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Trend of Antibiotic Resistance Pattern in Clinical Isolates of Salmonella Species

S Kalpana*; A Ganesh; Saramma Mini Jacob; Sivasangeetha

Department of Experimental Medicine, the Tamil Nadu Dr. MGR Medical University, Tamil Nadu, India.

***Corresponding Author(s): S Kalpana**

Department of Experimental Medicine, the Tamil Nadu Dr. MGR Medical University, Tamil Nadu, India.

Tel: 9445936151; Email: drkalpanaphd@gmail.com & depro1@tnmgrmu.ac.in

Received: Aug 16, 2023

Accepted: Sep 08, 2023

Published Online: Sep 15, 2023

Journal: MedDocs Microbiology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Antibiotic resistance; Salmonella; MIC.

Abstract

Background: Multi-drug resistance enteric fever is becoming an increasingly serious problem. This study's objectives were to estimate the frequency of Salmonella spp. isolations and to compare the isolates' epidemiological characteristics and patterns of antibiotic susceptibility.

Methods: A total of 200 blood culture samples from suspected typhoid cases were processed for the years 2019-2020. Antibiotic susceptibility pattern were screened for all the cases for Ampicillin (AMP 10 µg), chloramphenicol (C, 30 µg), Cotrimoxazole (COT, 25 µg), Nalidixic Acid (NA, 30 µg), Ciprofloxacin (CIP, 5 µg), Ofloxacin (OF, 5 µg), cefixime (CFM, 5 µg), Cefotaxime (CTX, 30 µg), ceftriaxone (CTR, 30 µg) and Trimethoprim (5 µg), CPM, Tetracycline (10 µg), Imipenem (10 µg), by agar dilution method, the Minimum Inhibitory Concentration (MIC) for above mentioned drugs at different concentration were also analysed.

Results: A total of 200 clinical samples were obtained from Hospital. Among the total Salmonella isolates 49.5% (29/40) were S. Typhi and 87, 43.5% were S. Paratyphoid A, 14, 7% were mixed infection both S.typhi and S.Paratyphi. MIC of antibiotics were also determined for isolates. The most common resistance pattern was identified for the antibiotic tetracylin and ceffazidime.

Conclusion: In our findings, we observed that Salmonella isolates had increased resistance to tetracyclin, ampicillin, ceffazidime and resistance to nalidixic acid. The need of continuous monitoring of antibiograms of enteric fever isolates in a region is highlighted in this study.

Background

Enteric fever is a severe bloodstream infection brought on by Salmonella enteric serovars Typhi (S. Typhi) and Paratyphi (S. Paratyphi) A, B, and C [1]. In underdeveloped nations, there are an estimated 11.9-20.6 million cases of typhoid and paratyphoid fever each year, with a recorded mortality of 129,000-223,000 [2]. In addition, a substantial portion of these cases and fatalities are centered in South Asia, where they show seasonal variation and peak from June to August due to rain [3]. The best treatments for enteric fever have been found to be antibiotics, including fluoroquinolones, ampicillin, co-trimoxazole, and chloramphenicol [4]. Without antibiotic therapy, the case fatality rate is reportedly 10%-30%, while with the right course of treatment, it drops to 1%-4% [2]. However, overprescription and careless use of these medications has resulted in the for-

Cite this article: Kalpana S, Ganesh A, Jacob SM, Sivasangeetha. Trend of Antibiotic Resistance Pattern in Clinical Isolates of Salmonella Species. Med Docs Microbiol. 2023; 1(2): 1002.

mation and spread of drug resistance, also known as Multidrug Resistance (MDR), in the virulent strains of Salmonella. MDR strains are to blame for failed treatments, a limited selection of medication regimens, and elevated severity and mortality rates [5]. Early drug resistance in Salmonella isolates first appeared in the late 1980s, when antibiotic resistance rendered the traditional first-line medications (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) ineffective, forcing clinicians to turn to fluoroquinolones, particularly ciprofloxacin [6]. However, the recent global spike in fluoroquinolone resistance could cause a catastrophic rise in infectious diseases worldwide [7]. However, the emergence of Nalidixic Acid-Resistant Salmonella (NARS) strains is now posing a threat to their long-standing usefulness. The establishment of nalidixic-resistance is thought to be caused by genetic factors, such as mutations in the DNA gyrase (gyrA and gyrB) and topoisomersase IV (parC and pare) genes. Therefore, the high frequency of NARS in our sample can possibly be attributed to genetic variables; however, addressing genetic factors was outside the purview of our study design. We also found that quinolone and nalidixic-acid resistant strains were common, as was the case in several earlier studies [8], while third generation cephalosporins (ceftriaxone, cefotaxime, and cefixime) and conventional first-line medications (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) were significantly more susceptible to infection. This type of uncommon reemergence of susceptibility may be caused by the long-standing obsolescence of conventional antibiotics. In addition, pathogenic strains have lost high molecular weight self-transmissible resistant-inducing plasmids as a result of evolution and mutation [9]. The development of third generation cephalosporin and azithromycin has been prompted by study findings that point to a growing burden of nalidixic acid-resistant bacteria with decreased susceptibilities to quinolones [10]. Due to the MDR strains' ability to develop drug resistance, these more recent drug generations are also threatened by them [11], as was noted in multiple earlier investigations. Salmonella spp. antimicrobial susceptibilities to first-line conventional antibiotics may be re-emerging, according to several research, which could aid in the fight against Nepal's expanding AMR [12]. The foundation of stopping the spread of AMR is adequate surveillance and strong infection controls [13]. The incidence and antibiogram of Salmonella spp. are poorly understood despite numerous prior attempts. There is a lack of trustworthy data and numerous scientific investigations.

