Brief Original Article

Re-emergence of susceptibility to conventionally used drugs among strains of *Salmonella* Typhi in central west India

Yashwant Kumar, Anshu Sharma, and Kavaratty Raju Mani

National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, 173204, Himachal Pradesh, India

Abstract

Introduction: Typhoid fever (enteric fever) is a global health problem causing high morbidity and mortality, especially in endemic areas such as India. The problem is exacerbated as the causative agent, *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi), rapidly develops resistance to drugs used in treatment. However, non-responsiveness of *S.* Typhi to quinolones has been reported simultaneously with the re-emergence of susceptibility to chloramphenicol. The present study investigates the re-emergence of sensitivity to conventionally used drugs among strains of *S.* Typhi in the central west part of India.

Methodology: We evaluated 128 *S.* Typhi isolates received at the National *Salmonella* and *Escherichia* Centre for chloramphenicol, ampicillin and trimethoprim susceptibility using standard methods. Minimum inhibitory concentrations were also evaluated.

Results: The proportion of *S.* Typhi isolates showing susceptibility to chloramphenicol, ampicillin, and trimethoprim was 95.3%, 94.5%, and 94.5%, respectively. These findings may help the health authorities in reconsidering the addition of these antimicrobial drugs into the treatment regime of typhoid fever and therefore may help combat the problem of increasing resistance to quinolones and cephalosporins.

Conclusion: The changing trends of *S.* Typhi resistance patterns necessitate reconsideration of conventionally used drugs in typhoid fever treatment in India. In the present study, *S.* Typhi strains exhibited increased susceptibility toward chloramphenicol, ampicillin and trimethoprim, therefore suggesting the possibility of their use for treatment of typhoid fever.

Key words: re-emergence; susceptibility; *Salmonella* Typhi

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Introduction

Typhoid fever (enteric fever) is of worldwide concern, especially in developing countries where it is endemic [1]. Antimicrobial chemotherapy against the causative agent *S.* Typhi emerged as an effective strategy to reduce morbidity and mortality due to typhoid fever [2]. Chloramphenicol was considered to be the gold standard of typhoid fever therapy after its introduction in 1948 [3]. However, strains of *S.* Typhi resistant to chloramphenicol, ampicillin and trimethoprim-sulphamethoxazole (TMP-SMZ) became prevalent in some Asian countries during the late 1980s and early 1990s and emerged as a significant therapeutic problem [4]. South-eastern China is believed to be the origin of this multidrug resistant strain of *S.* Typhi [5]. Due to the prevalence of these strains, the fluoroquinolone ciprofloxacin began to be used for the treatment of typhoid fever [6].

Several studies from different parts of India have reported the development of resistance of *S.* Typhi to various antibiotics [7,8]. In the central west parts of India, fluoroquinolones and third-generation cephalosporins are the mainstay of treatment for typhoid fever [9,10]. Rampant use of ciprofloxacin in the treatment of typhoid fever led to an increase in the minimum inhibitory concentrations (MIC) of ciprofloxacin in strains of *S.* Typhi [11]. Strains of *S.* Typhi showing *in-vitro* susceptibility to ciprofloxacin were reported to result in treatment failure due to increased levels of MIC [12]. In recent years there have been several reports indicating the re-emergence of susceptibility to drugs used in the past, such as chloramphenicol, ampicillin and trimethoprim [1,9,13-21]. In the present scenario of decreased clinical responsiveness of typhoid fever cases to ciprofloxacin, the reintroduction of historically useful drugs in the treatment regimen of typhoid fever would be of immense therapeutic importance. The aim of the present study was to study the changing antibiogram pattern of *S.* Typhi with respect to re-

Table 1. Antimicrobial susceptibility to chloramphenicol, ampicillin and trimethoprim

Susceptible isolates			
	Chloramphenicol	**Ampicillin**	**Trimethoprim**
Number of isolates (number of susceptible strains/total number tested)	122/128	121/128	121/128
MIC range (mg/L)	0.93 – 7.5	0.31 – 1.25	0.03 – 0.31
Resistant isolates			
	Chloramphenicol	**Ampicillin**	**Trimethoprim**
Number of isolates (number of resistant strains/total number tested)	6/128	7/128	7/128
MIC range (mg/L)	32 – 64	32 - 512	16 - 32

emergence of susceptibility to conventionally used antimicrobials.

