbody>
</table>

Controversy and Concern

The development of a new vaccine usually takes around 10–15 years. Mumps was the fastest vaccine which was developed in 5 year time. During this pandemic era there is lot of pressure by public and government agencies on the pharma companies to develop vaccine at a faster pace. Therefore, the vaccines are being developed in a very short period and on trial basis on smaller groups. As a result
when the vaccines are released for public use on mass scale, the side-effects which were unnoticeable during trial phase may become overt.\(^{39}\) RNA-based vaccines are a major new tool to combat pandemics like COVID-19 outbreaks as they require only viral genetic sequence information to initiate development.

In the past DNA and RNA vaccines have not been successful for human diseases and m-RNA based vaccines will be used for the first time in human being and that too in the pandemic period with trials done for less than a year duration.

The vaccine candidates using human adenovirus as vector such as Cansino Biologics (Wuhan, China) could reduce immune response as there is lot of pre-existing immunity to Adenovirus.\(^{31}\) Mutations of virus is another important factor that can result in having limited effectiveness of vaccines. There is also concern that vaccines may cause antibody dependent enhancement ADE (as was seen in Dengue Vaccines) or antibody dependent cellular cytotoxicity (ADCC).

Response to vaccines may differ in various age groups and in people with different status of immunity. During phase II trial Sanofi’s recombinant DNA derived vaccines did not yield adequate antibody titer in people aged 50 years or more.

The trial so far has been conducted in non-pregnant and younger population. Therefore, side effects and concerns in extremes of age and pregnancy are still to be decided.

Cost of vaccine, hesitancy, manufacturing capabilities, logistics, maintenance of cold chain, and transportation are other issues which may burden our country in terms of money and manpower in delivering vaccines to target population. (At the time of submitting of this article FDA has approved Tozinameran (BioNTech, Pfizer) on 11 December 2020, while on the same day Sanofi has postponed phase III trial due to poor antibody titer in people aged 50 years or more.)

**Conclusion**

This is an established fact that due to proper immunization of masses we have been able to either eradicate certain diseases like smallpox or polio or incidences of many communicable diseases like measles, pertussis, etc. have been brought to minimum. But due to lack of communication between people and HCP there has been apprehension and doubts in the mind of public at large which precludes them of using vaccines. Affordability of certain vaccines and Inertia on the part of HCP is another reason for underutilization of vaccination. The adverse effects of vaccines are so highlighted and dramatized by media (including social media) that it overshadows the efficacy of vaccinations. Need of the hour is to remove this misconception in mind of people with help of HCPs, Govt. agencies, media, etc. to help us overcome this menace as was done in case of polio and smallpox.

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Abstract
Critically ill patients are prone to many infections due to multiple interventions being carried out in them by means of indwelling catheters, endotracheal tube, etc. These infections lead to increased morbidity and mortality among critically ill patients. It is, therefore, imperative that these are recognized early and treated appropriately. More important than treating these would be to prevent them from occurring. This chapter deals with various infections in critically ill patients, their pathogenesis, diagnostic, and treatment guidelines and also the preventive guidelines.

Introduction
Critically ill patients are those who suffer from a critical illness that requires them to have intensive monitoring. This intensive monitoring entails them to have complete dependency on caregivers for their daily activities. These patients often are in coma/drowsy, mechanically ventilated, bed-ridden, and have many indwelling catheters. These factors make these patients prone to infections, which can be multi-drug resistant. This chapter deals with different infections that can occur in critically ill patients, their pathogenesis, and their preventive strategies.

Different Types of Infections Seen in Critically Ill Patients

Catheter-associated Infections
Pathogenesis and Risk Factors
Critically ill patients mostly have multiple catheters inserted. It may be urinary catheter, peripheral intravenous cannula, arterial catheter, or central venous catheter. These help in critical monitoring of the patient. In a certain survey in the United States, 87% of blood stream infections were associated with central lines. In the same survey, catheter associated urinary tract infection (CAUTI) was responsible for 95% of urinary tract infections. Mortality attributable to central venous catheter-related infections is 12–25% and mortality attributable to CAUTI is less than 5%.

A biofilm formation is critical in pathogenesis of catheter-associated infections. A biofilm is basically composed of a conditioning film (formed by the body fluid bathing the catheter) and the microbes that attach to it (sessile), which secrete an extracellular polysaccharide matrix on the condition film. This biofilm facilitates growth of microbes of different species and it is from here that some microbes become mobile (planktonic) and lead to infections. The biofilm leads to decreased penetration of antibiotics, local alteration in the microenvironment leading to decreased susceptibility to antimicrobials and antimicrobial resistance. Many microbiological culture results are only of planktonic organisms and these do not apply to sessile organisms embedded in the biofilm. It is because of this that sometimes antibiotics merely suppress the infection.
The intravascular catheters get infected on their outer surface by the microbes present on the skin, especially within 10 days of the insertion. So, short-term intravascular catheters like peripheral intravenous cannula, arterial catheters, and non-cuffed central venous catheters mainly get contaminated on outer surface. The common organisms that migrate to outer surface of catheters from skin are coagulase negative staphylococci and *Staphylococcus aureus*. The hub of intravascular catheters gets contaminated by microbes that chiefly come from caregiver’s hands. This especially occurs if the catheter is in situ for greater than 30 days or more. Intraluminal spread to catheter can also occur from a distant source (e.g., urinary tract) or from an infected infusate. Most common organisms implicated in these instances are *Stenotrophomonas*, *Candida*, *Pseudomonas*, enterococci. Immune compromised and elderly patients are more likely to develop contamination. Multilumen intravascular catheters and catheters inserted at femoral site are more likely to get contaminated. Internal jugular central venous catheter is more likely to get contaminated than the subclavian counterpart. Peripheral intravenous lines are more likely to get contaminated if inserted in lower extremities. Catheters inserted by non-sterile/improper techniques are more likely to get contaminated.²

The urinary catheter can also get externally contaminated either at the time of insertion or later by fecal matter. Intraluminal contamination can occur by reflux of microbes from the container bag or due to a break in collecting system. Commonly isolated organisms are *Escherichia coli*, *Pseudomonas*, *Enterobacter*, *Candida*, *Klebsiella*, and enterococci.² *Staphylococcus* and coagulase negative staphylococci have also been isolated. As with intravascular catheters, immune compromised patients are more likely to develop contamination. The other risk factors for developing bacteriuria in catheterized patients is longer duration of catheterization, diarrhea, female gender, renal insufficiency, improper catheter insertion technique, and improper catheter care.³

**Diagnosis of Catheter-related Blood Stream Infection (CRBSI)**

The Infectious Disease Society of America (IDSA) has published updated detailed guidelines for diagnosing intravascular CRBSI in 2009.⁴ The commonly used terminologies for intravascular catheter-related infections are catheter colonization, phlebitis, exit site infection, and blood stream infections. The details regarding all these can be read from the guidelines. From the guidelines it was noteworthy that catheter tips should be cultured only quantitatively and not qualitatively and that catheters to be cultured only after removal.

**Diagnosis of CAUTI**

The 2009 IDSA Guidelines define CAUTI in catheterized (urethral, suprapubic, and condom) patients and in those in whom catheter (urethral, suprapubic, and condom) has been removed in last 48 hours by the presence of symptoms and signs of urinary tract infection with no other identified source of infection and having 1,000 or more colony-forming units (CFU)/mL of 1 or more bacterial species.⁵

**Pulmonary Infections**

**Definitions, Etiological Agents, and Risk Factors**

Pulmonary infections are major causes of mortality in critically ill patients. Among intubated patients, ventilator associated pneumonia (VAP) diagnosis is made if pneumonia develops 48 hours after intubation.⁶ Any pneumonia other than VAP developing in hospital is called hospital-acquired pneumonia (HAP).⁷ VAP occurrence rates are quite different in different studies. This is due to use of different diagnostic criteria, variations in study population, differential evaluation of radiological images, and different methods of sampling. Fever, leukocytosis, purulent secretions, new infiltrates on chest x-ray/worsening infiltrates on chest X-ray carry high sensitivity in suspecting VAP, but at the cost of low specificity. Clinical Pulmonary Infection Score (CIPS) of greater than 6 strongly suggests VAP.⁸ Common risk factors for developing VAP are advanced age (>60 years), male gender, increased mechanical ventilation time (>2 weeks), prolonged hospital stay, altered consciousness, burns, comorbidities (diabetes, CAD, COPD, chronic renal failure, Hashimoto’s thyroiditis), prior antibiotic use (leading to MDR pathogen infection), placement of gastric tubes, invasive procedures in ICU (reintubation, tracheostomy, fiberoptic bronchoscopy), smoking, raised intra-abdominal pressure, hypoxia, and gene polymorphisms (single nucleotide polymorphisms of TNFα gene, ATG16L1 gene, and TREM 1 gene). Commonly
organisms causing early VAP (occurring within first 4 days of hospitalization) are Enterobacteriaceae and Staphylococcus aureus and those causing late VAP are non-fermenting bacteria and Enterobacteriaceae. Acinetobacter baumannii, Pseudomonas aeruginosa, E. coli, and Klebsiella are commonly isolated. Mortality from early VAP is nearly 19% and that of late VAP is 31.4%.6

Common organisms causing HAP are Pseudomonas aeruginosa, S. aureus, Klebsiella pneumonia, E. coli, S. marcescens, Stenotrophomonas maltophilia, and Acinetobacter baumanii.9

**Diagnosis of VAP and HAP**

Diagnosis of VAP should be made preferably by noninvasive sampling (endotracheal aspiration, spontaneous expectoration) rather than invasive sampling [protected specimen brush (PSB), bronchoalveolar lavage (BAL), and blind bronchial sampling (mini-BAL)]. Also, semiquantitative culture should be used for diagnosis. Diagnostic threshold for diagnosing VAP is more than 10³ CFU/mL by PSB or more than 10⁴ CFU/mL by BAL.

**Dermatological Infections**

Skin infections are also common in critically ill patients. In a study by Malheiro et al. in ICU patients, it was observed that immunosuppressive drug use, intravenous drug abuse, Type 2 diabetes mellitus, previous cutaneous or soft tissue infection (STI), HIV, cirrhosis, obesity, renal failure, etc. were risk factors for developing skin and soft tissue infections.10 They, however, included patients with necrotizing fasciitis, abscesses and cellulitis only. Fournier’s gangrene and cervicothoracic cellulitis were two most commonly observed fasciitis. Abscesses were commonest in cervical/thoracic region and cellulitis was observed in abdomen and lower limb. Myriad of organisms from E. coli to Staphylococcus aureus, Proteus, Klebsiella, etc. were isolated from different infection sites.

In a prospective analysis of ICU patients admitted due to surgical causes, 12.1% developed dermatological disorders out of which 28.8% had infectious dermatological lesions, 26% had dermatosis and 45.2% had maculopapular drug eruption was the commonest drug reaction observed.11

Almost similar findings (though with different proportions) were observed in patients admitted in Medical ICUs. The only difference from surgical ICU patients was that fungal skin infection was the most commonly observed infectious dermatological lesion. Mortality was significantly more in those who had dermatological lesions as compared to those who did not.12

**Treatment**

Treatment of any infection that develops in a critically ill patient depends upon the etiology, site, severity, and antibiotic sensitivity. Some infections like cellulitis, fasciitis may also require surgical intervention. Catheter removal might sometime need surgical assistance. Empirically started antibiotics must be according to the antibiogram of the hospital/institute.

**Prevention of Infection in Critically Ill Patients**

This is the utmost important part in critical care. Prevention is always better than cure.

**Prevention of CRBSI**

Centre for Disease Control and Prevention has laid down extensive guidelines for prevention of CRBSI. Some salient points are:

- Staff should be adequately trained and educated regarding insertion, care, and removal of catheters.
- Peripheral catheters to be inserted in upper extremities.
- Use midline catheters/PICC line if duration of intravenous therapy is likely to exceed 6 days.
- Do not remove gauze dressing at insertion site daily. Palpate the site for any tenderness or swelling. Remove peripheral catheter if there are signs of phlebitis.
- Use subclavian site for central venous catheter (CVC) insertion for nontunneled catheters to minimize infection. There is no recommendation for site for tunneled CVC insertion.
- Use CVC with minimum ports.
- Remove CVC as soon as its use is over.
Perform hand hygiene before and after catheter insertion, dressing, removal, guide wire change or catheter site palpation. This can be achieved by washing hands with soap and water or by applying alcohol-based hand rubs (ABHR).

Use maximum sterile barrier precautions (sterile gown/drape, sterile gloves, mask, and cap) while inserting arterial catheter, PICC, or CVC.

Insert CVC preferably by using ultrasound.

Clean skin with antiseptic (70% alcohol, tincture of iodine or alcoholic chlorhexidine gluconate solution) before peripheral venous catheter insertion. Prepare clean skin with more than 0.5% chlorhexidine preparation with alcohol before inserting CVC, PICC, or arterial catheter.

Use 2% chlorhexidine wash for patients daily skin cleansing.

Use a chlorhexidine/silver sulfadiazine or minocycline/ rifampin-impregnated CVC if the catheter is likely to be in place for more than 5 days and only if the Central Line Associated Blood Stream Infection (CLABSI) rate is not decreasing despite using a comprehensive strategy, which at least should include these three components: educating persons who insert and maintain catheter, using maximum sterile barrier precautions (as detailed in point 9) and more than 0.5% chlorhexidine preparation with alcohol for skin preparation before CVC insertion.

There is no need to use antibiotic prophylaxis while catheter is in situ. Also, there is no need to use anticoagulation.

Use antibiotic lock solution prophylaxis in patients who have long-term catheters and have history of multiple CRBSI despite optimal and maximal adherence to aseptic precautions.

Certain recommendations (apart from those mentioned above) in CDC guidelines were changed/addedin 2017. The details can be checked from CDC website.

Prevention of CAUTI

Insert catheter only for appropriate indication and leave in place for only as long as needed. Even for operative patients, use catheter only if required.

In males with bladder outlet obstruction/urinary retention, consider using external catheter in place of indwelling catheter. Intermittent self-catheterization can be done in patients with bladder dysfunction or with spinal cord injury.

Catheterization to be done by well-trained persons and hand hygiene to be practiced prior to insertion or after any manipulation of the catheter. Sterile technique to be used for insertion of catheter which entails using sterile gloves, gowns, and sponges and cleaning of periurethral area with appropriate antiseptic solution. There is no need to use antiseptic lubricants routinely. Catheter needs to be secured adequately after insertion.

Maintain a closed drainage system. Avoid kinking of catheter and collecting tube and ensure that collecting bag does not rest on the floor. Collecting bag to always be below the level of urinary bladder. Empty the container bag regularly using separate clean container.

There is no role of systemic antibiotic prophylaxis while catheter is in-situ. There is again no role of putting antiseptic/antimicrobial solution in collecting bag routinely. There is no recommendation for using urinary antibiotic (methanamine). Routine use of any catheter with antibiotic release cartridge installed in the catheter’s drain port is not necessary. There is no need to clean periurethral area with antiseptic solution daily when catheter is in place; cleaning of meatal area while daily bathing/sponging is sufficient.

Use antibiotic-impregnated catheters only, if CAUTI rate is not improving despite using a comprehensive strategy.

Silicone catheter might be preferable over other catheter materials in long-term catheterized patients who have frequent obstruction as it prevents encrustations. Hydrophilic catheter might be preferable to standard catheter in patients requiring intermittent self-catheterization.

Prevention of VAP

VAP can be prevented by following methods:

- Avoid intubation, if possible. Use noninvasive positive pressure ventilation (NIPPV).
- Minimize sedation. Try to give off to sedation once a day (awakening trial). Give spontaneous breathing trials. Try to club the two trials together.
- Provide early exercise and mobilization.
Use endotracheal tubes with subglottic secretion draining ports if intubation is likely to last for greater than 48–72 hours.

Elevate head end of the bed to 30–45 degrees.

Change ventilator circuits only if visibly soiled.

There are certain special approaches but they have insufficient data. These are selective oral or digestive decontamination, regular oral care with chlorhexidine, and use of prophylactic probiotics.

Certain approaches like using ultrathin polyurethane cuff endotracheal cuffs, saline, automated control of endotracheal cuff pressure, saline instillation before tracheal suctioning, and mechanical tooth brushing have low quality evidence to prevent VAP.

Conclusion

Infections in critically ill patients can increase the morbidity and mortality in these patients. Appropriate preventive measures can curtail occurrence of such infections.

References

Abstract

Sepsis syndrome, a dysregulated host response in infection, has multimodality and multifaceted treatment which has been changing over the decades since its recognition. Advances in management of the syndrome brought along with it many controversies and failures in treating it. The advances made in treatment kept changing with emergence of newer definitions, its assessment scores, role of infusion and combination empiric antibiotic therapy. The prognostic value of biomarkers to guide duration of antibiotics, fluid therapy, its role in endothelial glycocalyx protection along with antioxidant therapy and steroids is being evaluated.

Introduction

Sepsis is a life-threatening syndrome of a dysregulated host response to infection. Despite advances in diagnosis and treatment, sepsis still remains a significant cause of morbidity and mortality. Many aspects of the diagnosis and clinical management of sepsis require further study and remain controversial. Relevant literature and controversies regarding the overall evaluation and management of sepsis and septic shock need to be reevaluated time and again for management.

Newer Definitions of Sepsis and Its Impact on Diagnosis

The last two decades have seen ever changing definitions of sepsis, thus creating “more controversies” in the minds of bedside clinicians and emergency physicians. The changing clinical scenarios for the treating physicians at bedside further add to the already complicated issues of labeling the patient in sepsis. The effort to simplify the definition or criteria for defining sepsis, in recent times, has lead to increased diagnosis of this life-threatening syndrome of a dysregulated host response to infection. As of 1991, the initial definition stated that systemic inflammatory response syndrome (SIRS) to infection would be called sepsis. While subsequent revision of the definitions in 2001 specified the organ damage. Similarly, the new definitions 2016 defined it as a life-threatening organ dysfunction due to dysregulated host response to any infection. Later the role of Sequential Organ Failure Assessment (SOFA) was brought in where an organ dysfunction is assessed as an acute increase in total SOFA score by two points as a consequence of infection. Further adding to dilemma, the Sepsis-3 investigators introduced a new bedside index, called the quick Sequential Organ Failure Assessment (qSOFA). This was introduced to identify patients evolving into sepsis or could be in early sepsis in suspected infections. The qSOFA has just three easily measured physiological variables, that is, systolic arterial blood pressure ≤100 mm Hg, respiratory rate >21 breaths/min and altered mental status (GCS ≤ 13), with each receiving one point and range being zero to three. It is quite obvious that by these parameters there is a tendency to over diagnose sepsis in many patients having above
variables. As any two of these constitute a “positive” result and an indication that the patient is at risk of sepsis. The difficulty here is that it has a very low threshold (all that is needed is tachypnea and a slightly low blood pressure) to over diagnose sepsis. It needs to be understood that it is not a surrogate definition of sepsis but just an indication of increased risk.

Though this was a step to increase specificity of definition and has eliminated SIRS as was there in previous definitions, but it still has its own deficits. In absence of gold standard definition, the clinicians still adopt various parameters to diagnose sepsis by combining non-specific physiological and laboratory anomalies.

Similarly, the statement of definition as “life-threatening organ dysfunction caused by a dysregulated host response to infection” is more controversial in defining the terms like life-threatening, dysregulated, quantitative assessment of dysregulation.

Though the new Sepsis-3 definitions have reduced complexity, removed terms like severe sepsis, defined the role of qSOFA and has helped the emergency team categorize patients early into sepsis but still has major drawbacks. The controversies around microbiological confirmation, quantitative assessment of dysregulated syndrome, a syndromic approach, and over sensitivity of qSOFA remains to be further evaluated in bedside practices and epidemiological studies.

**Sepsis or Septic—Is there a Difference in Assessment**

The word “Sepsis” is being confused with “being septic.” As understood over the decades, sepsis by definition is bacteriological culture positivity of disease in any previously sterile body tissues or blood. While the word Septic signifies presence of any viral, protozoan, fungal, or any other illness leading to a diseased state. While SIRS also included non-infectious cause’s of sepsis syndrome. Simplicity of the term sepsis, defined as an invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic microorganisms can never be disputed, in spite of the fact that a microbiological confirmation is difficult at times. All the definitions of sepsis do not differentiate or distinguish between viral, parasitic, and other causes of sepsis. Similarly, regional infections, which necessitate the treatment variations, also need to be catered to especially when planning the health-care policies of the region. This will specifically target and reduce the mortality and morbidity in a particular region due to endemic diseases causing critical illnesses.

Abandoning terms like severe sepsis, septic shock, MODS, and septicemia have further widened the difference in concept of ideal or specific management strategies of these illnesses. Emergency physicians need to think more broadly and initiate appropriate management strategy on the basis of differential diagnoses, which is important factor in management and this has obviously been neglected in initial resuscitation strategies. In our endeavor to simplify criteria, there is a deficit in understanding disease pathophysiology and its impact on management strategies. The practical guidelines for the management in accordance with specific etiologies and organ involvement need to be considered and managed accordingly.

**Combination Empiric Antibiotic Therapy**

Combination of antibiotics is usually initiated on a clinical diagnosis of sepsis and this is usually done with broad spectrum antibiotics, in optimal doses. This is done with a view to have maximal coverage with a perspective to de-escalate once the organism is recognized and antibiogram available for further management. Though there are both advantages and disadvantages of using combination therapy, but no measurable survival benefit has been seen on routinely using a combination therapy except in few instances. The current recommendations for combination therapy as per Surviving Sepsis Campaign are only meant for the neutropenic patients, patients with suspected multidrug resistant organisms especially (Acinetobacter or Pseudomonas) and respiratory failure with sepsis. Though the combination therapy, for many multidrug resistant Gram-negative sepsis infections, is essential, but this cannot be followed as rule in light of studies suggesting that combination therapy was only effective if the drugs were effective in vitro too. Thus negating the suggestion that combination was always logical, as together the combinations overcome the so-called target attainment minimum inhibitory concentration (MIC) threshold, despite in vitro resistance. The definite advantage of combination therapy in form of a broad spectrum of activity, reduction in development of drug resistance, and synergism can never be denied but all this adds to the risk of toxicity, super infections, and additional cost.
Antibiotics Infusion in Sepsis

Different antibiotics differ in their mechanism of action, and in the case of $\beta$-lactams, the time above the MIC (T > MIC) (i.e., the duration of time that the antibiotic concentration in the relevant tissue space exceeds the minimal inhibitory concentration required to kill the bacteria) is the critical pharmacokinetic characteristic that determines its efficacy. This makes it pharmacokinetically more effective if it is administered as a continuous infusion to optimize the T > MIC. Dulhunty et al. showed that continuous infusion improved the pharmacokinetics but it did not improve the ICU survival rate or the statistically significant clinical cure.1 Similarly, the BLISS trial reached nearly, identical conclusions. A meta-analysis of 632 patients concluded that there was a statistically significant benefit of continuous infusion for both the clinical cure and its survival benefits. When this was analyzed by multivariate analysis the independent effect of continuous infusion was lost. Arguably it stands to reason that this intervention improves the clinical cure of infection, but as the survival of critically ill patient is a multifactorial variant thus the outcome may not be as expected in view of its dependency on various other factors.2

Biomarkers to Guide Duration of Antibiotics

Duration of an antibiotic therapy has always been based on the evidence of clinical course, cure, and the outcome of the infectious disease being treated. The duration of antibiotic therapy has been, as controversial as its initiation without a documented infection, and that too in critically ill patients. Though there is no conflict on the general principle that, it should be given for the shortest duration, but the exact duration of therapies have always been controversial except in few clinical conditions. The same holds for the patients being treated in critical care units.

The role of biomarkers in the last decade have changed the guidelines and treating principles, but the controversies in their potential use a sole criteria to guide a therapy also exist. The role of procalcitonin has been much studied, with over more than eight thousand papers defining its role in treatment of sepsis. The question which haunts any treating physician after the normal Procalcitonin report is whether the patient is infected and should an antibiotic be withheld. In case of abnormal reports even after adequate antibiotic therapy is whether the physician can de-escalate it. And obviously the answer stands to a good reason of clinical assessment.

As per recommendations of the Surviving Sepsis Campaign all biomarkers, including procalcitonin can be used by clinicians to assist him in withdrawal of antibiotics in septic patients especially those who have not been proven to have an infection on subsequent investigations after the initial therapy under septic bundle protocol therapy.

Procalcitonin

Among other biomarkers Procalcitonin has been advocated the most. Procalcitonin is up regulated by the cytokines that are secreted in bacterial infections in sepsis. Thus ideally it should not be used as guide in such patients, especially as a guide to decision-making regarding initiation of antibiotics. However, its use as in guiding de-escalation of antibiotic therapy after initial rise has more significance. All the studies, that is, PRORATA, ProGUARD, and SAPS trial’s found a non-significant reduction in days of antibiotic use in their intervention arms, while the impact on mortality showed no differences. However, in view of false positive levels in non-infectious inflammatory diseases and false negative in severe infections clinical decision and judgment should always be used to override the importance of serum levels.3

Fluid Therapy and Its Role in Endothelial Glycocalyx Protection

As controversies in fluid resuscitation in sepsis and septic shock exist even after three decades of active management, it is still eluding the question whether, under or over resuscitation is beneficial or not for sepsis management.

Endothelial glycocalyx, an endothelial cell surface layer composed of membrane anchored proteoglycans and heparan sulfate, essentially acts as a endothelial barrier and opposes leukocyte-endothelial adhesion.4 During sepsis, activation of heparanase in vessel wall leads to degradation of glycocalyx heparan sulfate thus furthering the endothelial dysfunction and injury caused due to sepsis. A hormone, released due to volume loading causing atrial stretching, that is, atrial natriuretic peptide furthers the degradation of endothelial glycocalyx, thus aggravating the septic vasoplegia. Though this phenomenon has not been explored much in humans,
but animal models have proved beyond doubt the amount of injury caused and the aggravation of the vasoplegia. Intravenous fluid resuscitation is associated with septic endothelial glycocalyx degradation. As expected, injurious effects of fluid therapy following resuscitation would worsen the outcome primarily by causing microcirculatory dysfunction. The potential beneficial hemodynamic effects of fluid resuscitation, thus being negated. Several recent randomized trials have demonstrated such worsening of sepsis after initial bolus intravenous fluids. The exact mechanisms by which such harm is caused need to be further evaluated and documented. Few studies also documented an association between volume of fluid used in resuscitation and intubation in high risk patients of heart failure, ESRD and Cirrhosis having sepsis. But similarly no differences in such events were detected in these patients who had received as per recommended guideline of 30 mL/kg of fluid for initial therapy.

**Steroids in Sepsis**

The immunosuppressive effects of steroids have harmful effect on severe sepsis but its role as anti-inflammatory and vasoactive substance cannot be denied. The controversies on the dose and duration are the most important issues in the management of sepsis. Though the harmful effects of high dose therapy have been well documented, but its therapeutic effects in low dose in sepsis are also less clear. The critical illness-related corticosteroid insufficiency has been well known. The ongoing efforts to prove the insufficiency as a diagnostic modality and the therapeutic options to correct have neither been proved or validated. The two studies, CORTICUS in 2008 and HYPRESS in 2016, evaluated the low-dose hydrocortisone versus placebo for their role in sepsis. Both the studies could not show any survival benefit over 28 days. Corticus trial did show faster resolution of shock but there was a non-statically significant increase in infections within the corticosteroid arm. Various other trials also lacked the uniformity in proving efficacy of steroids with respect to improvement in the severity of shock; along with ideal time of initiation of this therapy. Similarly, the duration and dose of therapy could not be ideally evaluated. The Surviving Sepsis Campaign guidelines in 2016 recommended the use of this therapy in septic shock refractory to fluid resuscitation. The meta-analysis of various studies showed reduction in 28-day mortality but such an effect could not be proven in all cause mortality in 90 days. Similarly, steroids therapy was associated with more-rapid resolution of shock and shorter duration of ICU stay. Therefore, in respect to above, an adjunct dose with 200 mg per day intravenous therapy was used in a study.

**Antioxidant Therapy with Vitamin C and Thiamine**

Protective effects of vitamin C against oxidative-stress-mediated cell damage and organ dysfunction in sepsis and septic shock are well known. Similarly, its deficiency has been observed in critically ill patients. During sepsis there is production of reactive oxygen species leading to endothelial injury and dysfunction, which is seen along with mitochondrial injury and both of these together lead to organ failure. Antioxidant activity of vitamin C helps in scavenging free radicals prevents production of the reactive oxygen species, acts as a neuroprotector, and helps as cofactor for synthesis of vasopressors, thus reducing the oxidant injury to vessel walls in sepsis. As humans cannot synthesize vitamin C, there develops a state of hypovitaminosis due to increased vitamin C consumption and reduced recycling, which needs to be corrected. This deficient state can only be restored efficiently with parenteral high-dose administration. Decrease in multi-organ failure and improvement in SOFA scores was documented in critically ill patients when treated with high dose of the antioxidants like vitamin C and E. Marik et al. in study concluded in 2017 that there was a significant decrease in mortality when vitamin C, hydrocortisone, and thiamine were used. Two prospective trials are currently underway that may shed more light on the efficacy of vitamin C as a treatment for sepsis.

Similarly, thiamine levels are reduced in 20–70% of the septic patients, and this is associated with worse outcome. Thiamine deficiency decreases the activity of enzyme involved in aerobic glycolysis, Krebs cycle, and pentose phosphate pathway. These in turn lead to reduced ATP production. ATP helps in maintenance of cellular pH and preservation of neurotransmitters both of which are important for cellular metabolism in critical illnesses. Thiamine also helps in protecting the kidneys from oxalate nephropathy due to high-dose vitamin C. Thus, thiamine...
played an important role in improvement of renal function without any impact on mortality.\textsuperscript{11}

Hydrocortisone and vitamin C, both together improved the endothelial barrier and microcirculation, and therefore together they increase the production of catecholamines and thus augment its vasopressor effects. Vitamin C also restores glucocorticoid expression of the vitamin SVCT2 transporter thus in turn improving glucocorticoid receptor function.\textsuperscript{12} And similarly both when combined showed higher in vitro barrier-protective effect after lipopolysaccharide exposure.\textsuperscript{13}

**Choice of Vasoactive Agents**

Several vasoactive agents can be used to support perfusion in septic shock. Catecholamines and vasopressin both function by stimulation of cardiac contractility and/or peripheral vasoconstriction, depending upon individual mechanism of actions. The Surviving Sepsis Campaign suggests norepinephrine, a potent agonist of both alpha and beta receptors, as the initial vasopressor of choice in septic shock. Norepinephrine is an endogenous catecholamine that stimulates alpha and beta receptors, while dopamine an endogenous catecholamine stimulates dopaminergic alpha, and beta receptors. Initiation of vasopressor therapy after initial fluid resuscitation is done to achieve an adequate mean arterial pressure (MAP). Norepinephrine has been used as a first-line vasopressor agent for a long now.\textsuperscript{14} As per the new recommendations, the vasopressors should not be delayed until fluid resuscitation is completed, but should be started rather early to achieve a target MAP of 65 mm Hg or more. During the recent decades, terlipressin, vasopressin V1A agonist, and even Ca\textsuperscript{2+} sensitizer have been increasingly used to treat septic shock. A meta-analysis of forty-three trials with 5,767 patients assessing seventeen treatment modalities showed vasopressor therapy with norepinephrine plus dobutamine to be most effective followed by norepinephrine plus epinephrine or terlipressin.\textsuperscript{15} The studies have proved beyond doubt that norepinephrine should be used as a first-line treatment, but when followed by addition of dobutamine it would reduce the 28-day mortality. The serious potential adverse effect of precipitating ventricular and supraventricular arrhythmias needs to be actively monitored during its use especially with individuals having low-cardiac output due to impaired contractility.

**Conclusion**

Sepsis a multifaceted disease needs to be managed with simpler and universal guidelines, which are appropriate and can be delivered at all categories of health-care system. Initial approach needs to be aggressive and time bound even if diagnosis is underway as per clinical bedside guidelines and the clinical scenario. Initial resuscitation with fluids and in responsive patients the fluid therapy needs to be modified. Role of vasopressor is well documented and it should not be denied early in treatment with its weaning as per documented response. Initial antimicrobial therapy with a goal to target pathogens found in the suspected disease followed by change in antibiotics as per antibiogram and susceptibility should be done. Antimicrobial therapy guided by the relevant biomarkers reduction should be administered for a total duration of 7–10 days. Though, this can be of shorter or longer duration as per the clinical scenarios. Antioxidants and steroids have been documented to improve the outcomes and should be administered till further studies evaluate their role.

**References**


**Abstract**

Viral hemorrhagic fevers (VHFs) are a group of severe multisystem syndromes that are caused by several distinct families of viruses. Five distinct families of RNA viruses Arenaviridae, Filoviridae, Bunyaviridae, Flaviviridae, and Paramyxoviridae are well known for various VHFs in different geographic regions. The recent ongoing pandemic of SARS-CoV-2 Coronavirus virus primarily presents with high-grade fever, cough, and breathlessness due to primary involvement of lungs. But COVID-19 has been found to have multisystem inflammation with Kawasaki Disease like presentation in children, human-to-human transmission, and thromboembolic phenomenon; therefore, it can be considered as emerging VHFs. The CDC-USA defines VHF as acute onset fever (>40°C) with any one of the following clinical finding: severe headache, muscle pain, erythematous maculopapular rash on the trunk with fine desquamation 3–4 days after rash onset, vomiting, diarrhea, pharyngitis (arenavirus only), abdominal pain, bleeding not related to injury, retrosternal chest pain (arenavirus only), and proteinuria (arenavirus only). VHF viruses spread in a variety of ways, but share some common pathogenic features. They have the potential for aerosol dissemination via respiratory droplets and are dependent on an animal or insect host for survival. These viruses are usually restricted to some geographic place of domicile of the host species. After the accidental transmission from the host, human-to-human transmission is possible in some viruses. The mortality rate is highly variable, 0.5% for dengue to 90% for Ebola virus disease. The VHF outbreaks cannot be easily predicted, as they are sporadic and irregular. The blood, urine, vomitus, pus, stool, semen, and saliva from the VHF patient are usually infectious. Barrier nursing practices (such as wearing personal protective equipment) help in reducing the risk of transmission to health-care workers. There is no specific treatment for majority of VHFs, except supportive care.

**Introduction**

The term “viral hemorrhagic fevers” (VHFs) depicts a group of diseases that are caused by several distinct families of viruses. In general, it is used to describe a severe multisystem syndrome. The VHFs is mainly caused by six distinct families of RNA viruses Arenaviridae (lassa), Filoviridae (ebola), Bunyaviridae (Crimean-Congo hemorrhagic fever, CCHF), Flaviviridae (dengue), Paramyxoviridae (nipah), and Coronavirusidae (COVID-19). The SARS-CoV-2 virus (family-coronaviridae) with the recent pandemic has shown to cause multisystem disease with respiratory illness as the primary organ involvement. The properties of multisystem involvement with Kawasaki like disease presentation, aerosol transmission, human to human spread, and various thromboembolic phenomenon reported from autopsy series, makes COVID-19 a new candidate for VHF. The SARS-CoV-2 virus has already spread to more than 216 countries and territories and was declared pandemic by WHO on March 11, 2020, it has already caused >550 million infections and 13 million deaths worldwide.

Though VHF viruses spread in a variety of ways, they share some common pathogenic features. They have the
potential for aerosol dissemination via respiratory route (except dengue), but they are dependent on an animal or insect host for survival. However, these viruses are geographically restricted to the place of domicile of the host species. After the accidental transmission from the host, human–human transmission is possible in some viruses. VHF may impair the blood clotting ability and can also damage the walls of small blood vessels. The mortality rate of viral hemorrhagic fever ranges between 0.5–90%, depending on the pathologic agent. The VHF outbreaks cannot be easily predicted, as they are sporadic and irregular.

### Clinical Criteria for VHF

The Center of Disease Control (CDC), USA, has made the VHF definition (2010). A person with acute onset disease with all of the following clinical finding:

- A fever >40°C, and
- One or more of the following clinical finding: Severe headache, Muscle pain, Erythematous maculopapular rash on the trunk with fine desquamation 3–4 days after rash onset, Vomiting, Diarrhea, Pharyngitis (arenavirus only), Abdominal pain, Bleeding not related to injury, Retrosternal chest pain (arenavirus only), Proteinuria (arenavirus only).

The predominant signs and symptoms noted in common VHF are listed in Table 1.

### Table 1: Predominant signs and symptoms noted in common VHF

<table>
<thead>
<tr>
<th>Disease</th>
<th>Signs and symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ebola virus disease</td>
<td>Fever, headache, muscle pain, fatigue, weakness, diarrhea, vomiting, abdominal pain, conjunctival injection, chest pain, hemorrhage</td>
</tr>
<tr>
<td>Marburg virus disease</td>
<td>Fever, chills, headache, muscle pain, maculopapular rash, nausea, vomiting, chest pain, sore throat, abdominal pain, diarrhea, jaundice, hemorrhage</td>
</tr>
<tr>
<td>Lassa fever</td>
<td>Fever, nausea, vomiting, diarrhea, retrosternal chest pain, sore throat, muscle pain, enlarged cervical lymph nodes, abdominal pain, bleeding, maculopapular rash, conjunctivitis, headache</td>
</tr>
<tr>
<td>Crimean-Congo hemorrhagic fever</td>
<td>Fever, headache, back pain, join pain, abdominal pain, vomiting, conjunctival injection, facial flushing, petechial rash, jaundice, bleeding, photophobia, sore throat</td>
</tr>
</tbody>
</table>

### Ebola Virus Disease

Ebola virus disease (EVD) is a rare, but deadly disease commonly affecting humans and non-human primates. The EVD viruses are mainly located in sub-Saharan Africa and their periodic emergence has caused several outbreaks in African countries. The EVD gets transmitted to humans through direct contact with an infected animal (bat or non-human primate) or a sick or dead person infected with the virus. Ebola virus was first described in 1976 near the Ebola River, which currently belongs to the Democratic Republic of Congo (formerly Zaire).

In 1995, an outbreak of Ebola hemorrhagic fever affected more than 300 people in and around the city of Kikwit, Democratic Republic of the Congo. The outbreak caused the death of approximately 80% of the patients and more than one-fourth of all the patients were health-care workers. The 2014–2016 outbreak caused a mortality rate of up to 80–90%, and the death of many health-care workers were due to human-to-human infection. On March 23, 2014, the World Health Organization (WHO) reported the EVD disease within the forested rural region of southeastern Guinea. It was the beginning of the West Africa Ebola epidemic, the largest in history. On August 8, 2014, WHO declared the Public Health Emergency of International Concern (PHEIC), which is designated only for events with a risk of potential international spread or that require a coordinated international response. Over the duration of the epidemic, the disease had spread to seven more countries: Italy, Mali, Nigeria, Senegal, Spain, UK, and the US.

### EBV: Ecology and Transmission

Humans get initially infected with EBV through contact with an infected animal, such as a fruit bat or non-human primate. This is called a spillover event. After the spillover event, the virus can spread from person to person through the following routes:

- Direct contact (such as through broken skin or mucous membranes in the eyes, nose, or mouth)
- Blood or body fluids (urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with or has died from EVD
- Objects (such as needles and syringes) contaminated with body fluids from a person sick with EVD or the body of a person who died from EVD
Infectious Disease

- Infected fruit bats or non-human primates (such as apes and monkeys)
- Semen from a man who recovered from EVD (through oral, vaginal, or anal sex)
- Handling and consumption of bushmeat (wild animals hunted for food)

There is no treatment for EBV and only supportive management can be adopted. Ebola survivors mostly complain of myalgia and muscle pain even after treatment.

**Dengue Fever**

Dengue fever, the most important mosquito-borne viral disease with global epidemic potential, occurs mainly in tropical and subtropical areas of the world. The virus is transmitted to humans through the bites of infected female mosquitoes of the species *Aedes aegypti.* It is a mild to fatal disease with no cure and only palliative care. The following factors have contributed to the emergence of dengue as the classic disease of 21st century: urbanization, increase in travel/trade, highly efficient and adaptive mosquito vector, thriving of larvae and adults in urban areas, inability to avoid day biting of *Aedes* vectors, and difficulty in effectively implementing environmental vector control.

A 30-fold increase in the dengue cases has been recorded globally during the past 50 years, and it is associated with substantial social and economic burden. An average dengue episode results in a loss of 14.8 days for ambulatory patients, at an average cost of USD 514. However, the mortality rate is less than 0.5%, and it is asymptomatic in nearly 80% of the subjects.

The first evidence of occurrence in India was reported in 1956 from Vellore district in Tamil Nadu. In 1996, one of the most severe outbreaks of dengue fever occurred in Delhi, with 10,252 reported cases and 423 deaths. In 2006, the country witnessed an outbreak of dengue fever with 12,317 cases and 184 deaths. During 2014, a total of 40,571 cases were reported, which increased to 1,29,166 in 2016 and 1,88,401 in 2017.

**Clinical Presentation**

The clinical presentation of dengue progresses through the following three phases:
- **Febrile phase (4–7 days after exposure):** Headache, eye pain, nausea/vomiting, myalgias, arthralgias, and macular rash
- **Critical phase (may develop following resolution of febrile phase, lasts 24–48 hours):** Shock, hemorrhage, organ failure, and acute respiratory distress syndrome (ARDS)
- **Recovery phase:** Clinical stabilization, may develop confluent rash

**Diagnosis and Management**

Initial diagnosis may be established by clinical suspicion. Serum RT-PCR or viral antigen testing within first week of illness, followed by enzyme-linked immunosorbent assay (ELISA), may assist in concluding the diagnosis.

The national vector borne disease control program (NVBDCP) 2014 guideline revised the case definition and made a new classification with moderate dengue (Flowchart 1) and guidelines also modified clinical management of severe dengue with bolus fluid regimen. The bolus infusion of 10–20 mL/kg crystalloid infusion and frequent monitoring drastically reduced mortality in dengue patients with profound shock (Flowchart 2).

**Chikungunya Fever**

Chikungunya virus is a self-remitting febrile viral illness transmitted through the bite of infected mosquito *Aedes aegypti.* The clinical presentation includes acute infection, high-grade fever, polyarthralgia (typically bilateral/symmetric, distal > proximal joints), and macular rash. The severe complications are meningoencephalitis, respiratory failure, renal failure, hepatitis, hemorrhagic, and heart failure/cardiomyopathy. The disease can be diagnosed by RT-PCR or serology. Testing for dengue and Zika can also be considered. Management includes supportive care and fluid therapy. Aspirin and other non-steroidal anti-inflammatory drugs should be avoided to reduce the risk of hemorrhage, until patient is afebrile for 48 hours, and there are no additional warning signs for dengue.

**Zika Virus Infection**

Zika virus is spread mostly through the bite of an infected *Aedes* species mosquito (*Aedes aegypti* and *Aedes albopictus*). Common symptoms of Zika infection include fever, pruritic rash, and arthralgia. The severe complications of the disease are Guillain-Barré syndrome and neurologic complications including encephalitis and transverse myelitis. The recent ongoing outbreak...
Flowchart 1: The NVBDCP dengue case definition

<table>
<thead>
<tr>
<th>Dengue viral infection</th>
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</thead>
<tbody>
<tr>
<td>Symptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Mild dengue</td>
<td>Moderate dengue</td>
</tr>
<tr>
<td>DF with high risk</td>
<td>DF with warning signs and symptoms/DHF Gr I and II with minor bleeding</td>
</tr>
<tr>
<td>Comorbid conditions</td>
<td></td>
</tr>
<tr>
<td>Infants</td>
<td>In infants, old age, diabetes, hypertension, pregnancy, CAD, hemoglobinopathies, immunocompromised patient, patients on steroids, anticoagulants or immunosuppressants</td>
</tr>
<tr>
<td>Close monitoring* and possibly hospitalization</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary level care</td>
<td></td>
</tr>
</tbody>
</table>

*Close monitoring: HR, Pt, Hb, fluid intake/output, Pulse rate, Respiratory rate, Blood pressure, sensorium

in Madhya Pradesh and Rajasthan (Oct-Nov, 2018) has claimed two lives. Diagnosis and management strategies are similar to that of dengue and chikungunya.

**Crimean-Congo Hemorrhagic Fever**

Crimean-Congo hemorrhagic fever (CCHF) is caused by a tick-borne virus (Nairovirus) belonging to the family *Bunyaviridae*. The disease is usually seen in Crimea, Africa, Europe, and Asia; and human-to-human transmission occurs through direct contact with infectious blood/body fluids. In India, the first confirmed case of CCHF was reported during a nosocomial outbreak in Ahmadabad, Gujarat, in January 2011. The outbreak claimed the death of 3 health-care workers due to multiple organ failure, specifically failure of the liver and kidney. During the period of 2012–2015, several outbreaks of CCHF infections were reported in the states of Gujarat and Rajasthan.

