 DOI: 10.18231/2455-6807.2017.0024

Phenotypic and genotypic characterization of multidrug-resistant salmonella in a tertiary care hospital

S. Ramalatharani[1,*], J. Pavithra Devi[2]

[1]Assistant Professor, [2]Associate Professor, Dept. of Microbiology, K.A.P.V. Govt. Medical College, Tiruchy

***Corresponding Author:**
Email: drpavithramd@gmail.com

Abstract

This is a prospective laboratory based observational study on genotypic and phenotypic characterization of multidrug resistant salmonella conducted over a period of 1 year in a tertiary care hospital. About 2280 blood samples from patients suffering from PUO and suspected cases of enteric fever were received from various departments like medicine, paediatrics, septic labour ward, clean labour ward and general surgical ward. The main goal of the study was to identify resistance patterns as well as the mechanisms contributing to resistance in salmonella. Enteric fever due to *S.Typhi* was seen to be 4 times more than *S.paratyphi A. 144 samples were positive for* S*almonella Typhi among 2280 samples. Out of which, 16 isolates showed multidrug resistant & from these, 10 drug resistant plasmids were detected.* By transformation process, plasmids were transformed into *E.coli*. Antimicrobial testing confirmed multi drug resistance in these 10 transformed *E.coli* (100%). These results confirm that plasmids are responsible for drug resistance in all the strains of MDR Salmonella. It is by careful selection of appropriate antibiotics according to antimicrobial testing will avoid the emergence of MDR Salmonella in the future.

Keywords: Enteric fever, Multi-drug resistance, Plasmids, Transformation, Ampicillin, Co-trimoxazole, Chloramphenicol.

Introduction

Human infections with *Salmonella* are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta and humans are the only known reservoir for *Salmonella Typhi*. Food materials also get contaminated via polluted water or by the food handlers. Typhoid fever is a major cause of morbidity and mortality with an estimated global incidence of 21.6 million cases and 216510 deaths per year. In developing countries, its annual incidence ranges from 12 to 622/100000 persons. *Salmonellaenterica* serovar *Typhi (S. Typhi*) is responsible for the majority of cases followed by *S. enterica* serovar *para typhi A (*S. *paratyphi A*) that causes 20% of the cases. In the last two decades, the worldwide emergence of multidrug-resistant strains of *Salmonella* has led to virtual withdrawal of chloramphenicol and its replacement with fluoroquinolones and third-generation cephalosporins.[(1)]

Chloramphenicol was the first antibiotic introduced in 1948 against *Salmonella Typhi* and was found very effective. However, resistance was developed two years after its introduction and it caused a major problem until 1972.[(2)] Ampicillin and co-trimoxazole were the effective alternatives till the development of multidrug - resistant strains.[(3)] The presence of nalidixic acid resistance is a marker for decreased susceptibility to fluoroquinolones and should be tested for when dealing with multidrug-resistant strains. Since the fluoroquinolones – ofloxacin and ciprofloxacin were used as alternatives in multidrug-resistant strains, which concomitantly showed resistance to nalidixic acid strains. Hence, the dose of fluoroquinolones has to be increased.[(4)]

Third generation cephalosporins – ceftriaxone, cefixime and azithromycin, a macrolide antibiotic are the drug of choice for multidrug-resistant typhoid fever.

Contributing factors for multidrug-resistant *Salmonella* T*yphi* are overuse and misuse of antibiotics, inappropriate prescribing practices along with the intrinsic microbiologic plasmid mediated factors.

