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Prevalence of Faecal Carriage of *Salmonella* Serotypes and Their Antimicrobial Susceptibility Pattern among Asymptomatic Food Handlers

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Abstract

Salmonella enterica serotype typhi is a gram-negative bacterium that is responsible for typhoid fever and has been a burden on developing nations for generations. Typhoid fever is a bacterial infection due to a specific type of Salmonella that causes symptoms. They may vary from mild to severe, and usually begin 6 to 30 days after exposure. Often there is a gradual onset of a high fever over several days. A hospital based, prospective, cross-sectional study, was conducted in Microbiology laboratory-KMC hospital Ambedkar Circle from October 2012 to March 2014.The study included screening of 200 food handlers working in the mess of seven hostels, and in the canteens of three hospitals, for Salmonella carriage.

Blood cultures were performed using the BacT/Alert system, Bio'Merieux. Isolates obtained from blood cultures were identified by the automated Vitek II system, Bio'Merieux. Studying the hygiene standards of the food handlers is necessary because, a good hygiene can reduce the transmission rate of various infections, especially enteric fever. RAPD is a rapid typing method based on random amplification of polymorphic DNA segments. Here, a short oligonucleotide of arbitrary sequence is used to prime DNA synthesis by accessing random segments of genomic DNA at low stringency to reveal polymorphism. Between 16 clinical isolates, 6 various Random Amplification of Polymorphic DNA (RAPD) were observed. Clinical S.Typhi isolates and isolates from healthy carriers are genetically different.

Introduction

Typhoid fever has been a threat to the mankind for ages unbound and still continues to be. It is an infectious disease caused by *Salmonella* enterica group. Disease due to *salmonellae*, both typhoidal and non-typhoidal, remains a major public health challenge especially in developing countries. Inadequate treatment of enteric fever has a mortality rate of 30%, and with prompt diagnosis and treatment the mortality rate is 0.5% [1]. Asymptomatic carriage and drug resistance of typhoid bacilli have been the major problems of typhoid infection. Asymptomatic food handlers intermittently shed the bacilli in their faeces and urine, thus contaminating food and water sources. Detection of carriers among food handlers is important to prevent such public health catastrophies. Drug resistance among *salmonellae* also poses a significant problem in management of patients. The broad spectrum antibiotics like chloramphenicol, ampicillin and co-trimoxazole were the drugs of choice. Fluoroquinolone ciprofloxacin has become the first-line drug for treatment, especially since the widespread emergence of *Salmonella* isolates that are multidrug resistant (MDR) to the more traditional antimicrobial agents comprising chloramphenicol, ampicillin and co-trimoxazole. Third generations cephalosporins, including ceftriaxone have been the drugs of choice especially for enteric fever organisms that are resistant to fluoroquinolones. Of late, there have been reports of ceftriaxone resistance among non-typhoidal *salmonellae*. This study is being taken up to assess the prevalence of faecal shedding of *salmonellae* among food handlers in hostel mess and canteens in Mangalore and emerging ceftriaxone resistance among the *salmonella* isolates.

Materials and Methods

A hospital based, prospective, cross-sectional study, was conducted in Microbiology laboratory -KMC hospital Ambedkar Circle from October 2012 to March 2014.The study included screening of 200 food handlers working in the mess of seven hostels, and in the canteens of three hospitals, for *salmonella* carriage. Hence, in this context, stool specimen was collected from all the food handlers. All isolates of *salmonellae* obtained from blood cultures received at the Microbiology Laboratory at KMC Hospital Laboratory Services from October 2012 to March 2014 were included in the study. Data was collected from the food handlers using a structured preform containing the food handler's personal details and information regarding their health status. A consent form was provided both in English and Kannada, which was read by each food handler and consent taken accordingly. Each food handler was motivated to participate in the study and was enrolled by explaining to him/her the reason and purpose of the study. Enrollment was done by taking their signatures/thumb impression on the consent form. All the food handlers who were healthy and did not have fever at the time of sample collection or in the past three weeks, who had no history of typhoid in the past one year and no history of intestinal ailment in past 3 months were included in the study.

The food handlers with fever at time of sample collection or in the past three weeks, history of typhoid in past one year and history of intestinal ailment in past 3 months were excluded from the study.

Specimen collection

Stool was the specimen of choice for detecting the carrier status. Participants were clearly instructed regarding the method of collecting the stool specimen. Stool specimens were obtained from the food handlers in a sterile, dry wide-mouthed container, without admixture with urine. Five stool specimens were obtained on alternate days from each food handler.

