Review article

Multidrug-resistant typhoid fever: a review

Syed Ahmed Zaki[1] and Sunil Karande[2]

[1]*Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, India*
[2]*Seth Gordhandas Sunderdas Medical College and King Edward VII Memorial Hospital, Parel, Mumbai, India*

Abstract

Introduction: Multidrug-resistant typhoid fever (MDRTF) is defined as typhoid fever caused by *Salmonella enterica* serovar Typhi strains (*S.* Typhi), which are resistant to the first-line recommended drugs for treatment such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole. Since the mid-1980s, MDRTF has caused outbreaks in several countries in the developing world, resulting in increased morbidity and mortality, especially in affected children below five years of age and those who are malnourished.

Methodology: Two methods were used to gather the information presented in this article. First PubMed was searched for English language references to published relevant articles. Secondly, chapters on typhoid fever in standard textbooks of paediatric infectious diseases and preventive and social medicine were reviewed.

Results: Although there are no pathognomonic clinical features of MDRTF at the onset of the illness, high fever (> 104°F), toxaemia, abdominal distension, abdominal tenderness, hepatomegaly and splenomegaly are often reported. The gold standard for the diagnosis of MDRTF is bacterial isolation of the organism in blood cultures. Ciprofloxacin and ceftriaxone are the drugs most commonly used for treatment of MDRTF and produce good clinical results.

Conclusion: MDRTF remains a major public health problem, particularly in developing countries. Mass immunization in endemic areas with either the oral live attenuated Typhi 21a or the injectable unconjugated Vi typhoid vaccine, rational use of antibiotics, improvement in public sanitation facilities, availability of clean drinking water, promotion of safe food handling practices and public health education are vital in the prevention of MDRTF.

Key words: multidrug resistant typhoid fever; *Salmonella enterica* serovar Typhi; fluoroquinolone treatment; typhoid vaccine; plasmid-mediated resistance; chromosomal-mediated resistance

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Typhoid fever is caused by *Salmonella enterica* serovar Typhi (*S*. Typhi), a Gram-negative bacterium [1-4]. It continues to be a global public health problem with over 21.6 million cases and at least 250,000 deaths occurring annually [5-7]. Almost 80% of the cases and deaths are in Asia; the rest occur mainly in Africa and Latin America [8]. In developing countries such as India, the disease occurs with an incidence ranging from 102 to 2,219 per 100,000 of the population [9]. Several studies in areas of endemicity and outbreaks have shown that about one-quarter to one-third of pediatric typhoid fever cases are under five years of age, and that between 6% and 21% are under two years of age [10]. Varied presentations of typhoid fever are known in the paediatric age group, such as septicemia in neonates, as diarrhoea in infants, and as lower respiratory tract infections in older children [11-13]. Atypical presentations in older children include splenic abscess, liver abscess, cerebellar ataxia, meningitis, cholecystitis, chorea, palatal palsy, osteomyelitis, peritonitis, aphasia and even psychosis [14-22]. Due to these varied and atypical presentations, it is common for typhoid fever in children to be diagnosed late or even remain unrecognised. Also, no vaccine against typhoid fever is available commercially for children under two years of age [18]. To complicate matters further, in the last two decades, multidrug-resistant (MDR) *S*. Typhi strains have emerged and spread worldwide, resulting in high rates of morbidity and mortality [18-21,24-27]. This article reviews the historical perspective, global prevalence, clinical features, complications, diagnosis, and therapeutic alternatives

available to treat multidrug-resistant typhoid fever in children.

Definition of multidrug-resistant typhoid fever

Multidrug-resistant typhoid fever (MDRTF) is defined as typhoid fever caused by *S*. Typhi strains which are resistant to all the three first-line recommended drugs for treatment, *i.e.*, chloramphenicol, ampicillin, and co-trimoxazole (TMP-SMX) [18,19,25,28,29,30].

Historical perspective of MDRTF in children

In 1948, when chloramphenicol was discovered, it was the most effective and commonly used drug for typhoid fever [31]. Within two years, due to its rampant and indiscriminate use, chloramphenicol-resistant *S*. Typhi isolates were reported from England [31]. However, it was not until 1972 that chloramphenicol-resistant *S*. Typhi strains became a major problem, with outbreaks being reported in Mexico (1972), India (1972), Vietnam (1973) and Korea (1977) [32-36]. These strains were also resistant to ampicillin. Co-trimoxazole remained an effective alternative drug in treating these resistant strains until 1975, when resistance to it was reported in France. By the late 1980s, strains of *S*. Typhi resistant to all three first-line drugs were in existence [20,37,38]. The epidemic of MDRTF in the late 1980s compelled pediatricians throughout the world to use ciprofloxacin, despite a lack of data regarding its safety for use in children [20,26,28,36,39]. Fortunately, follow-up studies done in children found it to be safe, effective, and less expensive with a very high sensitivity pattern [40,41]. Thus, fluoroquinolones became the drug of choice for the treatment of MDRTF worldwide [18-20,26,28,36,39]. However, this was soon followed by reports of isolates of *S*. Typhi showing resistance to fluoroquinolones, with the first case being reported in 1992 in the United Kingdom [37]. Subsequently, similar cases were reported from several other countries including India [19,28,42-44]. With the development of quinolone (nalidixic acid) resistance, third-generation cephalosporins were used for treatment, but sporadic reports of resistance to them also followed [24,28].