**Kyasanur Forest Disease**

Kyasanur forest disease virus (KFDV) was first identified in 1957 when it was isolated from a sick monkey from the Kyasanur forest in Karnataka (formerly Mysore) state, India. Since then, around 400–500 human cases per year have been reported. KFDV is a member of the virus family *Flaviviridae*. Hard ticks (*Haemaphysalis spinigera*) are the reservoir and rodents, shrews, and monkeys are common hosts for KFDV. The disease is endemic to South Asia and the human transmission may occur after a tick bite or contact with an infected animal. No person-to-person transmission has been reported.
The disease begins with chills, fever, and headache. Severe muscle pain with vomiting, gastrointestinal symptoms, and bleeding problems may occur 3–4 days after initial onset of symptoms. Patients may experience abnormally low BP, low platelets and red blood cells, and leucopenia. However, after 1–2 weeks of symptoms, some patients recover without complications.

**Nipah Infection**

Nipah virus (NiV) infection is an emerging zoonotic disease of public health importance in the WHO Southeast Asia region. The possible routes of transmission include consumption of fruit contaminated by the saliva of infected bats, from direct contact with infected bats or their feces/urine. NiV was first recognized in 1998–1999.
during an outbreak among pig farmers in Malaysia and Singapore, and it was first recognized in India and Bangladesh in 2001. In July, 2018, a total of 19 NiV cases, including 17 deaths, were reported from two districts in Kerala state (Kozhikode and Malappuram).14

**COVID-19**

The recent outbreak of COVID-19 in Wuhan city, Hubei province, China caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) mainly leads to the respiratory illness. The virus is transmitted mainly by respiratory droplets15 and causes mild symptoms (asymptomatic/fever/cough/myalgia/sore-throat/anosmia) to severe symptoms (ARDS, shock, respiratory failure, multi-organ dysfunction) and deaths. The COVID-19 can cause some cutaneous manifestations which can be grouped into five major clinical patterns:

- Acral areas of erythema and edema with vesicles or pustules (pseudo-chilblain),
- Vesicular eruptions,
- Urticarial lesions,
- Maculopapular lesions,
- Livedoid or Necrotic lesions.

This is significant as it tells about the severity with pseudo-chilblains in mild disease to livedo-necrosis in severe disease.16 The current epidemiological update by WHO for COVID-19 (30th October, 2020) showed 4,48,88,869 confirmed cases and 11,78,475 confirmed deaths. The diagnostic laboratory studies are rPCR, rapid antigen testing, Antibody Testing (IgG,IgM), viral culture, computed tomography, chest X-ray. Laboratory tests of serum LDH, CRP, Serum Ferritin, D-Dimer, and CBC may be helpful in severe diseases with cytokine storm. The various antiviral therapies are only approved as emergency use authorization with no survival benefit. Steroid and oxygen therapy have been found to be effective in reducing mortality in hypoxic patients with moderate to severe COVID-19 (SpO₂ <95%). The general measures for infection control: handwashing, wearing masks, social distancing, and isolation of infected individuals have been found to be effective in controlling the infection in hospital and society.

Differentiating COVID-19 from other tropical disease (dengue/chikungunya) is a challenge for clinician. Similarly, coinfection of COVID-19 with other VHF dengue fever etc. is another big problem in endemic regions of vector borne diseases (Table 2).18

**Management of VHF**

VHFs are a group of distinct RNA viral diseases and it needs individualized care. The EVD has the highest mortality rate, while COVID-19 disease has affected maximum number of countries and population. The blood, urine, vomitus, pus, stool, semen, and saliva from the VHF patient are infectious. Barrier nursing practices (such as wearing protective clothing) help in reducing the risk of transmission to health-care workers.

No specific treatment, except supportive care, is available in most of VHFs. Correction of coagulopathies is needed. Antiplatelet drugs and IM injections are contraindicated due to the risk of hemorrhage. Investigational treatment approaches include ribavirin for 10 days for *arenaviridae* and *bunyaviridae*, and convalescent plasma within 8 days of onset for alkhurma hemorrhagic fever. Upon percutaneous/mucocutaneous exposure to infected blood or body fluids, wash thoroughly with soap and water, and irrigate mucous membranes with water or saline. Medical surveillance for all potentially exposed persons is needed for up to 21 days in ebola infection. The surveillance measures include: reporting hemorrhagic symptoms, recording fever 2×/day, reporting temperatures ≥101°F (38.3°C), and initiating presumptive therapy.

Viral hemorrhagic infection can be prevented using N-95 mask or powered air purifying respirator (PAPR). Keeping the patient in negative pressure room and using personal protective equipment (PPE) while handling the patient are essential. The health-care workers should be trained on the use of PPE.

**Assessment for VHF Risk**

The following CALM (Consider, Act, Laboratory, Monitor) algorithm is used to assess the risk of VHF infection in travelers:

- **Consider:** Travelers return from a region endemic for and/or currently experiencing VHF outbreaks are considered infected.
- **Act:** Isolate the patient. Limit the health-care workers who enter the room. Appropriate PPE should be
TABLE 2 Differentiating clinical features between COVID-19 and other similar viral diseases

<table>
<thead>
<tr>
<th></th>
<th>COVID-19</th>
<th>Dengue</th>
<th>Chikungunya</th>
<th>Seasonal influenza</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation period and onset</strong></td>
<td>Ranges 2–14 days (onset of symptom average 5–7 days). Acute onset of low-to-moderate grade continuous fever</td>
<td>Ranges 3–14 days (onset of symptom average 4–7 days). Acute onset of high-grade continuous fever</td>
<td>Ranges 1–12 days (onset of symptom average 3–7 days). Acute onset of moderate-to-high grade continuous fever</td>
<td>Ranges 1–4 days (onset of symptom average 2 days). Acute onset of moderate-to-high grade continuous fever</td>
</tr>
<tr>
<td><strong>Clinical presentation symptoms</strong></td>
<td>Cough, dyspnea, fever, myalgia, headache, sore throat, diarrhea, abdominal pain, anosmia, ageusia, fatigue, confusion</td>
<td>Fever, headache, Nausea, vomiting, retro-orbital pain, myalgia, arthralgia, rash, bleeding</td>
<td>Fever rash malaise arthralgia myalgia red eyes</td>
<td>Fever, cough, sore throat, and nasal discharge, headache, myalgia and malaise</td>
</tr>
<tr>
<td><strong>Signs</strong></td>
<td>Tachypnea, decreased oxygen saturation, multi-organ involvement</td>
<td>Signs of hypotenison and shock, hemorrhagic manifestations (petechiae), positive tourniquet test</td>
<td>Swelling and tenderness of joints</td>
<td>Pharyngeal wall hyperemia, cervical lymphadenopathy</td>
</tr>
<tr>
<td><strong>Warning signs</strong></td>
<td>Respiratory distress SpO₂ &lt;94%</td>
<td>Persistent vomiting, abdominal tenderness, fluid accumulation, mucosal bleed</td>
<td>High grade fever, progressive increase of myalgia and arthralgia</td>
<td>Respiratory distress SpO₂ &lt;94%</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>ARDS arrhythmias acute cardiac injury shock pulmonary embolism shock acute stroke</td>
<td>Hypotensive shock, bleeding, organ involvement, metabolic derangement</td>
<td>Respiratory failure, cardiovascular decompensation, myocarditis, acute hepatitis, renal failure, hemorrhage, meningoencephalitis acute flaccid paralysis GBS</td>
<td>ARDS myositis rhabdomyolysis acute MI myocarditis pericarditis encephalitis myelitis GBS</td>
</tr>
</tbody>
</table>

References

7. **Ebola Virus Disease.** Available from https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease

Worn by all personnel entering the patient’s room. Immediately notify your state/local health department.

- **Laboratory:** Inform the laboratory. Decision to test for VHF should be made in consultation with relevant health department/CDC viral special pathogens branch.
- **Monitor contacts:** Facilities should maintain a log of all the persons entering the patient’s room, including full name and contact information.

**Conclusion**

VHF is a diverse group of illnesses caused by RNA viruses belonging to various viral families. Though, the diseases differ by geographic occurrence and vectors/reservoirs, they share some common clinical features. The diseases are considered as having international health risk due to their potential for aerosol dissemination and human-to-human transmission. Management is only through supportive treatment. Infection control in health-care workers and relatives is of utmost importance. Surveillance of returning travelers by CALM algorithm is important to prevent any outbreak in other geographic area.
Antimicrobial stewardship focuses on interventions to improve and measure the appropriate use of antimicrobial agents. To fight antimicrobial resistance, timely diagnosis of infections, justifiable use of drugs, and avoidance of antimicrobial overprescribing and overuse are of paramount importance. Novel biomarkers aid in this process and are thereby gaining prominence. Biomarkers may be included into clinical guidelines to make their roles clear. This chapter discusses various biomarkers specific to bacterial, viral, and fungal infections which can be used for point of care testing. Biomarker based clinical algorithms prepared using regional data will help in clinical decision-making, thereby aiding rational prescription of antimicrobials.

Introduction
Antimicrobial stewardship is defined as “coordinated interventions designed to improve and measure the appropriate use of antimicrobial agents by promoting the selection of the optimal antimicrobial drug regimen including dosing, duration of therapy, and route of administration.”

In the background of increased incidence of antimicrobial resistance throughout the world, antimicrobial stewardship programs are focusing on timely diagnosis of infections and the justifiable use of drugs. Frequently, ignorance of the etiology of infection leads to antimicrobial overprescribing and overuse. This diagnostic dilemma may also result in late initiation of antimicrobial therapy and poor outcomes. Recently new biomarkers have been used to help making a clinical diagnosis. Novel biomarkers are an important addition in the repertoire of antimicrobial stewardship program, to aid in timely diagnosis and appropriate antimicrobial use.

In one estimate from the USA it was observed that close to 50% of antibiotic prescriptions in the outpatient scenario were avoidable, thus leading to unnecessary expenditure, antibiotic resistance, adverse drug reactions, and secondary infections with Clostridium difficile. Rapid biomarker testing is need of the hour. However, till now the rules for integrating biomarkers in antibiotic prescription decisions are not well known. Defined reference values for bacterial infections are often absent, thus affecting the sensitivity and specificity of biomarkers. Use of biomarkers may be included into clinical guidelines to make their roles clear. This will be particularly helpful in the primary care setting where patient turnover is quite high and antibiotic prescription is widespread.

Biomarkers
The following biomarkers will be discussed further and their use particularly for suspected pneumonia or sepsis will be defined:
- Bacterial—C-reactive protein (CRP), procalcitonin (PCT)
- Fungal—1,3-beta-D-glucan (BDG), Candida albicans germ tube antibody (CAGTA), and Galactomannan (GM)
• Viral markers—Myxovirus resistance protein A (MxA)
• Others—Adrenomedullin (ADM), Triggering receptor on myeloid cells 1 (TREM 1), Urinary clusterin, etc.

**Bacterial Infection Biomarkers**

**C-reactive Protein**

An acute phase reactant that indicates inflammation due to multiple causes. Use of CRP in differentiating between bacterial and viral etiology has been studied widely. It has been proven that CRP is very sensitive in identifying infections of bacterial etiology and thus helps in taking decision on instituting antibiotics.6

In a study of patients in Vietnam suffering from acute respiratory tract infections it was found that using CRP threshold of 20 mg/L in adults, there was reduction of antibiotic prescription from 78% to 64%. Similarly, in primary care setting, rapid CRP testing with a threshold of 40 mg/L resulted in a significant reduction in antibiotic prescription.7,8

**Procalcitonin**

PCT is an amino acid prohormone of calcitonin. On exposure of body to bacterial toxins, serum PCT becomes detectable in blood. Interferon-gamma released in viral infection downregulates its release. Other inflammatory states usually do not affect the serum concentrations of PCT. False positive increase of levels have been seen in burns, post-surgery, trauma, and renal dysfunction. False negative results may occur in too early testing or in loculated infections.9

**Role in Respiratory Tract Infections**

PCT has been used as a tool to help in diagnosis of bacterial pneumonia when there is clinical dilemma or negative culture results. Serial PCT concentrations have helped in deciding escalation/de-escalation/discontinuation of antimicrobial therapy. Various guidelines (Surviving Sepsis guidelines and IDSA) recommend repeating PCT levels serially to decide duration of antibiotic therapy in critically ill patients.10 Standard reference value of PCT in adults is usually 0.15 ng/mL or less. PCT rises within 2–6 hours and peaks at 12–24 hours.7,11

**Role in Sepsis**

Meta-analyses have established PCT as superior to CRP for the diagnosis of sepsis.12 Serial PCT measurements should be taken in sepsis and levels of less than or equal to 0.5 µg/L should signal the need for discontinuation of antibiotics.

From available literature, use of PCT for antibiotic stewardship in respiratory infections (specially LRTI) has been found to reduce initial prescription of antibiotics by 40–50% in emergency patients and by 70–80% in ambulatory patients. It has also reduced antibiotic prescription in pneumonia (CAP) by 40–50%.13-15

**Fungal Infection Biomarkers**

Detection of invasive candidiasis is challenging. High cost of new antifungals adds to the problem. Traditional culture methods are unable to detect close to 50% of cases. Thus, fungal infection biomarkers may improve patient outcomes.36

**1,3-beta-D-glucan**

BDG is a component of the cell wall of most fungi including Candida species. Elevated levels in blood can act as a biomarker to diagnose candidemia or invasive fungal infection and thus rationalize antifungal administration. False-positive results can be found with intravenous immunoglobulin, intravenous albumin, certain hemodialysis filters, and with some antibiotics. Serial BDG assays have a greater negative predictive value thereby guiding discontinuation of empirical antifungal therapy.

**Candida Albicans Germ Tube Antibody**

*Candida albicans* germ tube antibody can be released during invasive candidal infection. Its sensitivity and specificity to detect invasive candidiasis has been reported as more than 75% and more than 90%, respectively.17 Although study results are variable, CAGTA with or without BDG can aid in diagnosis of invasive candidiasis. However, the utility of CAGTA on course of illness is debatable.18

**Galactomannan**

Blood assay for GM has improved detection of invasive aspergillus infection at an early stage, particularly among the immunocompromised. Sensitivity and specificity of this marker are more than 70% with cut-off value of 0.5.19 The IDSA guidelines recommend use of GM for prompt diagnosis of invasive aspergillus infection especially in
patients with hematological malignancy or post-stem cell transplant state.20

**Viral Infection Biomarkers**

**Viral (Myxovirus Resistance Protein A)**

Myxovirus resistance protein A is induced by interferons and is specifically elevated in patients with viral infections. It has the potential to allow rapid differentiation between viral and bacterial respiratory infections. In combination with CRP or PCT, elevated levels of MxA can help identify patients with likely viral infection, thus helping antibiotic stewardship activities.21

**Other Biomarkers**

**Adrenomedullin**

ADM is a peptide hormone. Its half life is very less; hence pro ADM (cut-off values 3.5-5.5 nmol/L) is often measured clinically. ADM has been used clinically as a mortality predictor and prognostic marker in inflammation and particularly in Community acquired Pneumonia.22 Increased ADM concentrations will indicate increased severity and mortality in SIRS and septic shock. Pro-ADM may be better in this regard.

**Triggering Receptor Expressed on Myeloid Cells 1**

TREM 1 is an immunoglobulin expressed on the surface of macrophages, neutrophils, and monocytes. Phagocytes release soluble TREM 1 (sTREM 1) on stimulation, which acts as a marker of infection specifically for pneumonia and sepsis.23 Measurement thresholds are 725 pg/mL to diagnose sepsis (sensitivity up to 70% and specificity up to 60%),23 and 5 pg/mL in the diagnosis of pneumonia (sensitivity of 98% and specificity of 90%).24

**Urinary Clusterin**

Its use has been studied as a biomarker of nephropathia epidemica (a type of hemorrhagic fever with renal syndrome) caused by Puumala virus infection.25

**Methicillin-resistant Staphylococcus Aureus (MRSA) Nasal Screens**

It can be used in de-escalation of MRSA therapy, mainly in patients with suspected or confirmed pneumonia. The ATS/IDSA guidelines endorse the routine use of MRSA nasal PCR screening for the de-escalation of MRSA coverage. This has been shown to allow a median decrease of 2.1 days of vancomycin therapy.26

**Other Potential Bacterial Biomarkers in Development**

These are Amyloid A, Liposaccharide binding protein, Interleukin-10, and nCD64.27

**Conclusion**

Point-of-care biomarker testing allows rational antimicrobial use. Stewardship programs should analyze regional data of local population for using biomarkers in clinical decision-making. More regional studies are required for preparing biomarker based clinical algorithms.

**References**

Abstract

Biological Warfare is the intentional use of biological agents to cause morbidity and mortality in humans. The Bioweapons, those used in wars and by terrorist groups are attractive because of their ability to produce wide range of diseases, low production costs, no easy accessibility by routine security systems, and their easy transport from one place to another. In addition, after development of novel technologies that are primarily designed for use in early diagnosis and treatment and decreases the burden of diseases and its consequences on humans health, but this development of medicinal novel technology has often been used in wrong direction to produce more Bioweapons which ultimately threaten the well-being of the whole mankind.

Introduction

From ancient ages to modern era, history reveals there were multiple number of biological wars among persons, among states, and among nations to empower over others. There was an intention behind use of biological agents like microorganisms, and toxins, generally of microbial, plant, or animal origin to produce not only disease and/or death in humans and damaging live stocks and crops but also to create fear, panic, and paralyze uncertainty.

To protect this there were developments of biodefense in the form of biological weapons convention from time to time, but still there is continuous research on development of bio-weapons by the nations. Now for protection against biological warfare each nation continued research along counter measures, including vaccines and antisera.

Definitions

Bioterrorism: It is an action by a non-state actor to achieve a political, ideological, or religious goal. (Desire to terrorize as much or more than causing casualties.)

Biocrime: Biological agent used by a person/group against a person/small group often for revenge or extortion.

Biological warfare: A state actor uses a biological agent as part of its armamentarium in waging war.

Biological agents: Biological agents (bio-weapons) are living organisms or replicating entities (viruses) that reproduce or replicate within their host to cause harm.

CDC category of biological agents:

- **Category A**: These are high priority agents that easily disseminated or transmitted from person to person with high mortality rates. With a potential for major public health impact, might cause public panic and social disruption.
- **Category B**: Second highest priority. These are moderately easy to disseminate. They have moderate morbidity rates and low mortality rates. These agents require specifically enhanced diagnostic capacity.
- **Category C**: Emerging pathogens that could be engineered for mass dissemination in the future because of availability, ease of production, and
dissemination and potential for high morbidity and mortality rates and major health impact. The category and agents are shown in Table 1.

**Anthrax (Bacillus anthracis)**
- The most misused agent in biological warfare
- Route of transmission: By cutaneous and inhalation
- Signs and symptoms:
  - Prodermal—fever, headache, tiredness
  - Cutaneous (95%)—pustules, eschar (Figs. 1A to C)
  - Pulmonary (5%)—cough, chest pain, dyspnea
- Diagnosis:
  - Skin biopsy for cutaneous lesions
  - Blood culture
  - ELISA, PCR
  - Chest X-ray- widened mediastinum (Hilar and mediastinal lymphadenopathy) with or without infiltrate and pleural effusion (Figs. 2A and B)
- Treatment:
  - Ciprofloxacin, Penicillin, Doxycycline
  - Treated for 60 days
- Prevention:
  - Vaccination—6 doses over 18 months, booster annually
  - Chemoprophylaxis—Cipro/Doxy 4 weeks before exposure
- Infectious form: Spores-Hardy, resistant to environmental conditions
- Relatively easy to weaponize

**Example:**
- September 2001, Anthrax used as bio-weapon through US Postal system
- 22 cases (18 confirmed)—11 inhalational + 11 cutaneous.
- 5 deaths (all among inhalational)
- Ames strain used (beta lactamase + cephalosporinase), but luckily susceptible to antibiotics
- Maximum amount of spore in a letter—2 g (100 billion to 1 trillion spores) (LD 50 = 10,000)

**Plague (Yersinia pestis)**
- Highly contagious. Pneumonic plague is most severe.

**Signs and symptoms:**
- **Pneumonic plague:** Due to inhalational exposure. Cough with blood tinged sputum.
- **Bubonic plague:** Due to infection through skin causes ulcers (Fig. 3), Fever, Chills, Nausea, Vomiting. Buboes (Fig. 3) (1–8 days).
- **Septicemic plague:** Usually from bubonic plague fever, chills, nausea, vomiting, bleeding in skin, ischemia in limbs.
Figs. 1A to C: Skin lesions in anthrax

Figs. 2A and B: X-ray findings in anthrax
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Misutilizing Medicine for Biological Warfare

Fig. 3: Skin lesions in plague

Fig. 4: Eruptions in small pox

Diagnosis:
- Clinical features: Microscopic examination of bubo fluid/sputum
- Cultures and PCR/DFA

Treatment:
- Gentamicin, Streptomycin, Doxycycline

Prevention:
- Formalin fixed vaccine, Flea control measures

Spread:
- Through bite of infected fleas
- Through droplet spread from pneumonic plague patients
- Through direct contact with non-intact skin

Weapon potential:
- Labile in environment (1 hour)
- Highly contagious, person to person spread
- Can be weaponized as aerosols (10 km)

Smallpox (Variola)

By 1980, close to whole world population was immune and not important as bio-weapon then. Now susceptible population (50%).
- High infectivity, can spread at a factor of 10–20
- Between 10–30% mortality in untreated

Signs and symptoms:
- Incubation period: Between 7–17 days (12–14)
- Fever, malaise, headache, backache, emesis, maculopapular to vesicular to pustular skin lesions. Centrifugal, same stage of development, hemorrhagic and malignant forms (5–10%) (Fig. 4)

Diagnosis:
- Culture, PCR, Electron Microscopy

Treatment:
- Supportive treatment—Cidofovir, Anti-vaccinia immunoglobulin

Prevention:
- Vaccinia immunization
- Weaponization—Infected fomites (historical use), Aerosol sprays

Tularemia (Francisella tularensis)

- Extremely infectious (10–50 by inhalation)
- Infection through non intact skin, mucous membrane, GI tract, Respiratory tract
- Vectors: Rabbits, ticks, water rats, deer.

Signs and symptoms:
- Incubation periods 1–14 days
- Ulceroglandular (75%) (Figs. 5 and 6) and Typhoidal (25%)
- Fever, chills, malaise, myalgia, headache, chest discomfort, dyspnea, skin rash, pharyngitis, conjunctivitis Hilar adenopathy on chest X-ray
Infectious Disease

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Figs. 5A and B: Skin lesions in tularemia

Fig. 6: Glandular lesion in tularemia

**Hemorrhagic Fever Viruses**
- **Arenaviridae**: Lassa, New World (Machupo, Junin, Guanarito, and Sabia)

**Bunyaviridae**: Crimean Congo, Rift Valley
**Filoviridae**: Ebola, Marburg

**Transmission:**
- Person-to-person transmission through direct contact with body fluids (Lassa, Ebola, Marburg)
- Aerosol sprays infectious (animal studies)
- Up to 90% mortality

**Signs and symptoms:**
- Fever, myalgia, prostration, and DIC with thrombocytopenia and capillary hemorrhage
- Maculopapular or erythematous rashes
- Leukopenia, temperature-pulse dissociation, renal failure, and seizures

Diagnosis should be suspected in anyone with temperature >38.3°C for <3 weeks who also exhibits at least two of the following: hemorrhagic or purpuric rash, epistaxis, hematemesis, hemoptysis, or hematochezia in the absence of any other identifiable cause.

**Diagnosis:**
- RT-PCR, Antigen isolation

**Treatment:**
- Supportive therapy: Ribavirin, IFα, Hyperimmune Ig

**Prevention:**
- No known chemoprophylaxis, no vaccines
- Strict isolation and PPE (N95 mask or PAPR)

**Botulinum Toxin (Clostridium botulinum)**
- One of the most potent toxins. Produced by Clostridium botulinum

**Diagnosis:**
- Gram stain, culture (blood, ulcer discharge, sputum), immunohistochemistry, PCR

**Treatment:**
- Streptomycin, Gentamicin, Doxycycline, Ciprofloxacin

**Prevention:**
- Chemoprophylaxis: Doxycycline, 100 mg PO bid × 14 days or Ciprofloxacin, 500 mg PO bid × 14 days
- Weaponization—Aerosol sprays
Toxin is labile in atmosphere (1% per min). Organism is easily destroyed (chlorine, heat)
Botulism can occur: infection in a wound or the intestine, the ingestion of contaminated food, or the inhalation of aerosolized toxin

**Signs and symptoms:**
- Incubation period: 12–72 hours
- Dry mouth, blurred vision, ptosis, weakness, dysarthria, dysphagia, dizziness, respiratory failure, progressive paralysis, dilated pupils

**Diagnosis:**
- Mouse bioassay and toxin immunoassay

**Treatment:**
- Supportive—Intubation, mechanical ventilation, TPN
- Equine antitoxin (only against A & B)

**Prevention:**
- Botulinum toxoid is available for high-risk workers, lab workers, military personnel.

**Examples of use:** Botulinum toxin was the primary focus of the pre-1991 Iraqi bio-weapons program. (19,000 l conc. toxin.) Aum Shinrikyo cult unsuccessfully attempted on at least three occasions to disperse botulism toxin into the civilian population of Tokyo.
- 1990—Outfitted a car to disperse botulinum toxin through an exhaust system and drove the car around Parliament.
- 1993—Attempted to disrupt the wedding of Prince Naruhito by spreading botulinum in Tokyo via car.
- 1995—Planted three briefcases designed to release botulinum in a Tokyo subway.

**Cholera (Vibrio Cholera)**
- Causes acute, potentially severe gastroenteritis
- Spread through contaminated drinking water

**Signs and symptoms:**
- Begins in 12–72 hours
- Watery rice water diarrhea, abdominal pain, cramps, dehydration, electrolyte imbalance, seizures and cardiovascular collapse in children

**Diagnosis:**
- Stool microscopy—dark field

**Treatment:**
- Fluid and electrolyte replacement
- Antibiotics—Doxycycline, Ciprofloxacin, Erythromycin

**Prevention:**
- Live vaccine—50% efficacy, 2 doses + booster
- Inactivated vaccine—rapid protection, 2 doses, 85% efficacy, 2–3 years

**Spread:**
- By contamination of drinking water supply. Easily destroyed by heat, boiling, chemical disinfectants.

**Corona Viruses**

They are mostly contagious in nature mainly causing three dangerous diseases: SARS, MERS, COVID-19.

**Severe Acute Respiratory Syndrome (SARS)**
- Caused by SARS-CoV corona virus.
  - In November 2002, it started from Guangdong province of Southern China, eventually reaching Hong Kong. From there it rapidly spread, causing infections in more than 24 countries.
    - Spreads—by aerosols

**Signs and symptoms:**
- Begins over 7 days
- Dry cough, chills, diarrhea, breathlessness, body ache, pneumonia
- Mortality rate of 9.6%

**Diagnosis:**
- RTPCR, cell culture

**Treatment:**
- Supportive—Corticosteroids, oxygenation, ribavirin, lopinavir and ritonavir, ET tube intubation
- Prevention—Hand washing, PPE wearing, using face mask (N95, 3-layered surgical)

**Middle East Respiratory Syndrome (MERS)**
- Caused by MERS-CoV corona virus
  - First recognized by scientist in Saudi Arabia after severe respiratory illness. Since then it has spread to other countries
  - Spread by—Aerosols
Signs and symptoms:
- Fever, cough, breathlessness
- Mortality rate—35.2%

Diagnosis:
- RTPCR

Treatment:
- Supportive—ET tube intubation, NSAIDs, oxygenation

Prevention:
- Hand washing, PPE wearing, using face mask (N95, 3-layered surgical)
- No vaccine available

**COVID-19**

In December 2019, CDC started monitoring the outbreak of a new corona virus named SARS-CoV-2, which started from Wuhan city of China and then spread to nearly every country leading the WHO to declare a pandemic.

Spreads by—Aerosols

Signs and symptoms:
- Fever, cough, breathlessness, loose motion, loss of taste and smell, body ache, chest pain
- Mortality rate—Varies among countries
- 6% in the US
- 3% in India

Diagnosis:
- Rapid antigen test, RTPCR

Treatment:
- Supportive—Azithromycin, doxycycline, ivermectin, HCQs, favipiravir, remdesivir, corticosteroids, LMWH, ET tube intubation

Prevention:
- Hand washing, PPE wearing, using face mask (N95, 3-layered surgical)
- Weaponization—It is the process of converting the biological agent into a usable weapon.
- Delivery device—Bombs, Missiles, Spray systems – Aerial, Aerosol based.
- Non-traditional—Food, water supplies, animals, insects.

**Treaties and Conventions**

*Geneva protocol*, 1925: Geneva Protocol for the prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare was adopted by international community on 17th June, 1925. It banned the use but didn’t prescribe the research, production, or possession. It was appeal by international Red Cross and Poland. It was customary international law. A no first use agreement only.
Biological weapons convention\textsuperscript{5,13} 1972: Eighteen-Nation Disarmament Committee in 1969. Convention on the prohibition of the development, production, and stockpiling of bacteriological and toxin weapons and on their destruction was signed on 10\textsuperscript{th} April 1972. Entered into force in 1975. First treaty to ban an entire class of weapons doesn’t address non-state actors, e.g., terrorists. No protocol to monitor compliance (Fig. 7).

Prevention Against Biological Warfare

It is necessary to be aware of common epidemiological clue for detecting early of a biological attack.

- At particular time there is a single cause of certain disease of unknown cause in a large area which may not have epidemiological explanation.
- Genetically engineered agent.
- High morbidity and mortality rates with the same or similar symptoms.
- In a particular geographical or seasonal distribution.
- Transmission through aerosols, food, water.
- Rare illness affecting large population or certain age group; with unusual trends of mortality and morbidity.
- Clustering of cases for treatment.

Primary Prevention

By creating a strong global norm that rejects development of such biological weapons.

Secondary Prevention

Early detection and prompt treatment of disease. There is important role of medical community by participating in disease surveillance and reporting, and thus providing the first indication of biological weapons use. In addition, continued research to improved diagnostic capabilities, therapeutic agents, and effective response plans\textsuperscript{14,15} will further strengthen secondary prevention measures.

Tertiary Prevention

Prevention of disability from disease.

Biological warfare with India:\textsuperscript{11,12}

- Nodal agencies—DRDO (MoD), NDMA, MoHA, MoHFW
- Indian Biodefence Program—started in 1973

Conclusion

Time and again Medicine has been misutilized for biological warfare to satisfy human ego and the desire to harm the mass by the knowledge and the commodities of healing. We misutilize our discoveries to injure and kill innocent people in the name of war instead of fighting against the microbe to prevent the diseases and save the suffering mass. The recent pandemic of COVID-19 has originated at Wuhan state of China from a virology lab with similar attempts and the whole world has suffered for a year and we don’t know what is there in future. At least now the pandemic should open the vision of our world leaders to resolve to stop such heinous suicidal act for the benefit of everyone.

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Abstract

Antimicrobial resistance (AMR) is said to occur when microbes are protected from effects of antimicrobial agents by evolving genetic or acquired mechanisms. Rising AMR is a global threat to public health across the globe. There are various causes of AMR in community, health-care system, and agriculture industry. The need of the hour is to identify and remove these causes, prevent the misuse of antimicrobial agents by antibiotic stewardship, and apply the knowledge of novel research and technology to address this growing menace.

“There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' [resistance].”

—Alexander Fleming, 1946

Introduction

The discovery of antibiotics was an important landmark in the history of medicine that revolutionized treatment and saved countless lives. Antibiotics are one of the most commonly prescribed drugs in clinical practice. Unfortunately, resistant strains of microbes have religiously followed the path of these "magic bullets" antimicrobial resistance (AMR) is the ability of microorganisms such as bacteria, fungi, or protozoans to grow despite exposure to antimicrobial substances designed to inhibit their growth. Reduction in or loss of an agent’s antibacterial effect is referred to as resistance, and the properties of or alterations in the bacterium that result in reduced antimicrobial activity are termed resistance mechanisms. AMR is one of the biggest public health problems of recent decades that poses significant challenge to prevention and treatment of various infections. It is a complex issue that affects different sectors of society and is multifactorial in origin. Coordinated action is required to minimize the emergence and spread of AMR.

Antimicrobials have made the management of infectious diseases easier thereby decreasing morbidity and mortality. If current trends continue, it is projected that, by 2050, AMR could result in over 10 million deaths per year and over 100 trillion USD in lost output globally.¹ World Health Organization (WHO) is coordinating a global campaign to raise awareness of antibiotic resistance and encourage best practices among the public, policymakers, health and agriculture professionals, to avoid further emergence and spread of antibiotic resistance.²

What is Superbug?

There has been emergence of microbes which are resistant to multiple antimicrobials are called as superbugs. They cause difficult to treat life threatening infections and require higher doses of antimicrobials along with alternative or adjuvant medications. They increase rate of hospitalization, duration of stay at hospital, and lead to economic burden on society and health-care system. Many of them are multidrug resistant infections such as Mycobacterium tuberculosis, Acinetobacter, Enterobacter
spp., Enterococcus, Escherichia coli, Pseudomonas, and Staphylococcus aureus. These infections are caused by a range of opportunistic pathogens (organisms that only cause disease in immunocompromised individuals). Device-associated infections, ventilator-associated pneumonia (VAP), and urinary tract infections (UTI) account for approximately 60% of all hospital-associated infections.

**Causes of AMR**

- **Community**—
  - Overuse of antibiotics—Over the counter availability of antibiotics in absence of valid prescription by a qualified medical professional
  - Inappropriate use/misuse of antibiotics
  - Inappropriate choices because of diagnostic inaccuracy
  - Inadequate dosing/timing and noncompliance
  - Self-medication
- **Hospital-acquired infections**—
  - Intensive and unjustified/irrational use of antimicrobial drugs
  - Emergence and spread of multidrug resistant nosocomial infections
  - Immunocompromised patients
  - Prolonged stay in hospitals/intensive care units, treatment of immunocompromized patients
  - Failure to implement effective infection control measures
  - Absence of antimicrobial stewardship programs
- **Environmental causes of AMR** (as shown in [Flowchart 1](#))
  - Overwhelming use of antibiotics in animal husbandry and agriculture. This results in the evolution of multidrug-resistant organisms that act as reservoirs. These organisms or their genes can spread to humans either through direct contact or through the environment.
  - Organic pesticides used in agriculture may persist in the soil.
  - Sewage-water contamination with soil and environment.
  - The excessive use of antiseptics and biocides leads to resistance against these compounds and cross-resistance to antibiotics.
  - Many antibacterial drugs are derived from natural products of environmental microbial species.

Exposure to antibacterial agents results in the selection of resistant strains within an otherwise susceptible bacterial population.

**Mechanisms of resistance**—Microbes have outstanding quality to survive among variety of environmental adversities. Plasmids are extrachromosomal DNA, mostly present in bacteria. They replicate autonomously. The size varies from a few base pairs to thousands base pairs. Many antibiotic resistance genes are present in plasmids. It is common that resistant bacteria have combinations of resistance mechanisms either within one category or among categories, and many plasmids contain more than one resistance gene. Thus, plasmid acquisition itself can in many cases confer resistance to multiple antibacterial agents.

Mechanisms of resistance have been shown in [Figure 1](#).

- Reduced penetration and permeability and increased efflux pumps—Some bacterial species are intrinsically resistant to certain group of antibiotics because of reduced penetration and permeability of cell membrane and increased efflux pumps. TetA efflux pumps specifically pump tetracycline out of the cell. Mutations in Porin (protein channels present incell membrane) lead to porin los or change in size and conductance or decrease in expression results in less concentration of antibiotic in the cell and can lead to AMR.
- Enzyme inactivation and chemical modification—Enzymes produced by certain bacteria inactivate the target site, such as β-lactamase enzyme, which prevent β-lactam to bind to target site. Another mechanism mediated by bacteria in which they acquire an
envelope that chemically modify the target of the antibiotics. Ribosomal methylation of erythromycin makes it ineffective and N Acetyltransferase modifies aminoglycosides.

- Modification of antibiotic target site—Mutations in the bacterial genes, which encode for the protein that is target of an antibiotic. Resistance to penicillin in streptococcus species is due to acquisition of mutation in penicillin binding proteins.

- Overproduction/replacement of target site—Sometimes there is overproduction of target sites by replication of bacteria, which is found in combination with mutation that lowers the ability of antibiotics to bind to bacteria, e.g., trimethoprim resistance in E. coli. Some of mutations cause replacement of target site of bacteria for a particular antibiotic and result in resistance. S. aureus is resistant to most of the β lactam antibiotics.

**Implications of AMR**

Management of critically ill, cancer chemotherapy, organ transplantation, orthopedic surgery, intensive care for preterm newborns are being affected.

- Economic burden on patient and health-care system.
- Infections caused by multidrug-resistant bacterial strains lead to increased societal costs in terms of mortality and loss of productivity.

**Antimicrobial Stewardship**

"Using the right antibiotic at the right time at the right dose for right duration."

Goal of antimicrobial stewardship to optimize clinical outcome while minimizing toxicity and emergence of resistance. Antimicrobial stewardship programs are the need of the hour to limit indiscriminant use of antimicrobials. These programs require leadership commitment, accountability, and drug expertise. They include systemic evaluation of ongoing treatment, tracking and monitoring of antibiotic prescription and resistance pattern and education to clinicians about AMR and optimum prescription.

Antimicrobial stewardship programs are typically multidisciplinary and often include infectious disease physicians, pharmacists, pharmacologist, clinical microbiologists, administrators, and epidemiologists. It is
for practice of promoting the selection of the appropriate drug, dosage, route, and duration of antimicrobial therapy.

**Solutions to Combat Antibiotic Resistance**

AMR is a major concern globally. Infections due to drug-resistant pathogens are becoming difficult and a challenge to treat. Following strategies can be adopted:

- Mitigation of emergence of MDR strains and prevent spread
- Strengthen National one health surveillance program
- Rapid and innovative diagnostic facilities to be made available
- Antibiotic stewardship programs should be mandatory in health-care systems
- Accelerate applied and basic research to develop new antibiotics and new treatment options
- Capacity building across the globe by improving international collaborations to prevent AMR
- Novel therapeutic options can be explored such as antimicrobial peptides, biologics, nanoparticles, polymer-controlled delivery

**Conclusion**

AMR is increasing due to overuse and misuse of antibiotics in human beings and animals. There should be societal commitment to fight against AMR. Health-care professionals should demonstrate dedication and accountability to optimize antibiotic prescriptions and patient safety.

**References**

Abstract
Chikungunya is a vector born disease transmitted to humans by the bite of infected female Aedes aegypti or Aedes albopictus. It causes a multisystem disorder with predominant involvement of musculoskeletal system. Chikungunya arthropathy can develop into chronic phase which may lead to disability and debilitating joint pain. Current treatment options include NSAIDs, Opioids, Anticonvulsants/Antidepressants, Colchicine, DMARDs, Methotrexate. Biologicals, Ultrasound, and Transcutaneous Electrical Current Stimulation have shown promising results. Newer researches are undergoing to provide evidence-based therapeutic options.

Introduction
The word chikungunya, in Makonda language, means “that which bends up” or “to become contorted.” It refers to the prostrated appearance of affected patients.1

Chikungunya virus (Fig. 1) is an alpha virus belonging to Togaviridae family. ChikV was first isolated in 1953, in Newala District, Tanzania. The vector-borne disease is transmitted to humans by the bite of infected female Aedes aegypti (tropics) or Aedes albopictus (temperate).2

Clinical Features
ChikV is a multisystem disorder with a predominant involvement of musculoskeletal system. Incubation period is 5–7 days:1

- Neurological manifestations are meningoencephalitis, myelitis, peripheral neuropathy, Guillain-Barré syndrome (GBS).
- Ophthalmology-keratitis, episcleritis, optic neuritis, uveitis, and renal detachment.
- Cardiac—uncommon, but serious arrhythmias, vasculopathy, myocarditis, pericarditis, or dilated cardiomyopathy1

- Sepsis and septic shock
- Renal failure
- Toxic hepatitis
- Pneumonitis
- Bullous dermatosis, alopecia3
Mother-to-child Transmission
A study of pregnant women concluded in Reunion Island, 2005–2006 epidemic showed that vertical transmission rate was 50% when ChikV infection occurred during intrapartum period (2 days either side of deliver). Cesarean section did not prevent this transmission.4

Chikungunya Arthropathy
Clinical manifestations can be divided into:
- Acute phase (<3 months)
- Chronic phase (>3 months)
  Acute phase is further divided into viremic (5–10 days) and subacute post-viraemic phase (6–21 days).
  Viremic phase is characterized by sudden onset high grade fever, severe polyarthralgia, myalgia, conjunctivitis, exanthema.5
  Subacute phase is in which fever settles but articular symptoms persists.
  There is symmetrical, peripheral polyarthralgia involving small, medium, and large joints, which tends to be more intense in morning and worsens with physical activity.6

Chronic Phase
Prevalence of ChikV arthritis progressing to chronicity is 4.1–78.6%. This phase is similar to rheumatoid arthritis (RA), spondyloarthritis.7 Various joints are involved in chikungunya. There have been many studies studying involvement of joints (Fig. 2).

Chikungunya has been reported with changes similar to rheumatoid arthritis. Similar case was reported by jose kennedy Amaran (Fig. 3).16 Study from Colombia reported 25% patients remained symptomatic for joint pain after 20 months follow-up.

Similar findings were reported in a meta analysis, chronic inflammatory rheumatism (CIR) was present in 25% cases and chronic arthritis in 14%.8

In study involving 121 patients from Martinique Island in Caribbean, 21% patients progressed to seronegative RA in 1 year, 37% had flare of underlying degenerative arthritis, 35% had relapse of previous inactive spondyloarthritis and 7% had fibromyalgia.9

An observational study from Kerala found 57% patients had chronic polyarthralgia, 19.5% chronic tenosynovitis after 15 months of ChikV.1

Risk Factors for Developing Chronicity
- Comorbidities: RA, diabetes mellitus
- Age more than 45 years
- High viral load (>10⁷/mL) during acute phase
- In a study involving 140 patients with ChikV, smoking, and female gender were identified as main risk factors for severe joint pain in acute disease as well as chronicity as similar to RA
- Genetic predisposition
- Viral persistence
- Auto immune diseases5,10

Immunopathogenesis of Chikungunya Arthropathy
The mechanism of chikungunya arthropathy is unclearly understood.

In acute phase, there is an intense viremia, which is associated with activation of Type I IFN & IL-6. Proinflammatory cytokines and chemokines are activated.

This strong immune response clears the virus by CD+4 T cells, NK cells and macrophages and within 7–10 days of acute infection virus levels become undetectable.

For this reason, ChikV PCR for diagnosis after 7 days is not useful.2

IL-17 is also implicated in chronic joint disease, which upregulates IL-1,6, TNF alpha, RANK-L. RANK-L osteoprotegerin ratio is disturbed, which further increases bone erosions. Whether ChikV persists in synovial tissue in chronic phase is unclear.7

Sixteen patients from Reunion Island epidemic were studied for persistent viral infection by synovial fluid RTPCR in 10 patients and tissue biopsy in 6 patients. All samples were negative suggesting viral replication is not the cause of chronic articular disease.11

In a study conducted by Dr Chang on 38 patients during 2014–2015 Colombian epidemic, no evidence of viral persistence was found by synovial fluid RTPCR, mass spectrometry, and viral culture.8

Diagnosis
- Clinical: ChikV should be suspected in endemic areas or in travelers from affected areas or in patients presenting with high grade fever and joint pain
Fig. 2: Joints involved in chikungunya as observed in various studies.

Fig. 3: A 50-year-old woman with CCA and synovitis of right 3rd PIP and left 2nd PIP joints. She had acute Chik F 3 years prior and subsequently developed CCA.
**Complete blood count:** Anemia, leukopenia, lymphopenia, mild thrombocytopenia

**Biochemistry:** Increase in liver enzymes and serum creatinine levels

**RT-PCR:** Sensitive from 0 to 7 days after which it is unreliable

**IgM ChikV:** Detectable in 5–10 days after onset of infection, maximally positive after 3 weeks. May remain positive up to 2–3 months

**IgG:** Positive after first week of infection and remain positive for years

As a general rule, serological markers (IgM & IgG) should not be checked in first week of infection:

- RA factor, anti CCP antibodies, HLA B27, ANA in chronic arthritis
- MRI—greater sensitivity. Can show synovial thickening, bone marrow edema, effusions, tenosynovitis (Fig. 4).

**Treatment**

**NSAIDs**

PCM remains the initial choice for fever and arthralgia. NSAIDs should be avoided until dengue has been ruled out as dengue can be complicated by hemorrhage.

**Corticosteroids**

Low dose prednisone starting at 5–10 mg OD followed by gradual tapering over weeks can be used for intractable symptoms in subacute phase and chronic phase. Maximum dose of steroids recommended is 0.5 mg/kg. Use is not advised in acute phase.

In an uncontrolled case series during 2005–2006 Indian Ocean pandemic, short-term corticosteroids showed improvement in arthritis and tenosynovitis and decreased disability in patients with CCA. Long-term use is discouraged due to side effects.