Processing

Media used

Nonselective Differential medium- MacConkey's agar

Selective media-Hektoen Enteric agar, Deoxycholate citrate agar (DCA)

Enrichment broth-Tetrathionate broth, Selenite F broth

Media used for biochemical identification: Oxidation-fermentation glucose; Nitrate broth; Triple iron sugar; Glucose; Lactose; Arabinose; Dulcitol; Mannitol; Xylose; Indole; Methyl-red; Voges-Proskauer; Citrate; Urease; Lysine iron agar; PPA; Ornithine

Media preparation

All media were purchased from Hi-media Laboratories, in dehydrated form and were prepared according to the manufacturer's instructions.

The stool specimen was processed as follows **(Figure 1)**:

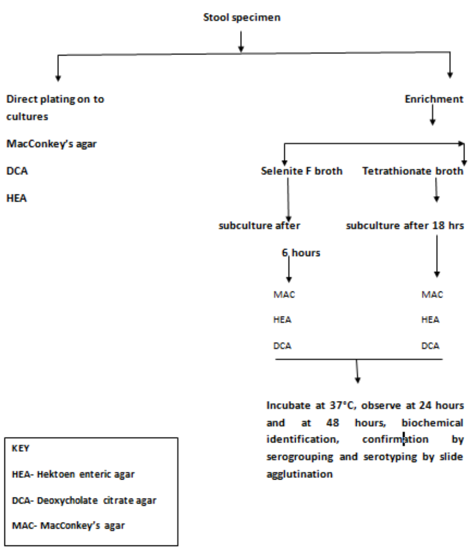

Figure 1: Flow chart showing processing of stool specimen.

Test	*S.Typhi*	*S.Paratyphi A*
OF TEST	Fermentative	Fermentative
Nitrate	Reduced	Reduced
TSI&H2S	K/A+H2 S	K/A
Motility	Motile	Motile
Indole	Negative	Negative
Methyl-red	Positive	Positive
VP	Negative	Negative
Citrate	Not utilised	Not utilised
PPA&Urease	Negative	Negative
Ornithine	Non decarboxylate	Decarboxylated
LIA	Decarboxylated	Non decarboxylate
Lactose	Not fermented	Not fermented
Arabinose	Not fermented	Fermented
Dulcitol	Not fermented	Fermented

Table 1: Biochemical differentiation between enteric fever causing pathogens.

Blood cultures

Blood cultures were performed using the BacT/Alert system, Bio'Merieux. Isolates obtained from blood cultures were identified by the automated Vitek II system, Bio'Merieux.

All isolates of *S.Typhi* and *S.Paratyphi* A from food handlers and from blood cultures were confirmed by slide agglutination with polyvalent group specific antiserum and by serotyping with type specific O and H antisera.

Confirmation of *Salmonella* isolates was done by slide agglutination test with specific antisera.

Antibiotic susceptibility test: All isolates of Salmonellae obtained from routine blood cultures, and those obtained from the stool specimens of food handlers were subjected to antibiotic susceptibility testing by Modified Kirby-Bauer disc diffusion method in accordance with CLSI criteria and by the automated Vitek II system, Bio'Merieux. The sensitivity patterns were recorded accordingly. Ampicillin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), Ciprofloxacin (5 µg/disc), Nalidixic acid (30 µg/disc), Ofloxacin (5 µg/disc), ceftriaxone (30 µg/disc), and Cefotaxime (30 µg/disc) were the discs tested against the isolate.

MIC for ceftriaxone: MIC for ceftriaxone was done for all the blood and stool isolates of *Salmonella*. MIC was determined by agar dilution method. ATCC *E.coli* 25922 was used as control.

Biotyping: Biotyping of *S.Typhi* and *S.paratyphi* an isolates was done by fermentation with l-Arabinose and d-Xylose. Each tube was observed for fermentation of sugars (by change of color of media to pink) and for production of gas (seen as bubble in inverted Durham's tubes). Organisms were classified as biotype I (Arabinose-, Xylose+), biotype II (Arabinose-, Xylose-), biotype III (Arabinose+, Xylose+) and biotype IV (Arabinose+, Xylose-).

Detection of bacterial adherence (biofilm formation)-Procedure: Biofilm was checked for *salmonella* serotypes to compare this property between faecal and clinical isolates of *salmonella* and to see whether there was a correlation between boifilm production and no. of days taken for defervescence after starting specific antityphodal therapy. Therefore those clinical isolates of *salmonella* for which clinical history was available from medical records department were selected for biofilm formation study, along with faecal isolates from healthy carriers. Biofilm forming ability was checked using the microtitre plate adapted from O'Toole and Kolter, with some modifications.

S.Typhi isolates selected for biofilm study were grown in Trypticase soy broth till stationary phase.

