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Original Research Article

Multi-drug Resistant *Salmonella enterica* Subspecies *enterica* Serotype Typhi: A Diagnostic and Therapeutic Challenge

Anees Akhtar[1*], Indu Shukla[2], Fatima Khan[2] and Anjum parwez[3]

[1]Department of Microbiology, FH Medical College, Tundla, Agra, India
[2]Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, India
[3]Department of Medicine, Jawaharlal Nehru Medical College, AMU, Aligarh, India
**Corresponding author*

A B S T R A C T

Salmonella enterica serovar Typhi, the human-specific, causative agent of typhoid fever, causes an estimated 21 million new cases and 216,000 deaths every year. Accurate diagnosis to differentiate typhoid fever from other conditions is often difficult, both in the clinic and in the laboratory, but is imperative for effective treatment selection. Isolation of the causative organism remains the most effective diagnostic method in suspected typhoid fever. PCR-based tests for detecting the causative pathogens of enteric fever have developed rapidly over the last decade. In addition to diagnostic dilemma, the emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol and co-trimoxazole, has further complicated the treatment and management of enteric fever. The present study was conducted to evaluate the different diagnostic methods like Blood culture, immunocheck antibody test and Polymerase chain reaction (PCR) and drug resistant pattern. Typhoid fever is one of the most common infectious diseases in developing countries including India. Out of 104 patients 12 (11.5%) were blood culture positive. All the culture positive and three other cases 15 (14.4%) were reactive by immune-check typhoid antibody test. *dH flagellin* gene was detected in 27(25.9%) cases. *dH Flagellin* gene amplification in blood detected the highest number of cases. On antimicrobial susceptibility testing all the 12 isolates was consistently susceptible to azithromycin, ceftriaxone and cefoperazone sulbactum. Enteric fever is a diagnostic challenge for the clinicians as well the microbiologists. Azithromycin, is a good treatment option for *S typhi* infections. Judicious use of antimicrobials should be done to prevent the emergence of drug resistance.

Keywords

Multi-drug resistant *Salmonella typhi,* polymerase chain reaction (PCR), dh flagellin gene

Introduction

Salmonella enterica serovar Typhi, the human-specific, causative agent of typhoid fever, causes an estimated 21 million new cases and 216,000 deaths every year (Crump *et al.,* 2004). The accurate and rapid clinical diagnosis of enteric fever regions is obfuscated by the range of other common fever-causing infections including malaria, dengue fever, leptospirosis, melioidosis and the rickettsioses. Accurate diagnosis to differentiate typhoid fever from these conditions is often difficult, both in the clinic and in the laboratory, but is imperative for effective treatment selection. Even in highly-resourced western countries, physicians often start typhoid treatment empirically whilst awaiting confirmation of the diagnosis. Serological tests, predominantly the Widal test, are available but have very low sensitivity and specificity, and no practical value in endemic areas despite their continued use (Levine *et al.,*

1978). Isolation of the causative organism remains the most effective diagnostic method in suspected typhoid fever and blood has been the main sample for culture for *Salmonella* serovar Typhi since 1900 (Wain and Hosuglu 2008). The sensitivity of blood culture is highest in the first week of the illness and reduces with advancing illness (Kundu *et al.,* 2006). PCR-based tests for detecting the causative pathogens of enteric fever have developed rapidly over the last decade; however questions regarding the clinical utility and standardization of tests remain. Detection of bacterial DNA in whole blood by PCR assay is able to substantially decrease the turnaround time without bias from the inhibitory effect of antibiotics, yet the published PCR assays for diagnosis of enteric fever are in limited use.

