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Antimicrobial Susceptibility Pattern of Salmonella Isolates at Tertiary Care Hospital, Ahmedabad, India

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A B S T R A C T

Background and Objective: 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. Factors responsible for emergence of new epidemic strains of *Salmonella* are not understood, but it is possible that antimicrobial play a role in emergence and persistence of epidemic MDR strains. The MDR *S. typhi* is on the rise. The object of this study was to determine the susceptibility pattern of various drugs used for treatment of enteric fever. Total of 1020 blood samples obtained from suspected enteric fever patients during June 2015 to July 2016. The sample was processed on Bact/ALERT 3D and isolates obtained from subculture were identified by morphological, biochemical and serological means. Antibiotic susceptibility testing was carried out using modified Kirby–Bauer disk diffusion method. Out of 1020, 26 *Salmonella* were isolated with isolation rate of 2.55%. 21 were *Salmonella typhi* and 5 were *Salmonella paratyphi* A. Susceptibility pattern of *S. typhi* isolates were 85.71% to ampicillin, 100% to chloramphenicol, 90.48% to co-trimoxazole and 3rd generation cephalosporins, 57.14% to fluoroquinolones. There was higher number of *Salmonella enterica* serotype *typhi* isolates resistant to fluoroquinolones (pefloxacin and ciprofloxacin). However chloramphenicol was sensitive to all isolates. This study suggests chloramphenicol as a drug of choice for enteric fever and further monitoring of efficacy of older and newer antibiotics are desirable.

Introduction

Enteric fever is a global public health problem and is endemic in many developing countries, including India (Mathu *et al.*, 2011). Enteric fever is a systemic infection caused by the human adapted pathogens *Salmonella enterica* serotype Typhi (*S. typhi*) and *S. enterica* serotype Paratyphi (*S. paratyphi*) A, B and C (Crump and Mintz, 2010). Enteric fever remains one of the major public health issues globally, especially in Asia. According to recently revised global estimate, above 22 million cases of typhoid fever occur each year round the world while 90% of the sufferers are from the South East Asia (Rahman *et al.*, 2011). It predominantly affects children and young adults (Wasfy *et al.*, 2000) and if not treated appropriately has mortality rate of 30%, whereas, with proper treatment the

mortality reduces to as low as 0.5% (Stormaon *et al.*, 1997).

Drug resistance is fast becoming a major problem in the management of this infection and the emergence of multi-drug resistance has great implications for the therapy, for example, patients infected with such strains are more ill at presentation, have a longer duration of illness and higher mortality. However, there are no pathognomic features to distinguish such infections from infections with fully sensitive *S. typhi* at presentation (Kumar and Gupta, 2007). Chloramphenicol resistance became established globally in the *S. typhi* population after 1972 on plasmids of incompatibility group Inc H and Multi drug resistance (defined as resistance to all the first line antibiotics used to treat typhoid fever, i.e. chloramphenicol, ampicillin and co-trimoxazole) has been endemic, particularly in Indian sub-continent and South East Asian countries since 1984 (Bhutta *et al.*, 1991). Though initially, individual plasmids were known to code for multidrug resistance to each of these antibiotics, since 1988 a single plasmid was known to code for multidrug resistance. This plasmid belongs to incompatibility group H 11 and is highly permissible. In addition to Multi Drug Resistance (MDR) *S. typhi*, now resistances to fluoroquinolones have emerged as the newer challenges to the treatment of typhoid fever (Shrikala *et al.*, 1999). This study was undertaken to isolate and identify *S. typhi* and *S. paratyphi* and their antibiotic sensitivity pattern which are the major cause of enteric fever in the developing countries like India.

Materials and Methods

The study was carried out at B.J. Medical College, Civil Hospital, Ahmedabad during June 2015 to July 2016. Total of 1020 blood samples were obtained from patients suspected with enteric fever.

Isolation of *Salmonella*

The samples to be tested were inoculated in BacT/ALERT culture bottle which was inserted into the BacT/ALERT 3D for incubation at 37ºC and periodic reading for 7 days. Each culture vial contains a colorimetric sensor which can detect CO_2 produced by the growth of microorganism. The sensor was monitored by the instrument every 10 minutes for an increase in its reflected light, which was proportional to the amount of presence of growth. A positive reading indicated the presumptive presence of viable microorganism in the bottle. Once a positive culture bottle was detected, a Gram stain slide was prepared from the bottle and then a loopful of the positive blood culture bottle content was sub-cultured on blood agar, MacConkey agar, Chocolate agar and, incubated at 37ºC for 18-24 hours.