Cultures were diluted 1:100 with fresh Tryptic soy broth and 200 µl was inoculated into sterile flat bottomed 96 well tissue culture plates. Each isolate was inoculated into 4 microtitre wells (ie. quadruplicate). Negative control wells contained broth only. The wells were sealed with paraffin.

The tissue culture plates were incubated at suitable temperature (37°C) for 48 hours.

The contents of each well were gently aspirated by using a micropipette.

Using the micropipette, the wells were washed with 200 µl phosphate buffered saline (pH-7.2). Adherent organisms were fixed in place with Bouine fixative and stained with 1% Hucker crystal violet. Excess stain was rinsed off by placing the plate under running tap water.

After drying the dye bound to adherent cells was resolubilised with 200 µl of 33% (v/v) of glacial acetic acid per well.

OD of each well was measured at 570 nm

The test was repeated twice each time in quadruplicate, to ensure reproducibility and repeatability and the values were averaged.

The adherence capabilities of the test strains were classified under 4 categories based on the OD of bacterial films. The cut off optical density (ODC) for the microtiter plate was defined as 3 standard deviations above the mean OD of the negative control.

Antimicrobial agent	Sensitive (µg/ml)	Intermediate (µg/ml)	Resistant (µg/ml)
Ceftriaxone	≤ 1	2	≥ 4

Table 2: Interpretative standard chart for agar dilution method (CLSI 2014).

RAPD (Random Amplification of Polymorphic DNA): RAPD analysis was done to look for differences, if any, in RAPD patterns of faecal and blood culture isolates of *S. Typhi.*

Results

A total of 232 food handlers were enrolled in the study from both the hostels and canteens in Mangalore. Out of 232 food handlers, 159 food handlers were working in the hostel mess and 73 food handlers were working in canteens. The age of the food handlers ranged from 21 to 60 years. Majority (60%) of them were between 21 to 30 years of age. Twenty five percent of food handlers were between the age group [31-40], and 8% constitute the age group [41-50]. The age group [51-60] constitutes the least (7%). Males formed the majority amounting to 59% (137) of the population.

The occupational distribution was done based on the actual work done by the food handler in mess or canteen. Eighteen percentage of the food handlers were only cooking, 30% were only serving, 16% were both cooking and serving, 11% were cleaning tables/utensils and 26% were involved in sweeping and mopping. The literacy level of the food handlers was also assessed. Majority (37%) of them had completed their 1st and 2nd PUC. Twenty six percent were literate up-to 10th standard. Twenty two percent were literate up to 5th standard. Whereas only 4% were graduates (B.sc and B.com). Fourteen percent were illiterate.

Hygiene standards of the food handlers were also assessed. The overall hygiene of the food handlers was good (Ninety-eight percent of the food handlers washed their hands with soap and water after defecation, 94% had their finger nail cut short and 96% wore gloves while cooking and handling food).

Salmonella **from stool specimen (Figure 2)**

A total of 1160 stool samples were processed. The *salmonella* carriage rate was 0.4% (1 out of 232 food handlers). The serotype identified was *S.Typhi*. The food handler from whom *S.Typhi* was isolated was a 35 year old male with educational qualification up to 11th standard. He was working in the hostel as a cook cum server. He wore gloves while cooking and serving and washed hands with soap and water after toilet and had his fingernails cut short.

Figure 2: Key reactions of *salmonella typhi.*

Key biochemical reactions: Indole-Negative, oxidation-fermentation-Fermentative, manitol motility test- motile, ornithine-Non-decarboxylate, triple sugar iron- K/A H2S+, anaerogenic, Lysine Iron Agar: Lysine decarboxylated, urease: Not hydrolysed, PPA: Negative, CITRATE: Not utilized. The identification was confirmed by slide agglutination with Polyvalent A-G, O9 and Hd.

Figure 3: Biotyping of the faecal isolate of *S.Typhi*.

Biotyping of the faecal isolate of *S.Typhi* was also done and it belonged to biotype III. The isolate was sensitive to Ampicillin, Chloramphenicol, Cotrimoxazole, Ciprofloxacin, Ofloxacin, Cefotaxime, Ceftriaxone and resistant to Nalidixic acid. MIC for Ceftriaxone of this isolate of *S.Typhi* was 0.125 µg/ml.

