REVIEW ARTICLE

New and Emerging Challenges in Management and Control of Typhoid Fever

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Introduction

Enteric fever, generally known as typhoid, is an extremely common infection in developing countries where environmental hygiene is vastly compromised, and where children are most vulnerable to the infection.[1] Enteric fever is caused mostly by typhoidal *Salmonella*, including *Salmonella enterica* serotype *typhi*, and less frequently by serotypes *paratyphi* A, B, and C. It most frequently manifests as nonspecific, prolonged fever without localization. Clinically the differential diagnosis for acute fever is extensive and includes a number of bacterial, viral, fungal, and parasitic infections, as well as noninfectious entities. An experienced physician should be able to rule out other causes of acute febrile illness on the basis of clinical history, examination and targeted laboratory tests before considering typhoid as the cause of acute fever without localization.

Microbiological characteristics

Salmonella enterica are gram negative, encapsulated, motile bacilli with flagellae, and belong to the family Enterobacteriacae.[2] Preliminary blood, bone marrow, urine or stool cultures on MacConkey agar yields white or colorless colonies that do not ferment lactose (NLF). There are no known animal reservoirs of typhoidal *Salmonella*, and the source of infection is organisms shed in the stool of infected humans that are acquired from contaminated food or water, or poor hygiene after using the toilet.[3] The most common mode of transmission remains municipal water contaminated with sewage. Chronic asymptomatic bacterial shedding in the stool with gallbladder disease may contribute as risk factor for carriage, although this risk has not been substantiated in poorer countries.[4]

Salmonellae have been grouped into six serogroups and more than 2500 serotypes, according to diverse surface structures. This system of classification has allowed for differentiation between *Salmonella enterica* and non-typhoidal salmonellae (NTS). NTS are present in other animal species as well as in the environment and cause episodic food- borne diarrheal outbreaks.[3,5]

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Gastric acid is protective against salmonella, and reduction in gastric pH due to prolonged use of H_2 inhibitors and proton pump inhibitors (PPI) are likely to favor salmonella infection. There is also an association of *Helicobacter pylori* infection with salmonella, both probably related to unhygienic food and water.[6]

Symptoms

7-14 days after ingestion of contaminated food or water, the bacteria invade and multiply in Peyer's patches in the ileum, during which the patient is either asymptomatic or has vague abdominal pain, diarrhea and/or constipation, and a feeling of being 'unwell'. Fever rises gradually over several days, rising up to 40^0 C with chills. There is a dull constant headache with generalized myalgia, nausea, occasional vomiting and dull, poorly localized abdominal pain. If infection persists untreated, the liver and spleen become palpably enlarged.[5] A useful clinical tip-off in examination in the adult is measurement of pulse and temperature simultaneously to appreciate relative bradycardia (normally, for each degree rise of body temperature above 100^0 F the pulse rate rises by 10 beats per minute); however, this feature is not consistent, as complications such as abscess or overt or occult bleeding may mask this clinical finding. Moreover, temperature: pulse dissociation, is not a feature of typhoid in children.[7,8]

If the infection remains untreated in the second to fourth week, the patient gets increasingly sick with higher fever, weight loss, progressive weakness, and complications may set in as the bacteria metastasize to various viscera. Gastrointestinal bleeding due to erosion of necrotic ileal Peyer's patches through the wall of enteric vessels may lead to a fall in hematocrit and reactive leukocytosis. Blood transfusions may be required, especially if a large blood vessel is eroded. Intestinal perforation, usually of the ileum, and not infrequently of the colon, may be an ominous complication requiring urgent surgical intervention.[9] The clinician should be on guard if the patient appears restless with further rise of temperature, along with tachycardia, hypotension, rise in white blood count, and the chest X-ray may reveal free gas under the diaphragm, indicating intestinal perforation. Feculent fluid may fill the abdomen.

There may be mental confusion, somnolence, or agitation and delirium, progressing to obtundation. Other complications include cholecystitis, hepatitis, pneumonia, acute kidney injury, and myocarditis. Children may develop typhoid meningitis or osteomyelitis.[10] Unrecognized and untreated typhoid carries a mortality of 10-30%, but with timely treatment the fatality rate

is less than 1-4%.[11]

Diagnosis

It is important to confirm the diagnosis through isolation of the organism on culture, which also provides sensitivity testing to antibiotics. Blood culture, if drawn before antibiotic administration and in sufficient quantity, gives a yield of 80% in the first 1 to 2 weeks.[12] The yield of positivity falls to below 40% if antibiotics are taken before phlebotomy is performed.[13] Bone marrow culture is likely to yield positivity even if the disease is prolonged, severe or complicated, because of higher bacterial concentration in the marrow. Culture yield from blood and bone marrow is considerably improved with use of Brain Heart Infusion broth medium.[13] Understandably, bone marrow aspiration is not readily acceptable to patients, but may be offered when the cause of prolonged fever remains elusive. *Salmonella enterica* can be isolated from stool in 30% cases, and in urine a low 1%.[12,13,14,15,16,17] In culture negative cases, PCR, if available, may be a useful tool for diagnosis.[18]