In this work, the antibiotic susceptibility profile of Salmonella spp. isolated from clinical samples taken at a tertiary care hospital in Chennai, Tamil Nadu, was examined. Additionally, this study sought to evaluate the MIC pattern, identify Salmonella strains that produce Extended Spectrum. Antimicrobial susceptibility pattern of Salmonella isolates by Kirby Bauer disc diffusion method. Determination of Minimum Inhibitory Concentration (MIC) of conventional and antibiotics against Salmonella by agar dilution method. Percentage of MDR detection and screening for quinolone and fluoroquinolone.

Material and Methods

The study was conducted in Department of Microbiology over a period of two years from 2019-2020. Blood sample was collected under strict aseptic conditions for culture. Totally 200 isolates from clinical samples from patients with suspected typhoid fever. Blood and stool samples was processed in the hospital for culture. The culture was further analysed in the department of microbiology, ESIC medical college and hospital, Chennai.

Procedure

Isolation & Identification of salmonella

Laboratory Processing and Identification of the Isolates BACTEC culture bottles inoculated with blood specimens were incubated at 37 ◦C for up to 5 days. Isolates showing growth on BACTEC were further inoculated on conventional culture media such as Blood Agar (BA), Mac Conkey Agar (MA) and Xylose Lysine Deoxycholate agar (XLD). The inoculated culture plates were incubated at 37 ◦C for 18–24 h. The BA plates were used for the observation of non-hemolytic smooth white colonies, MA for nonlactose fermenting colonies and XLD for red colonies with black center. Identification of the isolates was based on colony morphology, Gram staining, and biochemical tests including catalase test, oxidase test, Methyl Red (MR) test, Voges-Proskauer (VP) test, citrate utilization test, Triple Sugar Iron (TSI) test, and urea hydrolysis test (urease test). Serotyping of the isolates was further performed by agglutination method using Salmonella polyvalent antisera O, monovalent O:2, O:9, O:12 and Vi for confirmation of different serovars.

Antibiotic susceptibility test

The Antibiotic susceptibility testing of the isolates for routinely used antibiotics will be carried out by Kirby-Bauer disc diffusion method. Antimicrobial susceptibilities of the Salmonella isolates were tested by using modified Kirby–Bauer disc diffusion in accordance with the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI). The antibiotic discs used were Ampicillin (AMP 10 µg), Chloramphenicol (C, 30 µg), cotrimoxazole (COT, 25 µg), nalidixic acid (NA, 30 µg), Ciprofloxacin (CIP, 5 µg), ofloxacin (OF, 5 µg), Cefixime (CFM, 5 µg), Cefotaxime (CTX, 30 µg), Ceftriaxone (CTR, 30 µg) and Trimethoprim (5 µg), CPM, Tetracycline (10 µg),, Imipenem. (10 µg), In this method, broth culture of test organism (comparable to McFarland tube no. 0.5; inoculums density 1.5 × 108 organisms/mL) was uniformly carpeted on the surface of MHA. Then, the antibiotic discs were placed over the lawn culture of the test organism, and the plates were incubated at 37 ◦C for 18 h (or overnight).After incubation, the diameter of Zone Of Inhibition (ZOI) was measured and the results were interpreted as "Resistant" or "Intermediate" or "Susceptible" to that particular antibiotic based on the CLSI guidelines. Salmonella isolates showing resistance to three or more than three antibiotics of different antibiotic classes were called MDR Salmonella. For instance, Salmonella spp. resistant to amoxicillin, chloramphenicol and cotrimoxazole were indicated as MDR Salmonella. Control strains of Escherichia coli ATCC (American Type Culture Collection) 25955 was used to ensure the standardization of susceptibility testing.

Minimum inhibitory concentration studies for the drugs were done by agar dilution method.

Determination of Minimum Inhibitory Concentrations (MICs).