Salmonella from blood culture

Total number of Enteric fever isolates recovered from blood culture was 89. Among the 89, 60 (67%) were *Salmonella Typhi* and 29 (33%) were *Salmonella paratyphi* A. All the 89 isolates were subjected to antibiotic susceptibility testing by disk diffusion method. Among the 60 *S.Typhi* isolates, 59 (98%) isolates were sensitive to ampicillin and the same number of them ie 59 (98%), were sensitive to cotrimoxazole. Fifty seven (95%) isolates were resistant to nalidixic acid and only 3 isolates were sensitive to the antimicrobial. Fifty (83%) isolates were sensitive to ofloxacin and thirty four (57%) isolates were sensitive to ciprofloxacin. All (100%) isolates were sensitive to chloramphenicol, ceftriaxone and cefotaxime. Among the 29 isolates of *S.paratyphi* A, 27 (93%) isolates were sensitive to ampicillin and 19 (66%) isolates were sensitive to ciprofloxacin. Only 2 (7%) isolates were sensitive to nalidixic acid. MIC for Ceftriaxone was performed for all isolates. The majority of *S.Typhi* and *S.paratyphi* A isolates i.e., 63.3% and 66% respectively, had MIC of 0.125 µg/ml (**Figures 3 and 4**).

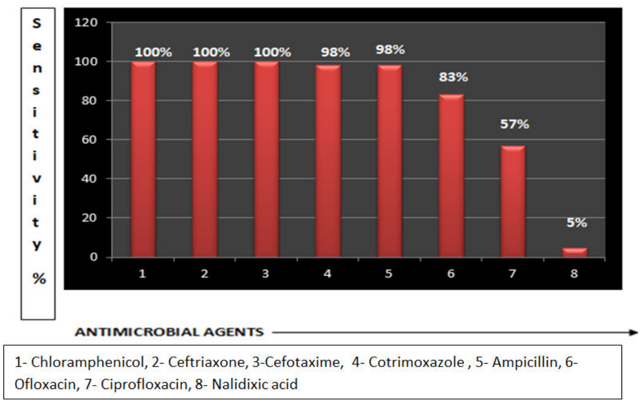

Figure 3: Antibiotic sensitivity pattern of salmonella typhi from blood culture.

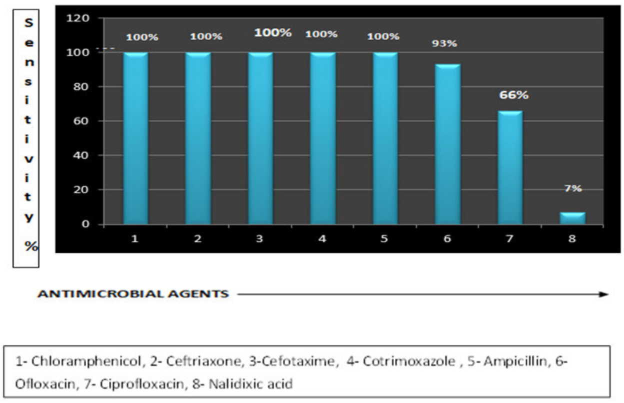

Figure 4: Antibiotic sensitivity pattern of salmonella paratyphi a from blood culture.

Biofilm

Sixteen clinical isolates of *S.Typhi* (from blood culture) were selected for study of biofilm production. These 16 were chosen since clinical details were available for these patients, from the hospital medical records. Biofilm production was studied for these 16 isolates and the single faecal isolate of *S.Typhi*, using the quantitative microtitre plate method and the isolates were classified as strong, moderate, weak and non-producers of biofilm.

Mean OD value	Biofilm formation	No. of strains
<0.084	Non producer (0)	0
0.084-0.168	Weak producer (+)	5(4 clinical+1 faecal isolate)
0.169-0.336	Moderate producer (++)	7
>0.336	Strong (+++)	5

Table 4: OD values of quantitative microliter plate method.

Of the 16 clinical isolates, 4 were weak producers, 7 were moderate producers and 5 were strong producers of biofilm. The faecal isolate was a weak producer of biofilm. An attempt was made to find out whether biofilm producing property had any significant relationship with the no. of days for defervescence after starting the treatment (**Table 5**). We did not find any correlation between biofilm forming capacity and no. of days for defervesence.

S.Typhi No.	Age/Sex	Duration of fever before commencement of treatment	Duration of fever after commencement of treatment	biofilm
1	36 y/M	7 days	5 days	Moderate
2	24 y/M	8 days	5 days	Strong
3	20 y/M	5 days	4 days	Strong
4	54 y/M	10 days	2 days	Strong
5	26 y/M	7 days	3 days	Moderate
6	18 y/M	10 days	5 days	Moderate

7	24 y/F	9 days	3 days	Strong
8	22 y/M	7 days	5 days	Strong
9	22 y/F	8 days	4 days	Strong
10	30 y/M	14 days	3 days	Strong
11	38 y/M	6 days	4 days	Weak
12	25 y/M	4 days	3 days	Weak
13	30 y/M	10 days	4 days	Moderate
14	55 y/M	10 days	4 days	Weak
15	30 y/M	9 days	5 days	Weak

Table 5: Representing fever duration and biofilm.