Several serologic tests have been in vogue for diagnosis of typhoid fever, the most widely used being the Widal test, which measures agglutinating antibodies against O and H antigens of S. *typhi* and paratyphi. Due to lack of standardization and its low specificity, the Widal test is frequently misinterpreted, and this test is now considered obsolete.[14,19]

Another popular serologic test, Typhidot®, which measures IgM and IgG antibodies against 50 kDa outer membrane protein of S. *typhi*, is purported to be a rapid diagnostic test with reported sensitivity of 67-98%, and specificity 85-90%. However, because of cross reactivity with other gram-negative bacteria as well as background antibody levels in the general population, the Typhidot® may remain positive indefinitely, even in uninfected people. A recent systematic review of studies of two widely used tests, Tubex TF and Typhidot[18, 20] concluded that the performance characteristics did not justify their use. Widespread prescription of these tests has, unfortunately, led to enormous misuse of antibiotics, rendering precious antibiotics redundant, incurring huge expenses to the patient and causing financial anxiety to less affluent patients. For suspected typhoid a single blood culture before starting antibiotics is the gold standard and the best and most cost-effective option. The accompanying sensitivity report is essential for guiding the physician toward antibiotic selection. This becomes all the more important in today's conditions of emerging resistant bacterial infections.

Routine laboratory tests are not specific but must be done to rule out sepsis, malaria, and prevalent viral infections. The white blood count is usually in the low to normal range, and ALT/AST may be 3-5 times upper limit of normal. Leukocytosis in typhoid is suggestive of a complication such as intestinal perforation, and a fall in hemoglobin is indicative of intestinal bleeding.[3,5]

Antimicrobial Selection

Prior to 1970s the drugs of choice were ampicillin, cotrimoxazole and chloramphenicol, and these were used effectively for cure for several decades.[21,22] Plasmid mediated resistance to chloramphenicol was first reported from Mexico, and subsequently from Vietnam, India, Bangladesh and South Korea. Ampicillin and cotrimoxazole were used increasingly until 1980s when multi drug resistance (MDR) to all three drugs became worldwide. A study from India in 2005 showed MDR typhoid as high as 66%.[23] Bhutta *et al*. reported increased mortality among children with MDR Salmonella infection.[1] As a response to increasing cases of typhoid in developing countries, fluoroquinolones were introduced, and ciprofloxacin became the drug of choice. In 1992 the first case of fluoroquinolone resistant typhoid was reported from UK, followed by reports from other countries. MIC ≥ 128 µg/ml was reported from Japan in 2002.[24] Nalidixic acid was used as surrogate for fluoroquinolone susceptibility; however, by 2011 15-36% resistance to fluoroquinolones was reported from several parts of the world.

Following resistance to fluoroquinolones, extended-spectrum cephalosporins, such as ceftriaxone and cefuroxime were used successfully in typhoid; however, reports of their resistance against *salmonellae* started appearing from South East Asia as early as in mid 1980s.25,26 Of growing concern was the fact that several regions were reporting carbapenem resistant NTS. In 2018 Klemm *et al*[27] identified 80 XDR S. *typhi* isolates by whole-genome sequencing and found large numbers of resistance determinants.

Several studies from various countries have reported *in vitro* azithromycin sensitivities against *Salmonella enteric*a, with MICs among human isolates ranging from 1 to 32 µg/ml.[28,29] Randomized clinical trials (RCTs) have established the azalide antimicrobial azithromycin to be an effective alternative oral treatment for uncomplicated enteric fever.[29, 30, 31,32] It belongs to the macrolide group of antibiotics, has excellent penetration into most tissues, and achieves very high concentration in polymorphonuclear leukocytes and macrophages. Hence it is considered to be an excellent choice of treatment against *Salmonella enterica*.

However, a case report from UK in 2010 reported failure of azithromycin against *S. paratyphi* with MIC 1: 256 µg/ml. The patient had acquired the infection in Pakistan.[33] There are no clinically validated MIC breakpoints for azithromycin against salmonellae, therefore no established guidelines exist for treating typhoid with azithromycin.[29]

The most common mechanisms of macrolide resistance are efflux of the antibiotics (extrusion of the drug from the cell) and target site modification by a post-transcriptional modification of 23S rRNA, or mutations in 23S rRNA of ribosomal proteins. Studies investigating azithromycin resistance mechanisms in

Salmonella are scarce, and it is recommended to conduct further studies. The detection of resistance mechanisms is becoming increasingly important as surveillance programs recognize their role in the global control of antimicrobial resistance.[27,28]