Global prevalence

The first multidrug-resistant strains emerged in Southeast Asia in the late 1980s and have since spread throughout the region [36]. Asian countries where MDRTF have been reported include China (1985), Pakistan (1987), India (1988), Malaysia (1991), Singapore (1994), Bangladesh (1994), Vietnam (1995), Japan (1999), Thailand (2001), Korea (2003), Nepal (2005) and Indonesia (2009); other countries include Kuwait (1996) and Jordan (2008) [1,36,38,45-53]. A recent multi-centric study conducted across five Asian countries (China, India, Indonesia, Pakistan, and Vietnam) that are endemic for typhoid reported the prevalence of multidrug-resistant *S*. Typhi strains ranging from 7% to 65% [54]. African countries that have reported MDRTF include South Africa (1992), Kenya (2000), Nigeria (2005) and Egypt (2006) [7,55-57]. Even developed countries such as the United Kingdom (1990), America (1997) and Italy (2000) have reported MDRTF; most of the cases were found among travellers who had returned from regions where MDR strains of *S*. Typhi had caused outbreaks or had become endemic [36,58,59]. Table 1 shows studies from different countries that have reported the presence of MDRTF [7,42,56,60-67]. Most of these studies are hospital based and may not reflect an accurate picture of prevalence in the community. Nevertheless, because these hospital-based statistics have documented a rapidly rising incidence of MDRST strains over successive years, it is reasonable to presume that they reflect the general trend prevailing in the community.

Experience in India

In India, MDRTF was first described in 1988 in Mumbai (formerly Bombay) and has since spread throughout the region in the last two decades [25,38,68-76]. In March 1990, an outbreak called *Dombivli fever* caused by resistant *S*. Typhi strains was reported in Mumbai [38]. A concomitant and steady increase in multidrug-resistant *S*. Typhi isolation was observed in Mumbai [38]. A similar outbreak of MDRTF was witnessed in New Delhi in the same year [76]. The *S*. Typhi strains in the above two outbreaks were sensitive to ciprofloxacin. A study in the mid-1990s in Bangalore showed resistance to ampicillin, chloramphenicol and co-trimoxazole to be as high as 95% [77]. In 1999, similar findings of high resistance to the above drugs were also reported from Manipal [78]. The strains remained uniformly sensitive to ciprofloxacin, norfloxacin and ceftriaxone. As ciprofloxacin had a high cure rate with no relapse or carrier state, it was considered to be the first choice for treatment of multidrug-resistant typhoid cases. However, over the next few years, studies from different parts of India

Table 1. Regions in the world reported to have multidrug-resistant typhoid fever

City/Country	Author	Study design	Year	Age group	Total cases of typhoid fever	MDR strains (%)
Asia						
Korea** [42]	Yoo *et al.* [42]	Retrospective study	1992-2000	All ages*	1530	21 (1.3%)
Ho Chi Minh, Vietnam	Wain *et al.* [60]	Prospective study	1993-1996	All ages*	369	313 (80%)
Karachi, Pakistan	Bhutta *et al.* [61]	Prospective study	1988-1993	< 14 years	1158	261 (23%)
Kuwait city, Kuwait	Dimitrov *et. al* [62]	Retrospective study	2002-2005	All ages*	101	43 (42.6)
Dhaka, Bangladesh	Naheed *et al.* [63]	Prospective study	2003-2004	All ages*	40	16 (40%)
Africa						
Cairo, Egypt	Srikantiah *et. al* [7]	Prospective study	2002	> 1 year	90	26 (29%)
Nairobi, Kenya	Mengo *et al.* [64]	Cross sectional study	2004-2006	All ages*	100	70 (70%)
Lagos, Nigeria	Akinyemi *et. al* [56]	Prospective study	2004	All ages*	41	25 (61%)
Kumasi, Ghana	Marks F *et al.* [65]	Prospective study	2007-2008	< 15 year	37	23 (63%)
Europe						
London, United Kingdom	Cooke *et al.* [66]	Retrospective study	2002-2003	All ages*	692	152 (22%)
North and Central America						
United States**	Lynch *et al.* [67]	Retrospective study	1999-2006	All ages*	2016	272 (13%)

*Proportion of pediatric cases not clearly mentioned in the studies
** Data derived from nationwide analysis of typhoid fever cases

documented an increasing resistance of *S.* Typhi strains to ciprofloxacin [68-70,79]. In 2000, a study done by Das *et al.* in Orissa found 2.5% of *S.* Typhi strains to be resistant to ciprofloxacin [68]. A prospective study done in Delhi by Kumar *et al.* at intervals of three years (1999, 2002 and 2005) found that the incidence of MDRTF sequentially increased from 34% in 1999 to 66% in 2005 [79]. Also, there was a gradual development of resistance to fluoroquinolones over these seven years. Later, several isolated reports of ceftriaxone-resistant *S.* Typhi strains were reported in different parts of India [8,79]. These observations of increasing resistance to ciprofloxacin and early evidence of resistance to ceftriaxone correspond with the global picture [19,24,28,42-44].