DISCUSSION

Demography

The demographic study showed that majority (60%) of the population were between 21 to 30 years of age. Abera et al. [28] in their study also showed that young adults formed the majority accounting for 96.6%. Males formed the majority amounting to 59% (137 of 232) of the population. In a study by Yousefi-Mashouf et al. [23] also males were predominant accounting for 85.5% of the food handler population and the females accounted for 14.5%.

Occupational distribution

Thirty percent were only servers, i.e., majority were only serving and 18% were only cooking. Sixteen percent were both cooking and serving.

Literacy

The level of literacy of the food handlers was also studied and showed that 37% of them had completed their 1st and 2nd PUC. Only 4% were graduates and 14% were illiterate. The majority were of low educational which agrees with the study by Abera et al. [28] where 29.4% were non-literate, 43.5% were 1-6 grade, 18% were 7-12 grade and 9.1% were more than 12 grade. In their study illiterate accounted for 29.4% whereas in our study illiterate accounted for only 14%.

Hygiene standards

Studying the hygiene standards of the food handlers is necessary because, a good hygiene can reduce the transmission rate of various infections, especially enteric fever. In our study 98% of the food handlers washed their hands with soap and water after defecation which is similar to the study by Abera et al. [28] where the food handlers' hand washing practices after toilet was 90.6%.

Ninety four percent of the food handlers had their finger nails cut short and 96% of the food handlers wore gloves while cooking and serving. These practices show that food handlers had awareness about food contamination and good hygienic practices.

The *salmonella* carriage

Chronically infected hosts are often asymptomatic and transmit disease to naive hosts via fecal shedding of bacteria, thereby serving as a critical reservoir for disease. Asymptomatic carrier state is one of the clinical manifestations of *salmonella* infection. The carrier state is a major concern because of its asymptomatic nature and being a huge reservoir of infection. The *salmonella* carriage rate in our study was 0.4%. The *salmonella* serotype isolated from stool was *S.Typhi.* It was sensitive to chloramphenicol, ampicillin, cotrimoxazole, ciprofloxacin, ofloxacin, cefotaxime, ceftriaxone, gentamicin, imipenem, merupenem, aztreonam, cefaperazone/sulbactum, piperacillin/tazobactum and resistant to nalidixic acid. The food handler from whom *S.Typhi* was isolated was a 35 year old male with educational qualification upto 11th standard. He was working in the hostel as a cook cum server. He wore gloves while cooking and serving and washed hands with soap and water after toilet and had his fingernails cut short.

Study	Authors	Year of publication	Salmonella isolation rate
Prevalence of Salmonella carriers among Food Handlers and Detection of Drug Resistance of Isolates in Hamadan. J Res Health Sci	Yousefi-Mashouf et al. [23]	2003	1.88%
Salmonellae carrier status of food vendors in Kumasi, Ghana.East African Medical Journal	Feglo et al. [24]	2004	2.30%
Multidrug resistant salmonella typhi in asymptomatic typhoid carrier among food handlers in Namakkal district,TamilNadu. Indian Journal of Medical Microbiology	Senthilkumar and Prabakaran [25]	2005	16.66%
Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town,Northwest Ethiopia. J Health Popul Nutr	Andargie et al. [26]	2008	NIL
Antimicrobial susceptibilities of salmonellae isolated from food handlers	Smith et al. [27]	2009	S.Typhi-6.8%

and cattle in Lagos, Nigeria. Int J Health Res			
Prevalence of Salmonella typhi and intestinal parasites among food handlers in Bahir Dar Town, Northwest Ethiopia. Ethiop. J. Health Dev.	Abera et al. [28]	2010	S.Paratyphi A-1.5%
			1.60%
Prevalence and pattern of bacteria and intestinal parasites among food handlers in the Federal Capital Territory of Nigeria. Niger Med J	Ifeadike et al. [30]	2012	42.30%
Bacterial Profile and Antimicrobial Susceptibility Pattern among Food Handlers at Gondar University Cafeteria, Northwest Ethiopia. J Infect Dis Ther	Dagnew et al. [31]	2013	1.30%
A study of salmonella carriage among asymptomatic food handlers in southern Ethiopia. International Journal of Nutrition and Food Sciences	Misganaw and David [32]	2013	0.93%.
Study		Year of publication	Salmonella isolation rate
Prevalence of Salmonella carriers among Food Handlers and Detection of Drug Resistance of Isolates in Hamadan. J Res Health Sci	Yousefi-Mashouf et al. [23]	2003	1.88%
Salmonellae carrier status of food vendors in Kumasi, Ghana.East African Medical Journal	Feglo et al. [24]	2004	2.30%

Table 6: Regarding *salmonella* carrier status various literatures were reviewed. Literature showing various isolation rates.

