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Prevalence of Antimicrobial Sensitivity and Phenotypic Detection of *Salmonella Enterica serovar typhi* in and around Chennai, Tamilnadu, - India

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ABSTRACT

The aim of the study was to evaluate the occurrence and diversity of multi drug resistant salmonella *enteric serovar typhi* existing in and around Chennai, India. Drug resistivity of isolates was carried out from the sample which was collected from the patients those are suspected to suffer from typhoid fever. A total of 2423 samples have been collected from various hospitals and organizations in and around Chennai. As soon as the sample collection all the isolates were cultured using various nutrient media to evaluate the cultural characteristics of the isolates followed by preliminary tests and biochemical tests using standard identification procedure to confirm the isolates for *salmonella enterica serovar typhi*. The total of 105 out of 2423 clinical isolates was confirmed for *salmonella enterica serovar typhi* and these isolates were further undergone to various procedures like Bio typing, Phage typing and serological tests (Agglutination) using standard identification procedure for phenotypic characterization. For antibiotic resistivity pattern an *in-vitro* test procedure using Kirby-Bauer method was conducted for the identified isolates using various antibiotics with different strengths. The specimens were collected from various age groups lying between <1 to 70. Outcome of the study shown that degree of *salmonella enterica serovar typhi* infection was more in 1-10 years (40%) followed by 11–20 (20%) and 21–30 (15%). In conclusion, the continuous surveillance of the development of resistivity is essential to alter treatment strategies aimed at maintaining that the useful life of the few remaining antimicrobials available to treat typhoid fever.

Keywords: *Salmonella enteric serovar typhi*, Multidrug resistance (MDR), Serotyping, Phage typing, Bio typing, Disc Diffusion Technique.

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INTRODUCTION

As per world health organization 16 to 33 million cases of typhoid fever are estimated every year and in that 5 to 6 lakhs deaths are occured[1]. In the year of 2000, it was estimated that over 2.16 million episodes of typhoid occur worldwide, resulting in 2,16,000 deaths, and that more than 90% of this morbidity and mortality occurred in Asia[2]. The mortality rate is mainly due to development of multi drug resistant strains, a naturally occurring phenomenon to microbes. The factors which leads to its resistivity's are severe use of broad spectrum drugs, sudden changes in its gene sequences, improper diagnosis and root cause analysis for infection, using medicaments without proper treatment from the less experienced medical practitioner and doctors, moreover un awareness and not considering the side effect which caused by the drugs to the patients who are using it. The ratio of resistant is gradually increased for the following extended spectrum antibiotics like cephalosporins and fluoroquinolones[3]. Sudden changes in domain of gyrA gene sequence which led to point mutation of *Salmonella enterica serovar typhi* which made the infection causing microorganism to became a resistance towards the antibiotic like fluoroquinolone. The resistance ability of antibiotics like cephalosporins is mediated by broad spectrum β-lactamase enzymes[4]. The aim of the research was to evaluate the prevalence of typhoid infection in and around Chennai, India. The identified microorganisms were completely studied for its phenotypic characterization including their susceptibility to commonly available drugs irrespective to sex and the age group of people.

MATERIALS AND METHODS

Sample Collection

A total of 2423 clinical samples were collected from patients those suspected for typhoid infection visiting to various hospitals, primary health care centers and clinical laboratory from in and around Chennai. Samples like urine and blood were collected and analyzed for colonial morphology and biochemical identification. The morphological characterization of isolates was carried out according to standard identification procedures[5]. The samples were sub-cultured on blood agar, Nutrient agar, MacConkey agar and Eosine Methylene Blue (EMB) agar plates using simple streaking techniques and the plates were incubated at 37˚C for 2 days at inverted position. Staining of isolates was done as per the standard identification procedure[6] to identify whether the isolates are gram positive or gram negative.

Biochemical tests

Biochemical tests like[5] IMViC, TSI, Oxidase, Catalase, Urease and Fermentation test for various sugars and Decarboxylase tests were performed.

Biotyping

Biotyping is now rarely used in many laboratories and it has been superseded by a range of DNA based molecular methods. *Salmonella enterica serovar typhi* divided into four biotypes - I, II, III, IV based on the fermentation of D– xylose and L– arabinose[7]. Biotype I is the most common among the four biotypes[8].