Table 2 highlights some of the regions in India which have reported MDRTF in the last fifteen years [25,68,69,70,71,73]. The results of these studies highlight the wide variation in incidence of MDRTF within the country. This could be due to various factors, including methodological differences in the studies, different geographical locations, lack of standardisation among the study population, and differences in standards of sanitation and hygiene.

Researchers' experience

We began encountering MDRTF in our institutes in the early 1990s. In 1991, of the 28 children with bacteriologically and/or serologically diagnosed typhoid fever treated at King Edward Memorial Hospital in Mumbai, only 18 (64.3%) responded to chloramphenicol or amoxicillin or co-trimoxazole. Ciprofloxacin was used in 19 (35.7%) cases with a 100% treatment success rate [39]. Blood culture grew *S.* Typhi in seven cases, of which five (72%) were

Table 2. Regions in India reported to have multidrug resistant typhoid fever (MDRTF) in the last fifteen years

Author	Duration of study	Year	Location	No. of typhoid cases	MDRTF (%)	Age group	Study population
Das *et al.* [68]	1 year	1997-1998	Orissa	715	115 (16.08)	All ages*	5410
Sen *et al.* [69]	2 year	2003-2005	Kolkata	195	28 (14.35)	All ages*	56,949
Kumar *et al.* [25]	2 year	2002-2004	Delhi	93	62 (66.6%)	< 12 years	Not available
Renuka *et al.* [73]	2 years	2000-2003	Delhi	472	183 (38.77)	All ages*	66555
Jog *et al.* [71]	2 years	2003-2005	Mumbai	73	5 (6.84)	All ages*	Not available
Madhulika *et al.* [70]	1 year	2002-2003	Pondicherry	157	61 (38.85)	All ages*	1296

*Proportion of pediatric cases not clearly mentioned in the studies

MDRTF. Since the mid-1990s, either ciprofloxacin or ceftriaxone has been used as the first-line drug for typhoid fever as it has been made available free of cost to the inpatients. The response to either ciprofloxacin or ceftriaxone, until today, has been excellent. We have also not observed any trend toward increasing fever clearance times with ceftriaxone. It is difficult to comment on the current prevalence of MDRTF in our inpatients. The reason for this is that most children who get admitted with a diagnosis of typhoid fever have already received oral antibiotics from their local general practitioner. The children are usually diagnosed as having typhoid fever based on a positive Widal test. We collect 50 and100 blood cultures each month from suspected typhoid cases of which less than 1% are positive for *S*. Typhi; all isolates have become sensitive to all five drugs (chloramphenicol, ampicillin, co-trimoxazole, ciprofloxacin and ceftriaxone) in the last five years.

Mechanism of drug resistance in S. Typhi

There are two main mechanisms of drug resistance development in *S*. Typhi. The first is a plasmid-mediated mechanism; the second is a chromosomal DNA-mediated mechanism [1,3,17-21,23,42,50,80,81]. Plasmids are extra-chromosomal, self-replicating circular pieces of DNA which can carry and transfer multiple resistance genes between bacteria [82]. Plasmids of incompatibility group (Inc) HI1 are important vectors of antibiotic resistance in *S*. Typhi. The first reported *S.* Typhi harboring an IncHI1 plasmid was isolated during a large outbreak of resistant typhoid fever in Mexico City in 1972 and was found to be resistant to chloramphenicol, tetracycline, streptomycin, and sulphonamides. Subsequently, MDR *S.* Typhi spread globally and, by 1998, IncHI1 plasmids could be isolated from MDR *S.* Typhi worldwide [23]. The chromosomal-mediated drug resistance phenomenon against fluoroquinolones has been reported recently as a result of selective pressure on the bacterial population due to their uncontrolled use. This has been attributed to a single point mutation in the quinolone resistance determining region (QRDR) of the topoisomerase gene *gyrA*, which encodes DNA gyrase [19,73,80,83]. However, other mechanisms such as decreased permeability and active efflux of the antimicrobial agent may also be involved [83].