**Opioids**

Weak opioids like codeine and tramadol can be used as adjunctive.

**Anticonvulsants/Antidepressants**

Pregabalin, amitriptyline, and gabapentin can be used for peripheral neuropathy and intractable pain.

**Colchicine**

Study by Rendel showed that colchicine used at 0.6 mg/kg/day in a patient with persistent ankle and wrist arthralgia showed significant improvement. After 2–3 days, there was resolution of swelling and improvement in joint pain. After 2 months, symptoms were resolved.

Patient continued treatment for 6 months with no adverse effects. Study suggested colchicine as a treatment option.
DMARDs

Study on 50 chikungunya patients by Gauri LA et al. shows that chikungunya patients who had persistent arthralgia on 5-year follow-up mimicked rheumatological disorder like rheumatoid arthritis in 70.58% cases. Therefore, disease-modifying anti-rheumatic drugs (DMARDs) can be effective.  

Methotrexate (MTX)

In a study from Reunion islands by Javelle et al. on a patient with post-chikungunya CIR with 21 months follow-up, 15 mg weekly MTX lead to clinical improvement in 75% (54/72) patients and 8% (6/72) achieved partial recovery while 9% (7/72) had radiographic worsening of joints. Remaining had to stop MTX due to side effects.  

In another small study, MTX given to patients with poor response to HCQ and SSZ combination therapy led to improvement in 93% cases after 3 months. Only 7% were symptomatic at the end of 2 years.

Bouqillard & Combe treated 19 patients with acute chikungunya with MTX, 13 patients (68.4%) had good clinical response.

Combination DMARD Therapy

Amaral et al. treated 48 CCA patients with 7.5 mg weekly MTX with dose escalations for refractory symptoms at 4 weeks. Final MTX dose was 9.2+3.2 mg/week.

MTX was combined with oral prednisone at 9 weeks with 6.1+2.2 mg for 9 patients (18%). Two patients received 400 mg daily HCQ with MTX and one received SSZ 1,000 mg. Pain VAS score decreased to 3.0 & 2.6 at the end of 4 & 8 weeks respectively from a baseline value of 7.7+2.0.

An open label study by Ravindran & Alias randomized patients on HCQ who had persistent arthritis (>1 year) patients were divided into two groups:

Group A: Received fixed dose combination (MTX 15 mg weekly + SSz 1,000 mg OD + HCQ 400 mg daily.

Group B: Continued to take HCQ 400 mg OD.

Both groups also took prednisolone for 6 weeks. At 25 weeks, combination therapy showed significant improvement in both disease activity & disability. VAS score was significantly less in combination therapy group.

Biologicals

No human trial to evaluate the efficacy.

Bougillard & Combe treated patients of acute Chik developing CIR, with TNF alpha inhibitors. These patients were refractory to initial MTX therapy. All Patients had good clinical response.

Ultrasound and Transcutaneous Electrical Current Stimulation

Ribeiro et al. reported the efficacy of ten sessions of continuous ultrasound with 1 MHZ OD applied from Monday to Friday followed by infrared laser at dose of 4J & 3S per hour. TENS burst with a pulse width of 250US and frequency of 2 HZ.

This association showed post-intervention improvement in quality of life, which was assessed by SF36 (medical outcome study 36) and VAS.

Ultrasound transmits heat by convection causing vasodilation and increased blood flow, hence increasing metabolic rate of the cell.

TENS stimulates large afferent sensory fibers that block first degree nociceptive fibers by releasing endorphins.

Laser therapy causes photochemical reactions within cells, increasing mitochondrial function and ATP productions, cell proliferation, and accentuating the healing process.

When to Give? What to Give?

Based on French, Brazilian, and WHO guidelines as well as the studies conducted in India, we can follow these points:

Acute Phase

Acute viremic phase (see Flowchart 1):

- Supportive care, hydration
- WHO recommend home treatment for non-complicated acute cases
- Common analgesics, weak opioids should be used
- NSAIDs to be avoided until dengue has been ruled out
Guidelines do not recommend use of steroids during acute viremic phase
- Use of heat generating rehabilitative procedures should be avoided in acute phase

Subacute phase (see Flowchart 2):
- NSAIDs, opioids, or adjunctive treatment for pain management
- Anticonvulsant/antidepressants like pregabalin and amitriptyline in cases refractory to opioids, NSAIDs.
- Use of prednisolone at 5–20 mg/day with gradual tapering
- WHO guidelines support use of HCQ 200 mg OD or CQ 300 mg Od for 4 weeks for resistant symptoms

Chronic phase (see Flowchart 3):
- Analgesics
- Weak opioids
- NSAIDs
- Oral corticosteroids may be used at 5–20 mg/day with gradual tapering
- HCQ with or without MTX or sulfasalazine (SSZ)
- Corticosteroid dependent disease—use of MTX at 10–25 mg/weekly is recommended
- SSZ 2–3 g/day with or without MTX. SSZ should especially be used in failure or contraindications to MTX
- Biological therapy (particularly TNF alpha) may be used after rheumatological evaluation or in patients refractory to treatment of corticosteroids or DMARDs
- A dictum is—“if it looks like RA, treat like RA” should be followed
  Rehabilitation intervention in all phases of ChikV is recommended as a complementary non-pharmacological measurement.

**Newer Therapeutic Approach**

**Ribavirin**
In studies by Ravindran et al., Ribavirin was used in a group of patient with chronic ChikV, analgesics discontinued, and Ribavirin started at 200 mg BD for 7 days. All patients reported improvement in pain.6

**Favipravir**, Ribavirin with a combination of IFN alpha, umifenovir (antiviral) and Suremein (anti-protozoal) have shown some benefit.5
Flowchart 2: Treatment algorithm for patients presenting in subacute phase of chikungunya arthropathy

Flowchart 3: Treatment algorithm for patients presenting in chronic phase of chikungunya arthropathy
Abatacept
T-cell costimulation blocker. In mouse models has shown a significant decrease in periarticular swelling and proinflammatory cytokines.\textsuperscript{2}

Pentosan Polysulfate
NOVEL glycosaminoglycan like molecule developed for the treatment of alpha virus infection. Treatment of ChikV infected mice reduced cartilage thinning and immunological infiltration of joints.

Intra-articular levels of proinflammatory cytokines were decreased and anti-inflammatory IL-10 increased through unclear mechanism.\textsuperscript{1}

Fingolimod
Sphingosine-1-phosphate receptor agonist used in multiple sclerosis. In ChikV infected mice, Fingolimod treatment decreased migration of CD4 T-cells into joints without affecting viral replication. However, its utility in CCA is unknown.\textsuperscript{1}

Curcumin
Turmeric derived compound used as food additive. Treatment exhibited antiviral properties through inhibition of virus binding to cells in vitro studies.\textsuperscript{22}

Pimozide and 5-Tetra Decyloxy-2 Fusoic Acid
Found to exhibit synergistic antiviral activity in studies causing genomic wide loss of function screen.\textsuperscript{22}

Neutralizing Monoclonal Antibodies-SVIR001
Neutralizing IgG MAB to E2 envelope glycoprotein inhibit entry, fusion, and egress of virus. Robust viral clearance and decreased cytokines and chemokines level have been observed.

Renders protection against multiple alpha virus including ChikV.\textsuperscript{22}

E2 Glycoprotein Mutation
Studies showed the presence of highly conserved amino acid on E2 glycoprotein, which promoted ChikV persistence in mouse joints.

Mutation of this conserved region allowed viral clearance in mice.\textsuperscript{22}

Prophylaxis with MDEF201
It is an adenovirus vectored IFN alpha.

Studies have shown decreased cytokines and decreased footpad swelling in mice treated with intranasal MDEF 201.

Prophylaxis with MDEF201 may have clinical potential in endemic areas of ChikV. Especially during outbreaks, it would be useful as a single prophylactic agent that could protect over several weeks to those at high risk.\textsuperscript{23}

Vaccine
- Recombinant measles and chikungunya vaccine (MV-Chik)
- Virus like particle (VLP) vaccine
  Vaccines induce neutralizing antibodies against E1 and E2, which may block viral entry into cells.

2018, CEPI (coalition for Epidemic Preparedness Innovation) reported that four vaccines are in phase I trial and two in phase II trial (VLP, MV-Chik).

Phase III field trials are complicated by inability to predict the geographical location and size of next outbreak.

Newer Mosquito Control Measures
- Biotechnology: Introducing insect toxin into fungus that infects mosquitoes
- Wolbachia: It is a gram negative bacteria affecting mosquitoes. Large field trial of Wolbachia is underway in Yogyakarta, Indonesia, with hope that transmission of DENV, ChikV can be decreased.

Conclusion
ChikV is an arboviral multisystem disease primarily affecting joints. Chikungunya arthropathy can develop into chronic phase, which may persist for months to years creating distress, disability and debilitating joint pain. Available research on treatment options is limited based on which use of DMARDs, corticosteroids, NSAIDs is advised. Newer researches are undergoing to provide evidence-based therapeutic options. Vaccine development is under phase II trial. Newer therapies have shown benefit in animal models and are being studies in human subjects. To prevent the economic burden that the disease causes, there is an urgent need to formulate universal guidelines and to test newer drugs as well as vaccines.
References

Abstract

Globally dengue is the most common arboviral disease being transmitted. Since past five decades disease is rapidly spreading in the world with a 30 fold increase in incidence. The common manifestations of dengue fever includes, fever, retro-orbital headache, generalized body ache, petechial rashes, and bleeding manifestations. Over the period of decades there has been occurrence of other manifestations which are different from typical dengue fever. These manifestations have been called as atypical manifestations and it has been observed that almost all the organ system of body has been affected by dengue fever. Focus of this article is to elaborate the atypical manifestations of dengue fever which also includes the expanded dengue syndrome. Knowledge of these manifestations is necessary to recognize the disease process, limit the complication, and prevent the morbidity and mortality associated.

Introduction

Worldwide dengue is the most common arthropod borne disease, which is being transmitted by the bite of mosquitoes. Since past five decades disease is rapidly spreading in the world, and incidence of dengue infections has been rise by approximately 30 times. There is estimated 100–400 million people gets infections each year. The first confirmed epidemic of dengue hemorrhagic fever (DHF) was recorded in the Philippines in 1953–1954 and in India it occurred in Calcutta in 1963. Although it is a preventable disease but it also has high mortality and morbidity. Due to lack of awareness among the health-care personnel, atypical presentation is often missed and goes unreported. Dengue virus belongs to the virus of genus flaviviruses of Flaviviridae family. There are various serotype of dengue virus are there namely DEN 1-4. The vector responsible for the spread of dengue virus is mosquito *Aedes aegypti* (*A. aegypti*).\(^1\) Infected *Aedes* mosquitoes, especially *A. aegypti*, transmit various serotypes to humans through their bites.

The dengue virus infection produces the myriad of clinical symptoms clinical ranging from mild asymptomatic to febrile illness of unknown origin (can be of viral origin), DHF, or dengue fever (DF), or dengue shock syndrome (DSS) (Table 1).\(^2\)

Infection in people who are never been infected is known as primary or first infection, usually causes classical dengue fever. Subsequent or secondary infection by different dengue virus serotype causes more severe illness like dengue DHF/DSS. Major pathophysiologic changes occurring in DHF/DSS are:

- Plasma leakage
- Rising hematocrit
- Thrombocytopenia, and
- Bleeding manifestations.

It’s an acute febrile illness with rash, arthralgia, leukopenia, headache, retro-orbital pain, hemorrhagic manifestations.\(^2\) As per WHO clinical diagnosis of DHF is made by the following criteria:\(^1\)

- Continuous spikes of high grade fever for 2–7 days
Infectious Disease

Positive tourniquet test or presence of bleeding tendency
- Platelet count <100,000/µL
- Evidence of hemo-concentration, i.e., 20% rise in hematocrit as per average for age, sex, and population, presence of ascites and pleural effusion

One of the life threatening complications is DSS, which is characterized by DHF (meeting all four criteria) along with circulatory failure. Circulatory failure is manifested by rapid and weak pulse, cold clammy skin and restlessness and narrow pulse pressure (<20 mm HG) or hypotension for age.¹

**Atypical Manifestations**
The classic presentation of dengue has been expanded with involvement of various organ systems. Due to diagnostic dilemma some atypical manifestation are often missed and being underreported. Henceforth, a new term Expanded Syndrome has been coined by WHO, in the revised classification in 2012 as shown in Flowchart 1.²,³ Atypical manifestations are correlated with neurological, renal, hepatic, cardiovascular, and other organs involvement (Table 2). It has been reported increasingly in DHF and also in dengue patients with no evidence of leakage of plasma. Most DHF patients who have unusual manifestations are the result of prolonged shock with organ failure or patients with comorbidities or coinfections.

**Neurological Manifestations**
The association between DHF and neurological disturbances were first described in 1976.⁴,⁵ Different
manifestations including neurological signs may occur due to neurotropicity, which is due to direct invasion of tissue, release of cytokines damaging the blood brain barrier, brain edema, capillary leakage can be leading to bleeding manifestations involving intracranial hemorrhage, and due to extended period of shock there could be hypoperfusion of brain, acute renal injury, liver dysfunction, and electrolyte imbalances like decrease in the sodium levels, i.e., hyponatremia and decrease in the blood glucose levels, i.e., hypoglycemia. Further unusual manifestation observed in the same study was that of spinal cord involvement including ATM, i.e., acute transverse myelitis, which was managed with steroid, i.e., methylprednisolone and IVIG. Muscle involvement also seen in the form of Myalgia cruris, which may be due to direct muscle invasion by virus causing damage to muscle or may be due to released cytokines. Another rare manifestation is dysarthria clumsy hand syndrome.

Gastrointestinal Manifestations

Gastrointestinal presentations of virus are such as febrile diarrhea, fulminant liver failure, inflammation of pancreas, i.e., acute pancreatitis, cholecystitis mostly acalculous, severe inflammation of parotid gland, i.e., acute parotitis. Severe pain abdomen is one uncommon manifestation seen in dengue. With such presentation, acute peritonitis, acute pancreatitis, or acalculous cholecystitis should be ruled out. Diverse involvement of virus in liver scales from advancing from meagre symptoms of increasing liver enzymes to a full-blown liver failure or fulminant hepatic failure. Elevation of liver enzymes (AST/ALT) is seen commonly in dengue infection. Elevation of liver enzymes like AST/ALT is commonly seen in patient with dengue fever and 9th day of the illness is the most crucial day where the liver enzymes tend to increase from the day of onset of symptoms and it gradually progresses toward normal within 3 weeks. Another illustration of acalculous cholecystitis introduces abdominal pain specifically localized to the upper quadrant in the right side, uninterrupted fever and positive elicitation of Murphy’s sign. Exact mechanisms are not known, it might be due to invasion by the virus in the gall bladder wall

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Atypical manifestations of dengue</th>
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<td>Neurological</td>
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<td>Encephalitis</td>
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<td>Encephalopathy</td>
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<td>Spontaneous splenic rupture</td>
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<td>Infarction of lymph node</td>
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<td>Gastrointestinal/Hepatic</td>
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<td>Acute renal failure</td>
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<td>Acute respiratory distress syndrome</td>
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<td>Pulmonary hemorrhage</td>
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whose NCV, i.e., nerve conduction velocity and EMG, i.e., electromyography were completely normal. Further unusual manifestation observed in the same study was that of spinal cord involvement including ATM, i.e., acute transverse myelitis, which was managed with steroid, i.e., methylprednisolone and IVIG. Muscle involvement also seen in the form of Myalgia cruris, which may be due to direct muscle invasion by virus causing damage to muscle or may be due to released cytokines.
giving rise to edema of gall bladder and microangiopathic injury, which itself is self-limiting. Cholecystectomy not recommended due to thrombocytopenia as postoperative bleeding. Except in case of peritonitis, gangrene and perforation where surgical intervention is required.

**Cardiovascular Manifestations**

Cardiac demonstrations of the virus are uncommon though cardiac rhythm abnormalities like AV blocks, Atrial fibrillations, dysfunction in sinus node and ectopic beats in ventricles have been noticed during DHF incidents. The physiopathology of cardiac leads to inflammation causing cytokine storm of both structural and functional integrity by the virus. If clinically and ECG findings are suggestive of myocarditis, CPK-MB is considered the most precious investigation to correlate, whereas tachycardia and volume depletion are suggestive of poor outcome.

**Renal Manifestations**

Dengue virus also affects renal system leading to acute kidney injury and it exhibits as acute tubular necrosis (ATN), which is a rare manifestation and mostly attributed due to shock. ARF in these individual is of pre-renal origin. There could be various methods but the approved ones are that of invasion by the virus directly into the renal system, i.e., kidneys or immune mediated. Management with appropriate fluid in hyperkalemia unresponsive to conventional treatment, decrease urine output, and uremia hemodialysis is to be used for management.

**Respiratory Manifestations**

Unexceptional manifestation in the respiratory system caused by the dengue virus is that of ALI, i.e., acute injury to lung and acute distress to the respiratory system, i.e., ARDS. These manifestations in turn lead to edema of alveolus and also there is increased permeability in the alveolar capillary membrane. Better outcomes can be achieved by early identification and treatment. If pulmonary hemorrhage occurs, it becomes the fatal complication.

**Musculoskeletal Manifestations**

*Dengue myositis:* It is manifested as break bone fever, severe muscle, bone, and joint pain. There is elevated SGOT, SGPT, and creatine phosphokinase. Direct invasion of muscles by viruses has not been proven and may appear to be myotoxic cytokines, particularly TNF.

**Coinfections**

It modifies the presentation of dengue clinically and results in delayed diagnosis and treatment of dengue shock.

**Malaria**

Malaria is the most common infection, which can be found with dengue infection. Because of the similar seasonal variations in the incidence of both infections and similar clinical presentation and even similarity in laboratory parameters sometimes the diagnosis of dengue can be very challenging and coinfection with malaria can be missed which can lead to fatal outcome. Among the four species of plasmodium, as per Indian studies *P. falciparum* is commonly associated. Its presenting features are headache, myalgias, backache, hypotension, hepatosplenomegaly. Suspicion and treatment of complicated malaria is necessary for prevention of fatal outcome.

**Zika Virus Disease**

It is confirmed after exclusion of dengue infection with serological tests. As compared to dengue virus infection, symptoms in Zika virus infection are mild fever, mild body ache, ill-defined rashes but no hemorrhage.

**Chikungunya**

*Aedes aegypti* is common vector for both Chikungunya as well as dengue. Arthralgia common in both, but in dengue it is self-limiting and latter can progress to disabling arthritis, which may persist for months. Also thrombocytopenia can be found in both infections.

**Conclusion**

The most effective way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes. Effective vector control strategies appear to be promising for dengue prevention and control. Risks associated with the disease can be assessed with the timely diagnosis and initiation of medical care. Recognizing expanded dengue syndrome in early stage is crucial for optimizing treatment strategies. A high index of suspicion of features of expanded dengue syndrome (EDS) is very important for targeting treatment option. Alteration in sensorium is most devastating atypical manifestation in case of severe dengue infection, which, if not recognized in time, may lead to fatal outcome.
Atypical Manifestations of Dengue

References


Abstract
Cholera and plague both are communicable diseases which are preventable by targeted multisectoral approach including improvement of sanitation, education regarding good hygiene practices, improvement of housing, and quality of life. As per the WHO, cholera cases have decreased globally and there is a significant downward trend in incidence. WHO has planned a global road map to reduce the cholera-related deaths by 90% till 2030. Plague can also be controlled by breaking the chain of transmission, proper application of insecticides, early identification, notification, isolation, and treatment by appropriate antibiotics.

Introduction
Cholera is often predictable and preventable public health problem, which poses a global threat. It is an indicator of lack of access to clean water and sanitation facilities. Good hygiene practices can eliminate cholera.1 Pandemics of plague occurred from time to time in the past, but nowadays it can be treated and prevented easily by use of effective antibiotics and standard measures for prevention.2

Cholera
Cholera is caused by toxin producing strain of Vibrio cholerae, which is curved shaped Gram-negative, highly motile with single polar flagellum bacterium. It causes acute diarrhea and is known as “Father of public health.” There are more than 200 serogroup of V. cholera. Of these, only two toxigenic serogroups O1 and O139 cause outbreaks. El Tor and classical are the two biotypes of serogroup O1. In comparison to classical strains, El Tor biotype causes more of asymptomatic cases and exists in environment for longer time. Classical strain was responsible for previous six pandemic and El Tor was responsible for seventh one starting in 1961. In 1992, O139 was identified as a cause of extensive cholera epidemic in various part of South Asia including India and Bangladesh. Humans become infected incidentally but, once infected, can acts as a vehicle for spread. There is no animal reservoir. Ingestion of contaminated water and food by human feces are the common means of acquisition of V. cholera. Risk factor include poor hygiene, poor water supply, lack of sanitation (peri-urban slums, refugee camps), etc.3

Epidemiology
WHO standard case definition: Suspected cholera case—when a patient aged ≥5 years develops severe dehydration or dies because of acute diarrhea in an area where the disease is not known to be present or when patient aged ≥5 develops acute watery diarrhea with or without vomiting in cholera epidemic area. Confirmed cholera case—any suspected case in which Vibrio cholerae O1 or O139 is confirmed by culture or polymerase chain reaction (PCR) test. Cholera endemic area—an area
where cholera confirmed cases have been detected in last 3 years due to local transmission. **Cholera outbreak** is defined by the occurrence of at least one confirmed case of cholera and evidence of local transmission or unexpected increase (in magnitude or timing) of suspected cases over two consecutive weeks, of which some are laboratory confirmed cases in an area with sustained (year-round) transmission.4

In 19th century, cholera spread across the world from Ganges delta in India and killed millions of people in seven subsequent pandemics. More than 40% of cholera cases reported annually to the WHO are from Africa, more than 35% from Asia, and more than 20% from the Americas.3 Researchers have estimated that every year, there are 1.3–4.0 million cases of cholera, and 21,000–143,000 deaths occurs worldwide due to the infection.5 In year 2017, 34 countries reported 5,644 deaths and 122,7391 cases which came down to 2,990 deaths and 499,447 cases in the year 2018. As per WHO, number of cholera cases decreased globally (60% in 2018) and significant downward trend is continued; however, outbreaks are still ongoing in various countries.

### Symptoms

Incubation period is 2 hours to 5 days. Most of the cases (around 75%) are asymptomatic and shed bacteria for 1–2 weeks in their feces. Those who are symptomatic, the majority have mild to moderate symptoms and few patients’ presents with sudden onset explosive, painless, non-bilious “Rice water stools”. It is not associated with blood and typically fishy, inoffensive odor. Stool of cholera patient contains higher concentration of sodium, potassium, and bicarbonate. It is usually associated with vomiting and severe dehydration even can be life threatening (**cholera gravis**).3 It can lead to hypotensive shock, renal failure and that can be fatal in 50–70% cases, if dehydration is not addressed properly. Other clinical symptoms are parallel to volume contraction (Table 1). Severity of illness depend on various factors including number of V. cholera bacteria ingested, neither exposed previously nor vaccinated, lack of passive immunity in new born (no breast feeding), pregnancy, immunocompromised conditions, malnourishment, reduced gastric acid production, and blood group O.

### Table 1
Clinical features and treatment of cholera according to severity

<table>
<thead>
<tr>
<th>Degree of dehydration</th>
<th>Loss of TBW</th>
<th>Clinical findings</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mild</strong></td>
<td>&lt;5%</td>
<td>Thirst</td>
<td>Fluid (Treatment of choice)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ORS required in first 4 hours is equal to weight in kg multiplied by 75 in mL.</td>
</tr>
<tr>
<td></td>
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<td>As for as daily requirement is concern, it should be given as much as patient can have desire to drink until signs of dehydration subsides</td>
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<td></td>
<td>Drug of Choice</td>
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<td></td>
<td></td>
<td></td>
<td>Azithromycin 1 gm single dose in adult (20 mg/kg in children) OR</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Erythromycin-250 QID for 3 days</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Second choice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Doxycycline in non-pregnant adult—300 mg single dose OR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tetracycline (non-pregnant adult)—500 mg QID for 3 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Third choice</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ciprofloxacin 500 BD in adult and 15 mg/kg BD in children for 3 days</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td>5–10%</td>
<td>Thirst, weakness, postural hypotension, tachycardia, decreased skin turgor, dry mouth/tongue, no tear</td>
<td>Fluid (Treatment of choice)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ringer lactate (Preferred) or Normal saline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 mL/kg in first 3 hours (in first 6 hours for children &lt;12 months old) to a total of 200 mL/kg in 24 hours</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>Further requirement of fluids depends on patient condition</td>
</tr>
<tr>
<td><strong>Severe</strong></td>
<td>&gt;10%</td>
<td>Unconsciousness, lethargy or floppiness, inability to drink, sunken eyes, weak or absent pulse, decreased urine output, hypotension or shock</td>
<td>Fluid (Treatment of choice)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ringer lactate (Preferred) or Normal saline</td>
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</tbody>
</table>

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<td></td>
<td>Ciprofloxacin 500 BD in adult and 15 mg/kg BD in children for 3 days</td>
</tr>
</tbody>
</table>

**Zinc Supplementation**

<table>
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<th>Clinical findings</th>
<th>Treatment</th>
</tr>
</thead>
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</tr>
</tbody>
</table>

**Recommended in children for 10 days**

- <6 month—10 mg/day
- 6 months to 5 years–20 mg/day
Cholera sicca, another form of cholera, presents with fluid accumulation in intestinal lumen, circulatory collapse and even death in absence of diarrhea. Fever is usually absent and muscle cramps are common due to electrolyte imbalance.\(^3\)

**Diagnosis**

Diagnosis requires high index of clinical suspicion with severe acute watery diarrhea. Detection of V. cholerae from stool by dark field microscopy or isolation from stool culture on selective TSBS agar (thiosulfate citrate bile sucrose) or TTGA (taurocholate tellurite gelatin agar) remains the standard test. PCR method is also becoming available for diagnosis. Several rapid diagnostic tests (RDTs) such as immune chromatographic lateral flow devices (dipsticks), which detect the presence of the O1 or O139 antigen in rice water stool samples (sensitivity—95% and specificity—65–85%). RTDs can be performed by semi-skilled workers and it provides point of care diagnostic facility.\(^3\)

**Treatment**

In severe cases, rapid rehydration (oral rehydration solution or intravenous fluid) is the primary treatment for cholera. About 90–95% of all cholera cases can be treated by oral fluid alone. Mild to moderate dehydration cases are treated by reduced osmolarity oral rehydration solution (WHO/UNICEF ORS). Composition of reduced osmolality ORS is given in Table 2. Oral rehydration solution is prepared by dissolving one standard sachet in a 1 liter of clean water. In absence of ORS, mixture consisting of table salt (one level teaspoon) and sugar (6 level teaspoon) dissolved in 1 liter drinking water may be safely used until the proper solution is available. Severe cases need urgent hospitalization and intravenous fluid resuscitation (typically 10–20 mL/kg/hour). Ringer lactate is fluid of choice as it also corrects the acidosis along with electrolytes.\(^3\) While transfusing the fluid regular monitoring for signs of dehydration including urine output, vitals, and chest examination are warranted. Though the rehydration therapy is the main stay of treatment but it neither reduces the duration of the disease nor excretion of bacteria in feces. Antibiotic are added in moderate to severe cases to decrease duration of illness, reduce volume needed for rehydration and hastens the clearance of the organism from the stool (Table 1). Oral supplementation of Zinc in children of 6 months to 5 years, reduces the severity as well as prevent the recurrence of diarrhea.\(^3\)

**Prevention**

Cholera can be prevented by access to potable drinking water, adequate sanitation, educating people regarding good hygiene practices (frequent hand washing with soap, proper disposal of excreta and avoiding consumption of food in unhygienic environment). Cholera vaccinations is a complimentary prevention and control measure (Table 3).\(^3-8\)

In 2018, WHO passed a global road map to reduce the cases of cholera by 90% till 2030 by focusing on three following strategies:\(^9\)

- Early detection of cholera cases and contains outbreaks rapidly
- Prevention of cholera recurrence by targeting multisectoral approach
- An effective mechanism of coordination for technical support, resource mobilization, and partnership at different levels

**Plague**

Plague is caused by *Yersinia pestis*, Gram-negative coccobacilli, bipolar in appearance (closed safety pin), non-capsulated, facultative anaerobic, non-motile, organism of family Enterobacteriaceae. Human acquires infection via bites of infected rodents, fleas, or infected domestic cats, direct contact with infected human, animal tissue or body fluids, inhalation of infected respiratory droplets from a patient with pneumonic plague or respiratory secretions from infected animals.
TABLE 3  Oral cholera vaccine

| Killed whole-cell monovalent vaccine with recombinant cholera toxin B subunit (WC-rBS) | Three bivalent whole-cell only vaccines (Biv WC), Based on serogroups O1 and O139 of V. cholerae | CVD 103-HgR (Vaxchora) |
| • Dukoral (First licensed in Sweden) | Schanchol (licensed in India in 2009), mORVAX (licensed in Viet Nam in 1997 for use in endemic region in 2009 for domestic use), Euvichol (licensed in the Republic of Korea), Shanchol and Euvichol produced for International markets, available in single dose vials | Approved by USFDA in 2016 for travelers to cholera endemic area |

- Vaccine contains a mixture of the recombinant B subunit (rBS) of cholera toxin (1 mg per dose) plus formalin/heat killed whole cell of V. cholera O1 (classical and El Tor, Inaba and Ogawa)
- Because of toxin, vaccine provides some protection to Enterotoxigenic E. coli (ETEC)
- To protect the toxin from gastric acid, it must be given with bicarbonate buffer
- WC vaccines do not contain the bacterial toxin B subunit and therefore do not protect against ETEC
- Vaccine is available in 3 mL, single dose vials together with the bicarbonates buffer (effervescent granules in sachets)
- Vaccine and buffer should be dissolved in 150 mL of water in children >6 years and adult, in 75 mL for children <5 years
- Intake of food and water should be avoided 1 hour before and after the vaccination

Primary immunization
- Not licensed for use in <2 years of age
- Children 2–5 years of age, 3 oral doses given 7 days—6 weeks apart
- Two doses are recommended in adults and children >6 years. Interval between the dose should be at least 7 days but not >6 weeks
- If second dose is not administered within 6 weeks after the first dose, whole schedule should be repeated
- Children age >6 years and adults—booster dose every 2 years. If booster dose is not administered in time, whole schedule should be repeated
- Two doses are given at an interval of 14 days in persons with age ≥1 year
- Approved for the use in 18–64 years of age

Booster dose
- Children 2–5 years—booster dose every 6 month of primary immunization. If booster dose is not administered in time, whole schedule should be repeated
- Children age >6 years and adults—booster dose every 2 years. If booster dose is not administered in time, whole schedule should be repeated
- No recommendation regarding booster dose exists
- No recommendation regarding timing and use of booster is currently available, probably because it provides long-term immunity after a single oral dose

Protection
- Provides 60–85% protection for the first few months
- 60% protection over 5 years among recipient of all age
- 40% protection among children ≤5 years of age
- Between 80–90% efficacious against severe cholera at 10 and 90 days after vaccination respectively

- If cold chain (2–8°C) is maintained. Self-life is 3 years. It stable only for 1 month at 37°C
- Self-life is 2 years at maintenance of cold chain (2–8°C) and remains stable for 14 days at 42°C
- Cold chain temperature (~25 to −15°C)

- Can be safely given in among populations with high rate of HIV infection and in pregnancy
- Safety in pregnancy is not established
Infectious Disease

of contaminated food and laboratory exposure are also the source of infection. During bioterrorist attack, primary pneumonic plague caused by aerosolized *Y. pestis* bacteria in non-endemic areas is a public health problem. 10

**Epidemiology**

- Three major plague pandemics have been recorded in human history: the “Plague of Justinian” in the 6th century, again in the 14th century (known as the “Black Death,” which killed up to one-third of the European population or an estimated 17–28 million people, and at the end of 19th century following the spread of infection from China.
- Plague is prevalent in all part of the world except, Oceania.
- Peru, Madagascar, and the Democratic Republic of Congo are the most endemic countries.
- Plague does occur in Asia, but is restricted to breeders and hunters since the reservoir consists mainly of gerbils in the steppe and marmots in the mountains.
- During epidemic season, cases of bubonic plague are reported every year in Madagascar.

**Symptoms**

Symptoms of plague usually develop after an incubation period of 1–7 days. Initial symptoms are nonspecific including high grade fever, headache, sore throat, body ache and generalized weakness. Specific symptoms depend upon types of plague. 11

**Bubonic Plague:** Most common form (80–95% cases) of plague. Apart from nonspecific symptoms, there is rapidly progressive, painful lymphadenitis (known as “bubo”). The common sites for lymphadenitis are regional lymph nodes, and near the site of flea bite. Suppuration over buboes can occur and it is differentiated from other conditions by absence of cellulitis and ascending lymphangitis. Case fatality rate in untreated bubonic plague is higher (50–90%) in comparison to treated cases (10–20%), mostly because of disseminated infection. In advanced stage it causes secondary pneumonic plague and meningitis due to spread to the lungs and brain, respectively. Bubonic plague patients can presents with abdominal discomfort.

**Pneumonic Plague:** Depending on the type of exposure it is of two types; Primary pneumonic plague—caused by the direct inhalation of bacteria into the lungs. It manifests earlier, having incubation period of few hours to few days. It starts with non-specific symptoms, then progresses to dyspnea, chest pain, cough, sputum production, hemoptyisis, tachypnea, and signs of hypoxemia and toxemia. Secondary pneumonic plague—It is more common form and is caused by hematogenous spread of bacteria to the lungs from a bubo or other source. Ten to fifteen percent of bubonic plague patients develop secondary pneumonic plague, if treatment of bubonic plague is delayed. Diffuse alveolar infiltrates of chest X-ray, and interstitial pneumonitis on computed tomography are typical of pneumonic plague. Untreated pneumonic plague is almost always fatal, and mortality is very high.

**Septicemic Plague:** It occurs in 10–20% of cases in advance stages of the disease. Patient is febrile having gastrointestinal symptoms and present as Gram-negative septicemia (hypotension, shock, disseminated intravascular coagulation, and multiorgan failure). Risk factors for septicemic plague include, age more than 40 years, diabetes mellitus and hemochromatosis. Their diagnosis is difficult because it occur without any preceding bubo.

**Other Types**

**Meningitis:** It can occur as primary manifestation as result of occult bacteremia or associated with bubonic, pneumonic, septicemia plague. It resembles to bacterial meningitis by symptoms and CSF examination (low glucose, increased protein with neutrophilic pleocytosis).

**Pharyngitis and Tonsillitis:** Manifest with nonspecific symptoms along with anterior cervical lymphadenitis. Patient with no symptoms can act as carrier for bubonic plague cases. In approximately 14% cases, involvement of GIT and urinary tract is also seen. Some patients may present with multiple organ failure and die without diagnosis, if symptoms are non-specific without bubo and there is no outbreak of plague in that geographical area.10,11

**Laboratory Investigations**

In plague endemic areas, neutrophilic leukocytosis (10,000–100,000/µL) with left shift, normal or low-normal platelets and symptoms (fever, unexplained regional lymphadenitis, and hypotension) with known contact with dead rodents is diagnostic clue. Blood culture is positive.
in 27–96% cases. *Y. pestis* can be cultured from pus from bubo in bubonic plague, from sputum or broncoalveolar lavage sample in pneumonic plague, from blood in septicemic plague and from CSF in meningitis. Peripheral blood smears, microscopic aspirate of bubo may show rod shaped organism with Wright-Giemsa stain and typical bipolar staining as “closed safety pin” on Wayson’s stain. Serologic testing of single titer of >1:16 of F-1 antigen of *Y. pestis* by passive hemagglutination test is diagnostic. Rapid diagnostic test can detect F-1 antigen of *Y. pestis* (0.5 ng/mL) within 15 minutes in serum and sputum in field testing.8,9 WHO case definition is given in Table 4.10

**Plague Outbreaks Management**12

- *Find out the suspected case:* During epidemic situation diagnosis of suspected case is based on clinical ground (acute febrile illness with painful lymphadenopathy) and in other situation rat falls (dead rats) provide a useful warning of a possible outbreak. Suspected case should be confirmed bacteriologically.
- *Notification* of each and every human or rodent plague case.
- *Isolation:* Isolation of suspected pneumonic plague patients is recommended until either the pneumonia is excluded or patient is treated for 48 hours with effective drugs, because they can spread the disease by aerosol generation.
- Maximum infectivity is in final stage of disease when patients are coughing sputum with plenty of blood or pus. Though simple cotton mask or surgical masks usually provides barrier protection against droplets but WHO recommends personal protective equipment’s for potential aerosol generating procedures and should include an N-95 face mask, a gown, gloves, and a face shield or goggle.8
- *Appropriate antibiotic treatment of cases:* It should be initiated within 24 hours, ideally after specimen for culture are obtained. It is continued for 10–14 days or a course can be continued until 2 days after fever subsided (Table 5).10,11,13
- *Surveillance:* Identification and monitoring of individuals having history of close contact.
- *Disinfection:* Disinfection by hand washing with soap and water, use of alcohol based hand rubs or house made disinfectant (10% diluted bleach).
- *Ensure safe burial practices:* Proper disposal of dead body of a suspected pneumonic plague patient should be ensured.

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>WHO case definition of plague</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suspected case</strong></td>
<td>Compatible clinical presentation and consistent epidemiologic features, such as exposure to infected animals or humans and/or evidence of flea bites and/or residence in or travel to a known endemic focus within the previous 10 days</td>
</tr>
<tr>
<td><strong>Presumptive case</strong></td>
<td>Meeting the definition of a suspected case plus Putative new or reemerging focus: ≥2 of the following tests positive</td>
</tr>
<tr>
<td></td>
<td>Microscopy: Gram-negative coccobacilli in material from bubo, blood, or sputum; bipolar appearance of Wayson or Wright-Giemsa staining</td>
</tr>
<tr>
<td></td>
<td>F1 antigen detected in bubo aspirate, blood, or sputum</td>
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<tr>
<td></td>
<td>A single anti-F1 serology without evidence of previous <em>Yersinia pestis</em> infection or immunization</td>
</tr>
<tr>
<td></td>
<td>PCR detection of <em>Y. pestis</em> in bubo aspirate, blood, or sputum</td>
</tr>
<tr>
<td><strong>Confirmed case</strong></td>
<td>Meeting the definition of a suspected case plus</td>
</tr>
<tr>
<td></td>
<td>Identification of an isolate from a clinical sample as <em>Y. pestis</em> (colonial morphology and two of the following four tests positive: phage lysis of cultures at 20–25°C and 37°C; F1 antigen detection; PCR; <em>Y. pestis</em> biochemical profile) or</td>
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<tr>
<td></td>
<td>A fourfold rise in anti-F1 titer in paired serum samples or</td>
</tr>
<tr>
<td></td>
<td>In endemic areas when no other confirmatory test can be performed, a positive rapid diagnostic test with immunochromatography to detect F1 antigen</td>
</tr>
</tbody>
</table>
Protect health workers: Training of health workers about infection prevention and control measures. Workers having history of close contact with pneumonic plague patients should take 7 days chemoprophylaxis. Disinfection, distancing, and PPE should be used appropriately.

Prevention

Prophylaxis: Post-exposure prophylaxis is recommended to individuals with unprotected close contact (face to face or 1–2 meter) with suspected or confirmed pneumonic plague and family members of a bubonic plague patient. Oral doxycycline 100 mg twice daily for 7 days or oral levofloxacin 500 mg once daily for 10 days are used for this purpose. In pregnancy double strength tablet of co-trimoxazole twice daily and in children trimethoprim 4 mg/kg twice daily has been used safely.11

Vaccination: A whole cell killed vaccine given at least a week before anticipated outbreak as its immunity starts after 5–7 days of first dose vaccine. Two subcutaneous doses of 0.5 mL and 1 mL, at an interval of 7–14 days are given. Booster dose is recommended 6-monthly for persons at continuing risk of infection as its immunity persists for 6 months. Vaccination is also recommended for travelers to endemic areas and those having increased risk of disease.

Control of Fleas: To break the chain of transmission (rodent->flea->man) proper application of insecticides (dust containing DDT 2%, BHC 2%, Malathion 5%, and Carbaryl 2%) are used. Rat borrows should be insufflated with the insecticidal dust. This must precede or coincide with anti-rodent measures.

Control of Rodents: Control of rodents is an important plague preventive measure. It improves with improvement of general sanitation, improvement of housing and quality of life.14

Conclusion

Though the number of cholera cases has been decreased in India, but the social, epidemiological, and ecological conditions in remote areas continue to favor the occurrence. World Health Organization has planned to reduce the cholera-related deaths by 90% till 2030. They focused on three important strategies; early detection of cholera cases and rapid response to contain outbreaks, prevention of recurrence by targeted multi-sectoral approach, and an effective mechanism of coordination for technical support, resource mobilization, and partnership at different levels. Like cholera cases, a number of plague cases are also reduced due to good availability of effective drugs, improvement of sanitation, improvement of housing and quality of life. Primary pneumonic plague caused by aerosolized Y. pestis is having high fatality rate. As Y. pestis has the capacity to infection through aerosol, it fits into the category of a potential agent of bioterrorism. Its outbreak should be identified as soon as possible and appropriate steps should be followed for its management.

### TABLE 5: Treatment of plague cases

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>30 mg/kg/day (up to total dose 2 gm) in two divided doses intramuscular</td>
</tr>
<tr>
<td>(Preferred-FDA approved)</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2 mg/kg loading dose followed by 1.7 mg/kg every 8 hourly intravenously (in children 2.5 mg/kg IV every 8 hours)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>200 mg loading dose on first day followed by 100 mg every 12 hourly orally or intravenously in adult and child ≥45 kg, child &lt;45 kg—2.2 mg/kg (maximum, 100 mg/dose) IV every 12 hours</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Oral 2 gm loading dose followed by 2 gm/day in four divided doses (not indicated in children &lt;7 years of age)</td>
</tr>
<tr>
<td>Levoﬂoxacin</td>
<td>Adult and child ≥50 kg—500 mg OD oral or intravenously, child &lt;50 kg and ≥6 months of age—16 mg/kg (maximum 250 mg/dose) oral or intravenously every 12 hours</td>
</tr>
<tr>
<td>Ciproﬂoxacin</td>
<td>400 mg IV every 12 hours or 500 mg orally every 12 hours in adult In children—15 mg/kg IV every 12 hours or 20 mg/kg orally every 12 hours</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>In adult and child &gt;2 years—100 mg/kg (maximum, 4 gm) in four divided doses (oral or intravenous), very useful in plague meningitis because of its ability to penetrate the blood brain barrier</td>
</tr>
</tbody>
</table>
References

1. World Health Organization. Available from https://www.who.int/health-topics/cholera
2. World Health Organization. Available from https://www.who.int/health-topics/plague
Abstract
Cryptococcosis is an opportunistic mycosis which was uncommonly identified before the HIV/AIDS pandemic. It is responsible for high mortality among people living with HIV/AIDS (PLHA) and also among other non-HIV immunocompromised people. Cryptococcus is one of the four fungal species accounting for almost 90% of fungal infection-related deaths in the world, the other three being Candida, Aspergillus, and Pneumocystis. C. neoformans is found in high concentrations in bird guano and C. gattii in decaying vegetation especially that of the Red River gum trees (Eucalyptus camaldulensis). The preferred sites of infection by C. neoformans and C. gattii are lungs and central nervous system. In an immunocompromised person, cryptococcus can disseminate widely involving skin, bone/joints, prostate, and eyes. The most severe clinical manifestation of cryptococcal infection is meningoencephalitis. There are three mechanisms by which infection can spread from the lungs to brain: one, by disruption of vascular integrity leading to passive hematogenous transport; two, by endothelial cells phagocytosing spores from blood stream and later expulsing them to circulation; and three, by movement of the yeasts from the bloodstream into CNS within macrophages using the Trojan Horse mechanism. Diagnosis of cryptococcosis is based on direct visualization of the yeast, histopathology of tissue specimens, culture, and detection of cryptococcal antigen (CrAg). Cryptococcal meningitis (CM) in PLHA is associated with high mortality. Timely diagnosis and optimal therapy are both required to prevent deaths. Treatment of CM is divided into three phases: induction, consolidation, and maintenance. Three drugs have been traditionally used in the treatment of CM: amphotericin B (AmB) or liposomal amphotericin (L-AmB), fluconazole (FLU), and flucytosine (5FC). A number of strategies have been evaluated and implemented to prevent CM in PLHA with low CD4 cell counts in resource-limited settings like starting preemptive fluconazole therapy in CrAg-positive patients with low CD4 cell count.