The isolation rate of salmonella from the food handlers in our study is comparable to the study done by Misganaw B and David W [32] in 2013 who also assessed the asymptomatic carriage of Salmonella among the food handlers in southern Ethiopia, which is similar to our study. The salmonella carriage rate in their study was 0.93%. The isolation rate in our study (0.4%) is slightly less than their study (0.93%). This could be attributed to the type of participants, sample size, and the difference in the technique. The difference can also be due to better hygiene standards and level of education (37% of the food handlers had completed 1st and 2nd PUC and 4% were graduates). Our study demonstrated a higher rate of isolation than the study done by Andargie G et al. [26] in 2008, as their isolation rate was nil. The present study demonstrated a much lower prevalence of salmonella carriage as compared to the study done by Senthilkumar B and

Prabakaran G [25] in Namakkal district, Tamil Nadu, India, where the carriage rate was 16.66%. Again this huge difference could be due to the level of hygiene maintained by the food handlers as 98% of them washed their hands with soap and water after defecation. Moreover, 96% of the food handlers wore gloves while cooking and serving and 94% of them had their finger nails cut short. Many studies from other countries also documented a higher prevalence of salmonella carriage than the present study [23,24,27,28,30,31].

No. of *salmonella* isolates in the blood culture

In our study the predominant serotype obtained in blood culture was *S.Typhi* (63%), followed by S.Paratyphi A (30%), which agrees with the study by Mohanty et al. [63] where S. Typhi was also the predominant serotype (75.7%) followed by S.Paratyphi (23.8%). Similarly, V Lakshmi et al. [90] in their study, out of 80 *Salmonella* isolates, 60 were identified as *S.Typhi* and 20 were identified as S. Paratyphi A.

Antibiotic susceptibility pattern

Total number of *Salmonella* isolates recovered from blood culture in our study was 89, of which *Salmonella* Typhi was the commonest serotype (67%) isolated, followed by *Salmonella* Paratyphi A (33%). All (100%) the isolates were sensitive to chloramphenicol. Ninty three (97%) isolates were sensitive to ampicillin and ninety five (99%) isolates were sensitive to cotrimoxazole. Only five (5.2%) isolates were sensitive to nalidixic acid and 82% isolates were sensitive to ciprofloxacin and all (100%) isolates were sensitive to ceftriaxone. Twenty *S.Typhi* and twenty *S.Paratyphi* A were biotyped using L-Arabinose and D-Xylose. *S.Typhi* biotype III was most common.

Salmonella infection can manifest as a mere gastroenteritis to severe systemic infection (Enteric fever). Hence, immediate antibiotic therapy can prevent the disease progression and mitigate the disease morbidity and mortality. But the organism has become resistant to most of the conventional antibiotics and is rapidly gaining resistance to higher level antibiotics. In 1948, and

Since then chloramphenicol has been the drug of choice for enteric fever [43-44]. The drug worked wonders and reduced morbidity and mortality due to *salmonella* infection to a great extent. In 1950, the first Chloramphenicol-resistant *S.Typhi* was isolated in UK [43]. In India, first chloramphenicol-resistant of *S.Typhi* was reported from Kerala in 197245. In India, the first multi-drug resistant (resistance to chloramphenicol, ampicillin and cotrimoxazole) *S.Typhi* was reported in 199044. In the same year of 1990, Jesudason MV and John TJ [46], reported 13 isolates of *S.Typhi* which were multidrug resistant. Since then studies from many parts of India documented multidrug-resistant *S.Typhi* [47-50]. Because of the multidrug resistant the therapeutic options for treatment of enteric fever included fluoroquinolones such as ciprofloxacin or ofloxacin, and expanded-spectrum cephalosporins such as ceftriaxone. Prabhakar H et al. [52] documented 61.4% of *S.Typhi* isolates to be multidrug resistant. Another study from Manipal by Ciraj AM et al. [56] showed 57.9% of the isolates to be multidrug resistant. Yismaw G et al. [66] in 2007 had also documented multi - drug resistance was in 84.7% of the *salmonella* isolates which in comparison to the above studies is a huge number. But in the same year of 2007, Sen B et al. [67] documented only 14% MDR isolates. In 2012, Menezes G A, Harish B N [76], from Pondicherry, documented only 22% multidrug Resistant (MDR) isolates of *S.Typhi*. Although the above studies have documented Multidrug resistant isolates, but our study did not document any multidrug resistant *salmonella*. Though multidrug resistance among *salmonella* was rampant, there is a decreasing trend which shows that there is resurgence of sensitivity to these conventional antibiotics.