Risk factors for development of drug-resistance in S. Typhi

Risk factors for the development of resistance in *S.* Typhi include overuse, misuse, and inappropriate antibiotic prescribing practices [20,84-86]. Factors such as patient and time pressures and diagnostic uncertainties are some of the main forces behind irrational prescription of antimicrobial combinations [87,88]. Easy availability of drugs at the pharmacy without a prescription, use of allopathic drugs by traditional medicine practitioners such as homeopaths, unani and ayurvedic practitioners, and uncontrolled use of antibiotics in agriculture, animal husbandry and fisheries has further aggravated the problem. Moreover, in some countries such as India, local production of many different antimicrobial drugs with questionable quality and potency control, coupled with poor compliance of patients to costly antimicrobials adds to the threat of antimicrobial

Table 3. Comparison of clinical features of infection with multidrug-resistant strains and sensitive strains of *S.*Typhi

Clinical feature	Sensitive strains	Multidrug-resistant strains	*p Value*
Fever > 104ºF (40ºC)	45%	88%	< 0.01
Toxemia	29%	44%	< 0.01
Abdominal tenderness	26%	35%	< 0.01
Abdominal distension	40%	90%	< 0.01
Hepatomegaly	38%	51%	< 0.01
Splenomegaly	48.8%	72.4%	< 0.01

Analysis of significance of clinical features done by chi square test)

Table 4. World Health Organisation guidelines for the treatment of multidrug-resistant typhoid fever in children

Susceptibility	Optimal therapy			Alternative effective drugs		
Uncomplicated disease	Antibiotic	Dose mg/kg	Days	Antibiotic	Dose mg/kg	Days
Quinolone sensitive MDR strains	Fluoroquinolone OR Cefixime	15 15-20	5-7 7-14	Azithromycin Cefixime	8-10 15-20	7 7-14
Quinolone resistant MDR strains	Azithromycin OR Ceftriaxone	8-10 50-75	7 10-14	Cefixime	15-20	7-14
Severe disease						
Quinolone sensitive MDR strains	Fluoroquinolone	15	10-14	Ceftriaxone Cefotaxime	50-75 40-80	10-14
Quinolone resistant MDR strains	Ceftriaxone or cefotaxime	50-75 40-80	10-14	Fluoroquinolone	20-30	14

Table 5. Mean defervescence time for different drugs used in the treatment of multidrug-resistant typhoid fever

Drug	No. of trials	Total patients	Children (%)	MDR strains in total patients (%)	Mean defervescence time in days for total patients
Ceftriaxone	13	393	60 (15.26)	41 (10.43)	6.1
Cefixime	4	160	100 (62.5)	90 (56.25)	6.9
Ciprofloxacin/ Ofloxacin	17	1049	25 (2.38)	56 (5.33)	3.9
Azithromycin	4	156	21 (13.46)	32 (20.51)	4.4
Aztreonam	4	101	63 (62.37)	31 (30.69)	5.8

(Modified from Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ (2202) Typhoid fever. N Engl J Med 347: 1770-1782)

resistance [87,88]. Antibiotics are unnecessarily prescribed for infections such as the common cold, cough and diarrhoea, which are usually of viral etiology and can be resolved by the immune system. Emphasis is placed on treatment instead of finding the causative organism and reaching a proper diagnosis. This leads to patients being treated with broad spectrum antibiotics, which results in the emergence of MDR organisms [84].

Defervescence of fever in typhoid usually begins after four or five days of starting treatment [89]. However, due to parental and time pressure or lack of knowledge, physicians change different antibiotics unnecessarily during the treatment of typhoid fever, which leads to the emergence of resistant strains. The involvement of host genetic factors is also implicated as a risk factor; for example, HLA-DRB1*12 is known to be protective against severe typhoid fever [90]. Also, it has been postulated that genetic variation within Toll-like receptor 4 (TLR4) may affect recognition of *S.* Typhi lipopolysaccharide [91]. This can alter the activation of innate immunity,

Table 6. Licensed vaccines against typhoid fever

Vaccine type	Live attenuated (Typhi 21 a)	Unconjugated Vi
Target age group	> 5 years	> 2 years
Number of doses	Multiple doses [1 capsule every other day , total of 3 capsules] Revaccination every 5 years	Single dose (0.5 ml) Revaccination every 2 years
Route of delivery	Oral, enteric coated capsules	Parenteral (S.C/I.M)
Efficacy	50-80%	64-72%
Duration of protection	At least 5 years	At least 3 years
Protective immunity	Cell mediated immunity, Antibody (non-Vi)	Anti-Vi antibodies

thereby detrimentally affecting the first line of defence against this pathogen.

Clinical features of MDRTF

There are no pathognomonic clinical features at the onset of the illness that can differentiate typhoid fever due to multidrug-resistant *S.* Typhi strains from those caused by sensitive *S.* Typhi strains [26,36,39,85.86]. However, some studies have reported certain clinical features to be more commonly associated with MDRTF (Table 3) [61,74,75]. It has been observed in some studies that, as compared with the children infected by sensitive *S.* Typhi strains, children with MDRTF are sicker and more toxic at admission [19,20,25,26,36,92]. Maximum cases of MDRTF are seen in children under five years of age [74,76]. This could be due to the association of typhoid fever outbreaks with malnutrition, commonly seen in children under the age of five in developing countries. The presence of malnutrition enhances susceptibility to typhoid infection by alterations in the intestinal flora or other host defenses [20]. Also, frequent diarrhoeal disease in children of this age with indiscriminate use of antibiotics provides an ideal milieu for the emergence of MDR strains of *S.* Typhi [74]. Other risk factors for young children may be their unhygienic habits and their dependence for food on adults, who may be the carriers of MDR strains [20]. No sex difference or seasonal variation has been noted for typhoid fever due to sensitive strains and MDR strains [74,76]. However, the presence of fever >104^{o}F, toxaemia, hepatomegaly, splenomegaly, abdominal tenderness and abdominal distension are seen in a significantly higher proportion of cases with MDRTF as compared to typhoid fever due to sensitive strains (Table 3) [61,74,75].