In addition to diagnostic dilemma, the emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol and co-trimoxazole, has further complicated the treatment and management of enteric fever (Jesudason and John, 1992; Mourad *et al.* 1993). In India, antibiotic resistance among *S. typhi* has been reported since 1960, and the first outbreak of multidrug resistant *S. typhi* (MDRST) was reported in Calicut (Agarwal, 1962). The period 1990 to the present has been a hallmark era in the history of enteric fever because of the emergence and dissemination of *S. typhi* strains carrying resistance to multiple clinically relevant antibiotics (Arora *et al.,* 2010; Saha *et al.,* 1992). Most developing countries have experienced outbreaks of multidrug-resistant enteric fever due to *S. typhi*. The incidence of multidrug resistant (MDR) *S. typhi* has been reported to be as high as 60% but then declined in Pune (1999), Nagpur (2001), Delhi (2004) and Calcutta (2000) (Sanghavi *et al.,* 1999; Chande *et al.,* 2002; Saha *et al.,* 2002; Walia *et al.,* 2005). Fluoroquinolones were very effective in early 1990s, but emergence of resistance to these drugs occurred soon (Malla *et al.,* 2005). An increase in the number of MDR and nalidixic acid resistant *S. typhi* globally (NARST) was noted, although all isolates remained sensitive to ciprofloxacin and ceftriaxone (Ackers *et al.,* 2000). Also sporadic reports of high level of resistance to Ceftriaxone in *S. typhi* and *S. paratyphi* have been seen and the relapse rate is 3–6% with this drug (Parry *et al.,* 2002; Jog *et al.,* 2008). Given the variation in the susceptibility patterns reported for *S. typhi*, it is important to constantly monitor it so as to provide suitable guidelines for treatment.

Therefore, we studied the sensitivity and specificity of blood culture for the diagnosis of enteric fever and also sensitivity pattern of *S. typhi* to chloramphenicol, ampicillin and co-trimoxazole, flouroquinolones, cephalosporins, azithromycin and some other drugs which have been frequently used in the treatment of enteric fever since last 5 years.

Materials and Methods

The present study was conducted in the department of Microbiology, J.N Medical College, AMU over a period of two and a half years from September 2011 to February 2014. The cases included patients attending the paediatric and medicine OPD or IPD with clinical features suggestive of enteric fever like fever with abdominal discomfort, headache, anorexia, nausea, and vomiting, abdominal discomfort with diarrhoea, soft enlarged spleen, coated tongue, toxic look and relative bradycardia. Patients with any other obvious focus of fever like urinary tract infection, otitis media etc and patients with prior antibiotic therapy were excluded from the study.

Blood culture and antimicrobial susceptibility testing: Blood for culture was collected taking all sterile precautions by veni-puncture. A total of 5 ml of peripheral blood from adults and 2 ml from paediatric patients was collected and transferred to brain heart infusion broth. Subcultures were done after 24 hours, 48 hours and 7 days on 5% sheep blood agar and Teepol lactose agar. All isolates were identified by standard biochemical procedures and serotyping was done as per Kauffnamm white scheme. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standards Institute guidelines (CLSI, 2014) for the following cefepime (30µ), ceftriaxone (30µ), cefotaxime (30µ), cefexime (30µ), cefoperazone-sulbactum (75/10µ), levofloxacin (5µ), amikacin (30µ), azithromycin (15µ), ciprofloxacin (30µ), chloramphenicol (30µ), ampicillin (30µ), nalidixic acid (30µ).

Immunocheck typhoid antibody test: Immunocheck Typhoid IgG/IgM rapid test was done for all the serum samples as per the manufacturer's instructions.

Polymerase chain reaction for detection of *S. typhi* from blood sample: DNA extraction was performed by using QIAamp DNA investigator kit (50). PCR mixture consisted of 50 µl master mix containing 1 µl sample, 10X buffer, primer mixture, dNTPs, and taq polymerase. The primers were designed based on published *dH flagellin* gene sequence using oligo computer program. A 486 base pair region was amplified using the following primers

RK1 (5' TGG GCG ACG ATT TCT ATG CC 3')

RK2 (5' TTT CGC GAA CCT GGT TAG CC 3')

Cycling parameters of PCR were set as follows: hot start 94 °C for 4 min followed by 30 cycles of melting at 94 °C for 45 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min. Analysis of amplified products was done by gel electrophoresis. Amplicons of 486 bp were consistent with *dH* flagellin gene amplification.

