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Original Article

Determination of Drug Resistant Patterns of *Salmonella spp* from clinical samples

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Abstract

Salmonella typhi, *Salmonella typhimurium* and *Salmonella paratyphi A* strains were isolated from 90 blood and 55 faecal samples of suspected typhoid fever and gastroenteritis patients. Antibiogram pattern of all the isolates against eight antibiotics such as Ampicillin, Cefuroxime, Chloramphenicol, Ciprofloxacin, Streptomycin, Tetracycline, Ceftriaxone and Ofloxacin were assessed by Kirby-Bauer disc diffusion method. A significant number of strains exhibited Multiple Drug Resistance (MDR) *viz*., 22 strains of *S. typhi*, 15 strains of *S. typhimurium* and 2 strains of *S. paratyphi A.* Among the 60 isolates of *S. typhi*, the prevalence of resistance to antimicrobials were; Ampicillin (91.66%), Cefuroxime (60%), Tetracycline (30%), Ceftrioxone (30%), Streptomycin (26.66%), Chloramphenicol (5%), Ciprofloxacin (6.66%) and Ofloxacin (3.33%). *S.typhimurium* also exhibited similar kind of drug resistant pattern. Different MDR patterns of *S. typhi* and *S. typhimurium* were noticed. Of the 105 isolated *Salmonella* strains, 21.9% exhibited resistance against one antibiotic, 40% against two antibiotics, 26.66% against three antibiotics and 11.42% against four antibiotics. Overall 38.09% of the *Salmonella* isolates are exhibiting MDR.

Key words: Prevalence, Salmonellae, Multi Drug Resistance, Antibiogram.

Introduction

Salmonella spp are Gram negative enteric bacilli that cause typhoid fever and gastroenteritis. Salmonellosis is one of the most common and widely distributed food-borne illnesses. Millions of human cases are reported worldwide every year resulting in thousands of deaths (Groisman, 2001). Typhoid fever caused by *S. typhi* and *S. paratyphi* is an important and persistent health problem in developing countries. In recent years, problems related to *Salmonella* have increased significantly. Antibiotic resistance of *Salmonella* has magnified the public health problem (Oreste and Mascaretti, 2003*).* It is estimated that ever year there are approximately 21.6 million cases of typhoid fever which result in 2 lakh deaths world wide (Curtis and Wheeler, 2006). Antibiotics such as Chloramphenicol, Ampicillin and co-trimoxazole were used as first line therapeutic drugs (Cooke and Wain, 2004). As Salmonella developed resistance to these antibiotics, Ciprofloxacin and Ceftrioxone were used as second and third generation drugs. More over the rate of resistance is increasing year by year. This situation leaves behind the fourth generation Quinalones as therapeutic option. But, the day is not far when wide spread resistance to these agents. Therefore, the present study is focused to evaluate the scenario of the drug resistance among *Salmonella* spp from clinical samples over a period of 2 years.

Materials and Methods

In the present work, all the clinical samples were collected before treatment from four towns namely Erode, Karur, Namakkal and Salem. Blood samples were collected aseptically by vein puncture and inoculated in MacConkey biphasic medium and the faecal samples were inoculated in Selenite-F broth medium. After incubation at 37°C for 48 hours, subcultures were done on Bismuth Sulphite Agar and MacConkey agar. Each isolate was identified by standard morphological, physiological and biochemical characterization methods specified in Bergey's manual of determinative bacteriology. All the isolates were serologically confirmed with antiserum obtained from King Institute of Preventive Medicine, Chennai. Some strains were serotyped by CRI, Kasauli, Himachal Pradesh.

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Antibiogram of all the isolated strains were tested by Kirby-Bauer disc diffusion method (1966) as per the recommendations of National Committee for Control Laboratory Standards. *Escerichia coli* ATCC 25922 was used as a control strain. The following eight antibiotics (Himedia) were used namely Ampicillin (10µg), Cefuroxime (30µg), Chloremphenicol (30µg), Ciprofloxacin (10µg), Streptomycin (10µg), Tetracycline (30µg), Ceftrioxone (30µg) and Ofloxacin (10µg) for the present study. Muller-Hinton agar medium was used to check the antibiogram pattern.