In 2001 the entire genome of a multidrug-resistant isolate of *Salmonella Typhi* was sequenced. *Salmonella* share more than 70-80% of genes with other bacteria like *E.coli*. Another feature of *Salmonella Typhi* genome is the presence of over 200 inactivated genes which are felt to be related to the adaptation of the bacteria to the human host and possibly its ability to invade human tissue. Drug resistance is encoded in a transmissible plasmid. The development of additional horizontal genes in the *Salmonella* Pathogenicity Islands (SPI) represented the separation of the *E.coli* and *Salmonella* lineages and allows for the targeting of intestinal epithelial cells by *Salmonella*. Genetic studies have shown that resistance is encoded on HI1 incompatibility plasmid and is transferable.[(3)]

Aims and Objectives

To study the prevalence of *Salmonella Typhi* at a tertiary care hospital and to identify the multidrug-resistant *Salmonella Typhi* among the positive isolates. To isolate the plasmids from these MDR *Salmonella* Typhi. To prove genotypically, the plasmid mediated drug resistance in these isolates by transformation of isolated plasmids into *E.coli*, later confirmed by disc diffusion and agar dilution method.

Materials and Method

The present study was conducted at GRH attached to Madurai Medical College. Ethical clearance was obtained. The period of study was one year and the study population consisted of 2280 patients of different age groups attending outpatient department and the patients admitted in different wards viz. Paediatrics, Medicine, Septic labour theatre, clean labour theatre and General surgical ward at Government Rajaji Hospital, Madurai. Blood samples were collected from the study subjects who had the following signs and symptoms.

Clinical features: Fever which is remitting and diurnal type, nausea, vomiting, abdominal pain, diarrhea, loss of appetite, headache, lethargy and confusion.

Clinical Signs: Rose spots, abdominal tenderness, splenomegaly, hepatomegaly.

Sample collection: Blood was collected using strict aseptic precaution. In adults, 5 ml of blood was drawn and transferred to the blood culture bottle with 50ml of brain heart infusion broth swabbing the cap with 70% alcohol. Similarly in children, 1-2 ml of blood was collected in 20 ml of sterile BHI broth in blood culture bottles. Collected samples were transferred to the microbiology laboratory within 1 hour.[5]

Processing of Specimens: In the microbiology laboratory, the specimen bottles were checked for any leakage or damage and incubated at 37ºC for 48 hrs.

The specimens were inoculated on Nutrient Agar, Macconkey Agar, Blood Agar plates. The plates were incubated at 37ºC for 24hrs.[5,6]

Identification of Bacteria

Colony morphology: On Nutrient agar plate, salmonellae colonies were large 2-3 mm grey white, moist, translucent, low convex, discrete, smooth surface with entire edges.

On Macconkey agar, colonies were 1-3 mm in size and were non lactose fermenting colonies. On Blood agar plates, non hemolytic colonies were observed. Colonies that were presumptively identified as *Salmonella* isolates were subjected to the following tests. GNB on Gram staining, positive Catalase test, Oxidase test-negative, Indole-negative, positive - MR test, negative – VP test, negative citrate test and negative - urease test. TSI agar media was alk/acid with minimalH_2S in case of *Salmonella Typhi* whereasH_2S is not produced by *Salmonella para typhi* A and profuse H_2S production in *para typhi B. Salmonella Typhi* were found to be fermentative in Oxidation – fermentation test.

Salmonella fermented glucose, mannitol and mannose forming acid and no gas in case of Salmonella Typhi and gas produced by *Salmonella Para Typhi A*.[5,6]

Slide agglutination tests: Slide agglutination tests were conducted with the specific anti *Salmonella* agglutinating sera containing *Salmonella Typhi* and *para typhi A & B* which are used for the rapid serotyping of *Salmonella* species. The agglutination was watched by naked eye.[5,6]

Antibiotic susceptibility test

Disc diffusion method: The species of *Salmonella* viz. *Salmonella Typhi* and *paratyphi A* were further subjected to antimicrobial susceptibility testing by the Kirbybaur disc diffusion method. The antibiotic discs used were ampicillin (10µg), co-trimoxazole (25µg), chloramphenicol (30µg), from Hi media. The inoculum was prepared and standardized to 0.5 McFarland turbidity. The inoculum was then spread across the surface of the Mueller Hinton agar plate of 9 cm size to give a confluent growth. Antibiotic discs were placed on the agar surface 24 mm apart. Three discs were kept on the surface of the plate at a time. After overnight incubation, the diameter of the growth inhibition was measured by an antibiotic scale (Hi media) and the sensitivity and resistance patterns were identified. The isolates which showed resistance to ampicillin, co-trimoxazole and chloramphenicol were taken as multidrug-resistant *S.Typhi and S.paratyphi A.*[7,8]