In our study only five (5.2%) isolates were sensitive to nalidixic acid and 63% isolates were sensitive to ciprofloxacin. Among the *S.Typhi* isolates in our study, 57% isolates were sensitive to ciprofloxacin and among *S.Paratyphi* A, 66% isolates were sensitive to ciprofloxacin. Nath G et al. [58] in 2000, documented only three ciprofloxacin resistant *S.Typhi* in their study. Chandel D S et al. [54] showed that 32% of the isolates had decreased susceptibility to ciprofloxacin. But Asna SM et al. [59] in 2003, in their study showed 100% sensitivity of *S.Typhi* isolates to ciprofloxacin, whereas in our study only 63% of the isolates were sensitive. Arora D et al. [74] in their study which was published in 2010, showed that 91% of the isolates were sensitive to ciprofloxacin which indicates an increasing trend in ciprofloxacin resistance, as in our study only 63% of the isolates were sensitive to ciprofloxacin.

Threlfall E J et al. [62] in their study showed that 49% of *S.Typhi* isolates were resistant to ciprofloxacin and 84% of *S.Paratyphi* A isolates were resistant to ciprofloxacin which is contradictory to our study where *S.Typhi* was more resistant to ciprofloxacin as compared to *S.Paratyphi* A.

In a study by Indian Network for Surveillance of Antimicrobial Resistance Group 85, the authors showed that only 8.3% of the *S.Typhi* isolates were sensitive to nalidixic acid, which is similar to our study. But Parry C et al. [53] documented 76% of *S.Typhi* isolates resistant to nalidixic acid. Similarly, Khanal B et al. [65] in their study also showed that 76% of the isolates were resistant to nalidixic acid, whereas in our study 94.8% of the isolates were resistant to nalidixic acid. *S.Typhi* was more resistant to nalidixic acid as compared to *S.Paratyphi* A. Similarly in another study 75% of the isolates were resistant to nalidixic acid [70]. Nagshetty et al. [73] from Gulberga University, Karnataka, showed 32% of the *S.Typhi* isolates were resistant to nalidixic acid. The authors suggest this as an indication of the emergence of Nalidixic acid-resistant S. Typhi that are resistant to ciprofloxacin [64].

Nalidixic acid resistance is a marker for predicting low-level resistance to ciprofloxacin among *S.typhi* and also an indicator of treatment failure to ciprofloxacin [90-92]. Hence, it is suggested that all *S.typhi* isolates should be screened for nalidixic acid resistance along with ciprofloxacin. Any isolate that shows resistance to nalidixic acid should be reported as intermediately susceptible to ciprofloxacin. The clinician should be advised to change the antibiotic [90].

In this era of fluoroquinolone resistance, there is re-emergence of sensitivity to chloramphenicol [72]. In our study all (100%) the isolates were sensitive to chloramphenicol which is similar to the study done by Neopane A et al. [70] where the author documented 100% sensitivity to chloramphenicol Gupta et al. in Chandigarh showed that 90% of the isolates were sensitive to chloramphenicol. In another study done by Takkar et al. [51] showed an increasing sensitivity of *S.Typhi* isolates to chloramphenicol. V Lakshmi et al. [90] in their study also showed an increase in sensitivity to chloramphenicol, being 60% in 2003 to 80% in 2004.This resurgence could be due to the restricted use of the antibiotic [51]. The less we use a particular drug, the probability of the organism becoming sensitive to the drug increases.

Apart from chloramphenicol, there is re-emergence of sensitivity to other first line antibiotics (ampicillin and cotrimoxazole) also. In our study, 97% of the isolates were sensitive to ampicillin and 99% of the isolates were sensitive to cotrimoxazole. Ironically, only 63% of the isolates were sensitive to ciprofloxacin. In 2000, Nath et al. [58] showed that resistance to chloramphenicol gradually reduced over the years with 50% in 1979 -1989, 54% in 1990-1998 and 31% in 1998. The pattern was similar for co-trimoxazole. P M Krishnan et al. [93] in their study also documented 86% sensitivity to chloramphenicol, 84% sensitivity to ampicillin and 88% sensitivity to cotrimoxazole.

This suggests re-emergence of chloramphenicol and co-trimoxazole sensitive strains [58].