Results

The isolated strains were identified by standard biochemical characters and confirmed serologically using anti sera for the collected 90 blood samples and 55 faecal samples. On processing 145 specimens, 105 *Salmonella* strains were isolated. Sixty strains of *S.typhi* and 5 strains of *S. paratyphi A* were obtained from blood specimen. Fourty *S. typhimurium* strains were isolated from the specimen faeces (Table-1). Age and sex wise perspective of *Salmonella* indicated that out of 105 positive cases, 74 were males , 31 were females and adults more than 21 years of age were highly infected (67/105) than children below 12 years (38/105). Therefore, the incidence of *Salmonella* is higher among adult male population. The percentage of age and sex wise distribution of *Salmonella* infection is represented in Table 2.

Table-1: Incidence of *Salmonella* strains from clinical samples

S. No.	*Salmonella* isolates	No of incidence	Percentage
1	*S. typhi*	60	57.14
2	*S. paratyphi A*	05	04.76
3	*S. typhimurium*	40	38.09

Table-2: Age and Sex wise distribution of *Salmonella* infections

Samples	Male in percentage		Female in percentage	
	Postive Adults (>12 years)	Positive Children (<12 years)	Positive Adults (>12 years)	Positive Childre n (<12 years)
Blood (90 samples)	50.76	23.07	16.92	9.23
Faeces (55 samples)	37.50	27.50	20.00	15.00
Total (145 samples)	45.70	24.76	18.09	11.42

Table-3: Antibiotic Sensitivity / Resistant patterns of isolated 105 *Salmonella* strains

S.No	Antibiotic	*S.typhi* Percentage			*S.typhimurium* Percentage			*S.paratyphi A* Percentage		
		S	I	R	S	I	R	S	I	R
1	Ampicillin(A)	6.66	1.66	91.6	7.5	-	92.5	20.0	-	80.0
2	Cefuroxime(Cu)	28.33	11.66	60.0	7.5	17.5	75.0	-	20.0	80.0
3	Ceftriaxone(Ci)	73.33	23.33	3.33	65.0	30.0	5.0	100	-	-
4	Chloremphenicol(C)	88.33	6.66	5.0	80.0	15.0	5.0	60.0	40.0	-
5	Ciprofloxacin(Cf)	86.66	6.66	6.66	80.0	15.0	5.0	40.0	60.0	-
6	Ofloxacin(O)	90.00	6.66	3.33	80.0	7.5	12.5	60.0	40.0	-
7	Streptomycin(S)	45.00	28.33	26.66	37.5	20.0	42.5	20.0	20.0	60.0
8	Tetracycline(T)	50.00	20.00	30.0	57.5	17.5	25.0	20.0	40.0	40.0

R – Resistant; S – Sensitive; I -Intermediate

Table- 4: Summary of Antimicrobial resistance profile of *Salmonella* strains (105)

No. of Antibiotics resistance of isolates	% of strains showing resistance
One	21.9 %
Two	40 %
Three	26.66 %
Four	11.42 %
Five & above	NIL

Antimicrobial sensitivity/resistance was interpreted according to National Committee for Control Laboratory Standard recommendations. *S.typhi* demonstrated high degree of resistance towards Ampicillin(91.66%), Cefuroxime(60%), Tetracycline(30%) and Streptomycin(26.66%). Least resistance was noticed against Chloremphenicol, Ciprofloxacin and Ofloxacin at the rate of 5%, 6.66% and 3.33% respectively. *S.typhimurium* exhibited high resistance towards Ampicillin (92.5%), Cefuroxime (75%), Streptomycin (42.5%) and Tetracycline (25%). Low resistance was noticed towards Ceftrioxone,