Agar dilution tests: Minimum inhibitory concentration tests are performed using an agar dilution method. Specific volumes of antimicrobial solutions are dispensed into premeasured volumes of molten and cooled Mueller Hinton agar. A series of plates, containing varying concentrations of each antimicrobial agent ampicillin 50µg/ml, chloramphenicol 25 µg/ml and co-trimoxazole 25 µg/ml were prepared in sterile distilled water to give a final concentration ranging from 100 – 1600 mg / l. Growth control plates without antimicrobial agents were prepared. The agar is allowed to solidify and then a standard number of test bacteria are spot inoculated onto each plate. After overnight incubation, MIC is read as the lowest concentration of the antimicrobial agents that inhibits the visible growth of the test bacterium. The shelf life of agar dilution plates is only one week and the plates were stored at 2 to 8º C. These isolates were kept separately and preserved in refrigerator at 4 to 5ºC for further studies. Genotypically plasmid profile analysis was done for all these isolated strains using gel electrophoresis method.[7,8]

Plasmid DNA isolation by alkaline lysis method (*Sambrooke* et al 1989):[9] Plasmids are non obligate, circular extra chromosomal bacterial replicons. Plasmid DNA isolates require separation of DNA from the chromosomal DNA from the bacterial cell as well as from the polysaccharides, lipids and proteins that constitute the cell. Subsequent manipulation especially enzymatic modification of the plasmid DNA requires that it be free of impurities.

Procedure: Sterile Eppendorff were taken and to which normal saline 1.5 ml / peptone water was added. A loopful of isolated colonies were added and incubated for 18-24 hours. It was mixed in vortex and micro centrifuged for 5 minutes at 10,000 rpm. After centrifugation, the supernatant was discarded and tubes

were drained on paper towel.0.1 ml of GTE mixture was taken (Glucose + Trischloride + EDTA) and added to the deposit, mixed in vortex and incubated for 5 minutes at room temperature. 0.2 ml of NaOH with sodium dodecylsulphate was added, mixed in a vortex and then kept on ice for 5 minutes. 150µl of potassium acetate was added, mixed in a vortex and kept on ice for 5 minutes. After which it was centrifuged for 3 minutes at 10,000 rpm. Carefully the supernatant was taken to a new eppendorff tube, 800 µl of 80% alcohol was added and kept for 2 minutes at room temperature. Centrifuged the tube for 1 minute at 10,000 rpm. A milky white thin layer or pellet was seen at the bottom. The supernatant was discarded with the help of a micro pipette. 1 ml of ice cold 100% ethanol was added to the pellet and kept for 3 minutes and centrifuged at 10,000 rpm for 3 minutes. The supernatant was removed and pellet was air dried. 30 µl of TE buffer was added to the pellet and plasmid was stored at -20°C.

Electrophoresis: The apparatus was washed with 1 x TAE buffer, 1% of agarose was prepared with 50 ml of TAE buffer. 3µl of ethidium bromide was added to the agarose solution (Use gloves while adding this) and was poured to the electrophoresis plate containing comb. The agarose slab was then fixed into electrophoresis apparatus. The plasmid was mixed with 6x loading dye (fermentas) along with a control and run at 100 V for 60 minutes and the bands were read in a gel-doc system.

Transformation of the resistant plasmids into competent cells *(E.coli):* [9,10] 500 µl of the plasmid DNA was added to 500 µl of one shot chemically competent *E.coli* (Invitrogen, CA) and mixed gently. The mixture was incubated on ice for 5 to 30 minutes. *(E.coli* shrinks by this method) Then the mixture was kept in water bath at 42 to 42.5°C for 30 to 50 seconds without shaking, so that the cells will expand. Then the tubes were transferred immediately to the ice. 250 µl of LB broth (Lysogenic broth) were added to the mixture(to enhance cell growth at room temperature). The tubes were tightly capped and shaken horizontally (200 rpm) at 37°C for 1 hour. Transformed mixture of the competent cells was kept separately. Antibiotic susceptibility testing of transformed competent cells (*E.coli*) by disc diffusion method using lysogenic broth agar and agar dilution method was done as described earlier.