Serological characterization (Agglutination)

Serological characterization was carried out as described by Hendriksen and Larsen method[9] and with some modification. Loopful of culture was placed at four places on slide and the following antisera were added to them. O9, O12, Vi, Hd. Mixed gently for two minutes and observed for positive and negative reaction. Mixed Poly O was placed on other slide and added with one drop of culture to serve as a positive and saline was placed on other side of slide to serve as a Negative Control.

Phage Typing

Phage typing of the clinical isolates was done as described by Anderson and Williams[10]. *Salmonella enterica serovar typhi* isolates were inoculated into 2ml of Difco broth to give a barely visible and incubated at 38.5^0C with shaking until the opacity were equivalent to approximately 5X10 organisms/ml and it attained in about two and a half hours. Standard loops with 3mm internal diameter were used throughout. One loopful of the incubated broth culture of the test strain was distributed to each of the various sections of the plate evenly spread into circles of about 1cm. The inoculated areas were allowed to dry and the typing phages were spotted on them with the standard loop without spreading. The drops of the phages were allowed to dry and the plates were incubated at 38.5^0C. First reading was taken after seven hours and the second reading was taken after 24 hours.

Antibiotic Sensitivity Test

Antibiogram resistogram pattern was performed on 105 clinical isolates of *Salmonella enterica serovar typhi* using Kirby Bauer technique. Muller-Hinton Agar (MHA) was used as said by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). Loopful of culture was taken from plate using sterile loop, with the help of sterile peptone water inoculum concentration of (1.5×10^8cfu/ml) was prepared which match with 0.5 McFarland turbidity standards using densi meter. With the help of sterile cotton swab cultures were spreaded on the surface of Mueller Hinton Agar plate and allowed for drying under the biosafety cabinet. Using antibiotic

disc dispenser antibiotics discs were placed onto the surface of the inoculated agar plates. The plates were incubated inverted position at 37˚C for 24 hours. With the help of zone reader the diameters of zone of inhibition were measured in millimeters after 24 hours of incubation period[11]. The susceptibility of isolates to various strength of antibiotics was done using tube dilution method to calculate the Minimal Inhibitory Concentration (MIC) as per clinical laboratory standards institute (CLSI, 2007). *Escherichia coli* ATCC 25922 were used as a standard strain.

RESULTS AND DISCUSSION

In olden days high degree of antibiotics spread where *Salmonella enterica serovar typhi* became a significant pathogen. Studies carried on important areas like isolation, identification and monitoring their susceptible patterns, which became major issues for the management of the infections caused by *Salmonella enterica serovar typhi*, before they are considered as severepathogen.[12] In the present study2423 clinical samples were processed, finally 105 isolates were found to be biochemically and serologically positive for *salmonella enterica serovar typhi*. The prevalence of typhoid infection found higher rate in outer Chennai area (10%) and comparatively lower degree in south portion of Chennai (3%). Due to increasing number of industries, inadequate treated water facility, improper drainage facility and low health consciousness the number of positive cases is high in urban outer areas which shown in Table1. Also the study reveals that the age groups of 01 to 10 years are more prone to get typhoid fever because they are not much developed in immunity, unawareness & unhygienic were the similar results was confirmed in another study were the rate of infection is high in children aged 5 to 12 years and 24.8% of cases were in children up to 5 years of age[13]details showed in Table 2. The biochemical tests are carried out and showed it in Table 3. Also the study reveals that 105 isolates are belonging to Biotype-1 by using of D-Xylose and L-Arabinose and it was showed in Table 4. On the basis of serological characterization we confirmed 100% isolates agglutinated with O9, O12, Hd and Vi-antisera antisera where as 92.97% isolates agglutinated with ‘Vi’ antiserum. 7.03% isolates were reported as Vi-negative. The results are showed in Table 5. On the basis of phage typing we confirmed that the proportions of different phage types are found range from 1% to 77%. Among the different type of phage, Phage type E1 was found in maximum portion followed UVS, A, E9, D8 & D2. These findings are concordance with different parts of the country[14]. The present study reports emergence and circulation of two new phage types (D2 and D8) which never been reported earlier. The results are showed in Table 6.

