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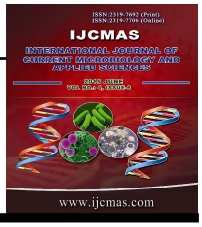

Original Research Article

Re-emergence of chloramphenicol sensitive isolates of Salmonella enterica serovar typhi isolates in India during 2013-14

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A B S T R A C T

Typhoid fever continues to remain a major public health problem, especially in endemic regions like India. Antimicrobial drugs are the mainstay of the treatment but the indiscriminate use of these drugs resulted in the emergence of MDR *Salmonella* isolates in the country during 1980s. Hence constant evaluation of the antibiogram of the causative agents of the infectious diseases like typhoid is mandatory for framing appropriate policies. A total of 286 isolates of *S. Typhi* were collected from clinical sources across different parts of India during 2013-14 and were tested for antimicrobial susceptibility according to the CLSI guidelines. MIC of resistant isolates to various antibiotics was performed by agar dilution method. Of the total isolates studied, one isolate was found to be resistant to all the antibiotics tested and 192(67.13%) isolates were found to be Multi Drug Resistant (MDR). An increase in the number of susceptible isolates (72.03%) towards chloramphenicol was observed. Simultaneously, for antibiotics like ampicillin, gentamicin there was an increase in the number of sensitive as well as intermediate isolates. MDR *S. typhi* continues to be an important public health issue in India. Hence a steady assessment of the antibiogram patterns is recommended.

Keywords

Antibiotic resistance, Typhoid fever, *Salmonella t yphi*

Introduction

Enteric fever is a major global health problem predominantly residing in Asian subcontinent. Due to its endemicity in the region, annually 22 million new cases of enteric fever are reported annually, with 200,000 deaths (Nagshetty 2010). *Salmonella enterica* serovar *Typhi* and *paratyphi* A are the predominant types of etiological agents responsible for enteric fever in India, particularly during summer (Jesudason and John 1990). Antimicrobial drugs have been the indispensible contribution of twentieth century for the treatment of such dominating infectious diseases. But the indiscriminate use and more misuse of these drugs has resulted in the emergence of multidrug resistant bacteria.

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 1990; 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). Since then MDRST has appeared throughout the world, especially in South America, the Indian subcontinent, Africa and Southeast Asia (Mourad et al. 1993). 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). Moreover, switching to new generation antibiotics as first line drugs for the treatment of typhoid has lead the bacteria to roll back to sensitivity against the old drugs like chloramphenicol (Kumar et al. 2009; Choudhary et al. 2013; Monica et al. 2014).

Hence the present investigation was under taken to evaluate the current status of antibiotic sensitivity among the *Salmonella enterica* serovar *typhi* isolates in India.

Material and Methods

Two hundred and eighty six isolates of *Salmonella* Typhi, received from different parts of the country from July, 2013 to June, 2014 at National *Salmonella* and *Escherichia* Centre (National Reference Laboratory), Central Research Institute, Kasauli, India constituted the material for the study. Ready-made media and biochemicals (Hi Media Lab. Pvt. Ltd., Mumbai, India) were obtained in their dehydrated form. Other chemicals used were of analytical grade (Merck Ltd., Mumbai, India). Glass- distilled water was used for preparation of media and reagents.

All isolates were identified by conventional biochemical tests and confirmed by serotyping (William 1994) using standard *Salmonella* agglutinating sera (Seiken Laboratories, Tokyo, Japan). Antibiotic susceptibility patterns of the isolates were determined by disc diffusion method using readymade antibiotic discs (Hi Media Laboratories, Pvt. Ltd., Mumbai, India), viz., ampicillin (10µg), chloraemphenicol (30µg), ceftazidime (30µg), cephotaxime (30µg), gentamicin (10µg), kanamycin (30µg), tetracycline (30µg) and trimethoprim (5µg), as per the CLSI guidelines and interpretative criteria (Popoff and Le Minor 1992). Minimum inhibitory concentrations (MIC) were determined by agar dilution test using purified antibiotic powders (Hi Media Laboratories, Pvt. Ltd., Mumbai, India).

The MDR *S. typhi* isolates showing reduced susceptibility for cefotaxime and ceftazidime were tested further for ESBL production. The isolates showing the zones of inhibition (ZOI) $\leq$ 27 mm and 22 mm for Cefotaxime and Ceftazidime respectively were also tested in combination with amoxicillin/clavulanic acid. The isolates showing an increase in ZOI by greater than or equal to 5 mm when evaluated in combination with amoxicillin/clavulanic acid were phenotypically considered as ESBL producers.

Results and Discussion

All the 286 isolates received during July 2013 and June 2014, were tested for their sensitivity towards eight antibiotics. Among them 65 (22.73 percent) isolates were observed to be resistant to all the antibiotics tested. Most of them (99.65 percent each) were found to be sensitive towards cefotaxime and ceftazidime. There was an increase in the number of intermediate

isolates against ampicillin (15.73%), gentamicin (13.64%), kanamicin (27.62%) as well tetracycline (30.42%). One isolate resistant against cefotaxime and ceftazidime was reported to be ESBLs producer.

A notable increase was observed in the number of sensitive isolates against chloramphenicol (72.03%) and ampicillin (61.88%) with MIC range 0.5 – 2 mg/L and 0.062 – 2 mg/L respectively.

Among the 286 isolates tested, one isolate was found to be resistant to all antibiotics used in the study and 192(67.13%) isolates were found to be Multi Drug Resistant (MDR).