About 25 to 200 µl from each transformation was spread on a prepared selective plate (Lysogenic agar) containing the following antibiotics, ampicillin 50 µg / ml, chloramphenicol 25µg / ml and co-trimoxazole 25µg / ml were added. The transformed mixture was spread evenly on to LB agar using 'L' rod and incubated overnight at 37°C. The plates were observed for resistant patterns. The antibiotic susceptibility test results of transformed competent cells were compared with the antibiotic susceptibility test of the previous MDR Salmonella isolates.

Results

The study was conducted over a period of 1 year and 2280 samples were collected. All the samples were sub cultured on Nutrient agar, Blood agar, Macconkey agar, confirmed by biochemical tests and slide agglutination test. Out of 2280 blood samples, 144 (6.31%) were positive for *Salmonella Typhi* by culture, biochemical tests and slide agglutination test and 43 cases (1.88%) were positive for *Salmonella paratyphi A*. Results are given in Table 1.

Chi-Square test (X^2) showed that there was a highly significant difference between typhoid fever cases and paratyphoid fever cases (X^2 =56.88; df = 1; p<0.001).Enteric fever due to *Salmonella Typhi* was seen to be four times more than that caused by *Salmonella paratyphi A* in this study. 187 Salmonellae isolates were subjected to antibiotic susceptibility test by disc diffusion and dilution methods using ampicillin, chloramphenicol and co-trimoxazole. By disc diffusion method, it was found that out of 144 *Salmonella Typhi* isolates, 16 (11.11%)showed resistance to all three drugs (ampicillin, chloramphenicol and co-trimoxazole) and out of 43 *Salmonella paraTyphi A*, none exhibited resistance to all three drugs. The results are depicted in Table 2.

The 144 isolates were also tested for antibiotic susceptibility test by agar dilution method and it was found that 16 samples were resistant for ampicillin (ranging from 100 – 400 mg/l), chloramphenicol (rangingfrom100-400mg/l)and co-trimoxazole dilution(ranging from 200 – 1600 µg/l) as shown in Table 3.

It was inferred that 11.11% samples were multidrug-resistant by agar dilution method. Plasmid isolation studies were done on these 16 isolates. Plasmids were isolated from 10 of these MDR isolates. This is depicted in Table 4

It was inferred that plasmids were detected in 62.5% of multidrug-resistant isolates.

The isolated plasmids were transformed to competent cells (*E.coli*) by transformation process successfully. Then the antimicrobial susceptibility of 10 transformed *E. coli* was performed against three antimicrobial compounds namely ampicillin, co-trimoxazole and chloramphenicol. The transformed *E.coli* were found to be resistant to all three antimicrobialagents→chloramphenicol, co-trimoxazole and ampicillin by disc diffusion and agar dilution method. This is depicted in Tables 5 & 6.

Out of 10 transformed competent cells (*E coli*), all the 10 isolates (100%) showed resistance to ampicillin, chloramphenicol and co-trimoxazole by disc diffusion method. Out of 10 transformed competent cells, all the 10 (100%) isolates showed resistance to ampicillin, chloramphenicol and co-trimoxazole by agar dilution method in dilution ranging from 100 to 1600 mgm/l. These results confirm the successful transformation of

E.coli with plasmid DNA of multidrug-resistant *Salmonella Typhi.*

Table 1: Comparison of positive isolates

S. No.	Organism isolated	Positive by Culture & Biochemical tests	Positive by slide aggluti nation test	Percentage (n= 2280)
1.	*Salmonella Typhi*	144	144	6.31
2.	*Salmonella paraTyphi A*	43	43	1.88