Identification and confirmation of *Salmonella*

The colony growths were identified by colony characteristics and bio-chemical reactions like oxidase test, catalase test, triple sugar iron agar, IMViC (Indole, Methylered, Voges Proskauer and Citrate) test, amino acid decarboxylation test, sugar fermentation test etc. Final identification of isolates was confirmed serologically according to Kauffman-White classification using *Salmonella* (somatic and flagellar) antisera like Poly O, O-9, and H-d.

Antibiotic sensitivity test

The clinical isolates were subjected to antibiotic sensitivity test on Mueller-Hinton agar, using modified Kirby Bauer disc diffusion method as per Clinical Laboratory Standard Institute 2015 guidelines. The panel of antibiotics included were Ampicillin (10 µg), Ciprofloxacin (5µg), Levofloxacin (5 µg),

Pefloxacin (5µg), Cefuroxime (30 µg), Cefotaxime (30 µg), Cefoperazone (75 µg), Co-trimoxazole (25 µg) and Chloramphenicol (30 µg).

The negative samples were incubated for next seven days and then subcultured, before finally declaring them as culture negative.

Results and Discussion

Total of 26 *Salmonella* were isolated from 1020 blood samples of suspected enteric fever patients.16 isolates were from male patients and 10 isolates were from female patients.

Culture positive patients fell into age group of 0-45 yrs. 12 isolates were from children of age group 0 to 15 yrs. 14 isolates were from adults of age group 16 to 45 yrs. Out of 26 *Salmonella* isolates, 21 were *Salmonella typhi* and 5 were *Salmonella paratyphi* A as shown in Figure 1.

Antimicrobial susceptibility pattern of *Salmonella typhi* and *Salmonella paratyphi* during our study period has been described in Table 1 and 2 respectively.

In the last fifteen years, the emergence of resistance to the antibiotics has lead to large epidemics. Typhoid is now encountered mostly throughout the developing world. Management of this serious disease becomes complicated due to the resistance of antibiotics used for the treatment of enteric fever. It is becoming difficult to control the spread of multidrug resistant (MDR) *Salmonella* (Parry).

Out of 1020 blood samples, 26 *Salmonella* were isolated with isolation rate of 2.55% which is similar to study done by Nilesh Patel *et al.*, (2015). In our study period, *S. typhi* outnumbered *S. paratyphi* A with almost 4 times higher rate of isolation in our region.

We have obtained blood samples from patients attending civil hospital, Ahmedabad. This hospital is referral medical hospital for the region and, many patients seek treatment here, after receiving some treatment at local level. This factor may interfere with organism isolation rate.

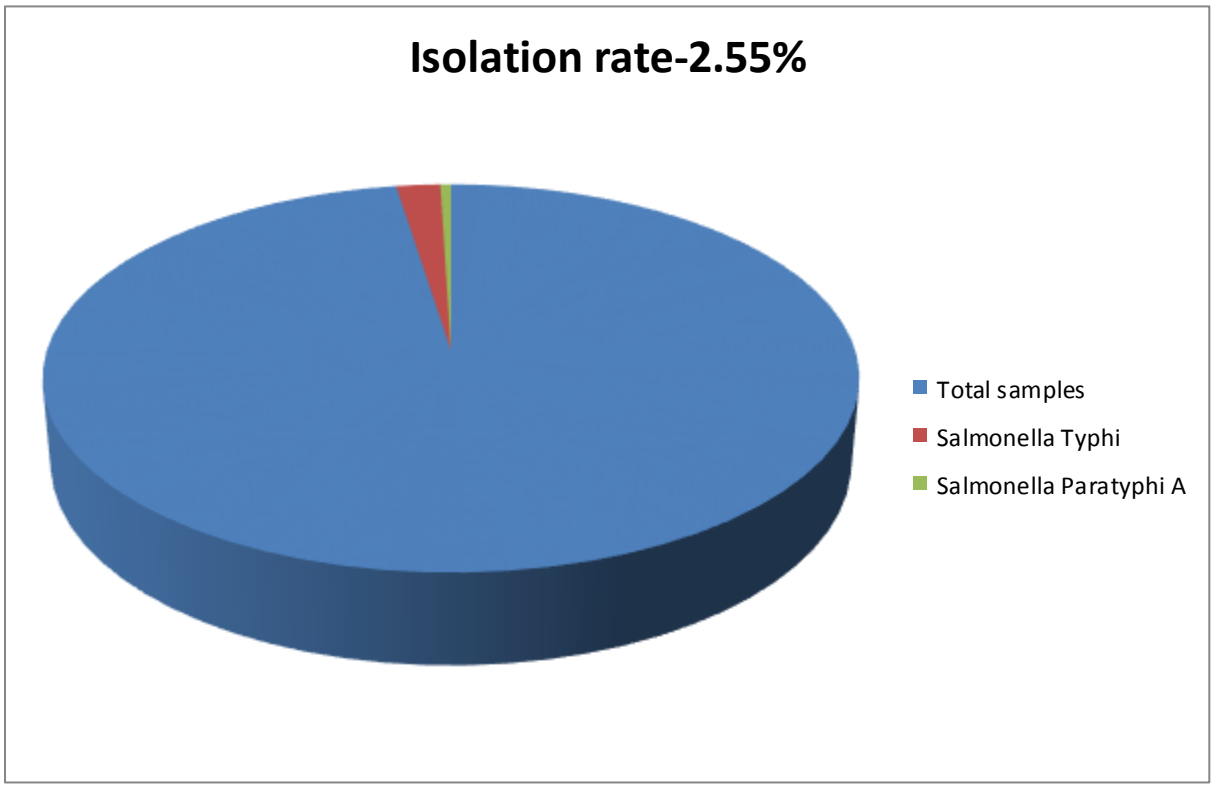
The highest incidence of typhoid occurs in 16-45 years age group. Males are affected more frequently than females (Parry). In the present study, 14 (53.85%) cases occurred between 16-45 years of age. 16 (61.54%) isolates were from male patients and 10 (38.46%) isolates were from female patients.