The 105 isolates were further subjected to antibiogram resistogram pattern using commercially available 11 antibiotics were tested based up on the Kirby-Bauer methods and the percentage of resistance to antibiotics was found Ampicillin (13%), Cephotaxime (5%), Tetracycline (40%), Cefuroxime (7%), Chloramphenicol (1%), Ciprofloxacin (85%), Nalidixic acid (94%), Streptomycin (10%), Trimethoprim (10%), Gentamycin (4%), and Kanamycin (10%). From these observations, it was noticed that the percentage of MDR strains sensitive to Chloramphenicol 98% and Cefuroxime 92% respectively. The total percentage of MDR strains were 5% from the total 2423 samples were observed. The 99 isolates out of 105 confirmed for (94%) of resistant to Nalidixic acid followed by fluoroquinolone drug Ciprofloxacin which shows (85%) of resistance. Similar observation was reported in another study were the Nalidixic acid resistance is an indirect marker of fluoroquinolone resistance of *Salmonella Enterica Serovar Typhi*[15]. Chloramphenicol (98%) and Cefuroxime (92%) which exhibits effective against isolated strains. The results are compared with Dr. ALM PGIMBS, university of Madras, Out of 176 *Salmonella Enterica Serovar typhi* isolates, (96%) were resistant to Nalidixic acid; increased sensitivity was reported for chloramphenicol (97.5%) Nalidixic acid showed only (2%) sensitivity by disc diffusion however cefuraxime showed 96% sensitivity[16]. The screening of Antibiotic sensitivity test was showed in Figure 1 and Table 7. The findings may be of immense importance to aid health care authorities to rationalize the policy of empirical treatment of typhoid fever. Looking at the challenging trends of antibiotic susceptibility of *Salmonella enterica serovar typhi* in addition to the endemic city of typhoid fever in India periodic survey needs to be done in antibiogram patterns.

Table1: Prevalence of *Salmonella Enterica Serovar Typhi* in Different Regions

S. No.	Locations	Total number of clinical sample	Total number of positively reacted samples	Percentage of positively reacted samples (%)
1	East Chennai	520	24	05
2	West Chennai	728	27	04
3	South Chennai	444	12	03
4	North Chennai	480	18	04
5	Chennai outer	251	24	10
Total		2423	105	

Table 2: Age Wise Distribution *of Salmonella Enterica Serovar Typhi* Strains

S. No.	Age	Number of isolates	Percentage of isolates (%)
1	Less than one year	1	1
2	1-10	42	40
3	11-20	21	20

4	21-30	16	15
5	31-40	10	10
6	41-50	8	8
7	51-60	5	5
8	61-70	2	2

Table3 : Morphological and Biochemical characteristics of *Salmonella enterica serovar Typhi*

S. No.	Characteristics	Observations
1.	**Morphology**	Gram Negative bacilli
	Gram staining	Positive
	Motility	
2.	**Fermentations tests**	
	Glucose (a/g)	+/-
	Lactose	-
	Sucrose	-
	Mannitol	+
3.	**Substrate utilization**	
	Citrate	-
	Triple sugar iron	H_2S ±ve
4.	**Decarboxylse test**	
	Lysine	+
	Arginine	-
	Ornithine	-
5.	**Enzyme tests**	
	Urease	-
	Catalase	+
	Oxidase	-
6.	**Miscellaneous tests**	
	Indole	-
	Methyl-red	+
	Voges-proskauer	-
	Cragie	+

Table4: Biotyping of *Salmonella Enterica Serovar Typhi*

S. No.	Sugars used	Observation	I	II	III	IV
1	D - Xylose	Positive	105	0	0	0
2	L-Arabinose	Negative				

Table5: Serological Characterization of *Salmonella Enterica Serovar Typhi*

Culture number	Observations
1 to 105	Agglutination with antiserum ‘ O9’ - positive Agglutination with antiserum ‘ O12’ - positive Agglutination with antiserum ‘ vi’ - positive Agglutination with antiserum ‘ Hd’ - positive

Table6: Phage Typing of *Salmonella Enterica Serovar Typhi*

S. No.	Phage type	Number of isolates	Percentage of isolates (%)
1	E1	81	77
2	UVS	14	13
3	A	4	4
4	E9	4	4
5	D8	1	1
6	D2	1	1

Table7: Antibiogram Resistogram Pattern of*Salmonella Enterica Serovar Typhi* Strains