Table 2: Resistant patterns by Disc Diffusion method

S. No.	Isolates	Nos.	Resistant (Zone of inhibition in mm)			%
			Ampi. (<13)	Chlor (<12)	CTZ (<10)	
1.	*Salmonella Typhi*	144	16			11.11
2.	*Salmonella paratyphi A*	43	-	-	-	-

Table 3: Resistant pattern of *Salmonella Typhi* by Agar dilution method (MIC values mg/l)

S. No.	Antibiotics	No. of strains tested	50	100	200	400	800	1600	3200	6400
1.	Ampicillin	144	-	5	4	7	-	-	-	-
2.	Chloramphenicol	144	-	5	5	6	-	-	-	-
3	Co-trimoxazole	144	-	-	4	6	3	3	-	-

Table 4: Plasmid isolation from multidrug-resistant *Salmonella Typhi*

S. No.	Total numbers tested for Plasmids	No. of plasmids isolated	Percentage (n=16)
1.	16	10	62.5

Table 5: Resistance of transformed *E.coli* to antimicrobial compounds by disc diffusion method

Antimicrobial agents	Resistant	% (n=10)
Chloramphenicol	10	100
Ampicillin	10	100
Co-trimoxazole	10	100

Table 6: Resistant pattern of transformed competent cells (*E. coli*) by agar dilution method (MIC in mg /l)

S. No.	Antibiotics	No. of strains tested	50	100	200	400	800	1600	3200	6400
1.	Ampicillin	10		2	3	3	2	-		
2.	Chloramphenicol	10		2	5	2	1	-		
3	Co-trimoxazole	10		2	1	2	3	2		

Discussion

The present study was carried out to know the phenotypic and genotypic characterization of multidrug-resistant *Salmonella Typhi.* Blood samples collected from patients, suspected of enteric fever and PUO at tertiary health care setting - Government Rajaji Hospital, Madurai. The drug resistant genes present on plasmids were demonstrated by isolation of plasmids and further characterization was done after transformation.

The study showed that enteric fever due to *Salmonella Typhi* was seen to be more common than that caused by *Salmonella paratyphi* A (6.31% and 1.88% respectively). In accordance with the study, Raveendran et al (2008),[11] Prabhakar et al (1996),[12] Sweta et al1 (2000)[13] and Madhulika et al (2004)[14] also found 1.66%, 2.7%, 3.32% and 12.11% *Salmonella Typhi* isolates respectively in their studies. Raveendran et al (2008)[11] explained that there were convalescent carriers in the population during their study period and Prabhakar et al (1996)[12] pointed out that the habits of hand washing techniques were lacking in their study group and they had the habit of sharing food from the same plate. Perhaps, these factors might have contributed to the increased incidence of *S. Typhi* in their studies. More isolation of *S.Typhi* in this present study may be due to close contact of children with asymptomatic carriers in the population because most of the cases in the study population were from endemic areas where carriers were

common. However, as carrier study was not conducted, it could not be proved.

In this present study, multi drug resistance was shown to exist in and around Madurai District. It was found that out of 144 *Salmonella Typhi* isolates, 11.11% of them were multidrug-resistant *Salmonella Typhi*. In accordance with this study, Jesudasan (1992),[15] Raveendran et al (2008),[11] OH et al (1994),[16] Uma Saberwal (1992)[17] also found 11%, 14.9%, 17.4% and 18.3% MDR isolates respectively in their studies. But, studies by Prabhakar et al (1996)[12] Ciraj et al (1991),[18] Ishaq et al (1990)[19] and Bhutta et al (1991)[20] found 61.4%, 57.9%, 30% and 20% MDR strains respectively in their studies and explained that the emergence of MDR might be due to the early use of effective antibiotics which resulted in a high rate of relapse. They also explained that the other reasons for emergence of MDR could be due to indiscriminate use of antibiotics, inappropriate prescribing practices and also due to intrinsic microbiological plasmid mediated factors. In this present study, plasmid mediated factor was proved to be the cause of emergence of MDR by Sambrooke et al (1989) method.[9]