Chloremphenicol, Ciprofloxacin and Ofloxacin. (Table- 3). Table 4 shows a detailed resistance pattern to antimicrobial agents. Starins susceptible to all antibiotics became less common. The proportion of the isolates resistant to multiple antibiotics has been considerably increased. The results show that above 38% of the salmonella isolates were multiple drug resistant i.e., resistant to three or more antibiotics. Among MDR isolates 22 strains of *S.typhi* (36.66%), 15 strains of *S.typhimurium* (37.5%) and 2 strains of *S.paratyphi A* were showed in Table 5, 6 and 7) Drug resistant patterns in isolated strains of *S.typhi* and *S.typhimurium* were studied and shown in Table 8. Five different drug resistant patterns were identified in *Salmonella typhi* (Pattern A- ACuST, Pattern B- ACuS , Pattern C- AST, Pattern D- ACuT and Undetermined Pattern) among which pattern B was predominant. Same patterns were exhibited by *S. typhimurium* but the pattern was undetermined.

Table 5 : Antibiogram Pattern of MDR Clinical isolates of *Salmonella typhi*

Isolate No	Antibiotics with Diameter of Zone of inhibition in mm									
	Ampicillin	Cefuroxime	Ceftrioxone	Chloremphenicol	Ciprofloxacin	Ofloxacin	Streptomycin	Tetracycline	No. of Resistance	Pattern
ST02	R (10mm)	R (13mm)	R (11mm)	S (23mm)	S (23mm)	S (21mm)	R (10mm)	S (20mm)	4	UD
ST10	R (12mm)	R (13mm)	S (22mm)	S (23mm)	S (22mm)	S (19mm)	R (11mm)	I (17mm)	3	B
ST11	R (12mm)	R (12mm)	S (21mm)	R (12mm)	I (20mm)	S (21mm)	S (20mm)	R (13mm)	4	UD
ST13	R (11mm)	R (14mm)	S (23mm)	S (22mm)	S (21mm)	S (17mm)	I (16mm)	R (14mm)	3	D
ST17	R (13mm)	R (13mm)	S (22mm)	S (22mm)	S (23mm)	I (15mm)	R (12mm)	R (12mm)	4	A
ST18	R (11mm)	R (14mm)	S (22mm)	S (23mm)	I (18mm)	S (20mm)	R (14mm)	R (12mm)	4	A
ST20	R (12mm)	R (12mm)	S (24mm)	S (25mm)	I (17mm)	I (14mm)	R (14mm)	I (16mm)	3	UD
ST32	R (11mm)	I (16mm)	S (23mm)	S (24mm)	R (14mm)	I (14mm)	I (16mm)	R (13mm)	3	UD
ST34	R (11mm)	R (10mm)	S (21mm)	S (23mm)	S (21mm)	S (20mm)	I (17mm)	R (11mm)	3	D
ST36	R (13mm)	R (13mm)	S (24mm)	S (22mm)	S (20mm)	S (23mm)	R (12mm)	R (14mm)	4	A
ST40	R (12mm)	S (19mm)	S (21mm)	S (23mm)	S (21mm)	I (15mm)	R (13mm)	R (14mm)	3	C
ST41	R (11mm)	R (12mm)	S (22mm)	S (22mm)	I (17mm)	S (18mm)	R (14mm)	I (17mm)	3	B
ST43	R (13mm)	R (12mm)	S (22mm)	S (21mm)	S (22mm)	S (20mm)	R (12mm)	I (15mm)	3	B
ST44	R (13mm)	R (14mm)	S (22mm)	S (22mm)	S (23mm)	S (20mm)	R (12mm)	S (20mm)	3	B
ST45	R (12mm)	R (14mm)	S (24mm)	S (21mm)	S (26mm)	S (21mm)	S (21mm)	R (14mm)	3	UD
ST47	R (12mm)	R (13mm)	S (25mm)	S (21mm)	S (27mm)	R (11mm)	S (20mm)	S (23mm)	3	UD
ST48	R (12mm)	R (13mm)	S (23mm)	S (23mm)	S (21mm)	S (19mm)	R (12mm)	S (19mm)	3	B
ST50	R (13mm)	R (13mm)	S (24mm)	S (23mm)	S (24mm)	S (25mm)	R (14mm)	R (13mm)	3	A
ST53	R (11mm)	R (14mm)	S (23mm)	S (26mm)	S (21mm)	S (22mm)	S (20mm)	R (22mm)	3	D
ST55	R (13mm)	R (11mm)	S (23mm)	S (22mm)	S (21mm)	S (22mm)	I (15mm)	R (16mm)	3	D
ST57	R (12mm)	R (12mm)	I (18mm)	I (14mm)	I (18mm)	S (16mm)	R (13mm)	S (21mm)	3	B
ST58	R (13mm)	R (12mm)	S (22mm)	S (23mm)	S (22mm)	S (19mm)	R (13mm)	S (20mm)	3	B