Complications and mortality due to MDRTF in children

The overall mortality reported during MDRTF epidemics is 7% to 16%, and is much higher than the figure of 2% seen in susceptible typhoid fever [20]. The increased incidence of complications and mortality in MDRTF is high due to delay in instituting effective antibiotic therapy; b) higher virulence of bacteria as a consequence of genes present on R-plasmid; c) much greater bacterial load in tissues due to resistance to conventional agents; and d) higher number of circulating bacteria [19,20,60,74,86,92,]. Gastrointestinal complications such as bleeding, intestinal perforation, paralytic ileus, hepatitis, cholecystitis and peritonitis have been described [25,75,76,94-96]. Respiratory system complications include bronchopneumonia and pleural effusion [25,75,76,95]. Central nervous system complications described are encephalopathy, meningitis, chorea, intracranial hemorrhage, cerebellar ataxia and seizures [25,75,76,95,97]. Renal complications include hypernatremia, hypokalemia, acute renal failure and glomerulonephritis [94,97]. Cardiovascular complications include myocarditis and peripheral circulatory failure [95,97]. Haematological complications include disseminated intravascular coagulation and bone marrow suppression [97]. Other complications described are parotitis, arthritis, subcutaneous abscess, subphrenic abscess and cutaneous ulcers [25]. As a result of these complications, MDRTF can sometimes mimic common endemic diseases such as malaria (chills and

rigors), viral hepatitis (poor appetite with jaundice), bronchopneumonia or meningitis. Hence a high degree of suspicion is required, as proper therapy instituted early in the course of the disease is of greatly beneficial [19,20,92].

Diagnosis of MDRTF

Due to the lack of specific clinical signs and possible presence of co-infections, the initial examination of a child suspected to have MDRTF should include a screen for the common endemic diseases which present as an acute febrile illness in that region, such as malaria, dengue fever, leptospirosis and acute viral hepatitis [22]. The investigations should include hemoglobin level, packed cell volume, complete blood counts with differential leukocyte count, peripheral smear for malarial parasites, liver transaminases, urine culture and blood culture. The gold standard for the diagnosis of MDRTF is a culture isolation of the organism with susceptibility testing of the isolates [3,4,10,18,19,80,92,98]. Blood cultures are the primary diagnostic method for MDRTF [3,4,10,80,92,98]. During the first week of illness, approximately 90% of patients have a positive blood culture, which decreases to 75% in the second week, 60% in the third week, and 25 % in the fourth and subsequent weeks until the subsidence of pyrexia [3,18,60]. The World Health Organization (WHO) recommends that between 10mL and15 mL of blood be taken from school children and 2mL to 4 mL from toddlers and preschool children to achieve an optimal isolation rate [28]. Blood should be drawn by means of a sterile technique of venous puncture and should be inoculated immediately into a culture bottle containing 0.5% bile broth with the syringe that has been used for collection. Dilution should be appropriate in order to adequately neutralize the bactericidal effect of serum and a ratio of at least 1:10 of blood to broth is recommended [28,98]. The culture bottle should then be transported to the main laboratory at an ambient temperature of between 15°C and 40°C. Blood culture bottles should not be stored or transported at low temperatures. If the ambient temperature is below 15°C, it is advisable to transport the culture bottle in an incubator [28]. Blood culture takes a minimum of 72 hours for a positive culture report [3,99]. If *S.* Typhi is not obtained from the first subculture, then it should be repeated every other day until growth is obtained. Cultures should be declared negative only after incubation for between 10 and 11 days [3,99]. The susceptibility tests should be performed against the following antibiotics: 1) first-line antimicrobials (chloramphenicol, ampicillin, trimethoprim/ sulfamethoxazole); 2) a fluoroquinolone; 3) nalidixic acid (for determining reduced susceptibility to fluoroquinolones); 4) a third-generation cephalosporin, and 5) any other drug currently used for treatment [28]. The appropriate break point recommendations for azithromycin against *S.* Typhi are still not clear and patients may respond satisfactorily to azithromycin even if isolates are not fully sensitive. Therefore, routine susceptibility testing against azithromycin is not recommended [28]. Clot cultures have not been found to be of superior sensitivity as compared to blood cultures in several clinical studies and hence are not advocated [98]. A failure to isolate the organism from the blood may be caused by several factors: (i) inadequate laboratory media; (ii) prior use of antibiotics; (iii) inadequate volume of the blood; (iv) the time of blood collection; and (v) incubation conditions [3,10,19,80,92,98].