Enteric fever is a major health problem in developing countries including India (Ochiai et al. 2008). Prompt institution of appropriate antimicrobial therapy can reduce mortality and morbidity associated with the illness. As *Salmonella typhi* shows varying trends of antimicrobial susceptibility collection of data that document antibiotic resistance patterns is critical and treatment of infection needs to vary on the basis of current patterns of resistance. This study describes the trends of antimicrobial resistance of serovar Typhi in India during 2013-14.

The increased number of chloramphenicol and ampicillin sensitive isolates is in concordance with other reports from India (Das and Bhattacharya 2006; Tankhiwale et al. 2003). The re-emergence of susceptibility to these two drugs is may be due to the emergence of de novo susceptible strains or the loss of high molecular weight self transmissible plasmid (Karmakar et al. 1991). This finding may be of immense importance for health authorities in deciding reintroduction of historically useful drugs such as chloramphenicol and ampicillin in treatment regimen of typhoid fever. The advantages of a return to the use of these drugs include their availability in the developing world, their cheaper cost and their well-established clinical efficacy (Sood et al. 1999). However, the risk of relapse and the development of carrier state were higher among patients treated with ampicillin than among those treated with chloramphenicol (Yew et al. 1993).

Resistance to trimethoprim among enteric organisms has dramatically increased in developing countries (Murray et al. 1985). Increased proportion of trimethoprim resistant isolates (Table 1) is of immense importance since it is a cheap and effective drug for the treatment of typhoid fever. Low resistance was found for gentamicin (9.44%) and kanamycin (9.79%) suggesting their possible use for the treatment of typhoid fever.

Resistance against cefotaxime and ceftazidime were found to very low while it was reported to be 2.2% for cefotaxime in other report from the country (Das and Bhattacharya 2006). This is an alarming development and it is of importance to limit unnecessary use of fluoroquinolones and third generation cephalosporins so that their efficacy against *Salmonella typhi* is not jeopardized further. Development of resistance to third generation cephalosporins may be due to the production of huge amount of chromosomal cephalosporinase due to the production of extended spectrum β lactamases (Pechere 1989). But further studies have to be carried out to determine the mechanism of resistance.

The MIC value of trimethoprim for intermediate isolates were found to be 10 µg/ml during study period (Table 3). Although CLSI specifies the interpretative criteria for disk diffusion susceptibility testing of nalidixic acid and trimethoprim for intermediate isolates there are no

corresponding interpretative criteria for dilution susceptibility testing. Therefore there is a need of interpretative MIC values for intermediate isolates for these antimicrobial drugs.

Further, test for the production of ESBLs among the cephalosporin resistant isolates once again proved the earlier findings to be true (Mehmood et al, 2012; Naiemi et al, 2008). ESBL producing microorganisms has gained attraction in recent years due to their ability to degrade third-generation cephalosporins even. It is a matter of great concern for microbiologists and physicians to consider other alternatives than these. The increase in the number of ESBL producers can jeopardise the drug therapy against typhoid fever.

Table.1 Antimicribial susceptibility pattern of 286 Salmonella enteric serovar typhi isolates between July, 2013 and June, 2014

S. No	Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
1.	Ampicillin(A)	177(61.89)	45(15.73)	64(22.38)
2.	Chloramphenicol(C)	206(72.03)	6(2.1)	74(25.87)
3.	Cefotaxime (Ce)	285(99.65)	0	1(0.35)
4.	Ceftazidime (Ca)	285(99.65)	0	1(0.35)
5.	Gentamicin (G)	220(76.92)	39(13.64)	27(9.44)
6.	Kanamycin(K)	179(62.59)	79(27.62)	28(9.79)
7.	Tetracycline (T)	108(37.76)	87(30.42)	91(31.82)
8.	Trimethoprim (Tr)	103(36.01)	6(2.1)	177(61.89)

Table.2 MIC values of Salmonella enterica serovar Typhi during 2013-14

Antibiotics	MIC range resistant isolates (µg/ml)	MIC range intermediate isolates (µg/ml)	MIC range sensitive isolates (µg/ml)
	2013-14	2013-14	2013-14
Ampicillin	32 – 4096	10	0.062 – 2
Chloraemphenicol	64 – 1024	16 - 32	0.5 – 2
Ceftazidime	32	16	0.004 – 0.25
Cephotaxime	64 – 512	16 - 32	0.004 – 0.25
Gentamicin	16 – 1024	8	0.12 – 1
Kanamycin	64 – 4096	32	1-2
Tetracycline	128 – 512	8	1 – 4
Trimethoprim	16 – 4096	10	0.015 - 1

The conjugation experiments conducted in the present study supported the earlier researches that multidrug resistance is plasmid mediated(Phillapa et al, 1998; Hermans et al, 1996) as all of the transconjugants were found to carry the donor profiles of antibiotic resistance. Almost every combination of resistance pattern was found and none of the isolate was found to be sensitive to all antibiotics used in the study, exhibiting the development of resistance against most of

the available drugs for the treatment of typhoid fever. The availability and affordability of effective drugs and the indiscriminate use of these drugs without prescription may lead to the selection of resistant strains of *Salmonella* Typhi. Three isolates of *Salmonella* Typhi was found to exhibit resistance to all antibiotics used in the study (Table 2). Presence of such strains in the country poses severe therapeutic problems in the treatment of typhoid fever.

Considering changing trends of antibiotic susceptibility of *Salmonella* Typhi in addition to the endemicity of typhoid fever in India, it is necessary to generate data on current antibiogram pattern from time to time. Although it appears that chloraemphenicol and ampicillin may again be used as the primary antibiotics for the treatment of typhoid fever in India, further monitoring is needed before a definitive statement can be made. Moreover, development of resistance to quinolones and third generation cephalosporins is a major cause of concern and health authorities should take appropriate steps to limit the indiscriminate use of these drugs.

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