UD- Undetermined pattern; ST- Strain identification code; R – Resistant; S – Sensitive; I -Intermediate

Table - 6: Antibiogram Pattern of MDR clinical isolates of *Salmonella typhimurium*

Isolate No	Antibiotics with Diameter of Zone of inhibition in mm									
	Ampicillin	Cefuroxime	Ceftrioxone	Chloramphenicol	Ciprofloxacin	Ofloxacin	Streptomycin	Tetracycline	No. of Resistance	Pattern
STM01	R (12mm)	R (11mm)	S (22mm)	S (19mm)	S (22mm)	S (16mm)	R (13mm)	S (20mm)	3	B
STM06	R (12mm)	R (12mm)	S (25mm)	S (20mm)	S (21mm)	S (18mm)	R (12mm)	S (19mm)	3	B
STM09	R (10mm)	R (13mm)	I (18mm)	S (26mm)	S (21mm)	R (12mm)	S (26mm)	S (19mm)	3	UD
STM10	R (13mm)	R (14mm)	S (22mm)	S (21mm)	S (22mm)	S (21mm)	R (14mm)	S (20mm)	3	B
STM12	R (11mm)	R (13mm)	S (23mm)	S (23mm)	S (23mm)	R (13mm)	I (17mm)	R (12mm)	4	UD
STM13	R (11mm)	R (14mm)	R (13mm)	S (21mm)	S (21mm)	S (21mm)	R (14mm)	I (16mm)	4	UD
STM14	R (12mm)	R (13mm)	I (18mm)	I (16mm)	S (22mm)	S (17mm)	R (13mm)	R (13mm)	4	A
STM16	R (13mm)	R (12mm)	I (17mm)	I (17mm)	S (23mm)	S (21mm)	R (12mm)	R (14mm)	4	A
STM19	R (10mm	R (12mm)	I (18mm)	S (19mm)	S (21mm)	I (14mm)	R (11mm)	I (17mm)	3	UD
STM21	R (11mm)	R (14mm)	S (21mm)	S (20mm)	S (24mm)	S (16mm)	S (18mm)	R (12mm)	3	C
STM23	R (11mm)	R (12mm)	R (14mm)	S (20mm)	S (23mm)	S (18mm)	S (23mm)	I (17mm)	3	UD
STM31	R (12mm)	R (11mm)	S (21mm)	S (21mm)	R (14mm)	S (24mm)	S (22mm)	R (13mm)	4	UD
STM33	R (13mm)	R (13mm)	S (24mm)	R (12mm)	I (19mm)	S (23mm)	I (15mm)	I (15mm)	3	UD
STM37	R (10mm)	R (13mm)	I (17mm)	I (16mm)	S (23mm)	S (20mm)	R (11mm)	R (12mm)	4	A
STM39	R (13mm)	R (13mm)	S (22mm)	S (25mm)	S (25mm)	S (20mm)	R (12mm)	S (20mm)	3	B

UD- Undetermined pattern; STM- Strain identification code; R – Resistant; S – Sensitive; I –Intermediate

Table 7: Antibiogram Pattern of MDR clinical isolates of *Salmonella paratyphi A*

Isolates No	Antibiotics with Diameter of Zone of inhibition in mm									
	Ampicillin	Cefuroxime	Ceftrioxone	Chloremphenicol	Ciprofloxacin	Ofloxacin	Streptomycin	Tetracycline	No. of Resistance	Pattern
SPTA3	R (11mm)	R (13mm)	S (25mm)	I (18mm)	S (21mm)	I (17mm)	R (12mm)	R (12mm)	4	A
SPTA5	R (12mm)	R (13mm)	S (25mm)	S (21mm)	I (17mm)	I (18mm)	R (10mm)	I (17mm)	3	UD

UD- Undetermined pattern; STPA- Strain identification code; R – Resistant; S – Sensitive; I -Intermediate

Table -8 : Drug Resistance patterns of MDR *Salmonella* isolates.