Ceftriaxone MIC

MIC for ceftriaxone was done for all the 96 blood isolates and the single stool isolate. For *S.Typhi* the MIC (ceftriaxone) fell between 0.03-0.125 μg/ml. For *S.Paratyphi* A the MIC ranged from 0.06 to 0.125 μg/ml and for *S.Typhimurium* the MIC was between 0.06-0.125 μg/ml. The MIC of the single stool isolate was 0.125 μg/ml.

When fluoroquinolone resistance emerged, third generation cephalosporins (ceftriaxone) became the drug of choice. But resistance to this drug is also emerging. In 1999, Saha et al. [57], documented a single ceftriaxone resistant Salmonella *Typhi*. In our study all (100%) the isolates were sensitive to ceftriaxone which is similar to the results obtained by Lin-Hui Su et al. [61] all the typhoidal salmonellae were sensitive to ceftriaxone. But in contrast to my study, Neopane et al. [70] documented two ceftriaxone resistant isolates of salmonella. Shetty et al. [81], in their study, documented a single isolate of S. Paratyphi A which was resistant to ceftriaxone. Hence, a constant check has to be kept on antibiotic susceptibility pattern as ceftriaxone resistance is coming up.

In a study by Gopal et al. [87] the MIC for ceftriaxone for S.Typhi ranged from 0.25 μg/ml to 0.125 μg/ml whereas in our study MIC for S.Typhi ranged from 0.03 to 0.125 µg/ml. By the MIC studies the strains are completely sensitive to ceftriaxone. Similarly in a study by S Qaiser et al. [89] the MIC of S.Typhi isolates ranged from 2 µg/ml to 0.015 µg/ml. In their study all were sensitive but three isolates showed a higher MIC value (2 µg/ml).

Bio-typing

In the present study, 16 isolates of *S.Typhi* belonged to biotype III. The faecal isolate also belonged to biotype III. In a study by P M Krishnan et al. [93], the authors showed that, *S.Typhi* biotype I was most common in Chennai. U. Madhulika et al. [96], from Pondicherry also found *S.Typhi* biotype I was the most common biotype.

Biofilm

We studied biofilm forming capacity among faecal and clinical isolates of *S.Typhi.* We found 31.25% of the clinical isolates were strong producers of biofilm, 43.75% were moderate and 25% were weak producers. The single faecal isolate was a weak producer. *Salmonella* carrier state is thought to be related to biofilm production on gall stones. Increased biofilm formation has been observed in patient faecal samples. Cholecystectomy is used to treat human carries, because *salmonellae* have been shown to produce biofilms on gall stones and in bile [38].

In this study, the faecal isolate from the healthy carrier did not produce a strong biofilm. This could be possible because the study was done in vitro and may not mimic conditions in vivo which may be much more conducive for biofilm formation. It has been shown that biofilm formation is bile dependent, with bile acting as a signal for biofilm formation to occur. Biofilm formation also depends on the medium used, presence of flagellae, presence of gall bladder stones and quorum sensing ability [11]. It has also been shown that the Vi capsular polysaccharide antigen of *S.Typhi* does not play a role in biofilm production. Furthermore, as we had only a single faecal isolate to perform the test in this study, we could not draw a statistically significant conclusion. Previous workers have shown that there was a direct correlation between biofilm production capability and the duration of *S.Typhi* clearance from typhoid patients. In this study, we attempted to find out whether biofilm producing capability was related to the no. of days required for defervescence after initiation of specific anti typhoidal treatment.

Among our 16 isolates defervescence ranged from 2-7 days. We did not find any correlation between biofilm formation and time required for defervesence.

RAPD

Genomic diversity among microorganisms is now widely being studied by molecular techniques. RAPD is a rapid typing method based on random amplification of polymorphic DNA segments. Here, a short oligonucleotide of arbitrary sequence is used to prime DNA synthesis by accessing random segments of genomic DNA at low stringency to reveal polymorphism. The bands thus generated produce a genetic fingerprint of the genomic composition of the organisms. Prior knowledge of nucleotide sequence of the organism is not necessary for this molecular method.

In this study we used RAPD for genetic analysis with an attempt to find strain similarities/differences between clinical isolates of *S.Typhi* (from blood culture) and the faecal isolate from healthy carrier.

Conclusion

Among the 16 clinical isolates, 6 different RAPD pattern were seen. The faecal isolate had a totally different pattern which did not match any of the clinical isolates of *S.Typhi.* This probably indicates some genetic differences between clinical *S.Typhi* isolates and isolates from healthy carriers, but due to small number (only one) of faecal isolates, we could not arrive at a definitive conclusion. Furthermore, we used only one primer for this study. Larger studies, with more numbers of isolates and with multiple primers should be done to arrive at a statistically significant conclusion.

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