Bone marrow aspirate culture is particularly valuable for patients who have been previously treated, who have a long history of illness, and for whom there has been a negative blood culture with the recommended volume of blood [10,80,98]. The overall sensitivity of bone marrow cultures ranges from 80% to 95%, and is good even in late disease and despite prior antibiotic therapy [18,80,98]. The enhanced isolation of *S.* Typhi from bone marrow can be explained by the larger number of bacteria found in the bone marrow, an amount ten times greater in volume in bone marrow than in blood,. This bacteria may be protected from the presence of systemic antibiotics [100,101]. However, if enough blood is cultured, it is possible to increase the sensitivity of blood culture to that of bone marrow culture. Thus the need for bone marrow aspiration, an invasive, difficult, and extremely painful procedure can be avoided [19,92,98,100,101]. If blood culture bottles or media are not available, direct plating of the buffy coat from 5mL to 10 mL of blood will allow the growth of isolated colonies within 24 hours of specimen collection [101]. Buffy coat is collected by centrifuging 5 mL of heparinised blood at 4,000 rpm for five minutes. Then, 0.1 mL of buffy coat can be inoculated into 10 mL of Oxgall or spread onto the surface of a Columbia agar plate containing 0.05% saponin [101]. Plasma from the buffy coat specimens could be used for biochemical and serological tests and for seroprevelance studies. The sensitivity of

stool cultures is between 30% to35% and urine culture is 7% to 10% [18,80,98]. These are usually positive after the first week of illness and hence they are of limited use in early phase of the illness [19,92,98]. The volume of stool cultured from each sample should be at least two grams, with higher culture volumes increasing the yields [101]. In addition to diagnosis, stool cultures are also important for monitoring for the carriage of *S*. Typhi after an apparent clinical cure, which is a risk factor for the families of the patients [100]. The commonly performed Widal test is positive after the first week of illness [3,4,10,98]. Although the test is inexpensive and easy to perform, it has suboptimal sensitivity and specificity with negative results in up to 30% of culture proven typhoid cases [3,4,10,18,19,80,92,98]. Moreover, it does not provide any information regarding antibiotic susceptibilities and hence is not useful in the diagnosis of MDRTF.

Newer and more rapid diagnostic modalities include the identification of a specific nucleic acid sequence of *S*. Typhi. The first evaluation of polymerase chain reaction (PCR) as a diagnostic tool for typhoid fever was conducted in 1993 by Song *et al.*, who successfully amplified the flagellin gene (*fliC-d*) of *S.* Typhi in all cases of culture proven typhoid fever [102]; since then, several studies have used this evaluation. These studies reported excellent sensitivity and specificity when compared to positive and healthy controls [103,104,105]. Other investigations have described multiplex PCR assays as a method for detecting clinically relevant antibiotic resistance genes frequently encountered with *S*. Typhi. Also with the advent of nested PCR, it is possible to amplify and detect specific gene sequences of *S*. Typhi within a few hours [104,106]. It has a sensitivity and specificity of 100% and may replace blood culture as the new gold standard. However, the cost and requirement of sophisticated instruments for performing these molecular tests is a major drawback in developing countries [98]. Other investigations such as complete blood count, erythrocyte sedimentation rate and liver function tests are non-specific and non-pathognomonic of MDRTF [18,19,92,98]. Serologic tests do not give any information regarding antibiotic susceptibility and hence are not useful in the diagnosis of MDRTF [28,98].

When to suspect MDRTF

MDRTF infection should be suspected in the following situations [20, 26,107]:

a) Failure to respond (*i.e.*, no improvement in general condition, loss of appetite, no defervescence of fever, or no reduction in toxic look) after five to seven days of treatment with a first-line antibiotic (chloramphenicol or ampicillin or trimethoprim/ sulfamethoxazole) for typhoid fever

b) Severe typhoid fever with shock or abnormal sensorium or other potentially life-threatening complications such as intestinal haemorrhage and/or perforation, disseminated intravascular coagulation, or myocarditis

c) Clinical deterioration or development of a complication during conventional antibiotic treatment

d) Household contact with a documented case or during an epidemic of MDRTF

Treatment of MDRTF

Children with MDRTF are more toxic at admission with a significantly higher incidence of life-threatening complications than those with susceptible *S.* Typhi strains [19,20,25,26,36,92].

Supportive treatment

A high grade fever and extreme anorexia aggravate the severe symptoms. Hence supportive measures such as the use of antipyretics, maintenance of adequate hydration, and nutrition play an important role in alleviating symptoms [19,28,89,92,101].