Methodology

The National *Salmonella* and *Escherichia* Centre (NSEC), Central Research Institute, Kasauli, India, has provided a service to the nation for the past five decades, and is a national reference laboratory formerly under the control of the World Health Organization. During the year 2008-2009, 128 isolates of *S.* Typhi were received by the NSEC from 14 hospitals within Maharashtra, and these constituted the material for this study. Maharashtra is the third largest state in India situated in the central west part of the country. This state is India's leading industrial state contributing 15% of the national industrial output and over 40% of India's national revenue. The records of all the isolates were retrieved from laboratory records of the department.

All media, antibiotics and biochemicals were obtained from Hi Media Lab. Pvt. Ltd., Mumbai, India. All isolates were identified as *S.* Typhi by conventional biochemical tests [22] and confirmed by serotyping [23] using standard *Salmonella* agglutinating sera (Seiken Laboratories, Tokyo, Japan). Antibiotic susceptibility patterns of the isolates were determined by the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretative criteria [24]. Antibiotic disks were used as follows: chloramphenicol (30 µg), ampicillin (10 µg) and trimethoprim (5 µg). *Escherichia coli* strain ATCC 25922 was used as control. The inocula were prepared by picking three to five well-isolated colonies of the same morphological type into broth and adjusting to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. MIC were determined by agar dilution test [25] using purified antibiotic powders.

Results

Of the 128 isolates of *S.* Typhi received at the NSEC, 122 (95.3%) were found to be susceptible to chloramphenicol (MIC 0.93 –7.5 mg/L), and 121 strains (94.5%) were susceptible to both ampicillin (MIC 0.31 – 1.25 mg/L) and trimethoprim (MIC 0.03 – 0.31mg/L) (Table 1). MIC ranges were found to be 32 – 64 mg/L, 32 – 512 mg/L, and 16 – 32 mg/L, respectively, for chloramphenicol, ampicillin, and trimethoprim in resistant isolates (Table 1).

Discussion

In the present study, re-emergence of susceptibility to historically used drugs within strains of *S.* Typhi was observed in higher proportions than those reported from different parts of the country [1,16,17-19], with the exception of one study from northern India which reported 96% sensitivity to chloramphenicol [26]. The results of the present study are in concordance with other studies from the central west part of the country, which also report sensitivity to chloramphenicol, ampicillin and trimethoprim [9,14,27,3], except one which reported 100% resistance to these drugs [28].

The re-emergence of susceptibility to these drugs may be a result of the emergence of *de novo* susceptible strains [17] or the loss of high molecular weight self-transmissible plasmids [29]. However, resistance may again develop if multiple drug resistant (MDR) strains are able to transfer their R-plasmids, encoding resistance determinants, to the strains sensitive to these drugs [30].

Antimicrobial drugs used for the treatment of typhoid fever are increasingly being used in the treatment of other diseases, leading to problems with antibiotic resistance due to acquisition by *S.* Typhi of resistance-encoding plasmids from other infectious bacteria [31]. Several reports have been published highlighting increased MIC of ciprofloxacin in *S.* Typhi in different parts of the world, including India, therefore resulting in treatment failures [32,33]. In some cases, higher values of ciprofloxacin MIC have been associated with resistance to another quinolone in nalidixic acid resistant *S.* Typhi (NARST) [34]; a high proportion of these strains have been reported from the central west part of India [35]. In the present scenario of fluoroquinolone resistance and the increasing number of reports on the re-emergence of susceptibility to old drugs in the central west part of India, the findings presented here may be of immense importance to aid health authorities to rationalize the policy of empirical treatment of typhoid fever. Furthermore, the return to the use of these drugs includes advantages such as their availability in the developing world, their lower cost and their well-established clinical efficacy [36]. It should be noted that the risk of relapse and the development of a carrier state have been found to be higher among patients treated with ampicillin than those treated with chloramphenicol [37,38].

Briefly, looking at the changing trends of antibiotic susceptibility of *S.* Typhi in addition to the endemicity of typhoid fever in India, it is necessary to perform regular surveys of antibiogram patterns. Although it appears that chloramphenicol, ampicillin and trimethoprim may become the primary antibiotics for the treatment of typhoid fever in some areas of the country, further monitoring of different parts of the country is needed before a definitive statement can be made.

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Corresponding author

Yashwant Kumar
National Salmonella and Escherichia Centre
Central Research Institute
Kasauli (HP) – 173204
India
Telephone: +91-1792-272059 Ext. 218
Email: yasht26@yahoo.co.in

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