Result and Discussion

Typhoid fever is one of the most common infectious diseases in developing countries including India. The disease is present more commonly in areas where healthcare facilities are limited with high rate of illiteracy and unhygienic living conditions are prevalent. In the present study a total of 104 cases were clinically suspected to have enteric fever. Fifty-five (52.9%) of these were males and 49 (47.1%) were females. Butler *et al.* (1991) also reported similar pattern of sex distribution in cases suspected of enteric fever, which could be due to greater exposure of males contaminated food and water outside the home. The clinical presentation of typhoid fever is notoriously variable, ranging from non-specific fever symptoms to fulminant Gram-negative sepsis with multisystem disease. The clinical diagnosis mainly relies on step ladder fever with rashes which are difficult to appreciate on the Indian skin, headache, alternate constipation and diarrhoea, abdominal pain, coated tongue. In our study fever was consistently present in all the patients, with headache being present in majority of them (88.5%). Vomiting, coated tongue, diarrhoea and constipation were present in 16.3%, 23.1%, 5.8% and 7.7% respectively (Graph 1).

Out of these 104 patients suspected of enteric fever clinically, only 12 (11.5%) were blood culture positive. Low concentrations of *S. typhi* in the blood of

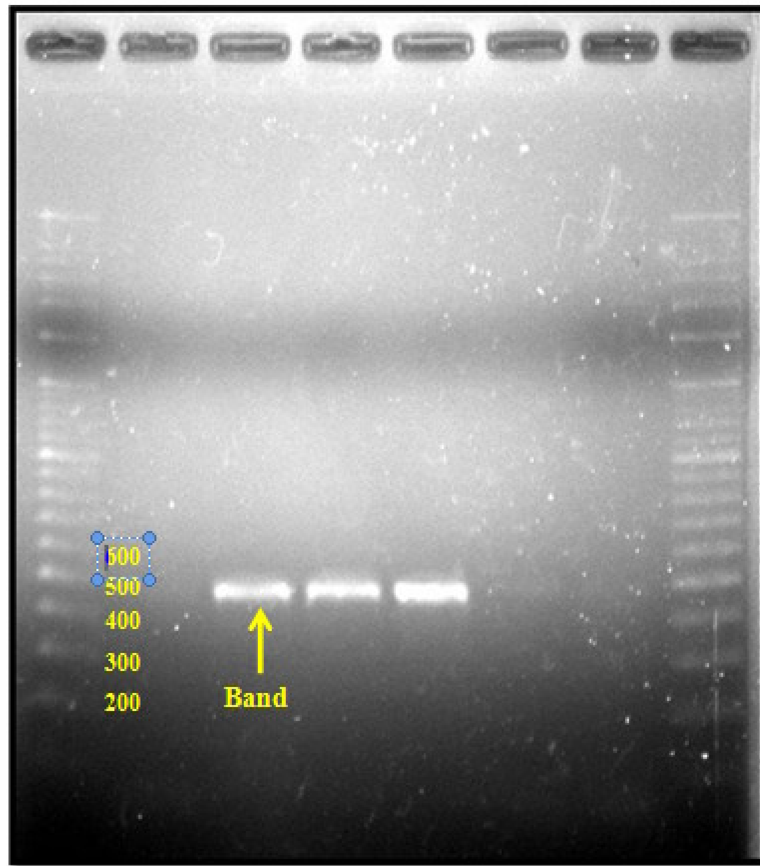
patients with typhoid fever (< 15 bacteria/ml) contribute to the moderate sensitivity of blood culture (Rubin *et al.,* 1989, Rockhill *et al.,* 1980). Our is a tertiary care centre, where most of the patients had already taken antibiotic treatment from quacks before attending the hospital may be a reason for low sensitivity of blood culture. The volume of blood taken and the laboratory methods used for isolation are also important factors determining the yields from blood culture (Wain *et al.,* 2008). All the culture positive and three other cases making a total of 15 (14.4%) were reactive by immune-check typhoid antibody test. Of these 15, 13 were reactive for both IgG & IgM and two were positive for IgM only. *dH flagellin* gene was detected in 27(25.9%) cases. *dH Flagellin* gene amplification in blood detected the highest number of cases (Figure 1). But only quarter of all the clinically suspected cases were found positive for enteric fever by all the methods used for diagnosing the same. This may be because of the non specificity of symptoms, which overlap with other endemic diseases prevalent in this region like malaria, viral hepatitis, dengue, leptospirosis, chikungunya etc.