S. No.	Antimicrobial agent	Sensitivity no. (%)	Intermediate no. (%)	Resistant No. (%)
1	Ampicillin (A)	86(82)	5(5)	14(13)
2	Cephataxime (Ce)	81(77)	19(18)	5(5)
3	Tetracycline (T)	58(55)	5(5)	42(40)
4	Cefuroxime (Cu)	97(92)	1(1)	7(7)
5	Chloramphenicol (C)	103(98)	1(1)	1(1)
6	Ciprofloxacin (Cf)	12(11)	4(4)	89(85)
7	Nalidixic Acid (Na)	6(6)	0(0)	99(94)
8	Streptomycin (S)	63(60)	32(30)	10(10)
9	Trimethoprim (Tr)	95(90)	0(0)	10(10)
10	Gentamycin (G)	90(86)	11(10)	4(4)
11	Kanamycin (K)	84(80)	11(10)	10(10)

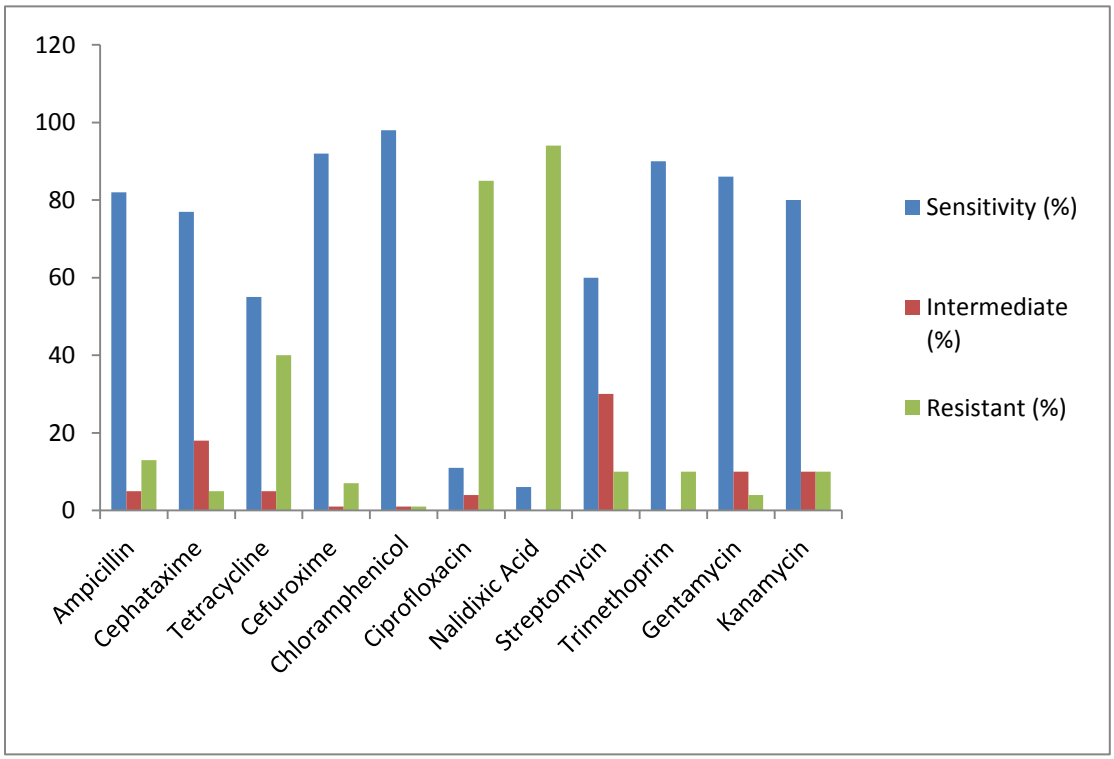

Figure 1: Antibiogram pattern of anti-microbial agents

CONCLUSION

In conclusion the present study clearly explained that, most of the isolates are sensitive to Chloramphenicol, Cefuroxime, Ampicillin, Gentamycin and Trimethoprim and exhibits resistance against Nalidixic acid and Ciprofloxacin. The results are highly comparative with earlier other similar studies and also results of phage typing shows that (E1, UVS, A, E9, D8, D2) the emergence and circulation of two new phage types (D2 and D8) which never been reported earlier. Routine collaborative typing studies have to conduct to elucidate current trend of circulating phage types in country along with their relationship with antimicrobial resistance. Continuous surveillance of the development of resistivity it is essential to alter treatment strategies aimed at maintaining that the useful life of the few remaining antimicrobials available to treat typhoid fever.

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