In this present study, a single plasmid of 120 kb was isolated from 62.5% of the MDR strains. Similarly, Karmakar et al (61.84%)[21] Jesudasan et al (59.5%)[15] and Mandal et al (60.2%)[22] also isolated plasmids of 120 kb from MDR strains. In all these studies, the samples were blood, the cases were suspected cases of enteric fever and the method of study followed was Amyes and Gould and Birnboim and Doly. Whereas Senthilkumar and Prabhakaran(2005)[23] isolated plasmids of 120 kb from 13.7% of MDR strains from stool samples, the cases were asymptomatic typhoid carriers and the method of study followed was Kado and Liu. Thus, the variation in the rate of isolation in different studies was shown to rely on the nature of sample, the selection of cases and the method of studies adopted.

Plasmid can be transferred to recipient cells mostly to *E.coli* strains by two methods, either transformation or by transconjugation. Studies conducted by Jesudasan et al (1992)[15] showed that the transfer experiments were carried out using *E. coli* K 12 as recipient and 90.9% showed transformation of plasmids and the method followed was Amyes and Gould. Also studies by Mandal et al (2003)[22] indicated the presence of R-plasmid in *S.Typhi* isolates by transconjugation method (Protocol of Jevanand et al). It was demonstrated by him that R-plasmids isolated from *E. coli* could convert antibiotic sensitive *S. Typhi* to antibiotic resistant *S. Typhi.* Transconjugation studies by Senthilkumar and Prabhakaran (2005)[23] showed that in conjugation studies,(Cappuccino & Sherman procedure) the isolates having R+ factor showed the transfer of drug resistance through conjugation and it was determined by the development of antibiotic resistance in recipients. Bader Jehan Farooqi (1993)[10] did both conjugation (Miller) and transformation studies. In transformation studies he used host *E.coli* HB 101 and the plasmids were transformed successfully. He also did conjugation experiments and found that the plasmids are conjugative in nature. In this present study, transformation process was carried out to transfer isolated plasmids into competent (*E.coli) cells.* (invitrogen, CA) and proved to be 100% successful. Moreover the transformation process of plasmid transmission is an easy technique, results can be obtained within a shorter period and cost wise it is also a cheaper method.

All the transformed competent (*E.coli*) cells were subjected to disc diffusion and agar dilution method. It was found that the transformed *E.coli* also showed resistance to all three antimicrobials as similar range as donor cells as in the studies of Bader Jehan Farooqui (1993).[10] Thus, the plasmid mediated drug resistance proved in this study may be due to various factors including the acquisition of plasmid encoded inactivating enzymes like beta lactamases and chloramphenicol acetyl tranferase.

Conclusion

The study revealed 11.11% of *S.Typhi* to be drug resistant for 3 drugs. 62.5% of these showed plasmids, same transformed to competent cells (*E.coli).* The transformed cells showed 100% resistance to the three antimicrobials. Thus, confirming that a single plasmid is responsible for carryover of the drug resistance in all strains of MDR *S.Typhi*. This study proved the emergence of MDR *Salmonella Typhi* at GRH, Madurai and it was plasmid-mediated. It is a signal for the clinicians to prevent future epidemic of MDR *Salmonella* in the community by choosing proper antibiotics by drug sensitivity, to avoid improper usage and drug abuse during clinical management.

References

1. Gaind. R Paglietti. B et al. "Molecular characterization of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi and ParaTyphi A causing enteric fever in India" Journal of Antimicrobial Chemotherapy (2006) 58(6):1139-1144.
2. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections Clin. Microbiol. Rev. July 2015 28:4:901-937.
3. S. Mirza S. Kariuki et al, "Analysis of Plasmid and Chromosomal DNA of Multidrug-Resistant *Salmonella enterica* Serovar Typhi from Asia" Journal of Clinical Microbiology(2000) vol 38 no 4, pp1449-1452.
4. Harrison's principles of Internal Medicine (2008) 17th edn. Vol I pp: 956-961.
5. Bailey and Scott's Diagnostic Microbiology. 2007. XII edn. pp:323-330, 216-241,784-786.
6. Color atlas and Textbook of Diagnostic Microbiology 1997 Vedn pp:172-234.
7. National Committee for clinical laboratory standards (NCCLS). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. 4th edition approved standard, M7-A4, Wayne (PA) NCCLS: 1997.