Majority of the cases were culture positive (66.7%) during the first week of fever, 25% had fever of more than seven days i.e. 2nd week and 8.3% showed growth on culture during the 3rd week of fever. The sensitivity of blood culture decreases with increasing duration of illness (Wain *et al.,* 2008). Similar pattern was seen for the rapid immunocheck Typhoid antibody test. All the isolates found positive by blood culture showed the presence of *dH flagellin* gene. Considering the polymerase chain reaction as gold standard, the specificity of blood culture was 100%. However, 15 cases which showed the presence of the *dH* flagellin gene did not show any growth on culture. Thus, blood culture had a good specificity but poor sensitivity (44.5%). While Immunocheck detected 1 case, which was negative for the *dH flagellin* gene (i.e., specificity 93.3%).

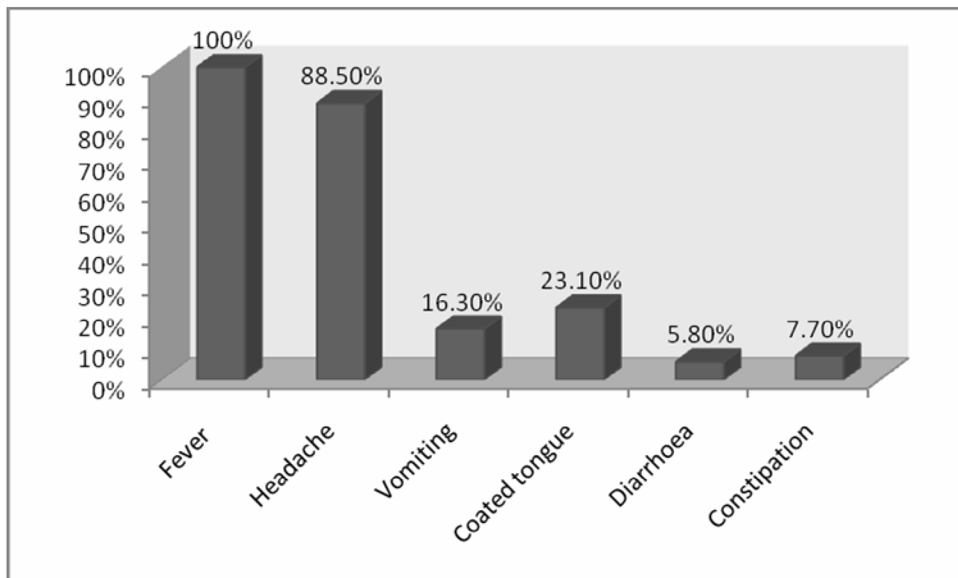
dH Flagellin gene detection rate was also highest in patients during the first week of fever followed by 25.9% during 2nd week but during the 3rd week also the detection rate of *dH flagellin* gene was significantly high as compared to blood culture. Thus, during the later duration of illness, when the sensitivity of blood culture decreases, then such expensive tests can have a role in diagnosis.

On antimicrobial susceptibility testing of the blood culture positive isolates it was noted that all the 12 isolates were consistently susceptible to azithromycin, ceftriaxone and cefoperazone sulbactum. Cefexime, ciprofloxacin, and cefepime showed susceptibility to 83.3%, 83.3% and 91.6% isolates respectively (Graph 2). Two isolates were resistant to nalidixic acid (16.7%), while 8(66.7%) were multidrug resistant (i.e., resistant to ampcillin, chloramphenicol and co-trimoxazole). Surinder *et al.* (2008) reported a sequential increase in multidrug resistance in *S. typhi* from 34% in 1999 to 66% in 2005. The uniform susceptibility to azithromycin, ceftriaxone and cefoperazone sulbactum suggests that these can be used as first line drugs for enteric fever in this region. Azithromycin has an additional advantage of oral administration and short course compared to the other two. There are also reports that azithromycin is effective both clinically and bacteriologically in treating even the MDR enteric fever (Girgis *et al.,* 1999). Enteric fever is a diagnostic challenge for the clinicians as well the microbiologists with a number of other tropical infections mimicking the clinical presentations.