Table.1 Antibiotic susceptibility pattern of *Salmonella typhi*

Antibiotic	Susceptible (%)	Resistant (%)
Ampicillin (10µg)	18 (85.71%)	3 (14.29%)
Cefotaxime (30µg)	19 (90.48%)	2 (9.52%)
Cefoperazone (75µg)	19 (90.48%)	2 (9.52%)
Ciprofloxacin (5µg)	12 (57.14%)	9 (42.86%)
Pefloxacin (5µg)	12 (57.14%)	9 (42.86%)
Co-trimoxazole (25µg)	19 (90.48%)	1(4.26 %)
Chloramphenicol (30 µg)	21 (100%)	0 (0 %)

Table.2 Antibiotic susceptibility pattern of *Salmonella paratyphi* A

Antibiotic	Susceptible (%)	Resistant (%)
Ampicillin (10µg)	5 (100%)	0 (0%)
Cefotaxime (30 µg)	5 (100%)	0 (0%)
Cefoperazone (75 µg)	5 (100%)	0 (0%)
Ciprofloxacin (5 µg)	5 (100%)	0 (0%)
Pefloxacin (5 µg)	5 (100%)	0 (0%)
Co-trimoxazole (25 µg)	4 (80%)	1 (20%)
Chloramphenicol (30 µg)	5 (100%)	0 (0%)



In our study, overall rate of resistance of 21 *S. typhi* isolates was 14.29% to ampicillin, 9.52% to cephalosporins, 42.86% to fluoroquinolones and 4.76% to co-trimoxazole. Five isolates of *S. paratyphi* A shows high level of sensitivity to all antimicrobials.

S. paratyphi isolates were 100% sensitive to ampicillin, fluoroquinolones, co-trimoxazole chloramphenicol and 80% sensitive to 3rd generation cephalosporins.

Susceptibility pattern of *S. typhi* isolates to ampicillin (85.71%) and chloramphenicol (100%) were similar to study conducted by Gordana Mijovic *et al.,* (2012) whereas sensitivity to fluoroquinolones was 57.14% which was similar to study in Kuwait by Dimitrov *et al.,* (2009). with sensitivity of 63.3%.

Sensitivity of *S. typhi* isolates to co-trimoxazole and cephalosporins were 90.48% which was similar to study conducted by Gordana Mijovic *et al.,* (2012). In conclusion, the findings of the

present study indicated that the first line antibiotics may still have a role to play in the treatment of typhoid fever. This study suggests Chloramphenicol as a drug of choice for enteric fever and further monitoring of efficacy of older and newer antibiotics are desirable.

Sensitivity of *Salmonella* isolates to all tested antimicrobial agents except to fluoroquinolones was been good over testing period. Resistance rate to pefloxacin was higher and this fact deserves attention. The surveillance of antimicrobial resistance in *Salmonella* spp. is very important. Also, it is important to maintain *Salmonella* active surveillance of resistance on an international and intersectoral level.

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