S.No.	Antibiotic Resistance	Antibiotyping								No. of Strains	
		A	Cu	Ci	C	Cf	O	S	T	*S. typhi*	*S. typhimurium*
1	Pattern A (A,Cu,S&T)	R	R	S	S	S	S	R	R	4	3
2	Pattern B (A,Cu&S)	R	R	S	S	S	S	R	S	7	4
3	Pattern C (A,S&T)	R	S	S	S	S	S	R	R	1	1
4	Pattern D (A,Cu&T)	R	R	S	S	S	S	S	R	4	Nil
5	Undetermined Pattern	R/S	R/S	R/S	R/S	R/S	R/S	R/S	R/S	6	7

A- Ampicillin; Cu- Cefuroxime; Ci- Ceftrioxone; C- Chloremphenicol; Cf- Ciprofloxacin ; O- Ofloxacin; S- Streptomycin; T- Tetracycline

Discussion

Salmonella is one of the most important food-borne pathogen. Development of MDR *Salmonella* is a serious health problem and more troublesome in the present therapeutic scenario. In the present study, the prevalence of *S.typhi*, *S.typhimurium* and *S.paratyphi A* were 57.14, 38.9 and 4.76% respectively. Surinder Kumar *et al*. (2008) reported that *Salmonella* isolates of infected patients had 80% infection by S.*typhi*, 9% by *S.paratyphi A* and the remaining 11% were infected by other groups including *S.typhimurium*. The rate of multidrug resistance was observed as 66%. Lakshmi *et al*. (2006) reported almost the same prevalence of 60 *S. typhi* and 20 *S. paratyphi A* from 80 blood cultures. *S. typhimurium* is the second most common *Salmonella* spp isolated from human in England (Cornelius *et al*., 1998).

Salmonella typhi and *S.typhimurium* isolates have shown varying degree of sensitivity against the selected eight antibiotics. *Salmonella typhi* isolates exhibited high level of resistance to Ampicillin (91.66%), Cefuroxime (60%) and high sensitivity to Chloremphenicol (5%), Ciprofloxacin (6.66%) and Ofloxacin (3.33%) which were quinolone drugs. Similar results have been documented by *S.typhimurium*. A very large number of *Salmonella* strains were observed to exhibit resistance to Ampicillin, Cefuroxime, Tetracycline, Ceftrioxone and Streptomycin which were used as drug of choice in the therapy of *Salmonella* infection. Such MDR incidence confirmed that indiscriminate use of antibiotics along with poor hygiene prevail in the study areas. Hence the sensitivity result reasserts earlier reports published by Senthil Kumar and Prabakaran, (2005).

Development of Multiple drug resistance in *Salmonella typhi* was 36.66% and 37.5% in *Salmonella typhimurium*. Kwai *et al*., (2000) studied that 50% of the *Salmonella typhi* isolates were MDR and they were commonly resistant to Ampicillin, Chloramphenicol, Trimethoprim and Sulfamethoxyzole. Sheory *et al*., (2003) stated the incidence of MDR *S. typhi* in Mumbai as 67.6% and 34% of *Salmonella typhimurium* were resistant to Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides and Tetracycline (CDC,1996). From table MDR *Salmonella* isolates (38%) resistant to three or more antibiotics were observed commonly in the study area.