Introduction
Cryptococcosis is an opportunistic mycosis which was uncommonly identified before the HIV/AIDS pandemic. It is responsible for high mortality among people living with HIV/AIDS (PLHA) and also among other non-HIV immunocompromised people. The ability to detect cryptococcal antigen (CrAg) has enabled us to estimate the global prevalence of the infection. Cryptococcal antigenemia is prevalent in about 6% (95% confidence interval 5.8–6.2%) of PLHA with CD4 cell count <100 cells/µL (severely immunocompromised). In absolute numbers this is a staggering 2,78,000 people. Of these, 2,23,000 developed cryptococcal meningitis (CM) in 2014 and 73% of these cases occurred in sub-Saharan Africa (SSA). It is estimated that 1,81,000 PLHA die of CM every year with 75% of these deaths occurring in SSA. About 50% of PLHA with CM die within a year of contracting infection mainly due to unsuccessful/inadequate treatment. AIDS-related CM causes 20–25% of AIDS-related deaths every year. Cryptococcosis has been increasingly recognized in non-HIV immunocompromised people. In countries with good health-care systems with equitable access to timely care,
25% of all CM-related hospitalizations and deaths occur in non-HIV patients. In the US, CM is the commonest cause of non-viral meningitis now. Different serotypes of *Cryptococcus neoformans* have been associated with CM in PLHA and those without HIV/AIDS. However, the boundaries are getting increasingly blurred now. *Cryptococcus* is one of the four fungal species accounting for almost 90% of fungal infection related deaths in the world, the other three being *Candida*, *Aspergillus*, and *Pneumocystis.* There are 37 species of Cryptococcus in the genus Filobasidiella of the phylum Basidiomycota. Most human infections can be attributed to *C. neoformans* or *Cryptococcus gatti.* Based on the capsular polysaccharide, glucuronoxylomannan (GXM), the yeast form of *Cryptococcus neoformans* is classified into four serotypes, A through D. Serotypes A and D of *C. neoformans* are responsible for most human infections. *C. neoformans var. grubii* (serotype A) accounts for 90% of human infections. It is worldwide in distribution and is especially associated with CM in people with CD4+ T cell deficiency (mainly HIV-infected and also in transplant recipients and those with rheumatological disorders requiring immunomodulatory therapy). *C. neoformans var. neoformans* (serotype D) is also global in distribution but is mostly identified amongst human cases in Europe. *Cryptococcus gattii* (serotypes B and C) was for long thought to be a tropical/subtropical fungus but it has now been discovered in high temperate zones like Northwestern US, Vancouver, and other parts of British Columbia in Canada. *C. gattii* causes disease among those with “normal” immune system. It is now also being recognized as a pathogen in PLHA, and people with other immunodeficiencies especially those with autoantibodies against granulocyte-monocyte colony stimulation factor (GM-CSF). CM in both HIV-infected and HIV-negative with immune deficiencies is plagued by IRIS which causes considerable morbidity and mortality. IRIS as a disease entity came into its own only in the course of the HIV pandemic when highly active antiretroviral therapy was introduced. About 10–25% PLHA without a known opportunistic infection (OI) starting ART develop IRIS. Most IRIS events occur within the first 2–3 months of initiating ART. But delayed IRIS has been reported with cryptococcal and CMV infections and these can occur many years later. Approximately 25% of CM patients receiving ART and antifungal therapy will develop IRIS and mortality associated with Cryptococcal IRIS (cIRIS) varies from 0% to 67%. In HIV-negative patients with CM, IRIS has been reported with reversal of iatrogenic immunosuppression especially with TNF-α inhibitor therapy. IRIS has triggered a deep study of the immunobiology of HIV-associated OIs and new insights have opened up new immunomodulatory interventions.

**Pathobiology**

*C. neoformans* is found in high concentrations in bird guano, especially in the droppings of pigeons and chicken. *C. gatti* is found in decaying vegetation especially that of *Eucalyptus camaldulensis.* Cryptococcus survives in the environment as a yeast (sexual form), producing hyphae with terminal basidiospores which may break off and then become aerosolized. Since their average diameter is 3 microns, they are small enough to travel right up to the alveoli. In most hosts, the infection is asymptomatic. A seroprevalence study done among children in New York revealed that 70% had antibodies against cryptococcal antigens. It seems that asymptomatic colonization of airways and latent infection of lungs and airways maybe common. Autopsy studies have shown granulomatous lesions in lung parenchyma and sub-pleural nodules containing yeast forms. Just as in tuberculosis (TB), a robust immune system keeps the infection from manifesting as clinical disease. The most severe clinical manifestation of cryptococcal infection is meningoencephalitis. There are three mechanisms by which infection can spread from the lungs to brain: one, by disrupting vascular integrity leading to passive integrity leading to passive hematogenous transport, two, by endothelial cells phagocytosing spores from blood stream and later expulsing them to circulation, and three, by movement of the yeasts from the bloodstream into CNS within macrophages using the Trojan Horse mechanism. On crossing the blood brain barrier, the fungal cells are released by vomocytosis. Just as in TB, hematogenous spread can occur during primary infection or due to reactivation when immunity is lowered. Severe disease can develop many years after primary infection. Pirofski and Casadevall proposed a mechanism which allows the host to tolerate infection for long periods without manifesting disease. They termed it the disease tolerance and damage response framework (DRF). This system mainly evolved to mitigate and avoid damage to the lungs and brain.
The DRF is based on the interplay of microbial and host factors that may either cause disease or produce a net benefit to the host. The highly dynamic and complex interaction between the fungal virulence factors and host's immune response can result in a variety of outcomes ranging from debilitating disease to colonization, latency and commensalism. *Cryptococcus sp.* have an array of mechanisms that enable them to survive, proliferate, and disseminate in a mammalian host. The fungus produces numerous enzymes which cause damage at a molecular level. These include proteases, phospholipase, urease, and nuclease. At a cellular level, cryptococcus can damage host cells by interfering with phagolysosome maturation, increasing permeability of phagosome membrane, interfering with organelle function, causing cytoskeletal alterations, cytoplasmic vacuolation and both lytic and non-lytic exocytosis. The first step in the protective response against cryptococcal infection is the production of proinflammatory cytokines, then followed by generation of an effective Th1/Th17 adaptive immune response and classical activation (M1 type) of macrophages. All these processes lead to fungal clearance. A robust response commensurate with fungal burden can lead to excessive tissue damage. A good example of this is cryptococcal IRIS. In immunocompromised patients, both inflammatory response and immune function are compromised enabling unbridled fungal replication resulting in high fungal burden. The ideal scenario is one where the host resistance mechanisms are balanced by the host tolerance mechanism. This leads to minimal/ or no clinical disease and probably fungal latency. The host resistance mechanisms, namely proinflammatory cytokine production, activation of dendritic cells (DCs), generation effective Th1/Th17 response, and M1 type macrophage polarization are balanced by cryptococcal and host disease tolerance mechanisms.

Cryptococcus-associated disease tolerance strategies can be grouped as metabolic adaptions to physiological conditions in the host, evasion, and interference with innate and adaptive immune responses. Cryptococcus can activate gene expression that enables it to survive in human cells at 37°C and also in nutrient-poor environments like the CNS. The fungus van produce meaning using host catecholamines. This reaction is mediated by fungal phenoloxidase. It is this ability to use catecholamines that gains the fungus a niche in the CNS. The capsule serves as a principal defense against innate immune mechanisms. The capsule conceals cell wall carbohydrate antigenic epitopes, inhibits antibody binding to fungal cell wall, and activates and depletes complement factors. The capsule modulates cytokine production and suppresses T cell proliferation. It also induces apoptosis of host cells. During active infection, cryptococcal cell capsule can enlarge leading to formation of giant “Titan” cells. The size of these cells varies from 50 to 100 microns and this large size inhibits phagocytosis. Fungal cells can release their capsular glucuronoxylomannan (GXN) and this causes shedding of L-selectin from neutrophils. Loss of L-selectin inhibits neutrophilic migration, endothelial adhesion, and extravasation into tissues. Other capsular factors can interfere with maturation and activation of neutrophils, DCs, and macrophages. The yeast cells secrete a number of enzymes that enable them to survive in the harsh environment of the phagolysosome. These are mainly involved in nitric oxide detoxification and neutralizing oxidative stress. Other enzymes and substances that enable the fungus to survive intracellularly include urease, phospholipase B1, laccase, melanin, and heat shock protein70 homolog Ssa1. The ability to escape from host cell without causing its lysis is the most important mechanism for persistence of cryptococcus in host tissue. This non-lytic escape from phagocytes is also called vomocytosis. This enables the organism to prevent the inflammatory response that would be associated with host cell death. *Cryptococcus sp.* are able to swing cellular immune response toward a non-protective Th2 response by two main mechanisms: cryptococcal urease recruits immature DCs to lymphoid tissue in the lungs, and host chiotriosidase cleaves fungal chitin and triggers CD11 b+ conventional DCs to undergo Th2 differentiation. *C. neoformans* secretes prostaglandin E2 (PGE2) that suppresses Th17 differentiation. A strong Th17 response is critical for a sterilizing immune response. In fact, this process facilitates latent infection.

The host immune response to cryptococcal infection that leads to disease tolerance is due to T regulatory (Treg) cells, IL-10 signaling, DC response, role of cryptophan pathway and T cell exhaustion. Tregs are involved in the production of anti-inflammatory cytokines like IL-10, and transforming growth factor-β. Severe pathology occurs when there are mutations in Treg-associated transcription factor fork head box protein P3 (FOXP3). CrAg-specific
Tregs can colocalize with Th2 effector cells in infected lungs and thereby limit inflammatory damage. IL-10 is an anti-inflammatory cytokine that is secreted by Tregs and DCs. It inhibits production of IL-1, IL-6, IL-23, IFN-γ, TNF-α in fungal infections. In PLHA with cryptococcal infection, high levels of IL-10 correlate with fungemia and dissemination. Therefore, IL-10 levels can be a surrogate marker of progressive or persistent cryptococcal infection. Among the antigen presenting cells, DCs are the ones that can present cryptococcal antigens most efficiently to T cells. Development of a Th1/Th17 immune response greatly depends on classical activation of monocyte-derived DCs (MoDCs). As mentioned earlier capsular characteristics can affect DC activation. The local cytokine and chemokine milieu also affects their activation. Immunomodulatory DCs have been known to evolve in the course of infection and they can lead to Th1/Th17 suppression, reduced macrophage activation and impaired fungal clearance. Indoleamine 2,3-dioxygenase (IDO) is an enzyme involved in tryptophan metabolism. It plays an important role in balancing the activity of Tregs and Th1/Th17 cells. Expression of IDO by DCs leads to a tolerogenic phenotype in experimental models. The same has not been demonstrated in humans with cryptococcal infection. Its significance is still to be explored. A state of chronic immune activation can lead to T cell exhaustion. One of the mechanisms that promotes this is the upregulation of cytotoxic T lymphocyte-associated protein-4 (CTLA-4). T cell activation and function depend on a double signal: the binding of antigen presented by MHC to T cell receptor and binding of CD80 and CD86 on antigen-presenting cell to CD-28 on T cell. CTLA-4 binds to CD80 and CD86 and thus blocks CD-28. In the absence of IFN-γ signaling from CD4 T cells in the pre-ART phase. But a buildup of partially primed myeloid cells occurs in the CNS during this time. When CD4+ cell reconstitution occurs with ART, activation of these myeloid cells occurs in the CNS during this time. When CD4+ cell reconstitution occurs with ART, activation of these myeloid cells with production of proinflammatory cytokines occurs. Myeloid cells that accumulate in CNS prior to IRIS are mainly proinflammatory intermediate monocytes. The final phase of IRIS is marked by immune dysregulation. In a normal response to a fungal infection classical activation of monocytes leads to increased fungal kill. However, this does not occur in immunocompromised people. Here the buildup is of intermediate monocytes with increased expression of programmed death ligand-1 (PD-L1). Their activity results in a powerful inflammatory response and less efficient fungal kill. Lymphopenia associated with HIV infection results in a homeostatic response to cryptococcal infection regulated by IL-6 and characterized by proliferation of naive T cells which resemble effector memory T cells. Later, during IRIS, these cells ramp up secretion of IFN-γ and push the immune activity toward a powerful Th1 response. Immune restitution also results in a robust increase of highly-differentiated memory CD4+ T cells with no inhibitory control. The net result is severe inflammation, tissue destruction, and functional impairment.

Cryptococcal IRIS

T cell depletion in HIV infection severely compromises adaptive immune response and this can lead to unbridled fungal dissemination to the meninges. Immune restitution with ART predisposes patients with CM to IRIS. Evolution of cIRIS goes through three stages: pre-IRIS paucity of inflammatory response, innate cell activation, and immune dysregulation. During the pre-ART phase both innate and adaptive immune responses are compromised. Migration of neutrophils into the meninges and their fungistatic activity is compromised. Poor antigenic clearance and risk for IRIS are linked to decreased levels of IL-6, IL-8 and TNF-α (innate inflammatory cytokines) and IFN-γ (adaptive inflammatory cytokine). Risk of cIRIS increases with higher expression of CCL2 and CCL3. Natural killer cells with increased expression of CXCR3 and CX3CR1 also participate in the initial response to cryptococcal infection in CNS. When ART is started, T cell immunity takes some time to develop. In this interim, antigenic burden causes a buildup of proinflammatory signaling by antigen presenting cells. This is demonstrated by increased IL-6 and CRP levels in weeks preceding development of IRIS. When ART associated immune restitution reaches a critical level, a powerful Th1 response and cytokine storm herald the onset of IRIS. Phagocytic killing of intracellular pathogens is also defective due to absence of IFN-γ signaling from CD4 T cells in the pre-ART phase. But a buildup of partially primed myeloid cells occurs in the CNS during this time. When CD4+ cell reconstitution occurs with ART, activation of these myeloid cells with production of proinflammatory cytokines occurs. Myeloid cells that accumulate in CNS prior to IRIS are mainly proinflammatory intermediate monocytes. The final phase of IRIS is marked by immune dysregulation. In a normal response to a fungal infection classical activation of monocytes leads to increased fungal kill. However, this does not occur in immunocompromised people. Here the buildup is of intermediate monocytes with increased expression of programmed death ligand-1 (PD-L1). Their activity results in a powerful inflammatory response and less efficient fungal kill. Lymphopenia associated with HIV infection results in a homeostatic response to cryptococcal infection regulated by IL-6 and characterized by proliferation of naive T cells which resemble effector memory T cells. Later, during IRIS, these cells ramp up secretion of IFN-γ and push the immune activity toward a powerful Th1 response. Immune restitution also results in a robust increase of highly-differentiated memory CD4+ T cells with no inhibitory control. The net result is severe inflammation, tissue destruction, and functional impairment.

Cryptococcal IRIS
Clinical Features
The preferred sites of infection by *C. neoformans* and *C. gattii* are lungs and CNS. In an immunocompromised person, cryptococcus can disseminate widely. They can involve skin, bone/joints, prostate, and eyes. A retrospective study of cryptococcosis in Denver, Colorado, USA, showed that patients with cryptococcosis had, apart from features of CM, respiratory symptoms, hyponatremia, prior lung disease, or history of corticosteroid therapy.

Respiratory System
The main route of entry of cryptococcus is through the respiratory tract. Clinical manifestations are varied and depend on immune status. In an immunocompetent person it can be just a symptomless colonization of the airways or a pulmonary nodule detectable only on a radiograph. In the immunocompetent, the infection is accidentally discovered on imaging in up to one-third of the cases. The radiological manifestation maybe a solitary nodule or multiple nodules without calcification or pulmonary infiltrates. Hilar lymphadenopathy, pleural effusion, and cavitary lesions are also described. The presentation is more florid and dangerous when the immune system is compromised. In this setting the manifestation can be a severe pneumonia or a life-threatening acute respiratory distress syndrome (ARDS). Isolated pulmonary cryptococcosis can occur in the absence of CNS involvement in the immunocompromised. In the presence of cryptococcal meningoencephalitis, pulmonary involvement is seen in 10–55% of PLHA. But here, neurological symptoms usually dominate. A Chinese study in HIV-negative subjects reported slightly different findings. In this study, 49.3% of the subjects with CM had evidence of pulmonary cryptococcosis. They usually presented with fever, cough, expectoration, and lower limb edema rather than with CNS symptoms. Subjects with lung infection were also younger (<30 years) than those who did not have lung infection. Serum CrAg is usually negative in truly isolated pulmonary cryptococcosis. Whenever cryptococcus is isolated from the lungs or other sterile body sites in an immunocompromised individual, a lumbar puncture to exclude CNS disease is recommended regardless of the patient’s symptoms or serum CrAg titer results. The only exception for a diagnostic LP would be an immunocompetent person without CNS symptoms. Here, infection can be presumed to be limited to the lungs.

Central Nervous System
The presentation of CM is usually subacute with symptoms developing over weeks. Clinical symptomatology may include fever, lassitude, headache, altered sensorium, and focal deficits like cranial neuropathies. In severely immunocompromised patients, fungal burden in the CSF can be very high (>1 million yeast cells/mL of SF). In these patients presentation is usually acute and features of raised intracranial pressure maybe present. Their CSF will also show high CrAg titers. A study from SSA showed that seizures occurred at presentation in 28% (231/821) HIV positive subjects with CM and 15.5% developed seizures during the course of illness. Seizures at presentation were associated with lower GCS scores, lower CD4 cell counts increased CSF opening pressures when compared to HIV+ patients with CM but without seizures. CSF fungal burden was higher in those with seizures at presentation than in those developing seizures during course of illness. *C. gattii* infections can cause cryptococcomas in the brain and can also cause obstructive hydrocephalus. In the lungs also it can cause mass lesions. Focal neurological deficits and cranial neuropathies due to cryptococcomas are common manifestations. These patients respond poorly to antifungal therapy and decompressive surgery may be required to relieve raised ICP. *C. neoformans* is less likely to cause such lesions.

In the absence of a definite microbiological diagnosis, TB meningitis (TBM) is the commonest differential diagnosis for CM. In both, fever, headache, and vomiting are common presenting complaints. Altered mental status is more common in CM than in TBM in HIV-negative individuals. Altered vision and hearing are commoner in CM and cough is more likely in TBM. CM is associated with corticosteroid use, pulmonary infection, hepatobiliary disease and diabetes. Those with TBM are likely to have TB elsewhere outside CNS or disseminated TB. In the Denver study, risk factors associated with CM were HIV infection, prior use of corticosteroids, malignancy, transplant status, lung disease, and active smoking.

In HIV negative children with CM, fever, nausea, vomiting, and headache are the commonest symptoms. Meningeal signs, altered consciousness, and fundal changes are common findings. CSF opening pressure is
Involvement of other organs is common with the lungs being the commonest. Among the elderly, those older than 65 years are more vulnerable to developing CM. There is a female preponderance and altered consciousness and cerebral infarction are more common than in the young.10

Skin
After pulmonary and CNS involvement, cutaneous manifestations are the commonest presentation of cryptococcosis. Usually the lesions are non-specific and similar to those seen in other infections. Primary cryptococcosis is uncommon and is due to direct inoculation of the fungus into the skin by injury or laboratory accidents. The lesions are single and occur at the site of inoculation. They can present as whitlow, papule, nodule, ulcerated lesion or cellulitis. In immunocompromised patients, dermal cryptococcosis is secondary to disseminated infection. Here the lesions tend to be multiple. Lesions often resemble those of molluscum contagiosum. They are flesh-colored papules/nodules with central umbilications. Pustules, acneiform lesions, abscesses, cellulitis, and granulomata have all been described. Regional lymphadenopathy and discharging sinuses may occur. Skin biopsy and cultures are required to culture and identify the fungus. Solid organ transplant recipients on tacrolimus are prone to cutaneous cryptococcosis. Tacrolimus inhibits a protein required for cryptococcal growth at 37°C. It acts by interfering with calcineurin signaling pathway in the fungus. However, at lower temperatures (<24°C), this inhibitory activity is lost making patients on calcineurin inhibitors prone to cryptococcal infection.43,44

Immune Reconstitution Inflammatory Syndrome
Antiretroviral therapy leads to immune restitution in PLHA. When this process occurs in a severely immunocompromised person, it leads to a rebound of pathogen-specific immunity. This manifests as a clinical entity called immune reconstitution inflammatory syndrome. When there is a “paradoxical” deterioration of a known OI or appearance of the OI in new sites or “recurrence” of OI while on ART, it is called paradoxical IRIS. In unmasking IRIS, ART leads to an accelerated manifestation of an occult infection. In the context of cryptococcosis another entity is described and that is post-infectious inflammatory response syndrome which is a clinical deterioration in a previously healthy individuals. All of them also have an exaggerated anti-cryptococcal immune response. Cryptococcal IRIS causes severe morbidity and is associated with a high mortality.14,45-47 Cryptococcal IRIS is a diagnosis of exclusion. Progressive infection secondary to suboptimal antifungal therapy, primary antifungal drug resistance or persistent immune deficits, other coexistent OIs (like TB), malignancy, and drug toxicity need to be excluded. Risk factors for IRIS include severe immune deficiency at baseline with rapid immune restitution with ART, high fungal burden at baseline and ineffective immune reponse in the host during initial infection. The commonest time period for cIRIS is within first 3 months of starting ART. Classical features of cIRIS in HIV infected patients with low CD4 cell counts include little or no meningeal irritation despite high cryptococcal load, extremely high serum and CSF CrAg titres, muted inflammatory response in CSF manifesting as low CSF WBC count, and high yeast burden on CSF microscopy and ease in culturing yeast from CSF or serum.48

Laboratory Diagnosis
Diagnosis of cryptococcosis is based on direct visualization of the yeast, histopathology of tissue specimens, culture, and detection of CrAg. Molecular methods, although available and extensively used for research purposes, are not used currently in routine clinical practice.

Direct visualization is traditionally by India ink staining. The fungus appears as a globular, encapsulated yeast cells which may or may not be budding. The yeast cell size varies from 5 to 20 μm in diameter. India ink staining is the most rapid method for diagnosis of CM and has been the method in vogue in low-income countries for a long time. The sensitivity is only 80–85% for AIDS-related CM, meaning that 1 in 7 diagnoses will be missed. Sensitivity falls to about 40% in patients with low fungal burden (<1,000 CFUs/mL of CSF). Detection of yeasts is operator dependent. Lysed WBCs can be mistaken for fungal elements.49,50

Histopathology
Cryptococcus can be detected from a variety of tissue samples like lungs, brain, skin, bone marrow, lymph nodes,
and other organs. Histopathology is more sensitive than India ink staining. Special staining techniques are available to visualize different fungal elements. Mucicarmine, Alcian Blue, and Periodic-acid-Schiff stain the fungal polysaccharide capsule. The melanin in the yeast cell wall is delineated by Fontana–Masson stain. Gomorri-methenamine-silver stain also enables visualization of the cell wall. Calcofluor binds to fungal chitin.

Culture

Cryptococcus can be cultured readily using routine fungal or bacterial culture media. CSF, sputum, and skin biopsy sample all yield the fungus easily. The sensitivity of CSF and blood cultures in adult PLHA with CM is 90% and 50–70% respectively. Volume of inoculum determines the culture sensitivity. It is 82% for 10 µL of CSF and 94% for 100 µL. Fungal culture remains relevant even today for clinical, therapeutic, and research reasons. For the clinician, cultures help confirm clearance of the yeast from CSF after induction therapy, and to distinguish between relapse of CM and paradoxical IRIS. Serial cultures have a log 10-linear clearance. This enables computation of early fungicidal activity (EFA). EFA is a useful marker of drug regimen potency and is also used as an efficacy endpoint in phase II trials. Quantitative cultures allow measurement of fungal colony forming units (CFUs) from CSF. The disadvantages of using culture for diagnosis are that results become available only in 3–7 days, and in a setting of low fungal burden, cultures can be negative.

Serology

Serological tests to detect cryptococcal capsular antigen has made diagnosis of cryptococcal infection easy and fast. CrAg has been detected in CSF and serum using latex agglutination and enzyme immunoassay (EIA) techniques for more than two decades. The sensitivity of CrAg-Latex for serum and CSF is 83–97% and 93–100%, respectively. The specificity for serum and CSF is 93–100% and 93–98%, respectively. For EIA, the sensitivity for serum and CSF is 94% and 100% respectively and the specificity is 96% and 98% respectively. Both CrAg-Latex and EIA have some disadvantages. The CrAg-Latex is a manual test and there is a high degree of subjectivity in its interpretation. Both require laboratory infrastructure, and refrigeration of reagents thereby making the tests expensive. CrAg-Latex has lower sensitivity for CrAg of serotype C (C. gattii).

EIA had a lower sensitivity for serotypes C and D. False positive test results are low but known (<1%). These are due to technical issues, contamination or infection with Trichosporon beigelli, Capnocytophaga canimorsus, and Stomatococcus mucilaginosus. False negatives can be seen early in infection due to a low fungal burden, due to poorly encapsulated organisms and due to prozone effect in the setting of extremely high antigen titers, which can be overcome with dilution. False-negative results in latex agglutination tests can occur in the early stages of infection when fungal burden is low and also because of improper storage of clinical sample.

The US FDA has approved a lateral flow immunochromatographic dipstick assay (LFA) for serum and CSF. The European Union has approved it for serum, plasma, and CSF. It can detect CrAg of all species of cryptococcus. The test uses a combination of two monoclonal antibodies. The first one is reactive one against CrAg of serotypes A, B, and C. The other is highly reactive against CrAg of serotypes A and D. The test can be done in five easy steps (as shown in Fig. 1) and takes 10 minutes to give the result. The test meets the World Health Organization’s (WHO) “ASSURED” criteria. It is affordable (i.e., cheap), sensitive (equal to or better than other CrAg tests), specific (similar to other CrAg tests), user-friendly (just as easy as the pregnancy test), rapid and robust (done in 10 minutes), equipment-free, and delivered (i.e., it is portable and light-weight with a long shelf-life and requiring no refrigeration).

CrAg LFA can be used both qualitatively and semiquantitatively. A schematic representation of CrAg LFA is shown in Figures 2A and B. The test has been validated in a large multicentric study in South Africa and Uganda. The test has a sensitivity and specificity of 99.3% and 99.1% respectively. The data provided by the manufacturers state that LFA has a sensitivity and specificity of 99.5% and 99% respectively and, positive predictive and negative predictive values of 98% and of 99.7% respectively with serum, plasma, urine, or CSF samples when compared to cultures. There is 100% concordance for serum and plasma tested by fingerstick LFA test and 100% negative predictive value for excluding CM. The finger stick LFA can show false negative result in a scenario of asymptomatic patient with a low fungal burden. Pipetting whole blood on to the LFA can increase diagnostic yield over direct application of blood to CrAg.
Figs. 1: Five easy steps to detect cryptococcal antigen using LFA. Step 1: add one drop of specimen to a tube. Step 2: add 40 μL (1 drop) of patient specimen to the tube. Step 3: insert the LFA strip into the tube. Step 4: incubate for 10 minutes. Step 5: interpret results.

Figs. 2A and B: (A) Schematic representation of lateral flow immunochromatographic assay for detection of CrAg. (B) Images of positive and positive and negative controls.
LFA sample pad. CrAg LFA is about 5 times more sensitive than CrAg-Latex when comparing semi-quantitative titers by serial dilution. This means that a specimen positive by CrAg-Latex at 1:8 dilution will be positive by CrAg LFA at 1:40 dilution. The semiquantitative assay reports the highest dilution that gives a positive result.

CrAg detection is used for detecting early CM, screening PLHA for cryptococcal infection, and for prognostication and therapeutic decision-making (when it is done semi-quantitatively).

**Detection of Early Cryptococcal Meningitis**
A study in Uganda investigated 1201 HIV positive individuals hospitalized for suspected meningitis. Fifty-six percent of these patients (671/1,201) had CM confirmed by CSF CrAg positivity. Four percent of the subjects with neurological symptoms and CSF analysis negative for CrAg were found to have serum CrAg positivity. Cryptoccus was identified by culture or PCR in 9% of these serum CrAg+ and CSF CrAg- patients. This subset of patients with neurological symptoms, serum CrAg positivity, and CSF CrAg negativity constitute the early cryptococcal meningo-encephalitis’ group. In-hospital mortality was 32% in symptomatic cryptococcal antigenemia and 31% in CM. These findings suggest that immunocompromised patients with suspected CNS infections should always have serum tested for CrAg especially when no CSF pathogen is detected and that in early cryptococcal meningoencephalitis, yeasts maybe present in brain parenchyma without meningeal involvement.

**Screening for CrAg**
CrAg appears peripheral blood many weeks to months before the onset of symptomatic disease. There is thus an opportunity for early detection and pre-emptive action. Serological prevalence studies for CrAg in PLHA have been done in various parts of the world. The serological prevalence of CrAg in ART-naive asymptomatic PLHA with CD4 cell count <100/µL is 6.8% (95% CI: 2.2–21%) and 4–12.9% in Africa and Southeast Asia respectively. In Brazil, the prevalence in hospitalized PLHA with CD4 cell count <200/µL is 3.1% and 11.4% in two different studies. The global average prevalence in severely immunocompromised PLHA is 6%. The mathematics of cryptococcal screening for PLHA has been worked out. There are 110,500 severely immunocompromised PLHA in Uganda at risk of cryptococcal disease. Screening them and pre-emptively treating 15,500 people with fluconazole would cost $822,600 which is 15% cost of treating meningitis that would occur otherwise. It would also lead to 40% better long-term survival. The low cost of LFA makes screening for CrAg compellingly cost-effective even in areas of prevalence as low as 1%. WHO recommends screening of all ART-naive PLHA with CD4 cell count <100/µL for cryptococcosis by CrAg testing in serum/plasma. It also recommends antifungal therapy if CrAg is positive to prevent development of disease.

**CrAg Titer**
Semi-quantitative estimation of antigenemia can be done by both CrAg-Latex and LFA by serial dilution titers. Unfortunately the test is not standardized and results can vary with kits from different manufacturers. The variability is more for the latex test. The CrAg LFA manufactured by IMMY is considered the gold standard diagnostic. Semi-quantitative assays are clinically useful because antigenic titers correlate with disease severity and mortality. Plasma/serum CrAg titers of <1:80 are unlikely to be associated with CNS disease. The probability of CNS involvement increases beyond a titer of 1:160 and there is near-universal concordance with CNS disease at titers of 1:1,280. This will enable risk stratification of patients into low risk (<1:160), intermediate risk (1:160 to 1:1,280) and high risk (>1:1,280) of infection. All those in high risk group should be considered to have disseminated disease regardless of symptoms. Rajasingham and colleagues pooled data from four cohorts and found that survival decreased as plasma CrAg titer increased from <1:160 to >1:2,560 (log rank P <0.0001). Worryingly, mortality rates were high in asymptomatic individuals with CrAg >1:160 despite their receiving pre-emptive fluconazole therapy.

Programmatic challenges exist to screen severely immunocompromised PLHA for CrAg. The strategy of ordering the test for people who have got a CD4 count report of <100/µL is not efficient and many patients will miss the test. This is known as the “provider-initiated testing” strategy. A study from South Africa showed that only 27% of those eligible actually got screened by this strategy. It also increases the work of the clinic staff because they have to raise another investigation form.
and draw another blood sample. The best strategy that has evolved is one known as “reflexive screening.” Testing for CrAg is a part of the laboratory protocol. Whenever the laboratory finds a CD4 test result of ≤100/µL, it will automatically run the CrAg test on the remaining blood sample. The laboratory will also report it in along with the CD4 count result and will also offer a brief explanation as to what to do with a positive CrAg report.

Other Tests

β-D Glucan (BDG): It has been long believed that Cryptococcus does not produce enough BDG to be detected easily. A study done in Africa showed that BDG can be detected in the CSF of PLHA with CM with a sensitivity and specificity of 89% and 85% respectively. Sensitivity increases to 98% when fungal burdens are high (>10,000 CFUs/mL). Unlike CrAg, BDG levels decline fast with treatment and thus and thus it can potentially be used to monitor therapy, detect relapse, and also differentiate relapse from cIRIS.

Uses of film array system of multiplex PCR have been tried for detecting common pathogens causing meningitis. Cryptococcal infection can be detected with a sensitivity of 96% when fungal burden is high (>100 CFUs/mL of CSF) but only with a sensitivity of 50% at low fungi burdens (<100 CFUs/mL of CSF). The high cost and sophisticated laboratory facilities preclude its widespread use.

Ribosomal DNA genes and their internal transcribed spacers (ITS) have been found to be a good common marker for bacteria and fungi. It is possible to detect inter- and intra-specific variations. Deep sequencing of rDNA amplicons from CSF can diagnose CM. Metataxonomics of ITS amplicons may be the test of the future for rapid diagnosis and genotypic recognition of Cryptococcus sp.

Treatment

Cryptococcal meningitis in PLHA is associated with high mortality. Some strategies to decrease morbidity and mortality include: early diagnosis of HIV infection and early initiation of ART; ART adherence and retention in care; CrAg screening and treatment approach; and improved CM care.

Despite the global coverage of ART being about 60%, CM remains a therapeutic challenge. The incidence of CM remains high and more than 50% of CM cases occur in ART-experienced patients. Timely diagnosis and optimal therapy are both required to prevent deaths. Treatment of CM is divided into three phases: induction, consolidation, and maintenance. The induction phase is meant to drastically reduce fungal burden in the CSF within 2 weeks and is critical for good outcomes. Three drugs have been traditionally used in the treatment of CM: amphotericin B (AmB) or liposomal amphotericin (L-AmB), fluconazole (FLU), and flucytosine (5FC). Of these FLU is the cheapest and most widely available drug. FLU monotherapy, even the highest doses, is associated with high mortality (50% during 10 weeks of treatment). Various 2-week induction regimes have been tried. The main aim was to reduce the serious adverse effects (SAEs) associated with AmB. The dose and duration of therapy have both been modified in various studies. For a long time AmB 1 mg/kg/d + 5FC 100 mg/kg/d for 14 days was accepted as standard induction therapy for HIV-associated CM. But delivering a 2-week course of AmB remains a challenge due to SAEs, and cost. Phase II trials have demonstrated that a shorter course of AmB is associated with fewer SAEs than a 2-week course without reduction in rates of fungal clearance by 2 weeks. This is attributed to long half-life of AmB in the brain. The Advancing Cryptococcal meningitis Treatment for Africa (ACTA) trial looked at survival rates at day 14 and day 70 of a 1 week course of AmB combined with either high dose FLU or 5FC, 2 weeks of oral therapy with 5FC and high-dose FLU and 2 week AmB combinations. The study showed that 1-week combination of AmB and 5FC had the lowest 10-week mortality of all regimens and that 5FC was superior to FLU as a partner drug to AmB (10 week mortality HR 0.62, 95% CI: 0.45–0.84, p=0.0020). A systematic review and meta-analysis has looked at data from 13 studies with 2,426 patients. It found that at 10 weeks a 1-week course of AmBd plus 5FC was superior to other regimes for induction treatment of HIV-associated CM. In the case of non-availability of AmB a combination of 5FC and high-dose FLU was the next best alternative. These two regimens have now been accepted as the standard first-line treatment regimens by WHO for HIV-associated CM. The standard WHO recommended treatment regimen for HIV-associated CM is shown in Table 1. The alternate regimens that can be used during the induction phase are: 5FC + high-dose FLU for 2 week or AmB + high-dose FLU for 2 weeks (high-dose FLU=1,200 mg/d).
Efforts to further shorten AmB exposure and thus cut down on cost and minimize SAEs are on. L-AmB is associated with fewer SAEs than AmB. The AMBITION trial was a phase II trial looking at efficacy of different doses and durations of L-AmB in treatment of CM. L-AmB in a dose of 3 mg/kg/d and FLU 1,200 mg/d were administered for 14 days in the induction phase in the control arm. This was compared with LAmB 10 mg/kg on D1 and 5 mg/kg on D3 + FLU 1,200 mg/d for 14 days and LAmB 10 mg/kg on D1 with FLU 1,200 mg/d for 14 days. The primary outcome was early fungicidal activity and it was non-inferior in all the three short course arms as compared to the control arm. The mortalities in all arms were comparable. Encouraged by its results, AMBITION II phase III trial has been rolled out. This study compares the new WHO first-line regimen of AmB 1 mg/kg/d + 5FC 100 mg/kg/d for 7 days followed by high-dose FLU 1,200 mg/d for 7 more days with a single dose of L-AmB 10 mg/kg on day 1 along with 14 days of 5FC and high-dose FLU. The results will be out in mid-2021.75

Management of Raised Intracranial Pressure
An intracranial pressure of >250 mm of water is associated with poor short-term survival in patients with CM.76 Repeated lumbar punctures improved survival in the COAT study.29 The raised ICP is due to a strong inflammatory response in the CNS. Though dexamethasone is known to decrease levels of TNF-α, it also decreases the rate of fungal clearance and causes poor outcomes.77 It is thus contraindicated in treatment of CM.

Advances in Initiating ART in PLHA with CM
Cryptococcal meningitis signals a severely immuno-compromised state in PLHA. But starting ART precipitately can do more harm than good. IRIS occurs in 15–20% patients initiating ART. The Cryptococcal Optimal ART Timing Trial (COAT) provided some definitive guidance to delaying initiation of ART in patients with CM for a minimum of 4 weeks after starting antifungals. This trial demonstrated improved survival in patients with CM in whom ART initiation was deferred for up to 5 weeks after diagnosis as compared with immediate ART (within 1–2 weeks).29 A Cochrane Review further concluded that there is higher all-cause mortality if ART is initiated early in CM in HIV-infected people in low- and middle-income countries.78 The 2018 WHO guidelines recommend that ART should be started 4–6 weeks after starting antifungal treatment.

Newer Adjuvant Therapies for Treatment of CM
Sertraline, tamoxifen, INF-α, and steroids have all been tried. Steroids are associated with slower fungal clearance and increased mortality and are thus not recommended. Sertraline adjunctive therapy led to faster CSF cryptococcal clearance, and decreased IRIS and relapses when compared to historical data.79,80 Neurapheresis is the extracorporeal filtration of yeasts from CSF in CM. A proof-of-concept study in murine models has demonstrated its efficacy. Human trials have not been encouraging.81,82

Newer Drugs
Flubendazole is an imidazole anti-parasitic drug. It acts against cryptococcus by binding to fungal tubulin. Though studies in mice were encouraging, the results in rabbit studies were disappointing due to poor CSF penetration. An oral formulation is under development and could still serve as an adjunctive therapy.83 VT1129 is a tetrazole which has also demonstrated in vitro activity against both susceptible and FLU-resistant cryptococcal species. It

### TABLE 1

<table>
<thead>
<tr>
<th>Medication and dose</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3 - 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB (1.0 mg/kg/d) + 5FC 100 mg/kg/d</td>
<td>X*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLU 1200 mg/d</td>
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<td>X</td>
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<td>FLU 800 mg/d</td>
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<td>FLU 200 mg/d</td>
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<tr>
<td>Treatment phase</td>
<td>Induction</td>
<td>Consolidation</td>
<td>Maintenance</td>
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*Therapeutic lumbar puncture and electrolyte supplementation are most critical during the first week of therapy.*74

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**TABLE 1** WHO-recommended first-line antifungal therapy for treatment of cryptococcal meningitis
acts by inhibiting fungal cytochrome P450 enzyme Cyp51 thereby inhibiting biosynthesis of ergosterol. The unique feature of this drug is its high selectivity for fungal enzyme and thus its lack of SAEs and drug interactions. Some more exciting drugs are under clinical trials and results should be out in 2021. These include an orally bioavailable form of AmB, and a glycoprophatidylinositol-anchored wall transfer protein (G wt 1) inhibitor which inhibits transfer of mannoprotein from the golgi complex to cell wall.

**Prevention**

A number of strategies have been evaluated and implemented to prevent CM in PLHA with low CD4 cell counts in resource-limited settings. A widely accepted one is the “screen and treat” approach. Here, serum CrAg testing is used to decide on starting preemptive fluconazole therapy in CrAg-positive patients. Fluconazole is administered in a dose of 400 mg BD for 2 weeks followed by 400 mg OD for 6 weeks. It is continued at a dose of 200 mg OD till CD4 cell count rises to >200 cells/µL. This approach is associated with a decreased incidence of CM and improved survival among those with advanced HIV disease. It has been successfully implemented in several resource-limited settings, with a baseline prevalence of asymptomatic cryptococcal antigenemia of 5–13%. The cost saving and survival benefit of screen and treat strategy has been validated by many studies when compared with standard of care or universal fluconazole prophylaxis, even at CrAg prevalences of as low as 0.6%. The WHO recommends implementation of CrAg screening and preemptive fluconazole therapy in ART-naive adults with a CD4 count <100/µL before initiating ART in endemic settings. This strategy has been incorporated into existing HIV-care programs of many countries in sub-Saharan Africa. It has been shown that fluconazole monotherapy is inadequate to prevent CM deaths in asymptomatic CrAg positive with undiagnosed cryptococcosis. This is because fluconazole monotherapy is suboptimal for treatment of CM. In all asymptomatic CrAg positive PLHA, every effort should be made to diagnose cryptococcosis. Investigations should include CSF examination and blood cultures. A suggested approach is to give combination antifungal therapy for all CrAg positive patients or at least those with CrAg >1:160. A Thai study showed that in settings where ART is widely available and PLHA with CD4 count <100/µL are regularly started on ART and are CrAg negative, primary prophylaxis with FLU offers no mortality benefit and may not be necessary. Primary anti-cryptococcal prophylaxis is not recommended in high-income regions like Europe and the USA, where ART is widely available and CrAg prevalence in population is low. There is some data that “screen and treat” would be cost-effective, even in resource-rich settings, although this is currently not part of standard practice. Another vexing issue that has risen because of the widespread use of primary prophylaxis for CM in Africa is that of emerging fluconazole resistance in Uganda.

**Secondary Chemoprophylaxis**

Suppressive or maintenance therapy is offered to all CM patients on completion of intensive and consolidation phases of treatment because relapse rate of CM is 50% in the first year. Fluconazole at 200 mg daily is the suppressive therapy of choice. WHO recommends continuing till CD4 count rises to more than 200/µL. IDSA recommends that suppressive therapy can be stopped after 12 months if CD4 count is more than 100/µL and there is virological suppression sustained for ≥3 months (undetectable or very low viral RNA). Therapy should be restarted if CD4 dips below 100/µL.

**Outcomes**

HIV associated CM has a 3-month mortality of 70% in Africa. The major risk factors for mortality are: longer duration of symptoms, altered mental status, concomitant cryptococcal lung infection, disseminated disease, high fungal burden with low rate of clearance, low CSF WBC count (<20 × 10⁶/mm³), raised ICP on admission and lack of facilities to perform therapeutic lumbar puncture (LP) to control ICP and abnormal brain imaging. Mortality is higher in low- and middle-income countries than in high-income countries because of the high proportion of late testers and late presenters, CM being the presenting illness in HIV disease, severe immunosuppression compounded by anemia, malnutrition, delayed diagnosis due to lack of optimal laboratory services, non-availability of good combination antifungal therapy and lack of facilities for therapeutic LP. The study from the USA showed that seizures in HIV+ patients with CM were associated with increased 10 week mortality (adjusted hazard ratio 1.45, 95% CI: 1.11–1.89). Patients with seizures also had more...
cognitive deficits after 3 months when compared to those without seizures.\textsuperscript{37,100} TB confection with CM is associated with an increased hazard of death (HR 1.75, 95% CI: 1.33–2.32, p<0.001).\textsuperscript{101}

A study from Uganda and South Africa showed that the estimated hazard of death at 18 weeks was 10% lower for every 50 cells/µL increase in absolute CD4 cell count at the time of diagnosis. Mortality was lowest when CD4 cell count was between 50–99 cell/µL (mortality 35%) and higher if CD cell count was <50 cell/µL (47%) or if it was >100 cell/µL (40%). These findings were consistent with the DRF theory.\textsuperscript{102}

Cryptococcal antigenemia has a strong association with CM/mortality in PLHA with cryptococcosis and CD4 cell count between 100–200 cells/µL (Hazard ratio 10, 95% CI: 4.5–22.7, p=0.003). A high fungal burden of >100,000 CFUs/mm\textsuperscript{3} is also associated with increased mortality.\textsuperscript{56} The outcome of CM is bad even in Latin America. In one 15 year-follow up study, with 340 enrolled patients, 42% died during follow-up. More deaths were seen after starting ART than in treatment naive HIV-CM.\textsuperscript{104} Another study from the USA showed that CM led to hearing impairment, muscle weakness, and cognitive deficits.\textsuperscript{33,34}

In elderly patients, mortality directly correlates with age (>65 years). The presence of cryptococcaemia is the most significant prognostic factor in these people.\textsuperscript{105}

**Conclusion**

Cryptococcal disease in PLHA continues to be a big public health problem in low and middle income countries. Routine CrAg screening and pre-emptive antifungal therapy, can significantly reduce CM associated morbidity and mortality in these regions. Exclusion of cryptococcosis in asymptomatic PLHA with advanced disease and high CrAg titers is a good strategy. Combination antifungal therapy has made a significant difference in outcome of HIV-associated CM. The regimes are becoming more affordable, shorter, and safer. cIRIS continues to be a problem in immunocompromised persons with cryptococcosis.