Antibiotic therapy

Some important criteria for the selection of antibiotics in the treatment of MDRTF in developing countries are cost, susceptibility patterns and the availability and prevalence of antimicrobial resistance [28]. Per WHO guidelines, either fluoroquinolones or third-generation cephalosporins can be used in MDRTF, depending upon the sensitivity of *S*. Typhi strains to quinolones (Table 4) [28]. In quinolone-sensitive MDR strains, fluoroquinolones are recommended for treatment as they have many advantages over third-generation cephalosporins. Some of the advantages are lesser cost, availability of an oral preparation, shorter duration of therapy, and cure rates closer to 100% [10,20,26,28,89]. The fluoroquinolones commonly used in children for MDRTF are ofloxacin and ciprofloxacin (15 mg/kg/day) in two doses. Both are highly active and equivalent in efficacy. Norfloxacin

is not used due to poor bioavailability [28,89]. Gatifloxacin is a new fluoroquinolone which behaves slightly differently from other members of the group [101]. It binds at a slightly different site; thus treatment failures due to resistance developed by point mutations of the older binding site can still be averted. However, in cases of quinolone-resistant *S.* Typhi strains, third-generation cephalosporins are recommended as the first-line treatment [28]. Of the oral third-generation cephalosporins, cefixime (15-20 mg/kg/day) and cefpodoxime (10 mg/kg/day) are commonly used oral preparations. Of parenteral preparations, ceftriaxone (50-75 mg/kg/day) in one or two doses, cefotaxime (40-80 mg/kg/day) in two or three doses, and cefoperazone (50-100 mg/kg/day) in two doses are used [28]. Additional treatment with dexamethasone under strict supervision (3mg/kg for the initial dose followed by 1mg/kg every six hours for 48 hours) has been recommended for severely ill patients with shock, obtundation, stupor or coma [18,19,28,80,89,92]. This therapy lowers mortality from between 35% and55% to 10%. Dimitrov *et al.* found that patients with MDRTF had a longer duration of fever defervescence (8 ± 5 days) as compared to those with drug-susceptible typhoid fever (5.7 ±4 days) [62]. Table 5 shows the mean defervescence time after starting treatment with different antibiotics used in MDRTF. The data was compiled from a review of 57 randomized, controlled trials performed in adults and children with typhoid fever between 1964 and 2000. The research was conducted by using Medline (http://www.nlm.nih.gov/databases/databases_medline.html) and by searching the reference lists of articles about typhoid fever [80].

Recently, several studies have found that strains previously resistant to the first-line drugs (chloramphenicol, ampicillin and co-trimoxazole) are now showing decreasing resistance [28,109,109-111]. The withdrawal of selective pressure has probably resulted in the re-emergence of sensitivity to these first-line drugs [109]. A study done by Gupta *et al.* in 2007 in Punjab (India) found a very high sensitivity of 93.2%, 86.2% and 71.3% with chloramphenicol, cotrimoxazole and ampicillin, respectively [109]. Similarly, a re-emergence of chloramphenicol sensitivity was reported in 2008 by Prajapati *et al.* from Nepal [111]. Recent studies from Egypt have observed the re-emergence of chloramphenicol and ampicillin susceptibility and therefore suggest the reintegration of these drugs for the treatment of typhoid fever in Egypt [112]. Their cheaper cost and availability in developing countries, in addition to their well-established clinical efficiency, are among the benefits of re-using chloramphenicol or ampicillin [108]. Thus constant surveillance and vigorous audits of antibiotic sensitivity testing results are needed to determine whether the reintegration of these first-line drugs can be applied to a particular region.

Is there a need for vaccination after an attack of MDRTF?

There are no systematic studies which have measured the frequency of relapses and recurrences of typhoid fever in a community. However, an attack of typhoid fever does not provide long-lasting immunity from a future episode of the same illness. An episode of typhoid fever usually means that the child lives in an environment in which further exposure to infection is likely. There is also the possibility that early treatment could reduce the full force of immunity from developing; trials have shown that treatment of typhoid patients in the first two weeks of illness may inhibit the development of the protective anti-Vi CPS antibody response [113]. Taking into consideration the above points, the general recommendation is to administer the typhoid vaccine at least four weeks after full recovery from the illness [114,115]. As shown in table 6, two safe and effective vaccines are licensed and available for use in children: the live, attenuated oral vaccine, Ty21a, and the injectable subunit Vi polysaccharide vaccine [4,10,18,19,28,80,92,116]. Protection begins seven days after receiving Vi polysaccharide vaccine with maximum protection attained after 28 days [28]. In field trials conducted in Nepal and South Africa where the disease is endemic, the protective efficacy observed three years after a single dose was 55% [28]. In efficacy trials conducted in China using a locally produced Vi vaccine, 72% protection was obtained [28]. Household contacts should also be immunized with typhoid vaccine [114]. Since MDRTF is being reported in infants and toddlers, an effective and safe vaccination against *S.* typhi in children under two years of age is needed. A new modified Vi typhoid vaccine conjugated to a non-toxic recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), which can be used in children under two years of age, has been evaluated in Vietnam, but is not yet commercially available [18,80]. Also, the Novartis Vaccines Institute for Global Health (Sienna, Italy) is developing a bivalent conjugate vaccine by independent chemical conjugation of the

Vi polysaccharide of *S.* Typhi and the O polysaccharide of *S.* Paratyphi A to the carrier protein CRM197. This vaccine, which is envisaged to be effective and safe against both *S.* Typhi and *S.* Paratyphi A in children under two years of age, is still undergoing clinical trials [116].