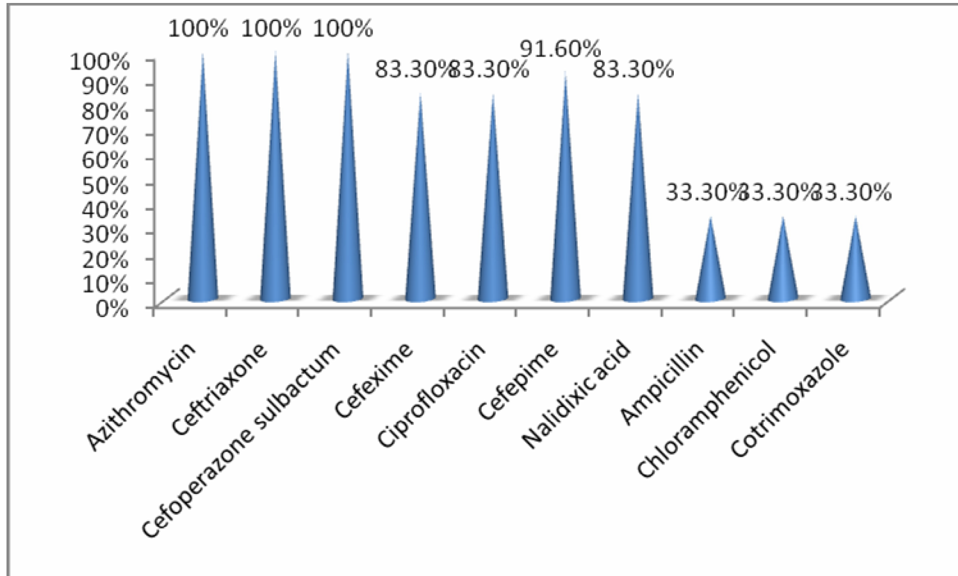
Figure.1 dH flagellin gene (486 bp) on Polymerase Chain Reaction



Graph.1 Clinical presentation of patients suspected to have enteric fever



Graph.2 Antimicrobial susceptibility pattern of *Salmonella typhi* isolates



Blood culture, with high specificity, is a traditional method of diagnosis during the first week of illness with the distinct advantage of bacterial identification with antimicrobial susceptibility, which plays an important role in epidemiological studies. When diagnosis is required in the later part of illness, amplification of *dH flagellin* gene is a good alternative, particularly in patients already on antibiotic treatment. In developing countries with low resources further investigation to develop rapid, cheap and reliable diagnostics for enteric fever are urgently needed.

Azithromycin, is a good treatment option for *S. typhi* infections. Judicious use of antimicrobials should be done to prevent the emergence of drug resistance to the options that are presently available. Combination therapy should be promoted instead of monotherapy.

References

Ackers, M.L., Puhr, N.D., Tauxe, R.V., Mintz, E.D., (2000). Laboratory-based surveillance of *Salmonella* serotype typhi infections in the United States: antimicrobial resistance on the rise. JAMA 283: 2668-73.

Agarwal, S.C., (1962). Chloramphenicol resistance of *Salmonella* species in India, 1956-61. Bull Wld Hlth Orgn 17: 331-5.

Arora, D., Singh, R., Kaur, M., and Ahi, R. S., (2010). A changing pattern in antimicrobial susceptibility of *Salmonella enterica serotype* isolated in North India. *African Journal of Microbiology Research*, vol. 4, no. 3, pp. 197–203.

Butler, T., Islam, A., Kabir, I., Jones, P.K. (1991). Patterns of morbidity and mortality in typhoid fever dependenton age and gender: review of 552 hospitalized patients with diarrhea. Review of infectious Disease, 13(1), 85-90.

Chande, C., Shrikhande, S., Kapale, S., Agarwal, S., Fule, R.P., (2002). Change in antimicrobial resistance pattern of *Salmonella typhi* in central India. Ind J Med Res 115: 248-50.

Clinical and Laboratory Standards Institute, (2014). Performance standards for antimicrobial susceptibility testing: Twenty fourth informational supplement: Approved standards M100-S24.Clinical

and Laboratory Standards Institute, Baltimore, USA.

Crump, J.A., Luby, S.P., Mintz, E.D (2004). The global burden of typhoid fever**.** *Bull World Health Organ*, 82:346-353.