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Abstract
Enteric fever is an important resilient community-acquired systemic bacterial infection, commonly seen in resource limited tropical countries with overcrowding and poor sanitation, causing significant morbidity and mortality, and thus posing a grave public health challenge to reckon with. Enteric fever includes infections caused by *Salmonella typhi* (typhoid fever) as well as those caused by *Salmonella paratyphi* A, B, and C (paratyphoid fevers). Its prevention, diagnosis, as well as management pose major challenges with limited options available for empirical treatment. Thus, there is an urgent need to monitor the burden of this disease and antimicrobial resistance to guide empirical therapy and devise newer diagnostic, as well as preventive public health strategies for containment of its spread.

Introduction
Enteric fever is an important differential of acute undifferentiated febrile illness in developing countries. It causes around 14.3 million infections annually with over 135,000 deaths worldwide. Its annual incidence in our country is 493.5/100,000 persons per years with 340.1/100,000 cases per years occurring in children of 2–5 years, the higher incidence in children reflecting active transmission in the community. The burden of typhoid and paratyphoid fever in India as per a meta-analysis in laboratory-confirmed enteric fever cases is estimated to be a prevalence of 7% for *Salmonella typhi* and 0.9% for *Salmonella paratyphi* A.

Human beings are the only reservoir of this infection and the transmission can occur either through direct contact or indirectly via contact with fecal contaminated food or water. Sexual transmission between male partners has also been reported. Both genders and all age groups are infected throughout the year with a peak during the summer and rainy seasons, that is, from May to October. Although the gastric acid serves as a barrier to the entry of *Salmonella* through the oral route, but an excess bacterial load along with the lack of gastric acid in persons on proton pump inhibitors or achlorhydria or *Helicobacter pylori* infection, allows bacteria to traverse through the stomach and reach the small intestine, where these are localized in the Peyer’s patches. However, once the barrier of Peyer’s patches is passed, these can reach different parts of the body, and cause enteric fever and its varied manifestations, which can involve any organ system of the body. Some factors which predispose to severe infection include—primary immunodeficiencies like chronic granulomatous disease, neutropenia, organ transplant recipients.

Incubation period can range from 6 days to 21 days (usually 2 weeks). The clinical presentation of typhoid and paratyphoid fever is the same except milder manifestations and shorter incubation in paratyphoid fever. Most cases of enteric fever are caused by the gram negative bacilli *S. typhi*; however, incidence of *S. paratyphi* A cases is rising in Asia, perhaps as a result of vaccination for *S. typhi*, and
it now accounts for up to one-third of enteric fever cases in India and Nepal.\(^1\,6\,7\)

**Clinical Features**

Fever is the most common presenting symptom in 90%\(^8\) patients and is prolonged (>4 weeks) in untreated patients. It is usually moderate to high grade and remittent nature, without touching baseline or continuous, which rises every 3rd or 4th day, in what is classically defined as step-ladder fashion. However, due to frequent use of antipyretics and antibiotics early in the course, this pattern may not be evident always. High-grade continuous fever is associated with toxemia. Non-specific abdominal symptoms, like vomiting, diarrhea, and occasionally constipation, may also occur during the first week of illness. Soft splenomegaly is characteristic (to differentiate from the firm splenomegaly seen in malaria) and can be easily missed. Patients with enteric fever usually have a coated tongue and relative bradycardia. Patient can also have jaundice because of liver involvement (*enteric hepatitis*), breathlessness, and wheezing (*enteric bronchitis*), obtundation, delirium and coma (*enteric encephalopathy*), bone marrow depression, cholecystitis, loose motions, that is, classically pea-soup diarrhea (*enteritis*), intestinal perforation, and skin rash (*Rose spots*—2–5 mm diameter macular lesions seen transiently during the second week of illness, considered to be due to bacterial emboli). Serious complications usually occur in the second week of illness. Intestinal perforation occurs in about 1–3% of hospitalized patients with enteric fever, with common site being terminal ileum (70–80%) followed by less commonly involved sites like jejunum, ceacum, colon, or gall bladder. Gall bladder may remain a preserved focus in partially treated patients of enteric fever, and lead to a carrier state, wherein the patient may keep shedding bacteria in stools for months or years, becoming a public health hazard. The prolonged asymptomatic carrier state can be seen in about 10–15% of patients.\(^9\)

Atypical manifestations of enteric fever have also been reported including ataxia, sensorineural deafness, meningism, Guillain-Barré syndrome (GBS), myocarditis, acute respiratory distress syndrome (ARDS), osteomyelitis, etc. Complications, like osteomyelitis, mycotic aneurysms, and soft tissue abscesses, are reported more often in paratyphoid infections.\(^10\) Renal complications, like glomerulonephritis, pyelonephritis, cystitis, and mild proteinuria, have also been reported rarely. Hematological involvement can lead to bone marrow suppression, disseminated intravascular coagulation (DIC) and hemophagocytic lymphohistiocytosis (HLH).\(^9\)

Reinfection may occur, if primary infection is terminated using early intervention with antibiotics.

**Differential Diagnosis**

Various tropical infections, like malaria, dengue, chikungunya, leptospirosis, and scrub typhus, are common differentials. However, continuous high grade fever, coated tongue, relative bradycardia, toxic looks, and soft splenomegaly should definitely be considered a pointer to enteric fever.

**Diagnostic Challenge**

Diagnosis of enteric fever poses an immense challenge as blood culture, which is considered the gold standard, has a low sensitivity of 40–60%.\(^11\) Moreover, in our country, delayed presentations with pre-exposure to inadequate and unnecessary antibiotic therapy prior to visiting a proper health-care facility hinders its performance. Though it is positive in the first week itself and also provides sensitivity testing for antibiotics, it takes longer time, thus delaying treatment. Newer commercially available rapid serological tests, like TUBEX TF\(^\text{®}\) (IDL, Sweden) and Typhidot\(^\text{®}\) (Malaysian Biodiagnostic Research, Malaysia), can also be used as point of care tests in an emergency setting for early diagnosis. The former is an antibody-based test based on the principle of inhibition reaction between host and in vitro antibodies that compete for *S. typhi* specific lipopolysaccharide, while latter is a qualitative enzyme-linked immunosorbent assay (ELISA) based test that detects immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against *S. typhi* outer membrane protein. They have sensitivity of 55–70% and specificity of more than 85%.\(^12\) These kits perform better among hospitalized patients than those evaluated in the community setting. Serum Widal test provides the titers of antibodies to somatic ‘O’ and flagellar ‘H’ antigens, and is helpful in the second week of fever onwards. A titer more than or equal to 1:160 is considered positive in endemic areas as in India, although rising titers in paired sera can be very helpful. Third week stool culture and fourth week urine culture may be helpful; however, untreated patients...
are more likely to develop complications in the third and fourth week of fever. In partially treated patients, blood culture may not be positive, but bone marrow culture may still be positive. Directed investigations may be dictated by the suspicion of various complications. Thus, the existing diagnostic tests suffer from limitations regarding time cycle, sensitivity, infrastructure need, etc, emphasizing the need for newer, more accurate, and rapid point-of-care diagnostic tests.

Complete blood counts usually suggest leukocytosis, but bone marrow suppression can be associated with normocytic normochromic anemia, leukopenia, and thrombocytopenia. Transaminitis with mild elevation of serum bilirubin levels may be observed. Kidney functions are normal, but insensible losses due to high fever and anorexia because of toxemia can result in prerenal azotemia.

Management Challenge

Mainstay for management of enteric fever is to give antibiotics along with use of antipyretics and maintenance of hydration and adequate nutrition. Conventionally, chloramphenicol 1–2 g IV 8-hourly used to be the gold standard of treatment; however, with advent of newer potent antibiotics in the 1990s and due to idiosyncratic complication of chloramphenicol, like aplastic anemia, its use was abandoned. Cotrimoxazole and ampicillin were other agents used. Use of aminoglycosides was precluded as they were injectable drugs with well-known neurotoxicity, nephrotoxicity, and ototoxicity. Multidrug-resistant (MDR) strains resistant to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol, soon became prevalent worldwide, though they are now decreasing with wider use of other antibiotics, but development of increasing resistance to fluoroquinolones as well as even cephalosporins is a growing challenge now.

Quinolones are an effective group, and ciprofloxacin, ofloxacin, lomefloxacin, and levofloxacin were all rampantly used with great effect. But due to rising fluoroquinolones resistance, ciprofloxacin is no longer the empirical choice of treatment in our country. Oral azithromycin 20 mg/kg once daily (to a maximum of 1 g daily) for 7 days has been recommended for treatment of uncomplicated enteric fever, and is considered to be equiefficacious to intravenous ceftriaxone (75 mg/kg/day to maximum 2.5 g/day) given for 7 days, with less chances of relapse and similar adverse effect profile. A 5-day course of azithromycin has also been reported to be efficacious.

In a study from Delhi, India, resistance to cotrimoxazole, chloramphenicol, ceftriaxone, and azithromycin were reported to be 6.1%, 13.8%, 16.1%, and 5.78%, respectively over a 7-year period. Multidrug-resistant S. typhi and S. paratyphi A were reported to be 2.73% and 1.91%, respectively. In a recent systematic review and meta-analysis, multidrug-resistant S. typhi was reported to be 9% and multidrug-resistant S. paratyphi as 2% in South Asia. Besides, fluoroquinolone non-susceptible S. typhi and S. paratyphi were pegged at 70% and 53%, respectively in South Asia. Resistance rates for various agents are highly variable from study to study and region to region, but resistance rates to azithromycin appear lowest among all the antibiotics.

There is a decrease in culture positive cases these days, which may suggest a decrease in the burden of disease, but can also be a reflection of the early empirical antibiotic use to treat typhoid fever in the community. There is a possibility that due to the emerging antibiotic resistant strains, under the selective pressure of antibiotic use, only patients who fail to respond to empirical therapy visit tertiary care hospitals. Thus, culture positive cases may represent only a small proportion of total number of cases in a community and this may lead to skewing of antimicrobial susceptibility data toward resistance.

Some treating physicians prefer to use two agents simultaneously to treat enteric fever. But this seems justifiable only in patients who have enteric fever with complications and in patients having no clinical improvement with monotherapy. It may be prudent to start with a single agent in uncomplicated enteric fever, and once susceptibility report is obtained decision on adding a second agent or switching to another agent can be taken. Role of various fixed drug combinations available still needs further evaluation.

Prevention and Control Challenge

Three vaccines are commercially available for typhoid fever, one live attenuated oral vaccine and two injectable
inactivated vaccines, with limited efficacy (50–72%). Ty21a, is an oral live attenuated S. typhi vaccine (given on days 1, 3, 5, and 7, with a booster every 5 years). It is not given in children less than 6 years. Vi CPS is a parenteral vaccine consisting of purified Vi polysaccharide from the bacterial capsule (given in one dose, with a booster every 2 years). It is not given in children less than 2 years. Third, is an injectable typhoid conjugate vaccine (TCV), consisting of Vi polysaccharide antigen linked to tetanus toxoid protein. It can be used in children from 6 months of age and adults up to 45 years of age. No vaccine for paratyphoid fever is presently available.12

Public health measures like availability of safe food and water, adequate sanitation services, and proper personal hygiene (WASH) are important to prevent enteric fever.12 The challenge of controlling typhoid fever is often compounded by the lack of adequate nationwide surveillance in the affected countries. Recently in India, a study titled "National Surveillance System for Enteric Fever in India (NSSEFI)" is being done to estimate the incidence of typhoid fever in age-specific manner in children between 6 months and 15 years.18

**Conclusion**

In this era of emerging antibiotic resistant strains, with no new drug in the horizon, there is an imperative need for continuous surveillance to inform clinicians regarding early diagnosis and better management of infections with use of effective antibiotic policies and also government policymakers for strengthening preventive strategies like adequate sanitation with safe food and water supply, along with development of new vaccines that are effective against both S. typhi as well as S. paratyphi A infections. Though challenges persist in all these aspects hindering implementation of facilities available, an integrated approach with a comprehensive policy framework is required for prevention, control, and elimination of enteric fever.

**References**


CHAPTER 168

Leptospirosis and Brucellosis—Can These Be Difficult to Diagnose?

Saurabh Srivastava, Amit Gupta, Indal Chauhan

Abstract

Zoonotic diseases are important for humans as they may cause disease in them and are recognized as important public health issue. Brucellosis and leptospirosis are important zoonotic diseases of public health importance. The clinical presentation of these disorders mimics many other common diseases. The present chapter summarizes the important manifestation of these disorders and there differentiating aspects with other diseases.

Introduction

Zoonoses are diseases transmitted from animals to man have been recognized as important public health issues for centuries. Brucellosis and leptospirosis are important infections in animal handlers causing wide range of similar clinical manifestation. Butchers and slaughterhouse workers are at high risk of contracting zoonotic diseases due to handling of animals or by ingestion of unpasteurized milk or milk products. Non-specific symptoms including fever lead to chronic disease due to misdiagnosis. Zoonoses should be suspected if there are infection’s protean manifestations along with appropriate exposure history. Hence, in this chapter, we seek to summarize brucellosis and leptospirosis infection in human.

Brucellosis

Livestock animals are mainly affected by brucellosis. It is caused by the small, Gram-negative coccobacilli of the genus Brucella. Main species causing disease in humans are Brucella melitensis (sheep and goat), Brucella suis (pigs), and Brucella abortus (cattle). The disease has worldwide distribution and is mainly seen in rural areas. Relapses can occur after primary infection and disease can also be chronic.

Pathogenesis and Modes of Transmission

Human transmission in brucellosis occurs through direct contact with infected animal, ingestion of unpasteurized milk and milk products, or carcasses of infected animal.

Human brucellosis is usually associated with domestic or occupational exposure to infected animals or their products. Veterinarians, shepherds, farmers, goatherds, and employees of meat-processing plants and slaughterhouses in endemic areas are occupationally exposed to infection. Laboratory workers who handle cultures or infected samples are also at risk. Travelers and urban residents usually acquire the infection through consumption of contaminated foods. The most frequently implicated sources of infection are dairy products, especially soft cheeses, unpasteurized milk, and ice cream, under exceptional circumstances raw meat and bone marrow can also be source of infection. Infections acquired through cosmetic treatments using materials of fetal origin have been reported. Person-to-person transmission is extremely rare, as is transfer of infection.
by blood or tissue donation. No evidence of increased severity and prevalence of *Brucellosis* in immunodeficient individuals and persons infected with human immunodeficiency virus, even though it is a chronic intracellular infection.

**Clinical Features**

The severity of brucellosis is related to infecting species, biotype, and host factors. *B. melitensis* and *B. suis* can cause severe disease in humans, with *B. abortus* usually being associated with milder disease. Human infection with *B. canis* is rare. Human brucellosis can be associated with acute, sub-acute, relapsing, and chronic manifestations. The incubation period is normally between 2 and 4 weeks, but it may be months.

Acute brucellosis can present as a non-specific febrile illness characterized by intermittent or remittent fever (39–40°C). The fever may be associated with chills, night sweats, joint and muscle pain, weight loss, fatigue, malaise, headache, and adenopathy mainly in children.

Waxing and waning type of fever is characteristic of sub-acute brucellosis and is also known as undulant fever. It is often low grade, persists for weeks, accompanied by arthralgia, arthropathy and diminished well-being. Undulant fever is more common in adults than in children and can involve specific organ systems. Intruterine infection, miscarriage, premature delivery, and spontaneous abortion are common during infection of human brucellosis in pregnancy.

**Localized Form**

Primary infection may progress to localized infection (even several months or years later):

- **Osteoarticular:** Sacroiliac joint, lower limbs joints, spine (vertebral osteomyelitis, intervertebral disk infection)
- **Genitourinary:** Orchitis, epididymitis
- **Pulmonary:** Bronchitis, pneumonia, pleurisy
- **Neurological:** Meningitis, encephalitis, polyneuritis

**Complications of Brucellosis**

- **Osteoarticular complications:** Affect 20–40% of patients. Sacroiliitis is the most common reported complication, especially when *B. melitensis* predominates.
- **Pulmonary complications:** Respiratory symptoms are reported by 15–25% of patients but radiological changes are seen in less than 10%. They range from flu-like symptoms to bronchitis, lobar pneumonia, interstitial pneumonia, lung abscesses, hilar lymphadenopathy, and lung effusions.
- **Genitourinary complications:** Complications from the genitourinary tract are rare. Acute orchitis or epididymo-orchitis with signs of systemic infection can occur in males and there can be pyelonephritis resembling tuberculosis, particularly in females.
- **Neurologic complications:** Depression is a common complaint, but invasion of the central nervous system occurs in only 2–4% of cases. It usually presents as acute or chronic meningitis. Encephalitis, polyradiculopathy, psychosis, and meningo-paravascular complications have also been described, as well as rarer complex abscesses.
- **Cardiovascular complications:** Endocarditis, although rare, is the main cause of death related to brucellosis. The aortic valve is more often involved than the mitral valve. Other complications include mycotic aneurysms, myocarditis and pericarditis.
- **Cutaneous involvement:** Cutaneous manifestations of brucellosis consist mainly of transient nonspecific lesions including erythema nodosum, petechiae, vasculitis, papules, and rashes.

**Diagnosis**

The diagnosis should be considered in a case of chronic febrile illness having epidemiological risk factors along with osteoarticular involvement (axial spine or sacroiliac joints), uveitis or other focal lesions described earlier. Confirmatory diagnosis is usually based on microbiologic culture of blood, tissues, or bone marrow. Blood culture, which is gold standard for diagnosis, is positive only in the acute phase and has sensitivity of less than 50–70%. Bone marrow cultures are more sensitive than blood cultures in acute brucellosis and remain positive even in later course of the infection, along with antimicrobial treatment.

Body fluids, such as cerebrospinal fluid (CSF) or joint fluid, show lymphocytosis, low glucose levels and high protein concentration. Elevated CSF adenosine deaminase levels are also present. Biopsied samples of tissues such as lymph node or liver may show noncaseating granulomas without acid/alcohol-fast
bacilli. In recent years, matrix-assisted laser desorption ionization time-of-flight spectrometry (MALDI-TOF MS) has emerged as a powerful tool in bacterial identification. The peripheral blood-based polymerase chain reaction detects bacteremia, to predict relapse, and to exclude “chronic brucellosis.”

Serologic tests are more sensitive than culture. The serum agglutination test (SAT) remains the best standardized and most widely used serologic test. Other serological tests are indirect immunofluorescence, Wright agglutination test, Rose Bengal, ELISA, etc. and provide presumptive diagnoses.

Malaria and tuberculosis are to be ruled out in endemic regions.

Radiography
Small erosions or destruction or joint space narrowing is seen in ankles, knees, hips, vertebrae, and sacroiliac joint. Chest radiograph is often normal but sometimes pleural effusion can be present.

Management (Table 1)
The goal of medical therapy is to control symptoms quickly, in order to prevent complications and relapses.

TABLE 1 Management of brucellosis

<table>
<thead>
<tr>
<th>Category</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children under 8 years on 12</td>
<td>Rifampicin + Co-trimoxazole or Gentamicin + Co-trimoxazole</td>
</tr>
<tr>
<td>Children 8 years and over</td>
<td>Rifampicin + Doxycycline or Gentamicin + Doxycycline</td>
</tr>
<tr>
<td>Adults</td>
<td>Rifampicin + Doxycycline or Doxycycline + Streptomycin or Gentamicin</td>
</tr>
<tr>
<td>Pregnant/breastfeeding women</td>
<td>Rifampicin occasionally Rifampicin + co-trimoxazole</td>
</tr>
<tr>
<td>Sacroiliitis</td>
<td>Doxycycline + Rifampin + Gentamicin—2–3 weeks</td>
</tr>
<tr>
<td>Nervous system infection</td>
<td>Doxycycline + Streptomycin + Rifampicin or Doxycycline + Co-trimoxazole + Rifampicin</td>
</tr>
</tbody>
</table>

Co-trimoxazole: PO for 6 weeks
Children < 8 years: 20 mg SMX + 4 mg TMP/kg 2 times daily
Doxycycline: PO for 6 weeks
Children ≥ 8 years: 1–2 mg/kg 2 times daily
Adults: 100 mg 2 times daily
Rifampicin: PO for 6 weeks
Children: 15–20 mg/kg once daily (max. 600 mg daily)
Adults: 600–900 mg once daily
Gentamicin: IM for 2 weeks
Children and adults: 5 mg/kg once daily
Streptomycin: IM for 2 weeks
Adults: 1 g once daily

High relapse rates reported with monotherapeutic approaches so multidrug antimicrobial regimens are the mainstay of therapy. The risk of relapse is not well understood and resistance is not a significant issue in treatment of brucellosis.

Same treatment is to be given for localized forms of the infection, but for a period of 6 weeks to 4 months depending on the focus.

Leptospirosis
Leptospirosis is an important zoonotic disease globally caused by spirochetes of the genus Leptospira. Leptospirosis affects almost all domestic and wild animals worldwide except Antarctica. Rodents (rat), dogs, and cattle are mainly affected. It is a disease of public health importance in tropics and is seen in agricultural workers as well as urban slum residents. Disease is endemic throughout the world; however, outbreaks superimposed on endemic disease activity are regularly linked to severe hurricane and flooding events.

Pathogenesis and Modes of Transmission
Human transmission of leptospirosis occurs by contact of moist soil or freshwater contaminated with urine of
an infected animal (indirect contact) or direct contact of blood, urine, and other body fluids or tissues of an infected animal through skin lesions or mucous membranes.

Leptospires are shed in urine as they colonize proximal renal tubules of mammalian hosts. They can survive for several months in the environment under moist conditions; factors affecting survival are the presence of warmth (above 22°C) and neutral pH (pH 6.2–8.0). Rodents are a particularly important reservoir. Some serovars appear to be preferentially adapted to select mammalian hosts; examples are the serovar *Icterohaemorrhagiae* which is primarily associated with the Norway rat, *pomona* with swine and cattle and *canicola* with dogs. However, a particular host species may serve as a reservoir for one or more serovars, and a particular serovar may colonize different animal species. Transmission of infection in humans usually occurs through contact with contaminated water or moist soil. Organisms enter humans through the mucosal surface of the nasopharynx, mouth, eye, or esophagus or through abrasions of the skin. Risks of leptospirosis transmission and exacerbation are increased due to migration of the rural poor to urban slums. Intense exposure to leptospires has been documented in workers of sugarcane, rice, and rubber plantation. Leptospirosis is also acquired by direct contact with the urine, blood, or tissues of infected animals but is less frequent.

**Clinical Features**

Severe, icteric illness occurs in less than 10% of symptomatic infections; however, subclinical infection is very common. Majority of cases of leptospirosis are mild, presenting as febrile illness, but disease can be potentially fatal leading to multiorgan dysfunction. The incubation period is usually 1–2 weeks but ranges from 1 to 30 days. Leptospirosis is classically described as biphasic.

**Acute phase (leptospiremic/septic/anicteric phase):** characterized by:
- High fever with chills of sudden onset (3–10 days duration)
- Headache
- Muscle pain (especially calf pain)
- Photophobia
- Ocular pain
- Bilateral conjunctival hemorrhage is very frequent
- Organism can be cultured from blood and detected by polymerase chain reaction
- May be associated with:
  - Gastrointestinal symptoms (abdominal pain, nausea, anorexia, vomiting)
  - Non-productive cough
  - Adenopathies
  - Hepatomegaly

**Immune phase:**
- The signs of the acute phase regress after 5–7 days then reappear for a few days but are usually mild (milder fever, less severe myalgia) and then disappear.
- Coincide with the appearance of antibodies, and leptospires can be cultured from the urine.
- Aseptic meningitis is the hallmark of immune phase of leptospirosis but is not associated with mortality. Cerebrospinal fluid pleocytosis can be demonstrated in 80–90% of patients during the second week of illness. Clinical signs and symptoms of meningitis are seen in only 50% cases.
- Uveitis is a late manifestation of leptospirosis. It is generally seen after 4–8 months of illness. Most frequently affected area is anterior uveal tract. Common symptoms of uveitis are photophobia, pain, and blurring of vision.

**Severe or Ictero-hemorrhagic Form (Weil’s Disease)**

The onset is the same but a few days later the symptoms worsen:
- Renal disorders (oliguria or polyuria or anuria)
- Hepatic disorder (jaundice—appears 5–9 day of illness, hepatomegaly)
- Widespread hemorrhages (ecchymoses, haemoptysis, purpura, epistaxis, etc.)
- Pulmonary signs (chest pain)
- Cardiac signs (myocarditis, pericarditis)
- Refractory shock
- Death occur from subarachnoid hemorrhage or gastrointestinal bleeding
- Eye signs: Conjunctival hemorrhage, scleral icterus, and conjunctival suffusion
- Leptospirosis-associated severe Pulmonary Hemorrhage Syndrome is as a widespread public health problem having a case fatality rate of about 50%
- Apparent critical threshold for severe outcomes such as SPHS and death is leptospiremia of 10,000 or more bacteria per milliliter of blood
Diagnosis

Leptospirosis may be difficult to distinguish from other infectious causes of fever, and a high index of suspicion is required based on the local epidemiology. Modified World Health Organization (WHO) Faine’s criteria (with amendment) 2012 Criteria for Diagnosis of Leptospirosis is summarized in Table 2. 17

Laboratory diagnosis is difficult to obtain. The routine investigation demonstrates:
- **Complete blood count**: Anemia, polymorphonuclear leukocytosis, or thrombocytopenia.
- **Urine**: Leukocyturia, proteinuria, possible microscopic hematuria.
- Enzyme markers of skeletal muscle damage are elevated in the sera of 50% of patients during the first week of illness, such as creatine kinase, aldolase, etc.
- Hyperbilirubinemia, prolongations of the prothrombin time, and modest elevations of serum alkaline phosphatase are typical. There is mild hepatocellular necrosis in Weil’s disease.
- Elevated markers of inflammation (C-reactive protein level, procalcitonin level, and erythrocyte sedimentation rate).

Serology

- **Between 0 and 7 days**: Real-time PCR (early diagnosis)
- **After 7 days**: Microscopic agglutination test (MAT); IgM ELISA test provides presumptive diagnosis
- **After 10 days**: MAT and IgM ELISA tests only.
  - **Culture**: Limited use (bacteria grow slowly, specific culture medium)

Radiography

The most common radiographic finding is a bilateral patchy alveolar pattern that corresponds to scattered alveolar hemorrhage. These abnormalities predominantly affect the lower lobes. Other findings include pleura-based densities (representing areas of hemorrhage) and diffuse ground-glass attenuation typical of acute respiratory distress syndrome (ARDS).

Management (Table 3)

Management should be started on high index of suspicion as early intervention may prevent the development of multiorgan failure and antibiotics are less likely to benefit once organ damage has occurred. 10

<table>
<thead>
<tr>
<th>Indication</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild leptospirosis</td>
<td>• Doxycycline 100 mg twice a day or&lt;br&gt;• Amoxicillin 500 mg thrice a day or&lt;br&gt;• Ampicillin 500 mg thrice a day</td>
</tr>
<tr>
<td>Moderate/Severe leptospirosis</td>
<td>• Ceftriaxone 1.5 million units IV or IM 6 hourly or&lt;br&gt;• Cefotaxime 1 gm IV 6 hourly or&lt;br&gt;• Doxycycline 200 mg IV loading followed by 100 mg IV 12 hourly</td>
</tr>
<tr>
<td>Chemoprophylaxis</td>
<td>• Azithromycin 250 mg PO once a week or&lt;br&gt;• Doxycycline 200 mg PO once a week</td>
</tr>
</tbody>
</table>

All regimens to be given for 7 days. Aggressive supportive care in leptospirosis essential and can be lifesaving.
### TABLE 4  Differentiating clinical features of various diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Symptoms</th>
<th>Physical and laboratory findings</th>
<th>Chest radiograph</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fever</td>
<td>Cough</td>
<td>Sputum</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Malaria</td>
<td>++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Influenza</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mycoplasma pneumonia</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Dengue</td>
<td>++</td>
<td>biphasic fever</td>
<td>–</td>
</tr>
<tr>
<td>Enteric fever</td>
<td>++</td>
<td>continuous fever</td>
<td>–</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>+ evening rise</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Legionellosis</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Rickettsial Disease</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>
Differentiating Brucellosis and Leptospirosis with Other Diseases

The differential diagnosis of these infections is broad, reflecting the diverse clinical presentations of the disease. When fever, headache, and myalgia predominate, influenza, and other common and less common viral infections (e.g., dengue and chikungunya) should be considered. Malaria, typhoid fever, Rickettsial diseases may mimic the early stages of disease and are important to recognize. Dual infections have been reported. In this light, it is advisable to conduct testing for other diseases also. Some common features to differentiate these infections with other common infections are shown in Table 4.

Conclusion

Brucellosis and leptospirosis have diverse clinical presentations, so these diseases have to be differentiated from many common disorders like influenza, malaria, dengue, typhoid, and rickettsial diseases. Although, management of these diseases is not difficult but early diagnosis is key to better outcome.

References

**Abstract**

*Klebsiella pneumoniae* is an important pathogen that belongs to family *Enterobacteriaceae*. Incidence of extended spectrum beta lactamases (ESBL) producing Gram-negative bacteria has gradually increased in last 2–3 decades. Problem is further compounded by carbapenemase producing Gram-negative bacteria. Carbapenem-resistant Enterobacteriaceae (CRE) has become a global threat recently. WHO in a policy statement in 2017 had mentioned that future development of antimicrobial agents should focus on CRE. Klebsiella is the most important pathogen of CRE family encountered all over the world.

**Introduction**

Edwin Klebb in 1875 had described Klebsiella, as a Gram-negative bacterium belonging to family *Enterobacteriaceae*. *Klebsiella* is part of microbiome in healthy individuals and colonizes mainly gastrointestinal system. It can cause severe infections in critically ill and immune-compromised patients. Klebsiella causes urinary tract infection, pneumonias, blood stream infections, liver abscesses, necrotizing fasciitis, meningitis and surgical site infections, mainly. Kaur et al. from a single tertiary care center in Delhi reported 69.3% mortality in patients suffering from colistin-resistant *K pneumonia* infections. Another study from South India reported high rates of resistance to Carabapenem, Minocycline, and Tigecycline in isolates of *Klebsiella* blood stream infections. Carbapenem resistance had increased during the study period. High resistance of Klebsiella to various antibiotics is also reported from China, Korea, Greece, other European countries, and the USA. Falagas et al. have shown that mortality due to carbapenem-resistant *Klebsiella pneumoniae* exceeds 60% despite the combination therapy.

*Klebsiella pneumoniae* is the main pathogenic organism in the genus. In some cases *K oxytoca* have been isolated from human clinical specimens. *Klebsiella* can be differentiated into various types by biotyping, serotyping, phage typing, bacteriocin typing and molecular typing. Serotyping is most commonly used and is based on capsule antigens.

**Pathogenicity**

Pathogenicity of *Klebsiella* depends upon these factors—

*Capsular antigens:* *Klebsiella* has a capsule made of complex acidic polysaccharides. Capsular antigens are essential to virulence and have been divided into 79 serotypes. Klebsiella strains of K1, K2, K4, and K5 are more virulent.

*Fimbriae:* Fimbriae (Pili) are filamentous projections on bacterial surface by which it attaches to the host surface.
Fimbriae help in developing biofilms and also help organism to adhere to medical devices. 

*Lipopolysaccharide:* Helps in evading host’s defense mechanisms.

*Siderophores:* Iron is an essential factor in bacterial growth. The level of free bioavailable iron is too low for bacteria growth. Klebsiella secretes high affinity, low molecular weight iron chelators, called siderophores. These siderophores in Klebsiella belong to two different groups. More common, the phenolate group with best known representative enterobactin, and hydroxamate type siderophores with best known representative, as aerobactin and ferrioxamine.5

Some terms like hypervirulent Klebsiella pneumoniae (hvKp) and hypermucoviscous K pneumoniae are also being used recently. hvKp was first recognized in Taiwan in 1986, although genomic studies suggest that it existed unrecognized as early as 1920s.7 Approximately 70% of hvKp isolates are capsular types K1 or K2. Fortunately less drug resistance has observed in hvKp. The siderophore, aerobactin accounts for more than 90% of hvKp’s total siderophore production. Clinical laboratories are unable to differentiate between classical K pneumoniae and hvKp. There is no commercially available assay that reliably differentiates the two. However, several biomarkers, including peg 344, iroB, iucA, MPa, mpA2, and quantitative siderophore production greater than 30 mg/mL predict hvKp strains.8

Hypermucoviscous phenotypes may also show increased virulence, as it may prevent them from host’s defense mechanisms.

**Role of Clinical Microbiology**

In isolates suspected to be carbapenemase producer Klebsiella, a double disc synergy test (DDST) on Muller-Hinton agar media may be done. Tests using inhibitors such as boronic acid or EDTA are being used more frequently to detect KPC or Metallo Beta Lactamase (MBL) carbapenemase respectively. The modified carbapenem inactivation method (Mcim) has replaced modified Hodge test recently, as it is easy to perform and is less expensive. Various automated systems may be employed to detect KPC producers. Various molecular methods, for example molecular antibiogram, have been used in identification of pathogens and most common resistant genes. Molecular assays though expensive, but help clinicians in de-escalation of therapy, avoiding unnecessary antibiotics, reducing length of stay and mortality.9

**Mechanisms for Antibiotic Resistance**

As early as 1970s, aminoglycoside resistant Klebsiella were noted. Since 1982, strains that produce ESBL, making them resistant to extended spectrum cephalosporins (hallmark being resistant to ceftazidime). These ESBLs were, SHV-5 in Europe and TEM-10 & TEM-12 in the USA. The first report of carbapenemase-resistant Klebsiella pneumonia in the USA was in 1996. Since then it has been widely reported in various countries of Europe, South America, and Asia. Carbapenemase produced may belong to Ambler class A (K pneumoniae carbapenemase) or class B (metallo-β-lactamases, MBL, New Delhi metallo-β-lactamases, NDM) and class D (OXA-48 like carbapenemases).9

Polymyxins serve as a last resort for treatment in CRE. Lately resistance to polymyxins is being encountered all over the globe. Polymyxin resistance has been reported as an independent marker for 14-day mortality in patients with KPC producing K pneumoniae. Polymyxin resistance is chromosomally mediated and takes place by addition of cationic groups to bacterial outer membrane. More recently a plasmid mediated gene (mcr-1) has been discovered, which confers resistance to polymyxins.10

As per clinical laboratory standards institute/European committee on antimicrobial susceptibility testing (CLSI/EUCAST) both microdilution should be used as a reference model for polymyxins susceptibility testing.

The SENTRY11 and SMART study12 reported that in India, most common gene foe encoding carbapenemase was NDM-1 followed by OXA-48 variants. However, a study from South India, done later reported equal distribution of NDM-1 and OXA-48 like genes.13

**Treatment**

The worldwide spread of multidrug resistant Klebsiella and KPC producing strains are causing a serious threat. In our country resistance to various antibiotics is steadily increasing. Problem is further aggravated by absence of a central collating body, ineffective antibiotic stewardship program, and rampant misuse of available antibiotics. Major pharmacological companies are not doing enough
research for discovering newer antibiotics. There is no gold standard treatment for KPC producing Klebsiella. The choice of treatment depends upon site and severity of infection, local antibiogram, and host factors. Various strategies to effectively treat these infections are discussed below.

**Combination Therapy**
Most of the retrospective studies have found that combination therapy is associated with lower mortality. Choice of the combination should be guided by local antibiogram. For KPC producing Klebsiella, it will be prudent to use polymyxins with carbapenems. However, this combination may not show good results, if MIC levels are more than 16 μg/mL. A study from ICU patients in North India showed polymyxins resistance in Klebsiella isolates in blood, urine, and sputum samples as 8.75%, 4.26%, and 4.4%, respectively.14

**Dual Carbapenem Therapy**
It refers to the use of ertapenem with either meropenem or doripenem. Ertapenem has highest affinity for carbapenemase and may consume most of it and allowing larger part of the other carbapenem to exert its bactericidal effect.15

**Triple Combination Therapy**
Triple combination of polymyxins, carbapenem, and tigecycline has been found to significantly reduce the mortality in infections due to KPC producing Klebsiella strains. The emergence of polymyxins resistant strains during the treatment is a serious concern.15

**Newer Agents**
Avibactam is a non-beta-lactam beta-lactamase inhibitor that has activity against class A and C beta-lactamases including KPC and AmpC. Its combination with ceftazidime has been approved by FDA in 2015 for complicated UTI including pyelonephritis. It may not be effective if MIC levels are more than 8 μg/mL. Meropenem with another beta-lactamase inhibitor vaborbactam is also being tried. Imipenem-cilastatin along with relebactam, another new beta-lactamase inhibitor is also being used. Plazomicin a new aminoglycoside with efficacy against multidrug resistant Enterobacteriaceae bacteria is also available now. Eravacycline, a semi-synthetic tetracycline with efficacy against KPC and NDM carbapenemase, is showing promise.15

**Conclusion**
To conclude, the management of multidrug and pan-drug resistant Klebsiella is a serious challenge to health care. Klebsiella is a major source of carbapenemase resistance in various species through horizontal transfer of plasmids and transposons, leading to emergence of multidrug and even pan-drug resistance. We need good infection control program, antibiotic stewardship, environmental cleaning, hand hygiene, and contact precautions to prevent spread of these deadly bacteria. Tracing carriers of KPC Klebsiella and eradicating through selective digestive decontamination may be an exciting option.16

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1110 SECTION 12 Infectious Disease

Infection with *Acinetobacter*—A Challenge for the Physician

Krishna Padarabinda Tripathy

**Abstract**

Acinetobacter species has emerged as one of the most significantly resistant pathogens causing healthcare associated infections. *Acinetobacter baumannii*, a Gram-negative coccobacillus constitutes 85% of the infections caused by Acinetobacter species in human beings. Prolonged length of hospital stay, invasive procedures, use of third-generation cephalosporins, etc. are some of the risk factors contributing toward the infection with this resistant organism. It has been increasingly recognized as an important cause of pneumonia, septicemia, meningitis, urinary tract, and wound infections and is associated with high mortality. Due to its inherently resistant property and aptitude to develop MDR and XDR strain, it has proven to be a therapeutic challenge for the physicians. This chapter highlights the infection and disease producing factors, mechanism of antibiotic class resistance and its treatment, and an unpublished data about *A. baumannii* in brief.

**Introduction**

In the modern health-care system, Acinetobacter has undoubtedly emerged as one of the most significant pathogens accountable for healthcare associated infections involving multiple organs. It is also known to cause community acquired infections in combat zone and disaster associated infections because of its ubiquitous nature. In 1911, Acinetobacter species were isolated from soil and first described as Micrococcus calcoaceticus. Thereafter, the genus was renamed multiple times for several years, and since 1950, it is referred as Acinetobacter. Earlier Acinetobacter was an organism of unconvincing pathogenicity, which during the last three decades has emerged as a serious infectious agent to the hospitals around the globe.

According to many studies worldwide, it has been found that *Acinetobacter baumannii* develops resistance to antimicrobials rapidly, resulting in multidrug-resistant and pan-drug-resistant strains, which may cause large outbreaks of healthcare associated infections. The World Health Organization declared that the ESKAPE organisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter species*) are known to effectively escape the outcome of antibacterial drugs. *A. baumannii* is amongst the most serious ones in the group. This Gram-negative coccobacillus because of its inherent mechanism of resistance and pathogenicity has become a threat to mankind and one of the significant therapeutic challenges for the physicians.

**Bacteriology**

These species are strictly aerobic, Gram-negative, non-motile, oxidase negative, catalase positive, non-fermenting coccobacillus that grows at 20–30°C and could be easily recovered on standard culture media. Amongst the Acinetobacter species causing infections in human beings, *A. baumannii* constitutes about 85% of the infections in humans. Other species in the genus causing occasional infections in human beings are *Acinetobacter nosocomialis, Acinetobacter pittii*. Molecular methods like Matrix assisted laser desorption-ionization-time-of-flight
mass spectrometry (MALDI-TOF-MS) and quantitative real-time polymerase chain reaction (RT-PCR) are often required to detect *A. baumannii* because it is difficult to identify it just based on phenotype.

Acinetobacter species that have been isolated from foods, arthropods, the environment, soil, and water are known to be its natural habitats. In our day-to-day practice, Acinetobacter culture positivity should be analyzed as colonization which is the presence of bacterium on a body surface (like on the skin, oral cavity, airway, or intestines) and not causing disease in the individual, in contrast to infection, where there is invasion of a host’s body tissues by disease-causing organisms. There are various risk factors for colonization and infection with the pathogen that is summarized in Table 1. It is hypothesized that *A. baumannii* species are ubiquitous in nature as they survive in dry as well as damp environment which promotes its environmental desiccation for weeks and causes fomite contamination in hospitals.

In the United States, the Centre for Disease Control have estimated 12,000 Acinetobacter infections every year out of which multidrug-resistant strains causes 7,300 cases with high mortality. The Indian Council of Medical Research estimates Acinetobacter species as the second most common isolated pathogen (45%) causing hospital-acquired infections after Pseudomonas species (52%). *A. baumannii* was found resistant to most antibiotics except colistin, it showed 70% non-susceptibility to almost every antibiotics tested. Invasive specimens from lower respiratory tract and blood had higher non-susceptibility rates against different categories of antibiotics in comparison to other specimens.

**Pathogenesis**

*A. baumannii* intrinsically is more virulent in humans as compared to other Acinetobacter spp. as it grows better at 37°C and resists macrophage uptake than other species. The important virulence factors and their role in pathogenesis is summarized in the Table 2.

**Mechanism of Resistance**

Acinetobacter infections have proven to be a therapeutic challenge to physicians as it is one of the most resistant organisms known and its treatment centers upon controlling the antibiotic resistance. It is an inherently resistant organism which has the aptitude to develop multidrug-resistant and extensive drug-resistant strains. This is one of the vital contributory factors in its capability to adapt itself to changes in environmental pressures. The common mechanisms of resistance are explained in Figure 1.

**AmpC cephalosporinase:** Acinetobacter-derived cephalosporinases (ADCs) are the genomic variants of *A.
Infection with Acinetobacter—A Challenge for the Physician

**Fig. 1: Mechanism of resistance in Acinetobacter baumannii**

baumannii which contains a non-inducible chromosomal AmpC cephalosporinase. Addition of promoter insertion sequence known as ISAba1 regulates the AmpC gene. Overexpression of AmpC cephalosporinase/ Aminoglycoside modifying enzyme (AME) and the presence of ISAba1 is intrinsically responsible for resistance to extended spectrum cephalosporin. Antibiotics like Cefepime and carbapenems, so far seems to have a stable response to these enzymes.

- **Porin channels:** Amongst the list of various mechanisms causing carbapenem resistance, the commonest are absence of penicillin binding protein 2 (PBP2) and production of naturally occurring oxacillinase (OXA). Some isolates have been observed to have added downregulation of porin expression, leading to reduction in entry of carbapenem.

- **Efflux pumps:** Overexpression of bacterial efflux pumps decreases the concentration of β-lactam antibiotics in the periplasmic space. Efflux pumps usually act in association with overexpression of AmpC β-lactamas or carbapenemases causing resistance in Acinetobacter. Efflux pumps not only remove β-lactam antibiotics, but can actively remove chloramphenicol, quinolone, tetracycline, tigecycline, and disinfectants.

- **Serine and metallo-β-lactamases:** It confers resistance to carbapenems as they as they exhibit strong hydrolytic activity against all β-lactam antibiotics except monobactams (i.e., aztreonam).

- **DNA topoisomerase mutations:** Resistance to aminoglycosides and quinolones in Acinetobacter infections is due to the point mutations in the gyrA and parC topoisomerases enzymes which are the bacterial targets.

- **PmrA and PmrB proteins:** Due to its overexpression, resistance to colistin occurs.

**Definitions in Antimicrobial Resistance**

- **Multidrug resistant (MDR):** When the isolate is resistant to at least three classes of antimicrobial drugs, that is, all penicillin and cephalosporins (including inhibitor combinations), aminoglycosides and fluoroquinolones, it is said to be “MDR Acinetobacter species.”

- **Extensively-drug resistant (XDR):** When the Acinetobacter isolate is resistant to carbapenems and shall also be resistant to three classes of antimicrobials mentioned above (MDR), it is said to be “XDR Acinetobacter species.”
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Pan-drug-resistant (PDR): When the XDR Acinetobacter isolate is also resistant to tigecycline and polymyxin, it is said to be “PDR Acinetobacter species.”

Clinical Manifestations

Pneumonia
Nosocomial pneumonias occur due to aspiration, prolonged hospital stay, and mechanical ventilation, which create an ideal environment for transmission of bacteria like Acinetobacter. It adheres and forms biofilms on the tube. The clinical manifestation is fever and increased sputum production. It is diagnosed through respiratory cultures. Imaging findings are non-specific and can include lobar consolidations, pleural effusion, or rarely cavitary.

Community-acquired pneumonia is not as common as nosocomial pneumonia but a serious manifestation when caused by A. baumannii. Majority occurs in regions with hot and humid climates. It is caused by a diverse range of strains and is distinct from hospital strains. Symptoms and signs are similar in both the types. Without appropriate initial antibiotic therapy, mortality rate has been reported to be as high as 64%.