Thirty nine strains of S. *typhimurium* out of 43 isolates revealed 5 drug resistant patterns identified in 1994-1995 (Kathleenglynn *et al*., 1998). Drug resistant pattern of the present work revealed that both *S. typhi* and *S .typhimurium* exhibited penta resistant patterns A, B, C, D and Undetermined pattern. Predominant resistance in *S.typhi* was observed in patternB where as *S. typhimurium* exhibited high resistant pattern which is undetermined.

Emergence of Salmonellae carrying stable resistance to multiple clinically relevant antibiotics is a public health problem in developing countries. Moreover, the trends in antibiogram of *Salmonellae* have been changing time to time. It underlines the need for the development of new measures to control the

same. The present work concluded that, the drug resistance in Salmonellae is at the increased rate particularly for the traditional drugs (First and second line). But the day is not far off when widespread resistance to even fourth generation drugs. Hence, there is a need to monitor the surveillance of antibiotic resistance and its patterns, better regulation of the use of antibiotics, important environmental sanitation and better education to the public can prevent the rate of infection and resistance.

References

Bauer,A.W., Kirby, W.M.M., Sherris, J.C. and Turck,M. 1996. Antibiotic susceptibility testing by a standard single disc method. *Am.J.Clin.Pathol*., 45: 493-496.

Centre for Disease Ccontrol (CDC), 1996. Outbreaks of Multiple drug resistant *Salmonella typhimurium*. 50: 701-704.

Cooke, F. and Wain J. 2004. The emergence of antibiotic resistance in typhoid fever. *Trav.Med.infect.Dis.*, 2 (2): 67-74.

Cornelius Poppe., Nonie Smart., Rasik Khakhria.m, Wendy Johnson., John Spika and John Presscott.1998. *Salmonella typhimurium* DT104: A virulent and drug resistant pathogen. *Can .Vet J*., 39: 559-565.

Curtis, T and Wheeler, D. J. 2006. Typhoid fever, eMedicine Opthamology. http:// emedicine. medscope.com/article/1204093-overview.

Groisman, E.A. 2001. Principles of bacterial pathogenesis. CA Academic Press. Pp-266-329.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley,J.T. and S.T.Williams. 1994. Bergey's Manual of Determinative Bacteriology. Ninth edn. Lippincott Williams & Wilkins. P-242

Kathleenglynn, M., Cherylbop, M.S., Wallis, D and Angulo, F.J.1998. Emergence of multi drug resistant *Salmonella enterica* serotype *typhimurium* DT104 infections in the United States. *J.Clin.Microbiol*., 6: 257-259.

Kwai Lin Thong, Zulfiqar,A., Bhutta and Tikki Pang,A. 2000. Multi drug resistant strains of Salmonella enterica sero type typhi are genetically homogenous and co-exist with antibiotics sensitive strains as distant, Independent clone. *Int. J. Infect. Dis*., 4: 194-197.

Lakshmi, V., Ashok, R., Susmitha, R., Shailaja, V. V. 2006. Changing trends in the antibiograms of Salmonella isolates at a tertiary care hospital in Hyderabad. *Ind. J. Med. Microbiol,*. 24(1): 45-48.

National Committee for Clinical Laboratory standards sub committee in Antimicrobial susceptibility testing, 1975. Performance standards for antimicrobial disc susceptibility tests. Approved standard: ASM-2.

Oreste and Mascaretti,A. 2003. Bacteria verses Antibacterial agents. An integrated approach. First Edn. ASM Press. pp- 329-349.

Senthil Kumar, B. and Prabakaran, G. 2005. Multi drug resistant *Salmonella typhi* in asymptomatic typhoid among food handlers in Namakkal District, Tamil Nadu. *Ind. J. Microbiol*., 23: 92-94.

Sheorey, H. S., Kaundiny, D.V and Deshpande, A. K., 1993. Multi drug resistant *Salmonella typhi* in Bombay. *Ind. J. Pathol. Microbiol*., 36(1): 8-12.

Surinder Kumar, Maher Rizwit and Nidhika Berry, 2008. Rising prevalence of enteric fever due to Multi drug resistant *Salmonella*: an epidemiological study. *J. Med. Microbiol.*, 57: 1247-1250.