WHO recommends that immunization of school-age children be undertaken wherever the control of the disease is a priority [28]. Such practices in the past have shown to be successful in controlling typhoid fever. Also, routine immunization conducted in several areas of Uzbekistan has resulted in a low incidence of the disease [28]. IAP has recommended that the government of India actively consider including the typhoid vaccine in the national immunization schedule. Several studies in typhoid-endemic countries in Asia have yielded data on the effectiveness of the Vi vaccine, the feasibility, acceptability and costs of large-scale community- or school-based vaccination and the population's demand for new-generation typhoid vaccines [117]. Demonstration projects using Vi vaccine and Ty21a have shown large-scale community- and school-based vaccination to be feasible and well accepted by populations in endemic countries. Evidence from China suggests that the programmatic use of Vi vaccine in selected areas largely controlled the disease within a four- to five-year period, reducing incidence to very low levels [117]. A meeting of experts was organized by the Novartis Vaccines Institute for Global Health in New Delhi in 2009 to discuss enteric fever in South Asia and the potential of new conjugate vaccines [116]. Considering the high prevalence of typhoid fever in Southeast Asia, the meeting emphasised on the necessity of the rapid introduction of typhoid vaccines into the national immunization programmes of typhoid-endemic regions.

Management of carriers of MDR S. *Typhi strains*

Patients infected with MDR *S.* Typhi strains are more likely to excrete the organisms in the stool; these patients serve as a source of infection to those they come into contact with [60]. There are three types of carriers: 1) the convalescent carrier, who continues to shed the bacilli in faeces for three weeks to three months; 2) the temporary carrier, who sheds the bacilli for over three months and under one year; and 3) chronic carrier, who sheds the bacilli for more than one year [3,4,28]. The risk for becoming a chronic carrier is under two percent in children [19,92]. The bacilli persist in the gall bladder or kidney and are eliminated in the faeces or urine [3]. Urinary carriers are less common and are often associated with some abnormality of the urinary tract [3]. Follow-up examination of the stools (and urine when indicated) should be done for *S.* Typhi between three and four months after the patient's discharge and again after 12 months for detection of carriers [4]. The Vi antibodies are present in 80% of carriers and are a good screening test [4]. Ciprofloxacin (15mg/kg/day in two divided doses) for four to six weeks can be used for the treatment of chronic carriers of quinolone-sensitive multiresistant strains [18]. There are no guidelines for the treatment of carriers with quinolone-resistant *S.* Typhi strains. Possibly, cefixime (15mg/kg/day) or azithromycin (8-10 mg/kg/day) are safe and effective drugs which can be considered for the treatment. Chronic carriers who cannot be decolonized are treated with cholecystectomy if cholelithiasis or cholecystitis is present [18].

Conclusion and future research strategies

Outbreaks of MDRTF require costly and widely unavailable drugs for effective treatment. This is an added burden to the health-care sector in developing countries. Emphasis must therefore be placed on disease prevention, which can include both short- and long-term measures that must be followed strictly. Short-term measures include vaccination of the high-risk population in endemic areas and rational and judicious antibiotic prescribing practices by health professionals [4,10,18,19,80,92]. It is necessary to control the sale of antibiotics without prescriptions. Also, antibiotics should be used more judiciously in outpatient and inpatient departments in hospitals and by private practitioners. Undergraduate medical students should be taught in depth the rational use of antibiotics. Regulation of antimicrobials for use other than in humans is also required. These issues require the collective action of governments, the pharmaceutical industry, health-care providers and consumers. A typhoid vaccination programme in schoolchildren along with the school-based administration of Td (typhoid and diphtheria) or, with the advent of new conjugate vaccine Vi vaccine, as part of the expanded program of immunization, should be considered. The impact of drug resistance may improve the cost effectiveness of mass vaccination programs in typhoid-endemic countries such as India. Other important preventive measures include improvements in sanitation, availability of clean drinking water, promotion of

safe food handling practices, and public health education [4,10,18,19,80,92]. Isolation and characterization of MDRST from all regions of the world for effective epidemiologic surveillance and control should continue with intensive scrutiny of *S.* Typhi strains from developing countries. Follow-up studies on children with MDRTF are essential to learn the rates of relapse and carrier state. In view of the re-emergence of sensitivity to first-line drugs, large-scale systematic studies are required to determine whether these drugs can again be used for the treatment of typhoid fever in developing countries.

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Corresponding author

Syed Ahmed Zaki
Assistant Professor, Department of Pediatrics
Room 509, new RMO quarters
Lokmanya Tilak Municipal Medical College and General Hospital
Sion, Mumbai 400022, India
Phone: 09321594488
Email: drzakisyed@gmail.com

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