Bloodstream Infections
Bloodstream infections due to A. baumannii occur mostly in the presence of central venous catheter or due to dissemination secondary to extensive pneumonia. Patients may present with septic shock and disseminated intravascular coagulopathy.

Skin and Soft Tissue Infections
Acinetobacter species are known to colonize the skin flora and it is important to differentiate colonization from nosocomial infections. Trauma-associated A. baumannii skin and soft tissue infections may be due to orthopedic external fixator devices, gunshot wounds, etc. There have been several recent reports regarding the occurrence of necrotizing fasciitis due to A. baumannii. The skin infection due to this species may evolve from an edematous peau d'orange appearance to sandpaper-like, to a necrotizing process with hemorrhagic bullae.

In war-zones or after a traumatic injury to soldiers, Acinetobacter has been a significant cause of skin & soft tissue infections. These infections are also found following natural disasters (floods, earthquakes, etc.).

Urinary Tract Infections
Health-care associated urinary tract infection which is a major source of A. baumannii isolates occur mainly as catheter-associated infections or with percutaneous nephrostomy tubes. Community-acquired A. baumannii urinary tract infections are reported in post-renal transplant and nephrolithiasis patients.

Other Miscellaneous Infections
Meningitis: Majority of the cases of A. baumannii meningitis have been reported in post-traumatic injuries and post-neurosurgical procedures.

Osteomyelitis: Occurs typically postsurgical or trauma related. Acinetobacter can cause keratitis due to use of contact lenses, and few cases of native and prosthetic valve endocarditis have been reported.

Our institutional experience (unpublished): In our institute KIMS Bhubaneswar, a recent study was done for 1 year (July, 2018–June, 2019). Around 455 patients grew A. baumannii infection in various cultures, out of which 348 (76.4%) found in ICU patients and 107 (23.6%) in non-ICU patients. Respiratory tract infection was the commonest infection in ICU as well as non-ICU settings, followed by septicemia in ICU and skin and soft tissue infection in non-ICU settings. In ICU setting, 8% of isolates were pure MDR, 68% were XDR, 7% PDR, and 17% were sensitive to all classes of antibiotics while in non-ICU patients, 14% of isolates were pure MDR, 52.3% XDR, 9% PDR, and 24% were sensitive to all classes of antibiotics. This states the seriousness of infections caused by MDR and XDR A. baumannii organisms in a health-care setting and the spread of pan drug resistance even in non-ICU areas.

Figure 2 depicts the pattern of drug resistance as per the isolates obtained from different site of infections.

Treatment
Acinetobacter is considered amongst the most resistant organisms and initiating effective empirical therapy is challenging to the physicians. Definitive therapy should be based on antimicrobial susceptibility test results.

Empirical Therapy
Antibiotic susceptibility pattern is an important factor, which guides clinicians regarding the therapy in an intrinsically resistant organism. In A. baumannii
infections, empirical therapy is advisable to initiate with carbapenems, as the probability of \textit{A. baumannii} being resistant to cephalosporins (a common first-line antibiotics) is more. After the sensitivity reports therapy is modified accordingly.

**Treatment of MDR \textit{Acinetobacter} Species**

Here the preferred drugs are Carbapenems, if susceptible. Usually, imipenem is given at the dose of 500 mg q6h and meropenem at the dose of 2g q8h. The prolonged infusion over 3–4 hours is better than bolus injections.

**Treatment of Extensive and Pan-drug Resistant \textit{Acinetobacter} Species**

The resistance rates of carbapenems in \textit{A. baumannii} infections have been rising substantially globally. However, there is no consensus suggesting optimal anti-microbial treatments for such strains. The options available are: Tigecycline is a glycyclcline antibiotic and is another drug that can be used clinically against MDR \textit{A. baumannii}; Tigecycline is usually found to have a low MIC (2 g/mL) for \textit{A. baumannii} strains, the serum concentration of the drug is also low, and the outcome of ventilator-associated pneumonia and bacteremia associated with \textit{A. baumannii} clinical trials have shown inferior results compared to other alternative agents.

Minocycline is a tetracycline that has good antimicrobial activity and it may even act against the strains resistant to other tetracyclines (including tigecycline). Cross-resistance has been reported with minocycline. Minocycline therapy has high treatment success once given in combination therapy.

Aminoglycosides (amikacin and tobramycin) are not of abundant use against \textit{A. baumannii} due to its toxicity and lack of lung penetration. Inhaled tobramycin can be an adjunct as inhalational therapy in \textit{A. baumannii} pneumonia.

Polymyxins (Colistin and Polymyxin B) being a cationic detergent disrupts bacterial cytoplasmic membranes. It was previously abandoned for its nephrotoxicity and neurotoxicity. Currently the nephrotoxicity rates are reportedly 36%, and neurotoxicity is rarely seen these days due to modified formulations. Although the dosage differs from patient-to-patient, polymyxin can be given at the dose of 1.5–3 mg/kg q12h in MDR-Acinetobacter infections.

Colistin can be given inhalational and intravenous route. For combination therapies, colistin is a key component. An important side effect of inhalational colistin is bronchoconstriction.

The rising rates to resistance are to the last-line drugs, that is, tigecycline and polymyxin against \textit{A. baumannii} are highly reported and is of substantial concern. There is a critical need to find alternative therapeutic options for this.

**Combination antimicrobial therapy:** Combination therapy appears to be promising in prevention of emergence of resistance and in improving clinical outcomes. Its key component to treat MDR \textit{A. baumannii} is Colistin. Many colistin-based combined therapies, including colistin-rifampicin, colistin-minocycline, colistin-carbapenem, colistin-sulbactam have been found to act synergistically in vivo or in vitro against \textit{A. baumannii}.

Although polymyxin causes dose-related nephrotoxicity, Polymyxin B combination therapies when used with amplified doses of antibiotics such as carbapenem, minocycline, and tigecycline have been found to attenuate the development of polymyxin resistance.

**Future treatment options:** In view of increasing rates of emergence of multidrug resistance and lack of newer class of antibiotics, alternative strategies are in pipeline to control and treat \textit{A. baumannii}.

- **Bacteriophage:** Bacteriophages therapy is being re-evaluated as an alternative management option to help counteract antibiotic resistance due to its high
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**Fig. 3:** Source of infections and the control measures to prevent infections

- Physical separation of *A. baumannii* positive patients from other patients.
- Chlorhexidine baths of every patient in ICU.
- Health-care workers should take contact precautions and maintain hand hygiene.
- Disinfection of equipment between patients.
- To follow antibiotic stewardship.

**Infection Control and Prevention**

Source of infections and the control measures to prevent infections is summarized in **Figure 3**.

**Conclusion**

Acinetobacter species have emerged as one of biggest challenge to physicians because of its inherent resistant strains and ability to cause extensive resistance to even the newer antimicrobials. To decrease the spread and emergence of resistance of *A. baumannii* infections, it is important to promote rationale use of antibiotics, and take adequate control measures to prevent the establishment of drug resistant endemic strains.

**References**

Abstract

Scrub typhus is an arthropod born acute infectious disease that is caused by intracellular Gram-negative organism Orientia tsutsugamushi. This disease is mainly reported from states of the Himalayan terrain, Eastern and Western Ghats, and the central part of India. Scrub typhus is transmitted by some species of trombiculid mites which are found in areas of heavy scrub vegetation. The mites feed on infected rodent hosts and subsequently transmit the parasite to other rodents and humans. The main target tissue of O. tsutsugamushi is the vascular endothelium in human, leading to generalized vasculitis and perivascular inflammatory changes, and results in end-organ damage of many vital organs including nervous system, cardiovascular system, kidney, lung, and other organ. Scrub typhus presents with fever, headache, myalgia, cough, abdominal pain, conjunctival redness, altered consciousness, apathy, shin pain, lymph node enlargement, hepatosplenomegaly, macula-papular rash and eschar. Currently, the drug of choice for treatment of scrub typhus is doxycycline. Other drugs used to treat scrub typhus, includes tetracyclines, chloramphenicol, rifampicin, azithromycin and quinolones.

Introduction

Scrub typhus is an arthropod born acute infectious disease that is transmitted to humans by Trombiculidae family. It is caused by intracellular Gram-negative organism Orientia tsutsugamushi. This disease was first described in 1899 in Japan. People of all ages are affected by this disease including those at extreme of age. The term "scrub" is related to the vegetation (land between woods and clearings) where vectors are proliferating. The name scrub typhus is misleading, as the disease can be contracted in many other habitats, including forest, beaches, large gardens, and plantations. The word “typhus” is a Greek word which means “fever with stupor” or smoke.1 “Tsutsuga” means small and dangerous and “mushi” means insect.

Scrub typhus is mainly distributed in the tsutsugamushi triangle, which is bounded by Australia in the south, Japan in the east, Afghanistan in the south, Japan in the north. The disease is mainly prevalent to south-eastern and eastern parts of Asia including India, Myanmar, Nepal, Sri Lanka, Thailand, and other areas in the region.2 More than hundred crore people of world are at risk for scrub typhus and an estimated ten lakhs cases occur annually.3 In India, scrub typhus is reported from states of the Himalayan terrain, Eastern and Western Ghats and the central part of India. Number of Leptotrombidium deliense is affected by rainfall, which may be the cause for a higher case during the Monsoon time as shown by Gurung et al.4 In India, the agent of scrub typhus is O. tsutsugamushi. Its antigenic structure is different from other Rickettsiae. Although our country India is a part of tsutsugamushi triangle, Scrub typhus grossly remains under diagnosed owing to the non-specific variable clinical presentation except eschar, poor specific diagnostic facility and very low index of suspicion by the physician.
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VECTOR AND HOST

*L. deliense* and *L. akamushi* are the main vectors of scrub typhus, which are found in countries of the Southeast Asian region including India. This disease is spread through the larval mites or “chiggers.” The natural hosts for scrub typhus are small rodents including *Rattus* subgenus. The field rodents and the vector mites act as a reservoir and because of these two the infection continues in nature.

**TRANSMISSION AND PATHOGENESIS**

The causative organism of scrub typhus is *O. tsutsugamushi*, which is an intracellular Gram-negative bacterium. Humans are accidental hosts and the infectious agent is transmitted through the skin by the bite of larval stage of infected trombiculid mites or chiggers. Disease occurrence is more in rainy season and commonly seen among persons who engage in occupational or other work related to contact with mite-infested area. The mites have a life cycle of four stages: adult, egg, larva and nymph. Only stage that can transmit the disease to humans is the chigger or larva. The chigger takes up tissue fluid and lymph. Very high number of *O. tsutsugamushi* are there in the salivary glands of the chigger and these are transmitted into their host when they take up tissue fluid.

Transovarial transmission is seen in mite where they pass the infection on to their eggs. Likewise, transstadial transmission is also seen in which the infection transmitted from egg to the chigger and adult.

The strain of organism and condition of the host are two most important factors that decide the severity of disease. The pathophysiology of scrub typhus is not fully understood, though in general it is thought to be due to vasculitis either localized or disseminated. The principal target site of *O. tsutsugamushi* is the vascular endothelium in human. This organism acts at vascular endothelial cells leading to generalized vasculitis and perivascular inflammatory changes, and results in end-organ damage of many vital organs. Both humoral and cellular immune response are seen against *O. tsutsugamushi*.

**CLINICAL FEATURES**

The average incubation period of *O. tsutsugamushi* in humans is around 10 days and can vary between 6 and 21 days. Main symptoms of scrub typhus are pyrexia, headache, muscle pain, dry cough, and abdominal discomfort. Patients may present with subacute/chronic pyrexia and fever of unknown origin. The severity of the symptoms varies widely, depending on the strain of organism and condition of the host.

A small vesicular lesion at the chigger feeding site is usual first sign of scrub typhus, which usually changes into an eschar or an ulcer with local lymph node enlargement. An eschar is a typical lesion with black necrotic center and an erythematous border and is mainly located in the groin, axilla, genitalia, and neck (Fig. 1). The eschar is found in patients suffering from scrub typhus ranges from 7% to 80% in different study. Eschar is the single most important clue for diagnosis on physical examination. The detection rate of eschar depends on multiple factors like the skin color of the infected person and site of the lesion.

Sudden onset fever is the most common symptom accompanied with conjunctival redness, severe headache, altered consciousness, apathy, myalgia, shin pain, and more characteristically lymph node enlargement and hepatosplenomegaly. Fever is usually of high grade and may be with shaking chills.

A maculopapular rash appear at end of first week which initially seen at the trunk and later on spread to the limbs. This rash is not well appreciated among dark skinned individuals.

Systemic symptoms and signs related to major organ system like the central nervous system, cardiovascular system, kidney, pulmonary, and digestive systems become evident by end of second week. Major organ system involvement may produce serious complication in the
form of myocarditis, pneumonia, meningitis/encephalitis, acute renal failure, and gastrointestinal bleeding. Acute respiratory distress syndrome development is more commonly seen in patients of scrub typhus who have high leukocyte count and late treatment. Meningitis, meningoencephalitis, or encephalitis is common form of neurological involvement seen in scrub typhus.10

According to Kim et al., older age (≥60 years), scrub typhus patients without an eschar, and laboratory parameters such as leukocyte counts >10,000/mm and serum albumin level ≤3.0 g/dL are potential indicators of complications.11 Untreated patient remains febrile for about 2 weeks and have a long convalescence of around 6 weeks thereafter.

**Differential Diagnosis**

Differential and undifferentiated fever are two form of acute febrile illness (AFI). In differentiated fever there is obvious presence of focus of infection or inflammation, while in undifferentiated fever there is no obvious focus of infection and the symptoms and signs are quite nonspecific. In patient with undifferentiated fever several diagnostic possibilities are considered, especially in the tropics.12 The differential diagnosis of scrub typhus includes typhoid fever, dengue fever, malaria, other rickettsioses, anthrax, leptospirosis, and hemorrhagic fevers. It is considered as one of the causes of fever of unknown origin (FUO) in endemic areas.

**Diagnosis**

Febrile illness along with eschar on physical examination almost confirms the diagnosis. Leukocytosis and decreased platelet count may be seen. Altered liver function and kidney function test may be seen in large number of cases. Ultrasonography may reveal hepatosplenomegaly.

The lab diagnosis of scrub typhus is either confirmed or supported by identifying the microbe in cell culture, presence of antigen by immune-histochemical methods or the antibodies by the indirect immunofluorescence assay (IFA) and detecting organism’s nucleic acid using polymerase chain reaction (PCR).

As antigen identification tests have low sensitivity/ specificity and require tissue specimens, serological tests to identify antibodies are the better diagnostic tool in real clinical scenario as they are simple and easy to perform.13 After infection IgM antibodies against *O. tsutsugamushi* appear in the body at the end of first week while IgG antibodies appear at the end of the second week. The Weil-Felix (WF) test has better specificity but poor sensitivity and is based on the identification of antibodies to various *Proteus* species, which contain cross-reacting antigenic epitopes to antigens from members of the genus *Rickettsia*. The WF test is said to be positive when there is high titer of 1:320 or more or a fourfold rise in titer.

The standard serological test is the indirect IFA for the detection of IgM antibodies. This test has many demerit which consist of retrospective nature, availability of trained technician and equipment which may not be possible in many labs.14 At present most lab use the enzyme-linked immunosorbent assay (ELISA) for the presence of IgM antibodies in scrub typhus as it gives an objective result and has sensitivity almost equal to that of IFA.15 Rapid diagnostic kit to detect scrub typhus IgM antibodies have sensitivity in range of 34.7–96.7% and specificity between 93.3–99.7%.15

The samples from which causative microbe of scrub typhus is isolated are buffy coat of blood, defibrinated whole blood, plasma, tissue, skin biopsy, and arthropod samples. It takes around 4 weeks to detect rickettsia in cell culture. Molecular methods using PCR is possible from skin rash specimen, eschar, lymph node biopsies, or blood and is less time consuming. Nested PCR method is more sensitive than doing single PCR. This is a less time-consuming method and takes around 1 day.16

A positive IgM ELISA in the suspected patient with defervescence within 2 days of prescribing doxycycline or scrub IgM ELISA seroconversion on convalescent sera with other causes of acute febrile illness ruled out after proper investigations also favors scrub typhus infection.12

**Management**

There are several drugs to treat scrub typhus, including tetracyclines, chloramphenicol, rifampicin, azithromycin, and quinolones. Currently, the drug of choice is doxycycline, a member of the tetracycline family, and several studies have proved its effectiveness. The recommended therapeutic dose for scrub typhus in adult is doxycycline 200 mg/day for 7–15 days and tetracycline 2 g/day for 7–15 days in divided doses. But many case reports of natural resistance make it difficult to prescribe suitable antibiotic.17 The use of azithromycin in pregnant
women is believed to be safe for both mothers and fetuses, and it is classified as category B by the US FDA Pregnancy Category. Azithromycin is an effective drug for the management of scrub typhus as it efficiently penetrates human white blood cells and macrophages, which are target area for of *O. tsutsugamushi*. It should be given as short course because of its longer half-life.\(^{18}\)

Chloramphenicol can also be given in adults and its dose is 500 mg qid orally for 7–15 days. Rifampicin is also effective against *O. tsutsugamushi*.

Doxycycline is the drug of choice for prophylaxis and the usual prophylactic dose is 200 mg per week. Killed vaccines against scrub typhus was disappointing because of results in human studies were not as successful as they were in animal studies.

### Current Status in India

Earlier Scrub typhus was an endemic disease in many pockets of our country. But because of large scale use of insecticidal later, scrub typhus cases were reported in very low number from India. Recently there had been increase in number of cases of scrub typhus in India. Resurgence may be due to changes in the human behavior, unplanned urbanization, and deforestation, which result in displacement of vectors as well host rodents. Human in town may get bitten by the disease-causing mite larvae while moving in parks or during any other recreational activities such as camping in the affected vegetation area.\(^{19}\)

### Conclusion

Scrub typhus is an important differential diagnosis of undifferentiated fever cases. Eschar is most important clinical sign. High index of suspicion is needed to diagnose it as India is a part of endemic zone “Tsutsugamushi triangle”.

### References

Abstract
Burkholderia species contain the most versatile organisms that occupy a surprisingly wide variety of ecological niches. These infections can be very difficult to treat and, in some cases, lead to death as it is considered to be one of the most antimicrobial-resistant organisms found in the clinical laboratory. These bacteria are used for the purpose of biocontrol, bioremediation, and promotion of plant growth, but safety issues regarding human infections, particularly in patients with compromised immunity, are still questionable. This chapter provides an overview of the genus Burkholderia, its ecological diversity, the clinical manifestations observed, and the management protocols that will guide the physician to effectively treat this infection.

Introduction
Burkholderia species continue to be one of the notorious multidrug resistant organisms among the nonfermenting gram-negative bacilli (NFGNB). First described by William Burkholder, the species majorly include plant pathogens and soil bacteria with two important exceptions, Burkholderia mallei and Burkholderia pseudomallei, which are known to be notorious pathogens in humans and animals. Apart from being a primary pathogen in patients of Cystic fibrosis (CF), Burkholderia cepacia complex (BCC) has been found responsible for bacteremia in immunocompromised and chronically debilitated patients. There have been numerous documented outbreaks of BCC septicemia in intensive care units and patients with renal failure. Similarly, B. mallei and B. pseudomallei have been reported as the causative agent of diseases known to be Glanders and Melioidosis, respectively, both of which carry significant mortality and morbidity.

Taxonomy
William Burkholder, an American microbiologist, first discovered the organism responsible for bacterial rot of onion bulb at Cornell University in the year 1950 and was named as Pseudomonas cepacia. In the year 1992, Yabuuchi et al. created a new genus “Burkholderia,” and transferred P. cepacia and six other species belonging to rRNA group II of the genus Pseudomonas under the new genus. Since the discovery, the taxonomy of the new genus has undergone considerable changes and presently the genus Burkholderia consists of 22 species. All the members of the species possess a very large genome ranging between a size of 6 and 9 Mb, which renders the organism versatile and ubiquitous. In the subsequent years, Vandamme and colleagues described Burkholderia cepacia as a complex of closely related genomovars and genomic species and referred them collectively as BCC, which included ten different unique species. The members of the BCC are B. cepacia, B. multivorans, B. cenocepacia, B. stabilis, B. vietnamiensis, B. dolosa, B. ambifaria, B. anthina, B. pyrrocinia, and B. ubonensis. Based on comparative 16S ribosomal RNA and recA sequencing, multilocus sequence typing (MLST), and intermediate DNA-DNA binding values, recently seven novel species of Burkholderia have been identified and are proposed to be included under BCC.
members are; *B. latens*, *B. diffusa*, *B. arboris*, *B. seminalis*, *B. metallica*, *B. contaminans*, and *B. sabiae*.

**Epidemiology**

The ability of *Burkholderia* species to survive in extreme environmental conditions makes it ubiquitous. Because of minimal nutritional requirements, it can survive for months in media and solutions like antiseptics, sinks, water, and intravenous fluids. They also tend to reside on indwelling catheters, IV cannula, and nebulizers. Commercial use of BCC in agriculture as a biocontrol agent, in the bioremediation of toxic agents and plant growth promotion contributed to the increased incidence of human infection by the organism. Various reports have documented person-to-person transmission by a few strains of BCC and *B. pseudomallei*. Studies have shown that by the age of 18 years, 3.5% of patients suffering from CF harbor BCC. Among all other *Burkholderia* species, BCC is the most frequently isolated clinical pathogen followed by *B. mallei* and *B. pseudomallei*. *B. pseudomallei*, the causative agent of Melioidosis, is predominantly found in the regions of Asia, Africa, Northern Australia, and South America and is isolated from soil and water. It is also known to cause outbreaks in Thailand causing significant mortality and morbidity. *B. mallei* also share the same geographical territories as *B. pseudomallei* and primarily affects horses, causing equine glanders. It was one of the first used agent as biological weapon by Germany in the World War I. Human transmission occurs by percutaneous inoculation, ingestion, or inhalation from environment, contact with infected animals and even laboratory acquired infections have been reported. Few other strains like *B. gladioli* and *B. pickettii* have also been reported to cause nosocomial infections and multidrug resistant outbreaks in certain communities and hospitals posing an emerging threat to the society. However, little is known about the epidemiology of these particular species.

**Clinical Manifestations**

**BCC Infection in Cystic Fibrosis**

Owing to increased life expectancy in patients with CF, BCC has emerged as a major cause of morbidity and mortality. There is chronic colonization of the major airways with BCC in these patients and it may persist for months to years. Up to 20% of the infected patients rapidly deteriorate due to necrotizing pneumonia and sepsis, which may result in death. This fatal clinical decline in patients with CF is known as “Cepacia Syndrome” and it has not been observed with any other pathogens. This explains the varied outcomes amongst CF patients who are infected with same strain. Reports of “Cepacia Syndrome” have also been documented in non CF patients. There has been reported mortality of 75% in patients with CF who undergo transplantation.

**BCC Infection in Non-CF Patients**

BCC is recognized as an important pathogen in patients suffering from chronic granulomatous disease where there is inability of macrophages to produce reactive oxygen species due to mutations in NADPH oxidase complex. Mostly, it is a nosocomial infection via contaminated hospital equipment like disinfectants, antiseptics, topical anesthetics, and respiratory therapy equipment. BCC bacteremia in non-CF hospitalized patients is most often seen in patients with comorbidities such as diabetes mellitus, congestive heart failure, malignancy, and hemodialysis along with indwelling urinary catheters, central venous catheters, and endotracheal tubes. BCC pulmonary infection is mostly observed in intensive care patients who are on prolonged mechanical ventilation. Besides this, skin, soft tissue, and genitourinary infection have been reported in patients with burns or surgical wounds, after prostate biopsy, urethral instrumentation, and exposure to contaminated solutions.

**Melioidosis**

It is a disease of human and animals caused by *B. pseudomallei*. Every year approximately 1,65,000 people are infected by melioidosis, resulting in death of approximately 89,000 infected patients. The incubation period for the disease has been recorded to be as less as 1 day to up to a period of 62 years. Because of its prolonged incubation period the disease has also been referred as “Vietnamese time bomb.” One or more risk factors for the disease are found in up to 80% patients. Well-known risk factors for melioidosis include diabetes mellitus, heavy alcohol use, chronic pulmonary disease, chronic renal disease, glucocorticoid therapy and cancer. The spectrum of clinical manifestations is greatly varied ranging for acute fulminant septic illness to a chronic infection that may be confused with tuberculosis or...
malignancy for which it has been nicknamed as “The Great Imitator.”\textsuperscript{21} The disease can manifest as an acute infection seen in 85% of the patients as pneumonia, sepsis or localized abscess (Fig. 2); as chronic infection seen in 10% of the affected group, where it mimics symptoms of tuberculosis; and as a latent infection in 5% of the patients who are immunocompetent. The most common presentation is pneumonia (Fig. 3), while others are genitourinary infection, skin infection, overwhelming sepsis with abscesses disseminated in multiple internal organs (Fig. 4), septic arthritis (Fig. 5), and osteomyelitis. Mortality rates for melioidosis are approximately 40% in Northeast Thailand (35% in children) and 14% in Australia.\textsuperscript{22}

**Glanders**

Glanders is a highly contagious and fatal disease of the equine. It is caused by the organism \textit{B. mallei}.\textsuperscript{23} In India, it is a notifiable disease and more than 50% cases of equine glanders have been reported from Uttar Pradesh.\textsuperscript{23} The incubation period for the acute form of disease is 1–14 days, while for the chronic form, the incubation period may be to 12 weeks. Clinically, it may manifest as a local infection with nodules and lymphadenitis when transmitted by skin inoculation. Pneumonic forms are usually transmitted through inhalational route. Patient may develop septicemia with shock when organism disseminates from the skin or lungs causing significant mortality. The mortality of pulmonary form and the septicemic form of glanders has been as high as 95% without treatment and about 40–50% with treatment.\textsuperscript{24}

**Others**

Other members of the \textit{Burkholderia} species like \textit{B. gladioli} and \textit{B. pickettii} have been rarely found to cause infections in CF patients and hospital outbreaks. However, a little is known regarding the clinical features and etiopathogenesis of these two species.

**Diagnosis**

Identifying \textit{Burkholderia} species has been a tedious task because of poor laboratory proficiency throughout the world. A combination of selective media, biochemical analysis along with commercial kits, is being routinely used for species identification. For identifying BCC, three selective media are used; the \textit{P. cepacia} agar (PCA), the oxidation-fermentation polymyxin bacitracin lactose agar (OFPBL), and the \textit{B. cepacia} selective agar (BCSA). BCSA is the most recent and preferred selective media used. Apart from BCC, other members of the species like \textit{B. gladioli}, \textit{Ralstonia} spp., and \textit{Pandoraea} spp. can also be grown in BCSA. Similarly, out of various culture medias used for identification of \textit{B. pseudomallei}, the Ashdown’s selective agar (containing gentamicin), is the most commonly used. The characteristic “safety pin” appearance on Gram’s staining in a histopathological specimen also helps in identification of \textit{B. pseudomallei}. Use of automated
identification systems like API 20, Phoenix, Microscan, and Vitek 2 to identify BCC complex is also on the rise nowadays, but the identification is not trustworthy as many of the species of NFGNB are misidentified as BCC. Distinguishing *B. pseudomallei* from *B. mallei* on the basis of morphology and serologic tests is also a tedious task. Molecular identification has been a breakthrough recently where identification is performed by DNA based polymerase chain reaction, which identifies the differences in sequence of 16S rRNA gene or recA gene,
that help in distinguishing the species. Other methods of molecular identification like MALDI-TOF MS, multilocus restriction typing, pulsed field-gel electrophoresis, and BOX-PCR have been equally found effective in identifying species.25

Management

Burkholderia cepacia Infections

This organism is intrinsically resistant to many of the antimicrobial agents, which pose the greatest challenge in its management. The BCC isolates from CF patients have shown to be substantially more resistant. Therefore, combination drug therapy for serious infections has been suggested. Antimicrobials that are most effective and considered first line are Trimethoprim-sulfamethoxazole (TMP-SMX), Meropenem, and Doxycycline. Some strains are also susceptible to third-generation ureidopenicillins, advanced cephalosporins, and fluoroquinolones.16

Burkholderia pseudomallei Infections

Treatment is divided in two phases: the “induction phase” for 2 weeks with intravenous ceftazidime or a carbapenem (either Meropenem or imipenem), and the “eradication phase” with 12 weeks of oral antibiotics such as trimethoprim-sulfamethoxazole to eradicate the infection and prevent relapse. Alternative eradication therapies include doxycycline and co-amoxiclav but both therapies have a higher rate of relapse.21

Burkholderia mallei Infections

This organism causes human infection rarely, so there is limited information available regarding choice of antibiotics. Because of its similarities with B. pseudomallei, it is postulated that the drug susceptibility would be same. Susceptibility to macrolides, azithromycin, and clarithromycin have also been reported.16

Conclusion

Most of the Burkholderia species infections are hospital acquired and potentially pathogenic in individuals with impaired host defense, which can lead to septicemia and acute respiratory distress syndrome (ARDS) with requirement of ventilator and perfusion support if not intervened at the earliest. Treatment of this infection is really a challenge to physicians as patient needs long-term antibiotic therapy and recurrence of infection is very common even after prolonged treatment. As these infections carry a very grave prognosis, hence, high risk of suspicion is required in all cases of septicemia and ARDS particularly with impaired host defense. Prompt management of the infection and the underlying conditions will definitely reduce the mortality and morbidity to a great extent.
Abstract
Dermatophytosis is common fungal infection in human population. These keratinophilic fungi are restricted to the stratum corneum. The frequent strain causing infection earlier was Trichophyton rubrum; but at present, Trichophyton mentagrophyte is found to be common pathogen. Dermatophytosis requires prolonged treatment. Change in the fungal species, resistance, and steroid abuse leading to reinfection make the condition refractory to treatment. Details of treatment taken and family history are important. Appropriate combination of oral and topical antifungal agents and counseling for better adherence to treatment are needed in resistant and steroid misuse cases.

Introduction
"Ringworm" infection is frequently used terminology for dermatophytosis. This infection basically involves the keratinized tissue of the superficial layers of skin. There are three genera among dermatophytes: Epidermophyton, Microsporum, and Trichophyton. These fungi are keratinophilic and have capacity to invade the keratinous tissue of living animals. The involvement of epidermis, especially stratum corneum and tissue with higher keratin contents like hair and nails is common. These organisms are unable to penetrate deeper layers of skin or organs in immune-competent individuals and commonly confine to superficial non-living cornified layers. The clinical manifestations are basically dependent on response of the infected individual to dermatophytosis, which can vary between milder to significant clinical expression. These are mainly caused by reaction of host to the metabolic products of the organism, degree of virulence, anatomic location of the infection, and prevailing environmental factors.1

There can be lot of geographical variation in incidence and prevalence of these fungal infections. Several factors, like high humidity, hygienic practice of the individual, and risk proportion of individuals to acquire infection, can change from region to region. The dermatophytosis can have seasonal influence, with higher incidence in summer and lower in other seasons. Nearly quarter of global population is expected to have dermatophytosis as per WHO estimates. There can be 30–70% of general population who are asymptomatic carriers.2

Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum canis and Trichophyton tonsurans are frequently seen pathogens to cause cutaneous fungal infection. Tinea capitis is commonly caused by Trichophyton tonsurans. Tinea pedis, Tinea unguium, Tinea corporis, and Tinea cruris2 are commonly caused by T. rubrum.

These cutaneous fungal infections can produce significant impact on quality of life. They can be responsible for lot of social embarrassment, psychosocial discomfort, and impact on their work environment and hamper efficiency. The best way to reduce morbidity and restrict transmission is by diagnosing early and providing proper health education for prevention and adherence
Infectious Disease

Changing Scenario of Dermatophytes

There is an epidemiological transition of dermatophytosis in India. *T. rubrum* was found to be common pathogen in the past by many observers in India; however, at present it is significantly less prevalent. *T. mentagrophyte* is presently found to be frequent when compared to past.3

Antifungal Drugs: Mode of Action

See Table 1.

Treatment of Dermatophytosis

Common topical antifungal cream used (Table 2).4

Duration of treatment is given for 4–6 weeks or longer, depending on clinical severity and therapeutic response (Modified and reproduced with permission from Poojary SA).4

Common clinical situations for use of topical preparations:
- Infections during conception and first trimester
- Patients with renal, hepatic, and cardiovascular diseases
- Patients who are taking compelling medications for comorbidities with potential interaction

Common guidelines for improved therapeutic outcomes of using topical preparations:
- Medication should be applied over the lesion and surrounding skin, starting from outer aspect
- Choice of formulation can be variable, based on the region of the disease:
  - *Liquid preparations*: Web spaces, axilla, external genitalia, wet-lesions, and larger area of involvement
  - *Creams*: Dry lesions, lesions with prurigo
  - *Ointments*: Hyperkeratotic lesions
  - *Gels*: Over the facial region
  - *Nail lacquers*: Subungual lesions
  - *Shampoos*: As additional component in tinea capitis

TABLE 1 The Mechanism of action of antifungal drugs

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylamine e.g., Terbinafine</td>
<td>Inhibition of squalene epoxide</td>
</tr>
<tr>
<td>Azoles</td>
<td>Inhibition of 14–α-demethylase</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Disruption of mitotic spindle</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>Bind to fungal cell membrane ergosterol</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>DNA–RNA inhibitors</td>
</tr>
</tbody>
</table>

TABLE 2 Common topical antifungal creams used

<table>
<thead>
<tr>
<th>Antifungal class</th>
<th>Formulations with concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoles-Imidazoles</td>
<td>• Clotrimazole 1% cream</td>
</tr>
<tr>
<td></td>
<td>• Ketoconazole 2% cream</td>
</tr>
<tr>
<td></td>
<td>• Miconazole 2% cream</td>
</tr>
<tr>
<td></td>
<td>• Bifonazole 1% cream</td>
</tr>
<tr>
<td></td>
<td>• Oxiconazole 1% ream</td>
</tr>
<tr>
<td></td>
<td>• Sertaconazole 2% cream</td>
</tr>
<tr>
<td></td>
<td>• Luconazole 1% cream</td>
</tr>
<tr>
<td></td>
<td>• Eberconazole 1% cream</td>
</tr>
<tr>
<td></td>
<td>• Fenticonazole 2% cream</td>
</tr>
<tr>
<td>Triazoles</td>
<td>Fluconazole 0.5% gel</td>
</tr>
<tr>
<td>Alkyl amine</td>
<td>Terbinafine 1% cream</td>
</tr>
<tr>
<td>Benzyamine</td>
<td>Butenafine 1% cream</td>
</tr>
<tr>
<td>Morpholine</td>
<td>Amorolfin 0.25% cream, 5% nail laquer</td>
</tr>
<tr>
<td>Hydroxypyridinones</td>
<td>Ciclopiox 1% cream, nailaqaer</td>
</tr>
</tbody>
</table>

Source: Modified and reproduced with permission from reference 4.

Advantages of topical versus oral antifungals:
- Insignificant side effects
- Less chances of interaction with other drugs
- Can have better psychological effect with pharmacoeconomy

Disadvantages of topical preparations:
- Difficult to use in extensive infection
- Poor response due to inadequate quantity of application
- Inability to apply in difficult to reach areas, which leave a residual focus of infection4

Adverse Effects of Topical Antifungals

Topical applications are well tolerated with few adverse effects like pruritus, burning sensation, irritation, erythema, maceration, and fissuring.4
Common oral antifungals used are summarized in Table 3.

Clinical situation where the systemic antifungals are required:
- Involvement of scalp (Tinea capitis)
- Involvement of the nails
- Fungal infection at many areas of the body
- Tinea corporis with extensive involvement
- Tinea pedis with extensive involvement

Adverse Effects of Oral Antifungals
Gastrointestinal disturbances, altered LFT, headache, pruritus, and cutaneous allergic reactions are common. Erythema multiforme and toxic epidermal necrolysis can occur. Lupus erythematosus and psoriasis can be exacerbated.

Drug Interactions of Oral Antifungals
Terbinafine is predominantly metabolized by CYP2D6. Caution should be exercised while prescribing tricyclic antidepressants, SSRI, beta-blockers antiarrhythmics, MAO inhibitors, warfarin, cyclosporine, and rifampin. These are common drug interactions of oral antifungal agents.

Antifungals in Elderly
The presence of comorbidities and higher drug interactions warrants caution for antifungals for the elderly. Fluconazole and itraconazole being CYP3A4 inhibitors can cause several drug interactions and terbinafine is safe in elderly and can be the drug of first choice. Topical terbinafine, ketoconazole, or clotrimazole cream is preferred.

Fluconazole is the safest oral antifungal in hepatic and renal disorders. Topical terbinafine, ketoconazole, or clotrimazole cream is preferred in hepatorenal disorders.

The following are the challenges encountered in the treatment of dermatophytosis:
- Resistance
- Recurrence
- Reinfection
- Misuse of topical steroids
- Associated comorbidities
- Immunosuppression

Antifungal Resistance
Antifungal resistance can be either microbiological resistance or clinical resistance. Resistance as assessed by in vitro susceptibility testing shows resistance, with exceeding of MIC from susceptibility breakpoint. The resistance, which occurs naturally, is primary where the organism is not exposed to the drug previously. The genetic mutation basically contributes to secondary resistance and the organism would have been susceptible to the same agent previously.

Several factors can contribute to inadequate clinical response, it could be due to inadequate dose, improper adherence to therapy or altered genetic profile of pathogen, or the therapeutic agent selected may be

![Table 3: Common oral antifungals used](https://example.com/table3.png)
wrong. The resistance to griseofulvin and terbinafine in some dermatophytes have been reported in India. The clinical response and susceptibility assessment may not correlate; hence, it is not appropriate to use the term, “resistant” in the absence of these definitive criteria for these fungal infections. Assessment of in-vitro susceptibility to terbinafine, fluconazole, and griseofulvin as reported in many studies does not imply that there is an absolute resistance, the clinicians should think of using better dosage or prolonged treatment for adequate response. The antifungal drug susceptibility is said to be different in Indians, hence there is need to establish dose determination and MIC breakpoint guidelines by the Clinical Laboratory Standards Institute (CLSI).

Steroid Abuse in Tinea
Steroid modified tinea is a common problem in India. Permutation and combination of various antifungals, antibacterials, and topical potent corticosteroids are used. Nearly half of the sales of these combinations in India are due to self-medication or advice by unqualified persons. The combination of clobetasol propionate, ornidazole, ofloxacin, and terbinafine are frequently used in India. Irrational and non-supervised use has aggravated the problem. Long time and intermittent use result in erythema, telangiectasia, and atrophy. The clinical picture variable due to altered T-cell mediated immunity suppression. Ineffective elimination of the dermatophyte results in chronic, wide-spread, and ill-defined lesions. Constant itching and scratching result in lichenification. Improper central clearing due to topical steroid abuse results in various bizarre shapes mimicking Tinea pseudoimbricata. Supervised short duration of topical steroid is required to treat lichenification followed by antifungal treatment. Prolonged treatment needs counseling for adherence to the treatment.6

Recurrence
There is lack of uniformly acceptable definition of “recurrent dermatophytosis.” Whenever there is a protracted course with variable degree of persistence of lesions, these patients contribute for easy spread of infection to family members and other closely associated people. Common contributing factors are overcrowding, living together in congested accommodation, the use of tight footwear, tight-fitting clothes, and sharing of community bath and sports facilities.

Future Directions for Prevention of Refractory Dermatophytosis
The primary factor for emergence of antifungal resistance appears to be inadequate dose and improper combination. There is need of clear guidelines for dose recommendation for prevention and treatment.

Ghannoum and Rice7 suggested for intense clinical correlation with appropriate medication combination and acceptable dosage by using the pk/pd data and surveillance inputs. Early diagnosis with correct combination will help in reducing resistance and refractoriness.

Measures improve hand hygiene: Hand wash; clipping of nail; regular bathing, keeping the skin dry, use of proper shoes, cotton socks, and absorbent powder; avoidance of sharing of bathing facilities and cloths; avoidance of walking barefoot in public bathroom are important in preventing spread.8

Using appropriate combination of drugs will increase the fungicidal effect and increase the spectrum of activity and reduce refractoriness. Choose a combination of oral and topical antifungal with different mechanism of action for better clinical outcome.

Antifungal drugs in the pipeline that are active against azole-resistant isolates, like cationic peptide histatin will be important for revised treatment strategies. The combined use of azoles and cytokines will be useful in treatment of fungal infections in immune-compromised individuals.

Conclusion
- Cutaneous fungal infections are very common in humans. Dermatophytes have higher affinity to stratum corneum. The common strain is T. mentagrophytes among dermatophytes. Tinea corporis and cruris require 3–4 weeks of treatment.
- Fingernail requires 6–8 weeks; however, toenail requires 10–12 weeks of treatment.
- Patients with renal, liver, and cardiovascular diseases are treated with topical antifungal.

Contd...
Managing Dermatophytosis (Tinea Infections) in Today’s Scenario

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Contd...

- Steroid modified tinea in India is a result of topical antifungal used in combination with potent topical steroids and antibacterial that account for about 50% of the sales of all topical steroids in India.
- Careful history taking—treatment history, combination of oral and topical antifungal with different molecules that have varied mechanism of action and counseling is needed in resistance and topical steroid misuse cases.
- Drug interactions must be considered before starting any oral antifungals.

References

Rickettsial Infections are important public health problems and are often underdiagnosed or misdiagnosed leading to the burden of morbidity and mortality due to nonspecific symptoms and signs and absence of specific diagnostic tests. The present review addresses the epidemiology, clinical features, diagnosis, complications, and management of these infections, primarily for a practicing clinician.

Introduction

The rickettsioses represent one of the important causes of febrile thrombocytopenia worldwide. Rickettsial infections which were initially seen more often had reduced in incidence with extensive use of pesticides to control vectors. Tetracycline was one of the most commonly used antibiotics which also contributed to reduction of prevalence of rickettsial infections. However, the indiscriminate use of tetracycline has declined due to adoption of better antibiotic policies. Additionally, the urbanization of rural areas has exposed more of the population to potential sources of infection. These two factors have coincided with the re-emergence of this deadly scourge.

These diseases can be incapacitating and difficult to diagnose in resource-poor settings. Potential cases are often under-recognized or under-tested. Adding to the burden of morbidity and mortality is the dearth of adequate laboratory testing facilities and uniformity of reporting systems.

Rickettsioses present with non-specific signs. However, certain clinical features are indicative when present in a constellation, such as the presence of high-grade continuous fever, centripetal/centrifugal rash, generalized lymphadenopathy, decreased platelets, and the pathognomonic eschar (tachè noire) which is rarely seen in our setup. However, atypical presentations are also more prevalent in hyperendemic areas. Clinical presentation along with positive Weil Felix test is employed to make the diagnosis of rickettsiosis.

Structure and Genome

Rickettsia is small, Gram-negative coccobacillary form adapted to obligatory intracellular parasitism and transmitted by arthropod vector (lice, fleas, ticks, and mites) found in their alimentary canal. In vertebrates especially humans they infect vascular endothelium and reticuloendothelial cells. Rickettsiae are not evident on blood smear and do not stain with most of the conventional stains; however, it stains red with Giemsa stain. Rickettsial genome is 1–1.5 Mb and is a single circular genome.

The Rickettsiaceae Family

Family Rickettsiaceae is divided based on lipopolysaccharide group antigen into four genera (Flowchart 1).
Vectors
Transmission is by arthropod vectors like lice, fleas, ticks, and mites.

Distribution
The distribution is dependent on the arthropod host/vector. Tick vector dependent infections have limited geographic spread (except Antarctica).1,2

Risk Factors
People staying near mountains, bushes, and equatorial rain forests are at risk. Improper hygiene and rearing of domestic animals are also risk factors.3

Pathogenesis
Endothelial cells are primary target (exception Rickettsia akari). They cause damage to the cell causing cellular detachment. The cells in circulation when lodged in distal capillaries become the source of infection. Through antibody, mediated opsonization rickettsia can enter phagocytic cell.3

Clinical Features
The incubation period varies 2–21 days. Signs and symptoms are non specific. Fever is the most common presentation which starts abruptly and is of high grade in association with headache, muscle ache, apathy, drowsiness, photophobia, conjunctival suffusion, generalized lymphadenopathy (abdominopelvic, para-aortic, porta hepatis, splenic hilum), and hepatosplenomegaly.6,7

The rash: Though considered as characteristic feature of rickettsioses is not seen in all patients. It occurs on the third or fifth day of illness. It may be typical with macules, papules, hemorrhage, or petechiae or atypical asymmetrical localized to small area (Fig. 1). It may be centripetal or centrifugal in distribution mainly seen over the soles of the feet and palms of the hand.3,8

Eschar: It is vesicular lesion at the site of inoculation which ulcerates, heals with development of black necrotic scar associated with regional lymphadenopathy. Necrotic

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**Flowchart 1: Classification of rickettsial infections**

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**Fig. 1:** Rickettsial rash
eschar (Fig. 2) is seen in belt area, in groin, under the arms, sometimes over the neck. Even though it is the single most important clue for the diagnosis it is recognized in less than 50% even by an experienced physician. Sometimes multiple eschars are seen under the trouser belt. Scrub typhus patients with virulent strains have lower incidence of rash/eschar, and hence severity of clinical features depend on serotype and genotype of rickettsia.