8. National Committee for clinical laboratory standards (NCCLS). Performance standards for antimicrobial disk susceptibility tests, 6th edition. Approved standard A2 - A6. Wayne (PA) NCCLS: 1997.
9. Sambrooke J, E F Fritsch and T Maniatis. Molecular Cloning: A Laboratory Manual,1989; 2nd Ed. Vol 3; 6.3 – 6.6. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
10. Badar Jehar Farooqui. "Genetic Analysis of Multidrug-resistant Salmonella Typhi isolated in Karachi" Pakistan. 1993. PhD Thesis submitted to the University of Karachi, Pakistan.
11. Raveendran R, C. Wattal, A Sharma, J K Oberoi, K J Prasad, S Datta. High level ciprofloxacin resistance in Salmonella enterica isolated from blood. Indian Journal of Medical Microbiology. 2008,26(1):50–53.
12. Prabhakar H, Harjit Kaur, Madhanlal. Prevalence of Multidrug resistant Salmonella Typhi in Ludhiana, Punjab. Indian Journal of Medical Sciences. 1996;50:277-279.
13. Sweta, Chomal, Lina, Deodhar. Multidrug resistance in *Salmonella Typhi*. British Health Journal, 2000, July 4203.
14. Madhulika U, Harish BN & Parija SC. Current patterns in antimicrobial susceptibility of S.Typhi isolates in Pondicherry. Indian Journal of medical research, 2004;120:111-114.
15. Jesudasan MV and John T.J, Plasmid mediated multidrug resistance in Salmonella Typhi. Indian Journal of Medical Research 1992;95;66-67.
16. Oh HML, SK Chew and EH Monteiro. "Multidrug resistant typhoid fever in Singapore". Singapore Medical Journal, 1994;35:599-601.
17. Uma Saberwal, Chaudhary and Santosh Saini. "MDR Salmonella Typhi in Hariyana", 1989-1990 Indian Journal of Medical Research 1992:12-13. Pub med.
18. Ciraj A M , K S Seetha, B K Gopalkrishna, P G Shivananda,"Drug Resistance pattern and phage types of *Salmonella Typhi* isolates in Manipal" South Karnataka. Indian Journal of Medical Sciences. 1999,53(11):486–489.
19. Ishaq, M., Farooqui, B., Ashfaque, M.K. and Khan, M.A., "Therapeutic implications of ofloxacin in the treatment of typhoid fever caused by multiple drug resistant Salmonella Typhi". J.P.M.A. 1990;40:176.
20. Bhutta, Z.A., Naqvi, S.B. Razzaq, R.A., and Parooqui, B.J. "Multiple drug resistant typhoid in children: presentation and clinical features". Review of infect. Dis. 1991;13:832.
21. Karmaker S, Biswas D, Sheikh NM, Chatterjee K, Kateria VK, Kumar R. "Role of large plasmid of S.Typhi encoding MDR". Journal of Medical microbiology 1991:34 :149-51.
22. Mandal S, MD Mandal and NK Pal. "Plasmid encoded MDR of *Salmonella Typhi* and some enteric bacteria in and around Kolkata, India": A preliminary Study. JHAS 2004;3(4):1-7.
23. Senthilkumar B and G. Prabhakaran. "Multidrug resistant *Salmonella Typhi* in asymptomatic typhoid carriers among food handlers in Namakkal district" Tamil Nadu. Indian Journal of Medical Microbiology. 2005:23(2):92-94.

How to cite this article: S. Ramalatharani, Devi J.P. Phenotypic and genotypic characterization of multidrug-resistant salmonella in a tertiary care hospital. International Journal of Medical Microbiology and Tropical Diseases 2017;3(3):100-105.