Girgis, N.I., Butler, T., Frenck, R.W., Sultan, Y., Brown, F.M., Tribble, D., et al (1999). Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid fever in randomized trial in Egypt that included patients with multidrug resistance. Antimicrobial Agents and Chemotherapy 43(6): 1441-1444.

Jesudason, M.V., John, T.J., (1992). Plasmid mediated multidrug resistance in *Salmonella typhi.* Ind J Med Res 95: 66-7.

Jog, S., Soman, R., Singhal, T., Rodrigues, C., Mehta, A.,*et al.,* (2008). Enteric Fever in Mumbai –Clinical Profile, Sensitivity pattern and Response to Antimicrobials. *JAPI* 56.

Kundu, R., Ganguly, N., Ghosh, T.K., Yewale, V.N., Shah, R.C., Shah, N.K. (2006). IAP Task Force Report: diagnosis of enteric fever in children**.** *Indian Pediatr*, 43(10):875-883.

Levine, M.M., Grados, O., Gilman, R.H., Woodward, W.E., Solis-Plaza, R., Waldman, W., (1978): Diagnostic value of the Widal test in areas endemic for typhoid fever**.** *Am J Trop Med Hyg*, 27(4):795-800.

Malla, S., Kansakar, P., Serichantalergs, P., Rahman, M. and Basnet, S. (2005) Epidemiology of typhoid and paratyphoid fever in Kathmandu : two years study and trends of antimicrobial resistance. J Nepal Med Assoc 44, 18–22.

Mourad, A.,S., Matwally, M., Nour, Ei Deen A, *et al.* (1993). Multiple drug-resistant *Salmonella typhi*. Clin Infect Dis 17: 135-6.

Parry, C.M., Hien, T.T., Dougan, G., White, N.J., *et al.* (2002). Typhoid fever. *N Eng J Med* 347:1770-1782.

Rockhill, R.C., Lesmana, M., Moechtar M.A., Sutomo, A. (1980). Detection of Salmonella Cl, D, Vi and d antigens, by coagglutination, blood cultures from patients with Salmonella infections. Southeast Asian J Trop Med Public Health 11: 441-445.

Rubin, F.A., McWhirter, P.D., Punjabi N.H., Lane, E., Sudarmono, S. P., Pulungsih, M., Lesmana, S., Kumala, S., Kopecko, D.J., Hoffman, S.L. (1989). Use of a DNA probe to detect Salmonella Typhi in the blood of patients with typhoid fever. J Clin Microbiol 27; 1112-1114.

Saha, M. R., Dutta, P., Bhattacharya, S. K., *et al.* (1992). Occurrence of multi-drug resistant *Salmonella typhi* in Calcutta. *Indian Journal of Medical Research A*, vol. 95, pp. 179–180.

Saha, M.R., Dutta, P., Niyogi, S.K., Dutta, S., Mitra, U., Ramamurthy, T., *et al.* (2002). Decreasing trend in the occurrence of *Salmonella* enterica serotype *typhi* amongst hospitalized children in Kolkata, India during 1990-2000. Ind J Med Res 115: 46-8.

Sanghavi, S.K., Mane, M.P., Niphadkar, K.B., (1999). Multidrug resistance in *Salmonella* serotypes. Ind J Med Microbiol 17: 88-90.

Surinder, K., Meher, R., Nidhika, B., (2008). Rising prevalence of enteric fever due to multidrug resistant Salmonella: an epidemiological study. Journal of Med Microbiol 57: 1247-1250.

Wain, J., Hosoglu, S., (2008). The laboratory diagnosis of enteric fever**.** *J Infect Developing Countries*, 2(6):421-425.

Wain, J., To, S.D., Phan, V.B., Walsh, A.L., Ha, V., Nguyen, M.D., Vo, A.H., Tran, T.H., Farrar, J., White, N.J., Parry, C.M., Day, N.P., (2008). Specimens and culture media for the laboratory diagnosis of typhoid fever**.** *J Infect Developing Countries* 2(6):469-474.

Walia, M., Gaind, R., Mehta, R., Paul, P., Aggarwal, P., Kalaivani, M., *et al.* (2005). Current perspectives of enteric fever: a hospital based study from India. Ann Trop Paediatr 25: 161-174.