In India, three common groups of infections are seen the spotted fever group (e.g., \emph{R. akari}), the typhus group (e.g., \emph{R. typhi}), scrub typhus (\emph{Orientia tsutsugamushi}). The significant clinical differences between them have been studied by the author’s team.9,10

\textbf{Scrub typhus} was more likely to present with fever 7–14 days (p=0.03); splenomegaly (p=0.023), and eschar (p=0.014) were significant examination findings; in the laboratory, it presents with increased SGOT/SGPT (p=0.024), and decreased albumin (p=0.021). Spotted fever group had a preponderance toward fever less than 7 days (p=0.043), vomiting (p=0.03), and joint pain (p=0.049). Macular rash (p=0.04) was observed clinically. Pleural effusion (p=0.021) was a significant complication. Typhus group had fever more than 14 days (p=0.04), associated with cough (p=0.019), and abdominal pain (p=0.039). Hepatomegaly (p=0.045) was a significant clinical finding.10

Atypical presentation that may be seen in hyperendemic areas are: acute abdomen without fever, nausea, vomiting, diarrhea, and constipation.

Neurological complications of rickettsioses are encephalitis, aseptic meningitis, meningoencephalitis; respiratory symptoms like cough and breathlessness associated with ARDS and pneumonia and non-cardiogenic pulmonary edema (30–60%), gastrointestinal manifestations presenting as acute gastroenteritis, severe pain abdomen often mistaken for surgical abdomen; transaminitis with acute hepatitis and hepatic encephalopathy, coagulation factor consumption results in a DIC-like syndrome.7

Differential diagnosis of aseptic meningitis should include rickettsial meningoencephalitis in endemic areas, especially when associated with altered renal or hepatic functions. Acute kidney injury is associated with poor prognosis.3

Retinal vasculitis is commonly seen amongst females. It is asymptomatic in the early stage, resolves by 6 months. The prognosis is favorable.3

Without medications uncomplicated infection might recover within 14 days. Untreated cases carry a mortality rate of 30%. Persistent subclinical infection in convalescent patients occurs with \emph{Rickettsia prowazekii}, which later presents as recrudescent typhus or Brill-Zinsser disease.2,9

People travelling from disease endemic region might present with symptoms within few days of return. Since the incubation period for most rickettsial infections is from 2–21 days, symptoms which begins 20 days after travel from a disease-endemic area has less probability to be a rickettsial infection.8

\textbf{Investigations}

\textbf{Early stages}—lymphopenia; late stages—lymphocytosis (30%), Elevated ESR, decreased platelet count in less than 50% of cases.

Later—Deranged liver function test (50%): Increased serum bilirubin, increased ALT, AST (75–95%), reduction in albumin less than 3 gm% (seen in significant number of patients); reduced sodium, increased blood urea, and serum creatinine.

\textbf{Chest X-ray}: Pneumonitis, bilateral infiltrates, pleural effusion, and ultrasound abdomen: Hepatosplenomegaly.11

\textbf{Diagnosis}

\textbf{Early stages}: It is challenging to diagnose these infections and differentiate them from other infections like dengue,
malaria. The clinical manifestations of most rickettsioses present as a continuous spectrum. In the absence of definite pathognomonic signs, there are signs and symptoms highly suggestive of the etiology. However, not all cases present with the set of typical clinical manifestations that aid in prompt recognition. As such the clinical acumen and awareness of the treating physician has a great role in the diagnosis and management of these cases. In resource poor country, fever with rash, fever with thrombocytopenia and rash Weil Felix test clinches the diagnosis.11

**When to Suspect Rickettsial Infection?**

Any patient presenting with fever and rash, fever and eschar, aseptic meningitis, acute renal failure with eschar, increased vasculitis should be suspected of having rickettsial infection.

**Weil-Felix Test**

This test is easily available, cheap, and easy to perform. The results are available on the same day. The sensitivity is 46%, specificity is 100%, is positive in second week. OX2, OX19, and OXK strains of agglutinins of proteus bacteria are used in the test.3,8,11 Results must be interpreted in the correct clinical context (Table 1). Value of testing two sequential serum or plasma samples together with fourfold rise in antibody level is more important in confirming acute infection.3,8,11

**Indirect Immunofluorescence Assay/Immunoperoxidase Assay**

It is the gold standard investigation. Sensitivity is 89–100%, specificity is 100%, but major constraints being, the test is not easily available, costly, and takes more than 7 days for results. Immunoglobulin M rises at the end of first week; Immunoglobulin G rises at the end of second week.

**Immunohistologic Examination**

The only diagnostic test proved useful during acute illness is immunohistologic examination from cutaneous biopsy sample of a rash or biopsy of a lymph node. It is 70% sensitive, 100% specific. However, high expertise is needed to interpret biopsy result.3,11

**Polymerase Chain Reaction**

It is species specific and is positive in initial 7 days. It can be done on whole blood, eschar, or skin biopsy. It has a specificity of 100% for both PCR and Rt-PCR and sensitivity of 22–36% for PCR and 45–82% for Rt-PCR. Tissue sample shows higher sensitivity compared to blood sample.

**Culture**

Organism is grown on tissue culture Vero cell of kidney, egg yolk sac and isolation needs biosafety level III labs. However, culture is unnecessary, laborious and hazardous to lab personnel. Median time for reporting is 27 days.

**Treatment**

If cases are left untreated fatality may go up to 30%.1,3 When diagnosed properly it can be treated easily, but the difficulty lies in diagnosing rickettsial infection during initial phase of the disease when antibiotic use is very effective.12 Doxycycline is the treatment of choice. Dose 100 mg two times a day should be used for 7–10 days. Within 24–48 hours after initiation of antibiotic, patient improves drastically. If patient fails to respond within 48 hours, it is unlikely to be Rickettsial infection.12 Extremely sick patients (especially with MODS and ARDS) may require 10–14 days to respond to treatment. Relapse is usually seen if treatment is discontinued as soon as fever subsides. Discoloration of teeth is more common in children and depends on duration of treatment,13 other side effects are hypoplasia of the enamel, depression of skeletal growth, and limb hypoplasia. As such, doxycycline should not be used in children less than 8 years because of these above side effects. In pregnant women due to the adverse effects on the skeletal growth of fetus. Azithromycin 500 mg once daily is used instead in this setting.7

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Weil-Felix</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OX-19</td>
<td>OX-2</td>
<td>OX-K</td>
<td></td>
</tr>
<tr>
<td>Rocky mountain spotted fever</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rickettsial-pox</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Epidemic typhus</td>
<td>+</td>
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<tr>
<td>Endemic typhus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Scrub typhus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1** Interpretation of Weil-Felix test
If patients respond poorly to doxycycline or drug resistant serotypes: combination of doxycycline with rifampicin for 6–8 days or azithromycin with rifampicin (600 mg once a day) to be used. Rifampicin should always be used in combination as it leads to resistance when used alone. In India, rifampicin is avoided due to the prevalence of tuberculosis.

**Treatment in pregnancy:** Chloramphenicol is also an alternative drug and can be recommended for use in pregnancy in dose 500 mg four times a day for 7–14 days but has to be avoided in last trimester due to fear of bone marrow suppression and gray baby syndrome. Azithromycin is safer alternative in pregnancy. A macrolide, Josamycin, has been used with success in spotted fever group in pregnancy in a dose of 3 gm/day orally for 5 days. Doxycycline can be used in late pregnancy.

**Vaccine**

No vaccine is available for Rickettsial infection. An ideal vaccine should give protection to all biogroups in order to give acceptable level of protection. This complexity continues to hamper efforts to produce effective viable vaccine due to enormous antigenic variations among the biogroups.

**Recent Developments**

Rickettsiae have specific regions of oompA and oompB surface proteins and measures to detect antibodies against them, might prove beneficial in future treatment of rickettsiae.

**Prevention**

Preventing exposure to a vector infested habitat, wearing closed-toed shoes, long pants, long sleeved clothes, topical application of insect repellents, protected pets may help in prevention.

**Conclusion**

Rickettsial infections are some of the covert re-emerging infections of present times generally incapacitating and notoriously difficult to diagnose. Untreated cases have fatality rate up to 30%. When diagnosed properly it is often easily treated. Greatest challenge is difficult diagnostic dilemma posed by these infections early in clinical course when antibiotic therapy is most effective; hence, physicians and pediatricians have to include rickettsial infection in differential diagnosis of acute febrile thrombocytopenia.

**References**

Abstract

Adult immunization is less talked about area of health-care practice needing regular updation and implementation. It is an easy means of disease prevention which should find place as a separate topic in the medical curriculum. While the Guidelines for Immunization of Children are routinely updated and practiced clinically, the same is not true for adult immunization. The slowly waning immunity gained from vaccination in childhood, risk of exposure from frequent travels, changing lifestyles, age related immunosenescence, increasing comorbidities like diabetes and chronic kidney disease, organ transplantation and increasing use of immunosuppressive further necessitate implementation of adult vaccination measures. API guidelines recommend routine vaccination for Pneumococcal infection, Influenza, Human papilloma virus, Measles, Mumps Rubella, Diphtheria, Pertussis, and Tetanus in Adult Indian subjects. It is imperative for the related health-care associations to work together and devise a common adult vaccination schedule which can be followed by clinicians all over the country. Physician realization, public health education about its need and government initiatives in implementation would go a long way in strengthening the infection control measures and improve the communicable disease health-related indices.

Introduction

The recent pandemic of COVID-19 has exposed the vulnerability of human race to infectious diseases and re-emphasized that prevention is an essential part of management of communicable diseases worldwide. Vaccination forms an important part of primary prevention for control of morbidity and mortality related to infectious diseases. With increasing age, adult immunity declines (immunosenescence) and therefore there is need to address it through adult immunization.1 The susceptibility of populations has increased due to changing work environment and culture involving international travels and explorations. The increasing numbers of organ transplant recipients, diabetics, chronic kidney disease (CKD) subjects, indiscriminate antimicrobial use, emerging resistance, and growing susceptible population of subjects on chemotherapeutic agents or radiation therapy, further adds to these concerns. As on date the status of adult vaccination in India is mostly inadequate or incomplete. In contrast to pediatric vaccination guidelines, little importance is laid on Adult Vaccination in India, even when the mortality from vaccine preventable diseases is 350 folds higher in adults in comparison to children.2 Lack of awareness amongst general public, physician’s apathy, meager resources, and lack of political will are impediments to implementation of adult immunization in routine clinical practice. Organizations like API have stressed the urgent need to develop adult vaccination guidelines in India and its revision periodically.

Determinants of the vaccination needs of an individual are age, prior vaccination status, mutations in infectious agents, health or comorbid illness, lifestyle, travel, occupation, infection trends, disease burden in the
population and related health-care/immunization costs. Vaccine induced immunity is neither long lasting nor broad spectrum and declines with time, implying that childhood vaccination may need to be bolstered in adulthood to provide lasting protection. In countries where last dose of Diphtheria vaccine had been administered at less than 6 years of age, a resurgence of Diphtheria in adults more than 15 years age has been noted. Similar, increase in average age of many vaccine preventable diseases in to adult life has been observed. Further, adult individuals act as reservoirs of disease and pose a risk to the unvaccinated children and other family contacts in the household. Likewise, the role of adult vaccination gains importance in hostel facilities, residential institutions, factories, and organizations with residential campuses.

India contributes to 60% of Diphtheria, 40% of tetanus, and 44% of Japanese encephalitis cases of the world. However, age wise epidemiological data relating to burden of vaccine preventable diseases and efficacy of adult vaccination strategies in India is scant. Recent outbreaks of Measles (University in Karnataka, 2013-2014), Japanese Encephalitis (West Bengal 2014), Influenza A (Rajasthan 2015), Varicella (Tamil Nadu 2016-2017), Diphtheria (Assam 2015-2016), Hepatitis A (2015-2016) have alerted us to the relevance of adult immunization in India. In early 2018, around 133 outbreaks of measles, varicella, food poisoning, and diarrhea were reported. To aggravate the issue further, there is a lack of proper disease surveillance and under reporting of outbreaks of vaccine preventable disease in India. Herein we discuss the adult vaccination guidelines as recommended by various organizations for Indian population. The recommended vaccination schedules for clinical practice are mentioned in Tables 1 and 2.

**TABLE 1** Summary of adult immunization guidelines

<table>
<thead>
<tr>
<th>Organism/Disease</th>
<th>Vaccine</th>
<th>Age of administration</th>
<th>Dosage recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza</td>
<td>Trivalent/Quadridvalent</td>
<td>&gt;19 years onward; 0.5 mL IM 1 dose annually; can be given during pregnancy (FOGSI)</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>PCV 13 PPSV 23</td>
<td>&gt;50 years or &lt;50 years in high risk; PCV followed by PPSV 23 at 1 years, repeat every 5 years</td>
<td></td>
</tr>
<tr>
<td>Human papillomavirus</td>
<td>0.5 mL IM Merck4 strain/2 strain GSK</td>
<td>9–14 years 2 doses 0 day and 6 months (IMA) 15–45 years at 3 doses 0 day, 1 and 6 months, defer if pregnancy</td>
<td></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>0.5 mL SC</td>
<td>Individuals &gt;60 years (ISNI); 2 doses 2–6 months apart</td>
<td></td>
</tr>
<tr>
<td>Diphtheria, pertussis, tetanus</td>
<td>(Tdap/Td) 0.5 mL IM</td>
<td>Age group &gt;19 years; immunized Tdap once at 10–18 years; then Td 10-yearly (IMA, API, CDC), Non-immune 3 doses, 2 does 4 weeks apart followed by third at 6–12 months Tdap during each pregnancy (API &amp; CDC) IMA adds TT/Td early during pregnancy (2 doses); Tdap during 3rd trimester of pregnancy (1 dose)</td>
<td></td>
</tr>
<tr>
<td>Measles, mumps, and rubella</td>
<td>MMR 0.5 mL SC</td>
<td>19–60 years; 2 doses 1 month apart (ACIP); 1 dose if previously immunized Pregnancy should be deferred for 3 months after immunization</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2** Immunization schedule in special situations (routinely not used)

<table>
<thead>
<tr>
<th>Organism/Disease</th>
<th>Vaccine dosage recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A</td>
<td>1.0 mL IM; 2 doses in Adults (0 &amp; 6 months)</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>1.0 mL IM; 3 doses (0, 1–2, &amp; 4–6 months) FOGSI recommends in preconception or at high risk during pregnancy</td>
</tr>
<tr>
<td>Hepatitis A+B</td>
<td>1.0 mL IM; 3 doses (0, 1–2, &amp; 4–6 months)</td>
</tr>
<tr>
<td>Typhoid</td>
<td>0.5 mL IM; 1 dose every 3 years</td>
</tr>
<tr>
<td>Cholera</td>
<td>2 separate doses of oral vaccine 1–6 weeks apart for those aged over 6 years</td>
</tr>
<tr>
<td>Meningococcal</td>
<td>0.5 mL SC Bivalent/Quadrivalent; Adults (1 dose); At risk/asplenia 2 doses (ACIP and IMA)</td>
</tr>
<tr>
<td>Varicella</td>
<td>0.5 mL SC; &gt;13 years of age; 2 doses 4–8 weeks apart</td>
</tr>
</tbody>
</table>
Pneumococcal Vaccination

Pneumonia and invasive pneumococcal disease are two dreaded complications of pneumococcal infection, which account for significant morbidity and mortality, especially in the immunocompromised and the elderly (subjects >50 years of age) with a case fatality of 28%. The common serotypes accounting for 55% of inpatient admissions are 1, 3, 5, 19F, 8, 14, 23F, 4, 19A, and 6B. Further emerging resistance to penicillin and macrolides has underlined the importance of adult pneumococcal vaccination. The incidence of pneumococcal disease per lakh population is 8.8 in healthy adults, 51.4 in adult diabetics, 62.9 in subjects with chronic lung disease, and 93.7 in subjects with chronic heart disease. To reduce morbidity in high risk groups pneumococcal vaccination is recommended in subjects less than 50 years of age with chronic heart disease, lung disease, chronic liver disease (CLD), diabetes mellitus, chronic smokers, and alcoholics.

There are two vaccines available, viz. a PCV13 and PPSV23 name denoting the number of serotype antigens contained. The PCV13 carries a greater response, longer immuno logical memory, protection against nasopharyngeal carriage, but a lower protection against invasive pneumococcal disease in elderly.

A single dose of PPSV23 is recommended in immunocompetent adults over 65 years of age, to be repeated at every 5 years interval. An early institution of second dose is noted to produce hyporesponsiveness. Initial administration of PCV13 vaccine has been shown to amplify the response to subsequent administration of PPSV23. ACIP 2015 recommends a space of 1 year between the two vaccines irrespective of their order. In individuals who have already received PPSV23, vaccination with PCV13 can be undertaken after 1 year interval. A prior pneumococcal conjugate vaccine (PCV13) vaccination followed by unconjugated capsular polysaccharide vaccine (PPSV23) vaccination is recommended for high risk group with cochlear implant, CSF leaks, sickle cell hemoglobinopathy, asplenia, congenital, or acquired immunodeficiency syndromes.

Influenza Vaccination

Annual influenza vaccination by itself has been shown to reduce cardiac mortality by 19–45% and hospital admissions by 54%, which is comparable to benefit gained from CV risk reduction by smoking cessation or statin therapy. Influenza vaccines are available as trivalent (two strains of Influenza A and one strain of influenza B), quadrivalent (two strains of influenza A and two strain of influenza B) or live attenuated (lyophilized) nasal spray vaccines. Influenza vaccination is recommended in high risk subjects, viz. CKD, chronic obstructive pulmonary disease, heart disease, immunosuppressed subjects, diabetics, and hematological disorders. Single dose needs to be administered annually to gain protection. The timing has to be 2 weeks before the peak season for influenza from October to May.

Measles, Mumps, and Rubella

In 2018, around 55,000 cases of Measles and 1000 cases of Rubella were reported in India. Around 16% of Indian population was found to be susceptible to Rubella infection. Incidence of Mumps outbreaks has been reported mainly from hostel facilities, colleges, and schools in poor socioeconomic settings. A single dose of triple vaccine MMR in already immunized individuals and two doses separated at interval of 1–2 month in unimmunized subjects is noted to provide adequate protection.

Diphtheria, Pertussis, and Tetanus

Childhood protection through vaccination is not lifelong and has been found to increase the proneness of adults to Diphtheria Pertussis and Tetanus. India accounts for the majority of these cases in the world. A single dose of triple antigen vaccine followed by booster every 10 years is noted to provide adequate protection in already vaccinated individuals.

Human Papillomavirus Vaccination

Cervical cancer is the second most common cause of cancer and second leading cause of mortality in India. Human papillomavirus (HPV) virus accounts for 90% of cervical and anal cancers, 70% of vulval and vaginal cancers, and 60% of penile cancers. HPV serotypes 16 & 18 are noted to account for 77% of cervical cancers in India. HPV vaccine is recommended before the onset of sexual activity in the age between 9 and 24 years. In the age group of 9–14 years two doses of HPV vaccine are to be administered at 6 months interval. In those over 15 years of age three doses at 0, 1, and 6 months are advocated.
Hepatitis A and B Vaccination

While Hepatitis A virus (HAV) accounts for 10–30% of acute hepatitis and 5–15% of acute liver failure cases, hepatitis B virus (HBV) accounts for 20–30% cases of Cirrhosis and 40–50% cases of hepatocellular carcinoma. The high prevalence of chronic Hepatitis B carrier state in the population also increases the risk of transmission. Hepatitis A vaccination is indicated for candidates for kidney or liver transplant with chronic hepatitis B/C to decrease the risk of fulminant liver failure. Also vaccination for Hepatitis A is indicated in illicit drug users, homosexuals, animal handlers, persons with CLD or other hepatitis virus infections, recipients of clotting factor concentrates. Two doses of Hepatitis A vaccine administered at 6–12 months interval gives adequate protection. Hepatitis B vaccine schedule is 20 µg at 0, 1, and 6 months. Booster dose is not recommended in immunocompetent adults. Combination vaccines for HAV and HBV can also be administered in a three dose (0, 1, and 6 months) or four dose (0 day, 7 days, 21–30 days, and 6 months) schedule.

Meningococcal Vaccination

Three Meningococcal vaccine are available, viz. two quadrivalent vaccine (against four antigens A, C, Y, and W135) for polysaccharide (MPSV4) and conjugate (MCV4) or a bivalent vaccine (antigen A and C). While MCV4 produces lasting immunity, and reduces nasopharyngeal carriage it cannot be used in adults more than 55 years of age and does not provide protection against Meningococcus B strain. Therefore, MPSV4 may be preferred in subjects more than 55 years of age or those requiring single dose, viz. travelers.

Conclusion

The common perception that vaccination is meant only for children has to give way to the understanding that vaccination has to be continued well in to adulthood. Most of adults above 40 years of age have not received universal childhood vaccination, which was started in 1978 and could attain 65% coverage only in 2014. Hence, the realization that adult vaccination is urgently required needs to creep in to the community and a national program has to be carved to reduce the menace of vaccine preventable diseases. As much as two-thirds of Indian population is still unaware of need of vaccination in adulthood. Further the introduction of adult vaccination is cost effective as demonstrated in cost benefit analysis of HPV vaccine in India. The cost of adult vaccination is lower than the cost for childhood vaccination and implementation of many other secondary preventive measures (lipid lowering therapy, antihypertensives, antidiabetics, and bisphosphonate treatment). For the adult vaccination to be successful it needs to overcome the barriers of vaccine hesitancy (4Cs of complacency, convenience, confidence, and cultural acceptance) which can be achieved by widespread health education, evolving consensus on national guidelines and incorporating them in routine clinical practice. Although, as on date adult immunization is not being routinely observed in clinical practice, achieving complete adult vaccination to vaccine preventable diseases through widespread implementation by medical professionals, society’s acceptance, government realization, and making vaccines affordable would make this dream come true.

References

Neurocysticercosis (NCC) is most common central nervous system parasitic infection caused by Taenia solium. NCC is a common cause of adulthood epilepsy with significant disease burden in developing countries like India. The precise knowledge of epidemiology, clinical features, radiological correlation with stage of parasite is very helpful in effective management and reducing the burden of disease. Relational use of anti-parasitic drugs and steroids are effective in curing NCC. This chapter is focused on clinical and radiological finding and guideline on management of NCC.

Introduction

Neurocysticercosis (NCC) is caused by Taenia solium and this is the most common helminthic infection of the central nervous system (CNS). Taenia solium is a parasite and transmission of larva stage from pig is responsible for infection in human. NCC is most severe form of cysticercosis and this is a most common cause of acquired epilepsy in adult. NCC is associated with substantial morbidity due to epilepsy, strokes, and associated side effects of long-term drugs therapy for seizures. This disease is a major health problem in India and its requirement in epilepsy with NCC is around 3.48/1,000 persons. Prevalence of NCC is higher in northern part of India as compared to southern part of India, particularly in urban population. The health and economic impact of disease has not been assessed in depth. Past study has shown that Rs. 5,916 is the direct cost of treatment of solitary cysticercus granuloma per patient.

The important factors responsible for occurrence of disease in India are poor personal and social hygienic conditions. This increases scavenging pigs with use of partially cooked unhygienic pork. Further studies will require for measuring the impact of this disease on economy and human health in India. Worldwide estimation of NCC prevalence has relatively less studied and the disease is common in India, Indonesia, most of Southeast Asia, part of China, many sub-Saharan Africa, and regions of Eastern Europe.

Even with advancement there is still a gap between basic and practical aspects of disease focusing, especially diagnosis of active lesion and appropriate timing of antiparasitic treatment in NCC. This chapter is especially focused on better understanding of disease, diagnosis, and treatment in clinical scenario.

Etiopathogenesis

Taenia solium, also called tapeworm, is the causative parasite of NCC. Both human as well as pig both act as intermediate host for Taenia solium. Adult tape worm releases the thousand of eggs in human feces. The pig fed the eggs containing feces due to poor hygienic condition. The eggs lose their covering in pig's intestine and converted into oncospheres. These oncospheres distributed in muscles of pig via blood after penetrating intestine and form cysticercus. Consumption of uncooked pork that contained cysticercus in muscle of pig leads to
infection in human. In human stomach digestive enzyme leads evagination of their scolices. These scolices penetrate the gut and reach in brain and produce parenchymal cystic lesion (Flowchart 1). Recent studies also showed that there are person-to-person transmission of human cysticercosis is also equally important as environmental source. Distribution of cysts in brain parenchyma occurs mainly at watershed areas between gray and white matter. Other common sites of cyst lodgment in brain are choroid plexus and 4th ventricle, that give rise ventricular cyst. In brain parenchyma, cyst has two components, first is vesicular and second is scolex. The first vesicular part is a viable phase consists of a transparent membrane and clear vesicular fluid and with transposed scolex. The initial phase of cysticerci in brain is a colloidal stage, which may survive for many years and finally due to immune response scolex get mineralized and converted into calcification nodule. Inflammatory response of parenchymal cyst is very less and generally limited to only surrounding tissue. But meningeal cyst mostly provokes a severe inflammatory response in subarachnoid space and land up with thickened leptomeninges.

**Clinical Manifestation of Neurocysticercosis**

Clinical manifestations of NCC are varied from asymptomatic state to life threatening seizure and encephalitis-like stage due to cerebral edema. Symptomology is primarily depended up on location and number of lesion as well as parasite load. Higher the parasite load associated with strong immune response and more severe symptoms. Population-based studies have been shown that a substantial number of patients in endemic area were asymptomatic with lesion on imaging of brain.

Most common clinical presentation of NCC is seizures and detected in up to 80% of symptomatic individual. NCC is the leading cause of epilepsy in adult age 25 years and more. Initially it was postulated that only viable cystic stage is responsible for symptoms and not due calcified nodules. The recent studies have been suggested that seizures could occur in any stage of cysticerci. Headache may be associated with or without seizure and may mimic the migraine. Table 1 is depicted the stage of parasite and possible mechanism of clinical manifestations.

Focal neurological signs may be a presenting feature of NCC. This has been seen up to 20% cases of NCC. The disease course in focal neurological deficits is commonly subacute or chronic and may resemble brain tumor. Pyramidal tract signs are the commonest, but other signs like sensory disturbances, involuntary movements, abnormal muscle tone, signs of brainstem dysfunction, and aphasias may occur in few patients. Acute stroke is uncommon manifestation and occurs due to infraction in internal capsule, corona radiata,
and/or brainstem.\textsuperscript{18} Intracranial hypertension is a rare but dreaded manifestations; either due to mass effect or host immune response leading to arachnoiditis or encephalitis.\textsuperscript{19} Neurocognitive manifestations are though rare and ranging from psychaitrics symptoms to severe dementia.\textsuperscript{20}

### Diagnosis

Diagnosis of NCC is improved significantly with increased availability of computed tomography (CT) and magnetic resonance imaging (MRI). These investigations can be able to differentiate the stage of parasite in brain parenchyma and this may be helpful for selection of antiparasitic therapy. Table 2 shows the pathognomonic finding according to stage to parasite in neuroimaging.\textsuperscript{21}

The MRI imaging delineates better subarachnoid NCC, and hydrocephalus is the most common finding. The cause of hydrocephalus is due to diffuse leptomeningeal inflammation and thickening and occlusion of the foramina of Luschka and Magendie.\textsuperscript{17} Diagnosis of ventricular cyst is also better MRI imaging and this easily missed in CT scan. Contrast in MRI imaging shows the mobility of cyst within the cavities and this is called “ventricular migration sign.”\textsuperscript{17}

Serological method of detection of \textit{Taenia solium} specific antibody by enzyme linked assay may be helpful in diagnosis of NCC. The major disadvantage of serological assay is high false negative results. Due to poor sensitivity this cannot be used as diagnostic test but this is a very useful for monitoring of therapy, if positive in NCC.\textsuperscript{22}

Even with the advances in neuroimaging and laboratory immunological test, the diagnosis of NCC is still a challenging task for clinician. To improve the diagnosis accuracy of NCC, Del Brutto et al. developed a criteria based on clinical, radiological, immunological, and epidemiological data (Table 3). The diagnostic accuries of NCC are classified on the basis of these criteria into definitive diagnosis and probable diagnosis. Presence of major criteria is highly suggestive of the diagnosis but minor criteria though non-specific but required.

### Treatment

Because of variable presentation of the disease, a single approach is not possible for treatment. The factors affected the tailoring of treatment in NCC are location, numbers, and stage of cysticerci in nervous system.\textsuperscript{23} The basis of NCC treatment consist both antiparasitic treatment and symptomatic treatment. The preferred antiparasitic drugs are albendazole (15 mg/kg/day) or praziquantel (50 mg/kg/day) with standard duration of treatment is 10–14 days.

In Table 4 there are some peculiar point in treatment guideline given by Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH).\textsuperscript{24}

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Stage of parasite</th>
<th>Clinical manifestations</th>
<th>Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cysts</td>
<td>Seizures, hydrocephalus, focal neurological defect</td>
<td>Mass effects cyst on the brain parenchyma</td>
</tr>
<tr>
<td>Colloidal and granular cysts</td>
<td>Seizures, encephalitis like stage due to cerebral edema</td>
<td>Degeneration of parasite and host inflammatory response</td>
</tr>
<tr>
<td>Calcified nodule</td>
<td>Epileptogenic focus</td>
<td>Gliosis formation around dead parasites</td>
</tr>
</tbody>
</table>

**TABLE 2**

<table>
<thead>
<tr>
<th>Stage of parasite</th>
<th>Neuroimaging finding of brain parenchymal lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cysts</td>
<td>A well outlined small and rounded cyst with no abnormal enchantment on contrast imaging. Invaginated scolex has seen as eccentric hyperdense nodule and called “hole-with-dot” appearance</td>
</tr>
<tr>
<td>Colloidal and granular cysts (degenerating)</td>
<td>The lesions are ill-defined and may be single or multiple with surrounding edema. Contrast imaging showed a ring enhancing or a nodular pattern of enhancing</td>
</tr>
<tr>
<td>Calcified nodule</td>
<td>Non-enhancing hyperdense nodule (better detected in CT)</td>
</tr>
</tbody>
</table>
TABLE 3  Diagnostic criteria for NCC

Absolute criteria:
- Parasite visualization either by histopathology or funds examination of retinal or neuroimaging (scolex in cystic lesions)

Major criteria:
- Radiology imaging of CNS or spine strongly suggestive of neurocysticercosis
- Positive immunoblot serology for parasite
- Resolution of suspected cystic lesion either spontaneously or after anti-parasitic therapy

Minor criteria:
- Radiology imaging of CNS or spine strongly suggestive of neurocysticercosis
- Clinical features are indicative of neurocysticercosis
- Positive parasitic antibody or antigen in CSF
- Presence of extra-neuronal cysticercosis

Epidemiologic criteria:
- Patient from high prevalent area of cysticercosis
- History of frequent travel to high prevalent area of cysticercosis
- History of household contact with Taenia solium infection

Definitive diagnosis (any one):
- One absolute criterion
- Two major plus one minor or one epidemiologic criteria
- Probable diagnosis (any one):
  - One major plus two minor criteria
  - One major plus one minor and one epidemiologic criteria
  - Three minor plus one epidemiologic criteria

Conclusion

Neurocysticercosis is caused by helminthic infection due to Taenia solium.
Most common cause of adulthood epilepsy.
Environmental human-to-human transmission is also important mode of transmission.
Diagnosis is improved with neuroimaging, but accuracy required diagnostic criteria.
Effective antiparasitic treatment with adjuvant corticosteroid therapy is most effective treatment.
Detection of stage of parasite by neuroimaging is very helpful in selection of treatment.

References


Abstract
Rabies is one of the most typical zoonotic, fatal, and acute progressive neurological infections that has been well known since ancient ages. It is avertable deadly viral disease of Rhabdoviridae family, Genus Lyssavirus. The virus uses nerve cells for their multiplication and produces mutation in the nervous system. There is no treatment for rabies, but we can avoid this by taking preventive measures. Vaccination for dogs is also available. The purpose of this comprehensive overview is to summarize the rabies and its medical importance.

Introduction
Rabies is a disease transmitted to humans through infected animal bites (all mammals) mainly dogs (reservoirs of infection) pets (40%) and stray (60%).

Myths about Rabies in India:
- Some herbal extracts and concoctions will cure rabies.
- People also resort to witch crafts and religious practices.
- Washing of wounds can cause hydrophobia.
- Dietary changes can cure, that is, shift from vegetarianism to non-vegetarianism or vice-versa; stopping consumption of white things, etc.
- A single dose vaccine will prevent rabies.
- Vaccines are more effective if taken in empty stomach.
- One should avoid bathing or eating meat or eggs during vaccination.
- Gems and stones have magical properties against rabies.

Avoid:
- To watch dog for 10 days (practically not feasible) is risky and treatment should be initiated as early as possible.
- Keeping vaccine in freezer. If accidentally kept it should not be used.
- Cauterization of the wound.
- Suturing and bandaging the wound because it may inoculate the virus deeply in to the wound.
- Debridement of the wound too much as this may cause problem with wound closure and appearance.
- Storage of vaccine at room temperature.
- Exposure to the Sun light, heat, or dust (vaccine should be stored at +2–8°C)
- Mixing of IMR and ID schedule.
- Using IG and rabies vaccine in same syringe and at same anatomical sites (it should never be practiced).
- Vaccine should be diluted with the diluent provided.

Clinical Features
See Table 1.

Prophylaxis and Treatment
Questions to be Asked/to Start PET?
The animal which bit you was pet (immunized or non immunized) or stray.
Was the bite provoked or unprovoked?
Did you clean/wash the wound gently/thoroughly? (This helps wash away the virus.)

**Decision to Initiate PET?**

Should be taken well in time, as delay may increase the risk for developing clinical rabies. PET is nearly 100% effective when used appropriately. Factors that should be taken into consideration when deciding whether to initial PEG include:
- The epidemiological likelihood of the implicated animal being rabid.
- The category of exposure (I-III)
- The clinical features of animal.

**Diagnosis**

**Availability of the Animal for Observation and Lab Testing**

During life, the diagnosis is usually made on clinical grounds but rapid immunofluorescent techniques can detect antigen in corneal impression smears or skin biopsies.

But for most cases in developing countries, the vaccination status of the implicated animal alone should not be considered when deciding whether to give or withhold prophylaxis.

Examination of CSF often reveals mild mononuclear-cell pleocytosis with a mildly elevated protein level. The presence of rabies virus-specific neutralizing antibodies in CSF suggests rabies encephalitis, regardless of immunization status.

Detection of rabies virus RNA by RT-PCR is highly sensitive and specific. This technique can detect virus in fresh saliva samples, skin biopsy specimens, CSF, and brain tissues.

Direct Fluorescent Antibody Testing with rabies virus antibodies conjugated to fluorescent dyes is highly sensitive and specific. The test can be performed quickly and applied to skin biopsy and brain tissue samples. In skin biopsy samples, virus antigen may be detected in cutaneous nerves at the base of hair follicles.

**Golden rule:** It may be safest to assume that the animal that bites you has rabies and treatment includes—
- Local
- Rabies shots
  - Fast acting shot (Rabies immunoglobulin)
  - A series of rabies vaccines.

**Local Treatment (Wound Toilet)**

Wash the saliva containing rabies virus and clean the wound with soap/detergent (soaps are viricidal) and flush the wound under running water for at least 15 minutes. After cleaning, anti-microbial agents, disinfectant, sanitizer can be applied.

**Rabies Immunoglobins**

The protective antibody takes 7–14 days to develop after initial dose of vaccine; hence, RIG should be started as soon as possible because it provides activity against rabies virus by providing passive immunity, beginning immediately after administration and lasts for about 7–10 days during which period active immunity to rabies develops and protects the individual.
Severe multiple bites on head, neck, face hands, and genitalia in particular have a short incubation period of only 4 days. Thus, these individuals are vulnerable to rabies despite the timely and full course of any modern rabies vaccine and proper wound care. In these individuals only RIGs are lifesaving, as their timely and proper administration neutralizes the virus in the wounds and prevents its progression into CNS. RIG provides protection, which begins immediately after administration and lasts for about 7–10 days, during which period active immunity to rabies develops and protects the individual.

Often, this failure of modern cellular vaccines in most cases has been because RIG was not use in high risk category III cases

There are two types of RIGs:
- **ERIG (Equine Rabies Immunoglobulion):**
  - It is heterologous in origin
  - Produced from hyper immunized horses
  - Economical as compared to HRIG; hence, more affordable
  - The currently manufactured ERIG are highly purified with least adverse effects. Most of the ERIG available are F(ab’)2 fragment free from reactogenic Fc fragment
  - Dose is 40 iu/kg body weight up to a maximum of 3,000 iu (given after sensitivity test)
- **HRIG (Human Rabies Immunoglobulin):**
  - Homologus in origin
  - Longer half-life when compared to ERIG; hence, given in half the dose of ERIG
  - Does not require prior skin testing
  - HRIG are imported, expensive, and scarce.
  - Dose is 20 iu/kg body weight up to a maximum of 1,500 iu.
  - RIGs should be given as a single dose and should not be repeated.

**Vaccines**

Human rabies vaccines are made from inactivated or attenuated rabies virus and have gone through successive improvements since the time of Pasteur. The first rabies vaccine was developed by Pasteur, which was nerve tissue based and virus was inactivated by drying, but this vaccine had a risk of activation of the virus and allergic reaction to the presence of nerve tissue or myelin. Myelin-free vaccine prepared from neonatal mouse brains were introduced by Fuenzalide et al.

Subsequently purified duck embryo vaccine (PDEC) was developed, which was highly immunogenic rabies vaccine that can be used safely and effectively at low doses, both for primary immunization and for treatment after exposure. Subsequently, more second generation rabies vaccine were developed like human diploid cell vaccine (HDCV), vero cell, purified chick embryo cell (PCEC). PCEC vaccine with PM (Pit men-moore) strain.

**Advantages:**
- It is more advantageous than many other cell line based rabies vaccines.
- It is more readily scalable to large scale commercial vaccine production.
- The vaccine produced by the present process has a very large yield, efficacy safety as well as the process is much cost-effective than many of the other processes known for the preparation of rabies vaccine.
- It has unique stabilizing agents, which make the vaccine more stable even at accelerated temperature.
- This method provides vaccine with high yield, greater potency, and immunogenicity, which make the vaccine cost-effective and unique.

All modern anti-rabies vaccines are freely interchangeable. The safest vaccine, free of complications, is human diploid cell stream vaccine.

Vaccines can be given:
- In pregnant woman
- In lactating woman
- Along with other vaccine (EPI, i.e., expanded program on immunization) vaccines
- To a child with chicken pox or measles
- To HIV +ve or AIDS patients
- To patient with jaundice
- To patients who are on antimalarials or steroids or taking immunosuppressive drugs. Generally, vaccine should be avoided with these drugs but if cannot be avoided then vaccine on day 0 may be doubled and given at two sites
- With a higher potency

Indications for doubling the first dose (0 dose) of rabies vaccines:
- Patients who seek treatment after a delay of 48 hours or even months after having been bitten should be dealt in the same manner as it expose occurred recently.
- Patients with very high risks exposures on extensive bites.
- Immunodeficient patients, or those on immuno-suppression drugs, e.g., antimalarials, anticancer drugs, etc.
- Severely malnourished patients.
- Patients with underlying chronic disease like cirrhosis of liver.
- Patients where RIG is indicated but unavailable.

**Discussion:** Rabies is one of the oldest diseases known in recorded medical history. It is caused by a bullet shaped RNA rhabdovirus that is a member of the rhabdoviridae family, genus lyssavirus. Generally, rabies is transmitted by saliva from infected animal bites but may also be transmitted by scratches, secretions that contaminate mucous membranes, aerosolized virus that enters the respiratory tract and corneal transplants.

Rabies is a neglected disease of poor and vulnerable population whose deaths are rarely reported. It occurs mainly remote rural communities where measures to prevent dog to human transmission have not been implemented. Under reporting of rabies also prevents mobilizations of resources from the international community for the elimination of human dog mediated rabies.

**Prevention of Rabies**

This consists of:
- Elimination of stray dogs
- Registration and licensing of dogs
- Immunization of dogs and pets

Since dogs are the major reservoir of rabies transmission in India (Table 2), there population must be controlled. Way back in 1985, the dog population was estimated to be 80 million as per census by agriculture ministry. It appears this population might have grown substantially.

For killing of dogs strychnine had been in use for long time, its use is not advocated these days because drugs being very hazardous and in humane. Saturated solution of magnesium sulfate administered IV is the ideal and recommended method for killing dogs. After destroying the dogs, the concass should be hygienically disposed off. But this is not allowed.

The dog destruction used to be one of the pivotal activities at the primary health center in India. Sanitary inspectors used to destroy dogs in rural areas after obtaining the requisition from village panchayats. Now this has been stopped. Human way of dog destruction is in practice in municipal areas. No solution seems to be in hand to destroy stray dogs in rural areas, the menace continues to grow. Similarly, monkey menace has been additional burden in urban areas, besides dogs.

Municipal authorities hold the responsibility of dog catching licensing and dog destruction by humane way to prevent cruelty against animals.

Sterilization of dogs and monkeys has been attempt to reduce their population.

**Pre-exposure Immunization in Animals**

Pre-exposure immunization of pet animals against rabies is recommended.

**Single dose:** The vaccine is prepared as 20% suspension of brain from sheep infected with modified rabies virus. The virus in the brain suspension is inactivated phenol or B-propiolactone (BPL). It’s only used for pre-exposure immunization.

**Veterinary Antirabies Vaccine Multiple Dose**

This vaccine is also prepared from sheep brain infected with modified rabies virus. The vaccine is given in multiple doses and can be used for both pre- and post-exposure immunization of dogs, cats, and other domestic animals. This vaccine confers higher degree of protection than conferred with single dose vaccine.

**Epidemiological cycles:**
- **Urban:** As many as 99% of human cases in India are due to bites by urban rabid animals, which maintain a cycle amongst themselves. DOG→DOG→Other animals, and man being an accidental victim and dead end of infection.
- **Sylvatic:** Cycle propagates itself within wild animals who sometimes transmit the infection to urban populations.

**Table 2: Reservoir animals**

<table>
<thead>
<tr>
<th>Frequently</th>
<th>Sometimes</th>
<th>Occasionally</th>
<th>Not reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>Monkeys, Horses, Sheep, Cow, Buffalo, Donkeys, Pigs</td>
<td>Camels, Elephants, Foxes, Mongoose, Jackals, Bears</td>
<td>Bats, Rodents, Birds</td>
</tr>
<tr>
<td>Cats</td>
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animals as well as man. Bat rabies has not been conclusively reported from this country. No courier state has been convincingly demonstrated in dogs and in all practical purposes it is taken as non-existent.

Mode of transmission:
- Licks on damaged skin and mucous membrane or scratch
- Directly from the bite by Rabid animals
- Handling saliva of rapid animals or patient
- Organ transplantation particularly by corneal graft etc.
- Unboiled milk of Rabid infected cow
- Urine, tears, nasal secretions, and sweat
- Aerosol contamination
- Mechanical transmission
  After local multiplication at the wound site, the virus enters the nerves and travels at the rate of 3 mm/hours to the dorsal root of ganglion, then reaches the anterior horn cells of the spinal cord and brain, developing rabies encephalitis, and inevitable deaths.

  **Incubation period:** On an average 20–90 days, but 4 days to 8 years have been reported.

**Pathology**
- Minimal pathologic changes
- The brain is edematous and congested on gross appearance
- Histopathologically:
  - Perivascular cuffing and gliosis
  - Minimal neuronal damage (necrosis)
  - Presence of Negri bodies is pathognomonic

**Conclusion**
- A much feared disease known to man since ancient times is rabies.
- A deadly disease, but also a 100% preventable disease.

- Taking right steps at right time can reduce exposure to infection and prevent rabies.
- When in doubt about the degree of exposure to rabies risk, it is safer to over treat that to under treat.
- Criterion for “protection” after immunization—is that the rabies virus neutralizing antibody (RVNA) titer of ≥0.5 iu/mL of serum in the vaccinated person which is considered protective.
- Person receiving/completed anti-rabies immunization either pre-exposure or post-exposure can donate blood, but the recipient does not benefit from the transfer of rabies neutralizing antibodies due to hemodilution.
- Health education of public on prevention and control of rabies and management of bites and their response to bite at local level is crucial to save lives. This should be through primary health-care infrastructure besides mass media as also formal and non-formal education channels.
- In developing countries, where human rabies globulin may not be obtainable, 0.1 mL of vaccine may be given intradermally into eight sites on day 1, with single boosters on days 7 and 28.

**Suggested Readings**
5. Rabies immunoglobulin manual, IJCP academy of CME.
Biomarkers for Diagnosis and Prognosis of Severe Malaria

Manoj Kumar Mohapatra

Abstract

Biomarkers are different cellular, biochemical, or molecular products, which are measured in different biological samples to assess the diagnosis and prognosis of malaria.