The transitioning from drug susceptible to drug resistant salmonella almost every decade raises the specter of further drug resistant S. *typhi* and we may face increasingly untreatable infections. The first case of ceftriaxone resistant S. *typhi* was reported in November 2016 in Hyderabad, Pakistan.[27] The isolate was also resistant to ampicillin, cotrimoxazole, chloramphenicol, fluoroquinolones, and third-generation cephalosporins, leaving few options for treatment. The resistance to five classes of antibiotics is henceforth referred to as extensively drug resistant (XDR). Genetic classification of the isolate was done through whole genome sequencing, and the results revealed the bacterium to be of indigenous origin rather than of imported source. It was suggested that an MDR H58 clone acquired an ESBL plasmid from an E. *coli* or an enteric bacterial donor. The emergence of the MDR H58 clone is a watershed event in the evolution of drug resistant S. *typhi* and leads to a worrisome outlook for a common but potentially dangerous infection that affects vast swathes of both pediatric and adult populations in Pakistan. Clusters of XDR typhoid have emerged, as cases from both urban and rural populations are escalating, while physicians mainly from Sindh province continue to report cases of XDR S. *typhi,* especially among children from the lower socioeconomic class. Over one year from July 2017 to August 2018, 1221 culture proven S. *typhi* were diagnosed in the lab at The Indus Hospital in Korangi, Karachi, of which 627 (51%) were XDR. Children below age 15 years overwhelmingly accounted for these cases (oral communication, publication in progress.) Many children and adults have presented with jaundice, anemia, soft tissue abscess, intestinal bleed or perforation. The more toxic patients are managed with intravenous meropenem with or without azithromycin and at least a few have required surgical or radiological intervention.

For patients with invasive XDR *Salmonella* infection, carbapenems may represent the last resort. An article reports carbapenem resistant *Salmonella enterica* due to plasmid mediated Class A cabapenemase KPC-2.[34] The emergence of carbapenem resistance in *Salmonella*, will pose a serious clinical problem, owing to the lack of other therapeutic choices.

The rampant irrational use of antibiotics in Pakistan has contributed hugely to multidrug resistant bacteria- be it gram positive cocci, gram negative bacilli, fungi or Mycobacterium tuberculosis. Not only are second line drugs not as efficient as first line drugs in most instances, they are much costlier, and many have serious adverse events. Watching the progression of antimicrobial resistance, there is now a sense of foreboding among physicians about the future management of infectious diseases, especially in hospital settings. Total drug resistant S. *typhi* will become inevitable if we continue to misuse antibiotics. The use of azithromycin or other macrolides for viral respiratory infections must be strongly discouraged.

Typhoid vaccine

Three types of typhoid vaccines are licensed by WHO:

i) oral, live attenuated Ty21a vaccine: to be taken as 3 doses over a week for children above 6 years age.
ii) injection of unconjugated Vi polysaccharide (ViPS) vaccine every 3-7 years;
iii) typhoid conjugate vaccine (TCV);

i and ii need to be repeated every 3 years.[35] TCV (Typbar-TCV®), a Vi-tetanus toxoid conjugate vaccine manufactured by Indian company Bharat Biotech, is the first typhoid conjugate vaccine to achieve WHO prequalification. The vaccine, currently licensed in India and Nepal is recommended as a single, intramuscular dose, and elicits a robust immune response in infants as young as six months of age. Typbar-TCV® offers advantages over currently available typhoid vaccines, including the ability to provide longer-lasting protection, requires fewer doses, and can be administered to adults and children younger than two years of age, making it the first-ever typhoid vaccine to be approved for this age group.[35] WHO recommends its introduction for infants and children over six months of age in typhoid-endemic countries and it can be delivered through routine childhood immunization programs and affords better protection for younger children.[36] There is no effective vaccine against paratyphoid A currently available commercially. At the time of this writing, Typbar-TCV® is not available in Pakistan, but is being applied for registration and importing. Vaccinating the entire vast population against typhoid may not be a practical option but must be done at individual or community level as far as possible.

Summary

Typhoid fever is a common enteric infection in Pakistan, affecting all ages, but more so among children. It presents as high fever without localization, and untreated or poorly treated, may result in serious complications. Diagnosis must be sought within the first week or two through blood culture *before* antibiotic is administered. Because of rising drug resistance, isolation of the organism along with drug sensitivity testing is crucial for selecting correct therapy. Laboratories in good standing no longer perform serological tests such as Widal or Typhidot®, and the Medical Microbiology and Infectious Disease Society of Pakistan (MMIDSP) strongly urges its discontinuation from all laboratories. The greatest challenge to typhoid control lies with city municipalities, whose responsibility is to provide clean drinking water. Secondly, random and imprudent use of antibiotics among humans must be discouraged, and restriction

of antibiotics for animal use must be regulated in order to retain drug sensitivity against pathogens. Stringent individual infection control practices, along with extensive reach of conjugate typhoid vaccination will prevent the infection. Control of typhoid outbreak must be dealt with urgently.

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