Introduction

Malaria is an intricate parasitic disease caused by intraerythrocytic parasite of the genus Plasmodia. Five species, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*, are responsible for human malaria. Almost all deaths due to malaria are caused by falciparum malaria, and, despite all efforts, still it persists as a disease of high mortality. Improper diagnosis and development of drug resistance are two factors that make the disease difficult to control.

For a long time, detection of the parasite in peripheral blood smear has been considered as the gold standard of diagnosis but with a lot of limitations. Further, definite indicators of bad prognosis factors are necessary for prognosis of patients of severe malaria. Therefore, there is necessity of detecting different biomarkers for diagnosis and prognosis of malaria.

Biomarkers

Biomarkers may be defined as different cellular, biochemical, or molecular alterations, which can be measured in different biological samples to assess the diagnosis, prognosis, or therapeutic responses of a disease. There is no suitable classification of biomarkers. However, Frank and Hargreaves have attempted to classify the biomarkers into the following three types:

- **Type 0**: Measures of natural history of the disease and correlate with clinical outcome.
- **Type 1**: Determines the biological effect of therapeutic intervention.
- **Type 2**: Characteristic or variable that reflects how a patient feels/functions/survives, i.e., a clinical end point.

Biomarkers for Malaria

In malaria, host and parasite interaction is of paramount importance. Therefore, biomarkers related to the parasite may be used for diagnosis and from the host may be utilized for prognosis. When parasite enters the human body, parasitic proteins trigger the host immune systems, which are associated with malarial pathogenesis and severity. The molecules released during pathogenesis can be used as diagnostic biomarkers. *P. falciparum* severe infection has diverse effect on multiple systems causing multiorgan failure. Molecules released during complications like cardiac dysfunction, circulatory dysfunction, kidney dysfunction, and alteration of cell signaling can be used as prognostic biomarkers or biomarkers of severity.
Criteria for an ideal biomarker: An ideal biomarker should be:
- Less costly
- Easy to detect and evaluate
- Can be used in low-resource settings
- It can be used at the point of care
- It should have the power to distinguish different forms of the disease like symptomatic versus asymptomatic or uncomplicated versus severe malaria
- It should provide better prognosis
- It can guide treatment

Biomarkers used for diagnosis are HRP II (Histidine rich protein II), Parasite Lactate dehydrogenase, Hemozoin, Aldolase, Haptoglobin.\(^4\)

Biomarkers used for severity of disease are vascular dysfunction (angiopoetin-Tie-2 system), RBC membrane dysfunction (gamma GT), serum uric acid, eGFR, Procalcitinin, lipase, cardiac markers (PPARG coactivator 1 alpha, CPK-MB), Apoptosis markers (DAPK1; death associated protein kinase-1), hypoxia and cerebral malaria (HRP II, IFN-gamma, Lymphotoxin-alpha, chemokine CXCL10), neurological dysfunction (ApoE, apolipoprotein).

Biomarkers to assess protective immunity are antibodies to ICAM-1 binding \(P. falciparum\) erythrocyte membrane protein-1 (PfEMP-1) and to glycosylphosphatidylinositol (GPI).\(^5\)

Diagnostic Biomarkers

Histidine Rich Protein

\(P. falciparum\) synthesizes a unique set of soluble HRPs during the asexual erythrocytic development. There are three types of HRP, namely HRP I, II, and III in the order of discovery.\(^4\)

HRP-I is also known as knob-associated protein. It produces knob like protrusions on the cell surface of parasitized RBC that helps in cytoadherence of infected erythrocytes to venular endothelium. HRP-II helps in heme binding and heme detoxification by hemozoin formation. It is exclusively found in \(P. falciparum\). HRP-III, also known as small histidine alanine rich protein (SHARP) was found to have polymorphisms in gene repeats and shares homology with HRP-II.

Amongst these HRPs, HRP-II was found to be transported from parasite through the host cell cytoplasm to the circulation and can be detected in urine of infected patients. Its release in abundance makes it an important parasite antigen for diagnosis of malaria.

Parasite Lactate Dehydrogenase (pLDH)

Normally, the requirement of glucose for the metabolism of RBC is modest. But after the invasion, plasmodium consumes an excess amount of glucose for its growth and metabolism. The extra glucose has been taken from the blood by the parasite and metabolized by anaerobic glycolysis and glucose is converted to lactic acid and excreted to circulation. The final enzyme of this glycolytic pathway is lactate dehydrogenase, and hence is over expressed and can be used for diagnosis purpose.\(^5\)

Hemozoin

The parasite infects the RBCs and digests hemoglobin resulting in release of amino acids and toxic-free heme (ferrireprotoorphyrin IX), which is polymerized to hemozoin. It helps the parasite to survive from the heme toxicity. It plays a role as a visible marker in identifying parasites; therefore, popularly termed as malaria pigment.\(^3\)

Other Biomarkers

Apart from these, Aldolase and Glutamate dehydrogenase are two enzymes, which can be used for diagnosis purpose.

Haptoglobin

Haptoglobin (Hp) is an \(\alpha 2\) glycoprotein that binds rapidly to free hemoglobin (Hb). After rupture of parasitized RBC and hemolysis of non-parasitized RBC, free Hb binds to Hp forming a complex which is rapidly cleared by the mononuclear phagocytic system, resulting in a state of hypohaptoglobinemia, which can be used as a clinical and epidemiological biomarker of falciparum malaria.\(^6\)

Biomarkers of Severity and Prognosis

In addition to the diagnostic performance, biomarkers have been identified for the prognosis of severe malaria. Endothelial cell activation is crucial in pathogenesis of \(P. falciparum\) malaria. Activation of endothelial cells causes release of procoagulant and inflammatory proteins like tissue factor, Ang-2 (angiopoetin-2), I-CAM, and E-selectin.

Ang-1 and Ang-2 are ligands of the Tie-2 receptor which is expressed on endothelial cells. Ang-1 is constitutively produced and excreted into blood by pericytes and
smooth muscle cells and also stored in platelets. It binds to Tie-2 receptor, thereby acting as agonist resulting in anti-apoptotic and anti-inflammatory status of endothelial cell. Ang-2 is produced in endothelial cell and prestored in Weibel Palade Bodies (WPB) together with vWF. Upon endothelial activation, there is exocytosis of WPB, Ang-2 released and replace Ang-1-Tie-2 interaction. This interaction stimulates inflammatory response. Further studies based on this pathogenesis showed that decreased Ang-1 and increased Ang-2/Ang-1 ration are robust biomarkers to distinguish uncomplicated malaria from cerebral malaria. However, they do not correlate with parasitemia.7

PCT is a prohormone of calcitonin containing 116 amino acids with a molecular weight of 13 kDa. Under physiological conditions, calcitonin is produced and secreted from C-cells of thyroid gland after intracellular proteolysis to circulation with plasma half-life of a few minutes. Therefore, under normal condition PCT level is low (<0.5 ng/mL). The origin of PCT in infection is thought to be extrathyroidal and the predominance of PCT without increase in calcitonin indicates the presence of a constitutive pathway within the cell that bypasses the enzymatic conversion of PCT to calcitonin.8

High negative predictive value of S.PCT may be helpful for a rapid exclusion of critical malaria on admission. When S.PCT was ≤2 ng/mL the patients can be managed in the general ward or domiciliary treatment may be given; with 2–10 ng/mL the patients can be managed in the general ward with special attention or in high dependency unit (HDU) and can be shifted to ICU when necessary; and if more than 10 ng/mL the patients should be shifted to ICU for management.9 Different biochemical investigations that determine dysfunction of different organ systems can be used as biomarkers of severity.

Elevated level of gamma glutamyl transferase (GGT) was observed when there is increased RBC membrane damage contributed by oxidative stress. Markers of liver dysfunction like serum LDH and total bilirubin is significantly elevated in severe malaria. Serum lipase, a pancreatic enzyme which has been associated with acute pancreatitis, a rare complication of *P. falciparum* can be used as a biomarker.10 Cardiac biomarkers like PPARG coactivator 1 alpha and CPK-MB are significant cardiac biomarkers indicating of severe malaria and can be used as biomarkers.11

DAPK1 (death associated protein kinase 1) is known as a mediator of apoptosis and autophagy. When there is an extracellular signal trigger, DAPK1 is phosphorylated, which increases its catalytic activity and causes cellular death mediated by p53 pathway. When there is an increased parasite burden, there is inhibition of DAPK1 and p53, a strategy of parasite survival during metabolic stress. Therefore, decrease in apoptotic markers DAPK1 and p53 signify severe malaria.

Apo-E is a major lipoprotein in brain and has been seen dominant in several neurological diseases. Hence, several studies have been carried out to assess its correlation with cerebral malaria as a neurological biomarker.5

### Biomarkers of Protective Immunity

- **Antibodies to ICAM-1 binding PfEMP1-DBL beta:** Expression of diverse *P. falciparum* erythrocyte membrane protein-1 (PfEMP1) gene variants allows clonal antigenic variation and cytoadhesion to endothelium. Adhesion occurs via specialized PfEMP1 domains known as duffy binding like (DBL) and cysteine rich interdomain region (CIDR) and antibodies against them are significantly associated with protection against severe malaria. This class of DBL beta domain could be used as diagnostic antigens.3

- **IgG antibodies to synthetic GPI:** In malarial parasites, GPI is a glycolipid that is found both free and as an anchor sustaining many proteins on the parasite membrane including merozoites. In animal studies, it has been found that antibodies against GPI were able to delay mortality by *P. berghei*, by blocking toxic immune response. Further studies showed that GPI was found to be present across all stages of malarial parasite life cycles suggesting that antibodies against GPI could be able to prevent both erythrocytic and hepatic infection and block transmission of parasite from human to mosquito. These observations highlight that IgG to GPI can be potential biomarkers of immune status.3

### Conclusion

Unlike other diseases biomarker research in malaria is slow. However, recently biomarker research on malarial pathogenicity has taken a leap as evidenced by discovery of HRP-II, pLDH, aldolase, hemozoin, angiopoietin, procalcitonin, etc. Further, it is now important to distinguish complicated and cerebral malaria as early as possible so that treatment can be implicated to reduce the mortality.
References

Abstract
Malaria caused by *Plasmodium vivax* is often a life threatening disease leading to significant mortality in our society. Though severe malaria has been attributed to *Plasmodium falciparum*; however, mortalities and complications associated with vivax malaria cannot be overlooked. The ability of vivax to remain dormant in the form hypnozoites causing relapse and increasing complications by cytoadherence and rosette formation further adds to the disease burden. Chloroquine resistance is an emerging challenge in the management of patients with vivax malaria. Complications like hemolysis, cerebral malaria, ATN, and acute respiratory distress syndrome are also now attributed to *Plasmodium vivax* malaria.

Introduction
Malaria is a life threatening protozoal disease caused by Plasmodium parasite, which is transmitted to humans by bite of an infected female Anopheles mosquito. Of the five parasite species that cause malaria, two of them, *Plasmodium falciparum* and *Plasmodium vivax*, pose the greatest threat. World Malaria Report 2019 states that nearly half the population of the world was at risk of exposure to malaria in 2018.¹ An estimated 228 million cases of malaria were there worldwide in 2018 in contrast to 231 million cases in 2017 of which around 405,000 deaths globally due to malaria. Currently, India accounts for 4% burden of malaria globally and 87% of the Southeast Asia.² Most vulnerable group includes children less than 5 years of age accounting for 67% of deaths due to malaria worldwide.³ The most prevalent parasite in African region is *P. falciparum* (99.7%), whereas *P. vivax* remains as the prevalent parasite in America, accounting for 75% of all malarial cases.¹ In India, malaria is a major health problem with 0.88 million cases occurring per year. Severe Malaria (as per the WHO guidelines) accounts for approximately 400–1,000 deaths per year³ and most of them are due to *P. falciparum*.

*P. falciparum* known to cause Severe Malaria has been contributing to maximum morbidity and mortality worldwide whereas infection by *P. vivax* is always thought to be a benign disease, classically causing mild symptoms. But recently it has been seen that the infection by *P. vivax* no longer results in a non-critical illness having so many case reports showing its association with severe life threatening complications. There are historical evidences too, showing its severity which come from reports on neurosyphilis therapies in early 1990s reporting 5–15% mortality rates in America and European treatment facilities using *P. vivax* for treatment of syphilis indicating that it always had a severe outcome.⁴

*Plasmodium vivax*: Epidemiology
The most widespread infections of all malarial parasites is P. Vivas which affects majority of the parts of subtropical & tropical areas of the world. Nearly 2.5 billion people are affected by *P. vivax* infection in the world and 16 million clinical cases occur annually.⁵,⁶ Highest burden is borne by Southeast Asian countries and South America out of which 53% of cases occur in South East Asia with India alone bearing around 47% of the total burden.⁵,⁶ it has a wider
geographical variation and distribution due to its ability to stay dormant in liver cells and then cause relapses and its capability to survive in cold weather. As per the studies, it has been reported that many people in Africa are resistant to \textit{P. vivax} infection due to absence of Chemokine Duffy antigen receptor on RBCs but this theory has been put to question by many reporting cases in Duffy negative patients also.\textsuperscript{7,8}

In 2014, there were 2.14 million cases of \textit{P. vivax} worldwide, 18% of them occurred in India alone.\textsuperscript{9} In the recent past the number has been increasing accounting for nearly half of all the malarial cases. The incidence of \textit{P. vivax} varies across India with ten states accounting for nearly 89% of total cases.\textsuperscript{10} Out of these Jharkhand, MP, Odisha, UP, and Gujarat carry 64% of the total burden. It is becoming a problem mainly in urban settings of India probably due to construction work, migration, and ever growing slum areas, and resulting in peaks in morbidity and mortality. Within the Urban Malaria Scheme, 98% of all malaria cases were \textit{P. vivax} in 2014.\textsuperscript{10} Its transmission occurs throughout the year, but it peaks during rainy or post-rainy seasons.

**Risk Factors and Pathophysiological Mechanisms Leading to Its Malignant Course**

\textit{P. vivax} infection now poses a great threat with multiple reports showing severe clinical presentations and deaths. A meta analysis\textsuperscript{11} published in 2014 showed that \textit{P. vivax} as a major cause of “Severe Malaria” and warranted a prompt and early detection of its clinical manifestation resulting in early initiation of therapy so that it could be life saving. Many risk factors have been discussed related to its severe course. Children below 5 years of age are at risk of developing critical illness and ultimately dying within 1 year of their initial presentation.\textsuperscript{12} Genetic polymorphism varies and shows alpha and beta thalassemia patients having increased risk and Duffy negative and G6PD deficient patients having decreased risks of \textit{P. vivax} infection.\textsuperscript{13}

**Relapse**

The ability of \textit{P. vivax} to persist as dormant stage as hypnozoites in liver initiates infection in the blood resulting in frequent relapses ranging from weeks to months and years.\textsuperscript{14} The variation in time of relapse varies from one region to another and it is observed that in tropical countries the dormant period of relapse is usually 8–10 months.\textsuperscript{15,16} The exact mechanism how hypnozoites initiate relapses is still unknown.

**Increased Pyrogenicity**

In contrast to \textit{P. falciparum}, which is known to invade all stages of erythrocytes, and as a result parasitemia can exceed as high as 20–30%, \textit{P. vivax} is known to invade the reticulocytes rather than the erythrocytes, resulting in lower parasite biomass and parasitemia, which rarely exceed 2–3% in blood even in severe infection. \textit{P. vivax} in spite of having lower pyrogenic threshold has been observed to have higher endothelial activation, increased production of cytokines, and a proinflammatory response as compared to \textit{P. falciparum}.\textsuperscript{14} The main reason could be presence of higher levels of genomic content (GC) in vivax genome, and thus having higher contents of CpG motifs, which are recognized by receptor 9 leading to cell activation and inflammatory responses.\textsuperscript{17-19} Toxins contributing to increased pyrogenicity of \textit{P. vivax} have been found to be cholesterol or triglyceride fraction of plasma at the time of paroxysm of fever.\textsuperscript{14} The imbalance between pro- and anti-inflammatory cytokines production is related to severe clinical conditions in \textit{P. vivax}.\textsuperscript{20}

**Sequestration and Rosetting Phenomenon**

The classical pathogenesis of severe \textit{P. falciparum} infection leading to “Severe Malaria” shows sequestration of parasite in endothelial tissue causing microvascular obstruction, inflammation ultimately resulting in hypoxicemia, and ischemic damage in organs. Similar studies are now documenting that there is binding of vivax infected RBC to endothelial cells via receptors like ICAM 1 but to a much lesser frequency than the \textit{P. falciparum} infected RBCs.\textsuperscript{21} They also seem to be attached to glycosaminoglycans like chondroitin sulfate and hyaluronic acid.\textsuperscript{22} Severity of \textit{P. falciparum} is associated with the above phenomena, which is also seen in \textit{P. vivax} but it shows a moderate level of cytoadherence to endothelial cells and ultimately resulting in inflammatory responses in organs like lungs. In fact this phenomenon of sequestration and adherence of infected RBCs and accumulation of malarial pigment deposits to the intervillous spaces of placenta has been associated with pregnancy induced malaria leading to
anemia and intrauterine growth retardation although it appears to be less severe.14

Apart to adhesion phenomenon, it is also known that vivax too has a tendency to form rosettes, which has been mentioned in literature more than 20 years ago,23 although seen more frequently with falciparum. Rosettes in *P. vivax* are formed by interaction of infected RBCs containing trophozoites, schizonts, or gametocytes. The rosettes of *P. vivax* are stable even under high physiological shear stress and cause increased rigidity of infected RBCs thereby contributing to sequestration of *P. vivax* in the microvasculature.24

**Chloroquine Resistance**

The recommended drug in management of both *P. falciparum* and *P. vivax* is chloroquine, but now resistance has started developing to this drug especially in endemic areas. This is attributed to presence of polymorphism in chloroquine resistance genes pvcrt-0 and pvmdr-1 in South India.25

**Clinical Manifestations and Complications**

Fever is the main complaint of malaria and symptoms start to appear as soon as the blood stage infection starts with the invasion of RBCs by the merozoites. As the life cycle of *P. vivax* repeats every 42–56 hours, the paroxysm of febrile episodes occurs during similar intervals and classically the fever is termed as the tertian fever.26 Fever is usually intermittent, may go as high as 40 degrees in non-immune individuals and children associated with chills and rigors followed by profuse sweating and weakness. Other non-specific clinical features include abdominal discomfort, malaise, fatigue, and muscle aches. In few patients, a prominent headache, altered sensorium, or irritability may be observed. Headache may be very severe but usually signs of meningeal irritation like neck stiffness and photophobia are absent.

In areas of high-relapse rate, anemia has been observed to be the most common finding in both children and adults. The most likely causes involved are cumulative losses of infected RBCs, lysis of uninfected RBCs, and defect in production of red cells.27-30 It is said that for every one infected RBC in circulation, approximately 34 uninfected RBCs are removed and thereby leading to severe anemia. A higher inflammatory response, in the spleen leading to higher losses of RBC, seems to be its explanation in spite of low parasitemic index. Also its tendency to relapse usually at 3–4 weeks interval repeats the cycle before the hematological recovery takes place from previous infection. Inflammatory cytokines also result in dyserythropoiesis and bone marrow suppression.31,32 Usually a palpable spleen is found several day after infection but many of the otherwise healthy individuals have been found to have a palpable spleen indicating repeated infections in an endemic area. Mild hepatomegaly may be observed in children. Mild jaundice may be seen in adults and usually resolves in 1–3 weeks indicating uncomplicated infection.

*P. vivax* also has pulmonary complications and the spectrum ranges from cough, acute breathlessness, pulmonary edema to ARDS, and death. A meta-analysis published in 2017 showed that out of 49 studies 59.1% case studies reported acute respiratory distress syndrome (ARDS), 20.4% respiratory distress, 4% respiratory failure, 2% lung injury, and 2% studies reported pulmonary edema.33 They concluded that respiratory complications and ARDS occur in lower frequency in vivax than in falciparum, but mortality resembles that of falciparum. Female gender, presence of comorbidities, respiratory complications at time of hospital admission, and low-hemoglobin level were associated with deaths. Another meta-analysis was published in 201411 in which three studies showed higher incidence of ARDS among *P. vivax* as compared to *P. falciparum*.34-36 The presence of lymphocytes, neutrophils along with phagocytosed pigments in the pulmonary vasculature is the main cause of direct damage to lung parenchyma, diffuse inflammation, and alveolar damage.37

Acute kidney injury (AKI) remains a major cause of morbidity and fatality with incidence ranging from 15% to 40% in numerous studies.38,39 Mostly it is due to *P. falciparum*, but AKI has been reported in 12–20% *P. vivax* infection also, whether it is due to solely vivax or mixed infection is still debated. Mortality associated with *P. vivax* AKI is 10–15%, which is comparable with *P. falciparum*39 and 10–20% may require renal replacement therapy. Kidney complication are mainly due to hemodynamic dysfunction and immune response and clinically they present as oligoanuria, severe metabolic acidosis, and hypercatabolic state.40 A study conducted in 201741 discussing the clinic histologically profile on 30 patients showed fever in all cases, oliguria in 23%. Renal biopsy
performed among 6 patients revealed features of ATN in 4 patients, one had features of thrombotic microangiopathy, while one revealed acute cortical necrosis. Another study conducted showed that a high index of suspicion for diagnosing thrombotic microangiopathy should be kept if patient has persistent anemia, thrombocytopenia, jaundice, and non-recovering renal failure, such patients usually respond to plasmapheresis. The inciting event may be the lesion in endothelium of renal microvasculature.

Cerebral malaria a complication of \textit{P. falciparum}, characterized by diffuse meningoencephalitis, is seen to occur with \textit{P. vivax} also. The patient usually presents with signs and symptoms of acute febrile encephalopathy, seizures, and coma. Focal neurological deficits are unlikely. However, a case presenting with Status Epilepticus has been described. Though the exact pathogenesis remains elusive, but similar pathogenesis of sequestration of infected erythrocytes have been described. One pathogenesis regarding cerebral malaria is genetic variation resulting in variant interspersed repeats genes, the largest subtelomeric multigene super family found in \textit{P. vivax}. 

So it is understood that features of Severe Malaria can also be seen in \textit{P. vivax} infection as commonly found in \textit{P. falciparum} leading to multiorgan involvement. A study conducted in 2019 among 150 patients showed Severe Malaria among 42% of cases (as per WHO criteria) with mortality of 1.33%.

**Management and Treatment**

Main pillar in elimination of malaria is early diagnosis and treatment thereby reducing disease burden and preventing complications, and deaths. Malaria is a notifiable disease in India. For diagnosis, uses of light microscopy or immunochromatography-based rapid diagnostic tests (RDTs) are being used. The gold standard for diagnosis is direct visualization of \textit{P. vivax} parasites on Giemsa stained blood smears by light microscopy. It is a low cost with high sensitivity diagnostic test. Parasitemia is detected by thick smears and at least 200 fields should be examined well before concluding negative diagnoses. Thin smears are used to calculate the parasite density. The parasite density of \textit{P. vivax} is lower in areas where mixed infections are common making the diagnosis more difficult.

Since the early 1990s RDTs have popularized as diagnostic tools as they can detect one or more plasmodium species and are time saving without the requirement of a skilled lab personnel. This test has a high sensitivity at higher parasitic load and may give false negative at lower parasitic load. This test can be positive even 1 month after successful treatment. A new recently developed real time micro PCR based diagnostic device has been claimed to have high sensitivity and specificity.

**Treatment of \textit{P. vivax}**

The objective of treating malaria caused by \textit{P. vivax} is to cure both blood stage and liver stage infections thereby preventing recrudescence and relapse, respectively. The ability of \textit{P. vivax} to from hypnozoites possesses a great challenge in management of patients.

WHO Malaria Treatment Guidelines, 3rd edition, suggest treatment of all suspected and confirmed uncomplicated malaria patients with chloroquine or Artemisinin Combination Therapy (ACT). 

**Chloroquine Sensitive Malaria**

Oral chloroquine at total dose of 25 mg/kg (initial dose of 10 mg/kg on first day followed by 10 mg/kg on second day and 5 mg/kg on third day) \textbf{(Box 1)}.

ACTs are highly effective in the treatment of vivax malaria, allowing simplification (unification) of malaria treatment, that is, all malaria infections can be treated with an ACT. The exception is artesunate + sulfadoxine pyrimethamine, where resistance significantly compromises its efficacy.

**Chloroquine Resistant \textit{P. vivax}**

ACTs containing piperaquine, mefloquine, or lumefantrine are the recommended treatment, although artesunate + amodiaquine may also be effective in some areas.

**Box 1**

| Treatment uncomplicated \textit{P. vivax}, \textit{P. ovale}, \textit{P. malariae} or \textit{P. knowlesi} malaria |
|---|---|
| \textbullet{} In areas with chloroquine-susceptible infections, treat adults and children with uncomplicated \textit{P. vivax}, \textit{P. ovale}, \textit{P. malariae}, or \textit{P. knowlesi} malaria with either an ACT (except pregnant women in their first trimester) or chloroquine. | \textbf{Strong recommendation, high-quality evidence} |
| \textbullet{} In areas with chloroquine-susceptible infections, treat adults and children with uncomplicated \textit{P. vivax}, \textit{P. ovale}, \textit{P. malariae}, or \textit{P. knowlesi} malaria (except pregnant women in their first trimester) with an ACT. | \textbf{Strong recommendation, high-quality evidence} |
In pregnant or breastfeeding women, weekly chemoprophylaxis with chloroquine is recommended until delivery followed by completion of breast feeding. Treatment for future relapses can then be decided on the basis of G6PD status (Box 2).

**Severe Malaria**

All patients including adults, children, infants, pregnant women in all trimesters, and lactating women with Severe Malaria are to be treated with intramuscular or intravenous artesunate for at least 24 hours and until they are able to tolerate oral medication. Artesunate is prescribed at a dose of 2.4 mg/kg at 0, 12, 24, and 48 hours. Once a patient has received at least 24 hours of parenteral therapy and who can tolerate oral therapy, complete treatment with 3 days of an ACT should be advised. Intravenous quinine can be considered as an alternative agent at a loading dose of 20 mg/kg followed by 10 mg/kg in 8th hourly dosing only, if artesunate is not available.

Symptomatic management of ARDS, seizures, and AKI is to be done according to institutional protocols.

**Conclusion**

Managing patients with severe malaria should caution the mind of a clinician in view of *Plasmodium vivax* as a causative agent and potential drug resistance in the same. Patient may present with hemolysis, jaundice, acute renal failure, cerebral malaria, or acute respiratory distress syndrome. Use of artemisin-based therapy is the treatment of choice for severe and drug resistant malaria caused by *Plasmodium vivax*.

**References**


Abstract

Majority of emerging and re-emerging diseases are zoonotic. Viral and parasitic infections are more common causative agents for emerging diseases than bacteria. Zoonotic diseases are more likely in our country due to our usual habit of keeping livestock in close proximity.

After the last influenza pandemic in 1918, the prevailing COVID-19 pandemic is almost over 1 year in existence. Whole world is now reeling with this emerging infectious disease along with various re-emerging diseases in between.

Introduction

Since antiquity human population have faced several epidemics of emerging & re-emerging infectious diseases with a significant death toll. Plague (in 14th century), smallpox (in 16th century) and Spanish flu (in 20th century)—were responsible for huge death to the tune of 200, 56, and 50 millions respectively. With the advent of antimicrobials and effective vaccines in 20th century infectious diseases no longer remained the major threat for mortality, especially in developed countries. But for last few decades a slew of newer infectious diseases (Avian Influenza, NIPAH, SARS, COVID-19) have emerged. Some of them have epidemic potentials with significant morbidity and mortality.

Definitions

Emerging Disease

Infectious diseases are said to be EID when incidence of one Inf. disease has increased abnormally within the recent past or threatens to increase in the near future. Diseases that fall in this category are:

- New disease
- Old disease with new feature: Here new means in respect to new location or new population (prevalence in different age group), new feature (changed C/F or non-responsiveness to conventional therapy)

Re-emerging Infectious Disease

A past disease, which was once well controlled or eradicated, has shown its reappearance is considered to be re-emerging infectious disease.

Origin of Infectious Diseases

Majority of EIDs are zoonotic and over 30 new infections have emerged in last three decades globally and most common agents are viruses & prions (37%) followed by protozoa (26%). Many of them have epidemic or pandemic potentials with high fatality rate and ~60% of them are zoonotic in origin, especially wildlife in origin. In India, zoonotic diseases are more likely as livestock usually stay in close proximity to human dwelling.
Infections from Animal to Man—How?

To get into the depth of origin of these economies we have to look back to our changed behavior. Human encroachment to animal habitat through deforestation and use of terrain for their personal living and farming has lead to increased interface with wild animals. This is considered to be an important or potential factor for jump of some microbes (with or without mutation) usually found in animal kingdom, to human population. Many of them have epidemic potential and spread is facilitated across countries by fast travelling and wild trafficking.

Important Diseases

Important emerging and re-emerging diseases in India in this millennium that deserve mentioning are:

Virus:
- NIPAH (2001)—West Bengal
- Chikungunya (2005)—Andhra Pradesh
- Influenza-H1N1 (2009)—Andhra Pradesh
- CCHF (2011)—Gujarat
- COVID-19/SARS-CoV2
- Zika Virus Disease

Bacteria:
- Diphtheria (2001–2015)—Many states (Andhra Pradesh, Assam, Delhi, Gujarat, Karnataka, Nagaland, West Bengal, etc.)
- Plague (2002, 2004)—Himachal Pradesh, Uttarakhand
- MDR/XDR Tuberculosis

Protozoa:
- ACT resistant Falciparum Malaria

Abridged Information of the above Diseases are Put Forward Except Malaria and TB

Nipah Virus Disease

Nomenclature:
- Nipah virus (NiV) belongs to family Paramyxoviridae & Genus Henipavirus.
- Named according to its first discovery in Sungai Nipah village of Malaysia.

- It could be considered South Asian disease as all the outbreaks (more than ten) after 1999 have occurred in this region only.

Human affectation: In various ways:
- More commonly found in male with history of high exposure to pigs.
- Food borne by consuming fresh date palm sap contaminated with saliva, urine of fruit bats, natural host of NiV, while they feed on it.
- Human to human transmission is possible if patient has respiratory symptoms.9

Clinical feature:
- Male predominant disease with M:F = 4.5:1.
- Presents with fever (97%), headache (65%) with altered sensorium and abnormal brainstem functions (50%).
- Dizziness, vomiting, hypotonia, areflexia, dysautonomia (hypertension, tachycardia, excessive sweating) may be present.10
- Siliguri (West Bengal) cases in 2001 showed additional feature of acute respiratory distress with tachypnea in half of the cases, making human to human transmission possible (unlike Malaysian outbreak) with high fatality of 62.5%.11
- NIPAH virus alert in Kerala was again found in 2018.

Management and prevention:
- Mainly supportive and barrier nursing methods are advocated.
- Ribavarin—tried but inconclusive in vivo trial.
- Monoclonal antibody targeting NiV G glycoprotein was found to be effective in ferret models.
- Vaccine—a subunit vaccine against Hendra virus (used in horses), a member of Henipavirus, offers a great potential for NiV through cross protection.12

Chandipura (CHP) Virus

- Named as per its first documented place of emergence, Chandipura, Nagpur of India in 1965 and possibly it is sandfly borne disease.
- In 2003, Chandipura AES (Acute Encephalitis Syndrome) outbroke in Children in Andhra Pradesh with high fatality (55%) but luckily residual neurological deficit was rare in recovered children.13
- After this two more focal outbreaks were observed in Gujarat (2004) and Maharashtra (2007).
Chikungunya

Chikungunya is a self-limiting disease and prevalent in tropical and subtropical areas of Africa and Asia. Chikungunya means “to be bent over” as per Swahili language of Tanzania (Africa) where from the first case was reported.

Causative agent:
- Chikungunya virus (CHIKV) of arboviridae family of the genus alphavirus.
- Vector—day-biting infected Aedes Aegypti and Albopictus mosquito.
- Reservoir of infection—Non-human primates

Clinical features:
- High fever (102–05°F) with severe joint pain (patient becomes bent over) and skin rash.
- Usually follow acute self limiting course but around 10–15% of cases develop subacute (3 weeks to 3 months), or chronic Chikungunya.
- Chronic Chikungunya may present as RA or post-chikungunya-rheumatic musculosketal (pcRMSK) disorder.

After 1973, in 2006 re-emergence of chikungunya infection in India was found in epidemic form and affected 1.2 million Indians with high morbidity rate. From 2006 to 2012 chikungunya was reported from 22 States and union territories.

Diagnostic test:
- IgM against CHIKV

Treatment:
- NSAID for 1 or 2 weeks will suffice in majority of patients.
- Chronic cases may need anti-rheumatic drugs like hydroxychloroquine or methotrexate.

Influenza Virus (H1N1)

Influenza virus is a respiratory virus and is of 4 types—A, B, C, and D.

Type A Influenza Viruses has produced several pandemics in humans and is nomenclatured depending on which one of 18 different Hemagglutinins (HA) & 11 different Neuraminidases (NA) is present as surface protein. It originates either as Avian or Swine.

Influenza A Virus caused the largest and the deadliest pandemics before the occurrence of COVID-19 and four Influenza A Virus pandemics since beginning of 20th century:
- Spanish Flu (1918)—due to H1N1 with 50 million death
- Asian Flu (1952)—due to H2N2
- Hong Kong Flu (1968)—due to H3N2
- Mexican Flu (2009)—due to H1N1 pdm09

The Mexican Flu (2009) pandemic got spread rapidly to over 214 countries worldwide including India. Globally total death toll was 18,449.15

A total of 1,54,259 Indians were tested for H1N1 pdm09 influenza till August 8, 2010, with 23.4% being positive including 1,833 reported deaths. Maharashtra and Gujarat were worst affected states.16

Treatment:
- Supportive therapy
- Neuraminidase inhibitors—Block virus release from infected cells. Used both for prophylaxis & therapy:
  - Oseltamivir—Oral
  - Zanamivir—IV
  - Peramivir—IV/IM
  - Laninamivir—Intranasal

Crimean-Congo Hemorrhagic Fever (CCHF)

Name was derived from the first two places of its occurrence—Crimea (in 1944) & Congo (in 1956) caused by single stranded RNA virus of Bunyaviridae family.

Virus continues its life by animal (livestock)-tick-animal cycle.

Human gets infection by:
- Infected tick bites
- Handling of infected animal tissues (Slaughter house workers, farmers, veterinarians)

Clinical feature:
- Incubation period 1–9 days.
- High index of clinical suspicion is needed. Presents with fever, myalgia, headache, lymphadenopathy, and bleeding manifestation (varying from petechial rash to internal mucosal bleeding).17
- Fatality rate ranges from 9% to 50%.

India saw the confirmed outbreak of CCHF in 2011 in Gujarat.18 Total 42 cases were reported till 2013 with high death rate of 59.9%.
Important D/Ds:
DHF, leptospirosis, severe malaria, meningococcal infection, and other viral hemorrhagic fevers.

Diagnosis:
- RT-PCR—sensitive, specific
- Rapid test
- Antibody—antiviral IgG and IgM detection

Treatment:
- Supportive therapy
- Ribavirine—if instituted within 5 days possibly improves the prognosis

**COVID-19/SARS-CoV2**

Microbes in their quest to be ahead of human, they undergo some mutation to increase their capability of jumping to human from their animal source and human to human transmission as well to reach epidemic/pandemic potential. While this article is being written whole world is reeling under the threat of a zoonosis COVID-19/SARS-CoV2—biggest threat in last 100 years.

**Corona Virus-related Diseases**

Corona virus (CoV), a respiratory virus, normally causes diseases in mammals (including birds) like—camels, civets, bats, etc. This virus is transmitted to human through droplet/fomite & usually produces mild-to-moderate respiratory illness. But through mutation it has caused following diseases with high fatality rate:

- **Severe Acute Respiratory Syndrome (SARS)—**First noticed in 2002 in China, soon followed by worldwide spread with total reported 8,000 cases and 800 deaths. It was acquired from bat.†
- **Middle East respiratory syndrome (MERS)—**Acquired from dromedary camels (affected from bat). First noticed in 2012 in Saudi Arabia then spreading to other Arabian Peninsula and Republic of Korea. By 2016, over 1,700 cases detected with mortality of 35%.
- **Novel Corona Virus Disease (COVID-19/SARS-CoV2)—**This ongoing pandemic is being greatest threat of all infectious diseases in last hundred years. Discussed below briefly.

**COVID-19/SARS-CoV2 Disease**

In December 2019 in Wuhan, Hubei Province of China, an epidemic of respiratory illness with moderately high fatality of unknown etiology was noticed, later proved to be caused by *novel corona virus* (SARS-CoV-2/COVID-19) with human to human transmission.

**Origin of Novel CoV Infection**—Speculated to have originated from bats, a natural carrier of CoV, but bats remain in hibernation during winter. As low temperature prevails in December in China, so intermediate host was thought of. *The virus likely jumped to humans from pangolins (long snouted mammals often used in food and traditional Chinese medicine) along with possibly other multiple intermediate hosts.*

**Burden of COVID-19 patients**—Infection rate of COVID-19 per day is varying greatly from country to country. Many western countries are braced with 2nd wave of COVID.

Highest infection in a day in India till 20/12/2020 was on 11/09/2020 and it was 97,570. Little short of 1 lakh/day. Gradually with some fluctuation it then came down to 26,624 on 19/12/2020. Total burden of COVID as on 20/12/2020 is shown in Table 1.

**Vaccines update** (till December, 2020)—Researchers throughout the world putting in great efforts round the clock to contain the devastating situation of COVID-19 pandemicity by finding an effective treatment and/or vaccine against SARS-CoV-2. This has lead to an unprecedented public/private partnership to go through a fast tracked vaccine development process.

On August 11, 2020, Russia make COVID vaccine, Sputnik-V, is the world’s first vaccine as per bulletin of Ministry of Health of Russian Federation. Russia has started Phase 3 and Phase 4 (administration to general people) trial simultaneously though experts has raised their concern for safety profile (Table 2).

India too is progressing fast keeping pace with other countries. As per ICMR reporting on 20th August, 2020, two indigenously developed vaccine candidates—Bharat Biotech’s COVAXIN (both intradermal & IM), Zydus Cadilla’s Zycov-D have started human trial in India, so also Oxford Astra-Zeneca’s Covidsheild with its Pune based

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Total COVID-19 cases</th>
<th>Recovered cases</th>
<th>Deceased</th>
</tr>
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<tbody>
<tr>
<td>Global</td>
<td>7,66,26,187</td>
<td>5,37,51,202</td>
<td>16,91,942</td>
</tr>
<tr>
<td>India</td>
<td>1,00,47,131</td>
<td>95,95,711</td>
<td>1,45,669</td>
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</tbody>
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AstraZeneca is likely to get regulatory approval from the UK’s independent regulator by the end of this year for a rollout to begin in early 2021, according to the UK Oxford University/AstraZeneca vaccine company.

Trials of the Oxford vaccine show the following as per their claim:
- Stop 70% of people developing Covid symptoms. Could increase protection up to 90%
- The data also shows a strong immune response in older people
- It is given in two doses
- Trials with more than 20,000 volunteers are still continuing
  This may be one of the easiest vaccines to distribute, because it does not need to be stored at very cold temperature.
- It is made from a weakened version of a common cold virus from chimpanzees that has been modified to not grow in humans.

WHO and most nations globally are willing to prioritize vaccine recipients as follows:
- Health-care workers (HCW)
- People >65 years of age
- People with comorbidities (like DM, Cancers, CVD, COPD)

Clinical feature:
- Incubation period—2–14 days
- Asymptomatic—some patients
- Majority shows features of fever (45.4%), headache (70.3%), dry cough (63.2%), sore throat (52.9%), asthenia (63.3%), and some developed dyspnea. Anosmia (70.2%) and altered taste sensation (49.8%) may be early symptoms in some patients.
- Diarrhea may be the presenting feature of COVID-19 in ~20% of cases.

**Silent Hypoxia in COVID**

Silent hypoxia is a danger sign of COVID-19 infection. It means person’s oxygen level in blood cells and tissue may drop due to COVID-related diffuse pneumonia without any initial warning in the form of cough, dyspnea, tachypnea, etc. until patient develops ARDS.

So arterial $O_2$ saturation ($SpO_2$) detection at regular intervals is a must for detection of silent hypoxia and to initiate supportive therapy at earliest possibility.

**Treatment**

No full-proof definitive but supportive therapy available at present.

Aggressive isolation measures aimed at reducing transmission in the community is our best weapon.

The followings had been tried with mixed results:
- **Hydroxychloroquine (HCQ):** Antimalarial with in-vitro activity against SARS-CoV-2 and may have immunomodulating properties. WHO recommends for prophylaxis but not for therapy (directive on 25/05/2020).
- **Azithromycin:** Shows anti-SARS-CoV-2 activity in vitro.
- **Anti-influenza agent (Favipiravir, Oseltamivir) and broad-spectrum antiviral (Remdesivir), anti-HIV agent (Lopinavir/Ritonavir):** Showing varying degree of activity against corona viruses in vitro.
- **Convalescent plasma:** Tried in severe or immediately life-threatening COVID-19 infections with favorable result in some studies.
- **HFNO (High flow nasal oxygen):** HFNO in awake prone position is considered to be win-win position to avoid invasive ventilator in some patients of declining $SpO_2$.
- **Other therapies:** Anticoagulant (LMWH), Anti IL6 receptor inhibitor (Tocilizumab), Invasive Ventilator, ECMO (extra-corporeal membrane oxygenation) are other therapeutic options in some specific patients.
Case fatality: Differs in different countries. In India, till August 2020 overall mortality was ~1.56.

Mortality increases significantly in:
- Patients >65 years
- Patients with comorbidities (DM, renal failure, COPD, on chemotherapy, etc.)
- Patients needing ventilatory support

**Zika Virus**

Zika virus disease is caused by the Flavivirus, the same family of virus causing dengue. Several Zika virus outbreaks were found in both North & South America since 2015, but it has emerged in India just with only three (3) confirmed cases in 2017—as per report of Ministry of Health & Family Welfare, Govt. of India. All these three cases were reported from Bapunagar, Gujarat.

- **Transmission:**
  - Mainly by bite of infected Aedes mosquito.
  - Sexual activity and blood transfusion can sometimes lead to this disease transmission.

- **Clinical features:** Majority (80%) are asymptomatic, 20% of cases have low grade fever, myalgia, rash, and sore throat.
- Concern lies if pregnant women contract this disease in first trimester, which may lead to microcephaly and brain abnormalities of infant.

**Diphtheria**

We all know that exotoxin-producing *Corynebacterium diphtheria* is the causative agent. Its incidence has reduced to a great extent globally due to successful implementation of childhood vaccination. From 1 million before 1980s to 1 lakh in 1980 and reduced to 4.5 thousand in 2015. Diphtheria is a rare disease in developed countries but global burden contributed mainly by sub-Saharan Africa, India, and Indonesia.

In 15 years tenure (during 2001–2015) almost half of the global diphtheria cases were from India, and CBHI (Central Bureau of Health Intelligence) India reported 41,672 cases of Diphtheria in 10 years (2005–2014) with mortality of 2.2% cases.

Diphtheria is now considered EID as it shifts toward higher median age than its earlier preference for under five children. It could be related to lack of 100% coverage of three doses of diphtheria vaccination (80% coverage) and expected low coverage of booster dose after 10 years. This may lead to waning immunity against diphtheria among school-going children and adults. Some Indian study showed higher prevalence among muslim children and it was about 38% in Delhi as stated in a study.

**Plague**

We doctors now bear a wrong impression that plague is almost nonexistent in India and not getting proper emphasis in medical undergraduate and postgraduate teaching. So doctors are not properly aware of its consideration in differential diagnoses.

Plague infection in India continues to exist as enzootic in wild rodents and epizootic spread of plague from wild to commensal rodents facilitate human transmission. Thus, it leads to re-emergence/outbreaks of plague in India in 2002 (Shimla, HP) and 2004 (Uttarakhand) of this millennium.

**Causative Agent and Transmission in Humans**

Plague is a zoonoses (wild rodents) and caused by Gram-negative bacilli, *Yersinia pestis*, spreading between rodents through their fleas.

Ways of transmission to man:
- By bite of infected fleas
- Coming in contact with infected tissues of rodent
- Human to human transmission through droplets in pneumonic plague

**Conclusion**

This has been proved beyond doubt that significant percentage of infectious diseases are zoonotic in origin. In last three decades thirty (30) emerging infectious diseases detected globally, majority of them are viral in origin and approximately 60% of them are zoonotic especially wildlife in origin. In India zoonotic diseases are more likely as here domestic/pet animals are kept in close proximity to their residence.

Through awareness and/or legislation if we can make human contact with animals (domestic/wild) scientifically modified or restricted (in some situations) emerging and re-emerging diseases could be avoided globally to a great extent.

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