Minimum inhibitory concentration of all the eleven drugs were determined by agar-dilution method based on CLSI guidelines. In this method, MHA plates with various concentration of drugs (ranging from 0.032 µg/mL to 1mg/mL) were prepared and the test organisms were inoculated on the agar surface. After proper inoculation, the plates were incubated at 37 ◦C for 18-20 h. Following sufficient incubation, the results were interpreted as "sensitive" or "resistant" using breakpoints approved by CLSI. [Clinical and Laboratory Standards Institute.

Performance Standards for Antimicrobial Susceptibility Testing, 28th ed.; CLSI Supplement; Clinical and Laboratory Standards Institute: Wayne, PA, USA; Volume 38, p. M100.]

Data analysis

Data collected through the laboratory analyses were entered in Microsoft Excel. Data were analyzed by using Statistical Package for Social Science (SPSS) version 24.0. Descriptive statistics was used and Chi-squared ($\chi 2$) test was used to predict the relationship between the variables in which a p value of <0.05 was considered as significant.

Results

A total of 200 clinical samples were obtained from Hospital. Among the total Salmonella isolates 49.5% (29/40) were S. Typhi and 87, 43.5% were S. Paratyphi A, 14, 7% were mixed infection both S. typhi and S. Paratyphi. (Figure 1).

The highest rate of antibiotic resistance was observed towards tetracyclin (66%) followed by cefixime (48%), Nalidixic acid (32%) and ceffazidmee (30%). Most of the salmonella sps sensitive to Ofloxacin, (77%) imipenum, ceftriaxone (76%) and ciprofloxacin (82%). Most of the isolates were susceptible to all classes of antibiotics used, such as amoxicillin, chloramphenicol, and cotrimoxazole (Table 1 & Figure2).

Determination of Minimum Inhibitory Concentrations (MIC)

In this study, the MIC values for ciprofloxacin ranged from 0.032 µg/Ml-1 µg/mL. The test detected 45% (91/200) isolates with MIC of ≤0.032 µg/mL as susceptible strains. In contrast 23% (45/200) isolates had MIC ≥ 1 µg/mL and were labeled as resistant, whereas more than 60% isolates had MIC between 0.125 µg/mL–0.5 µg/mL, showing increased susceptibility to ciprofloxacin.

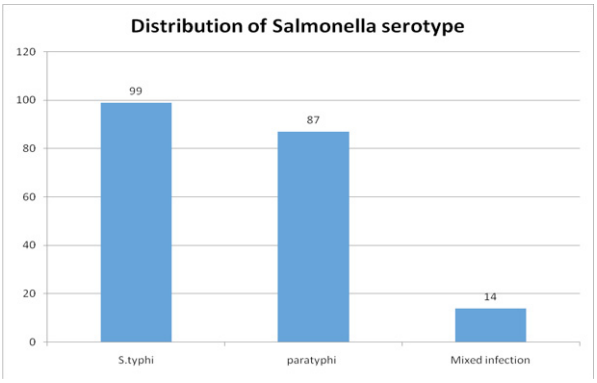

Figure 1: Frequency of Salmonella spp. in blood culture isolates.

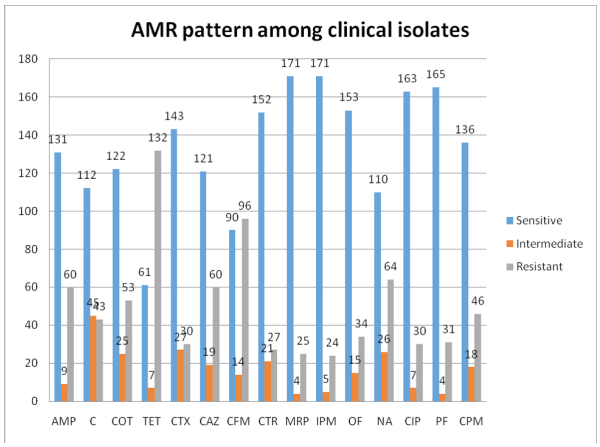

Figure 2: Frequency of Salmonella spp. in blood culture isolates.

Table 1: Antibiotic Susceptibility Pattern of Salmonella Isolates.

Drugs used for AMR	code	Total samples	Sensitive N(%)	Intermediate N(%)	Resistant N(%)
AMPICILLIN	AMP	200	131 (65.5)	9 (4.5)	60 (30)
CHLORAMPHENICOL	C	200	112 (56)	45 (22.5)	43 (21.5)
CO -TRIMOXAZOLE	COT	200	122M (61)	25 (12.5)	53 (26.5)
TETRACYCLINE	TET	200	61 (30.5)	7 (3.5)	132 (66)
CEFOTAXIME	CTX	200	143 (71.5)	27 (13.5)	30 (15)
CEFFAZIDIME	CAZ	200	121 (60.5)	19 (9.5)	60 (30)
CEFIXIME	CFM	200	90 (45)	14(7)	96 (48)
Ceftriaxone	CTR	200	152 (76)	21 (10.5)	27 (13.5)
MEROPENEM	MRP	200	171 (85.5)	4 (2)	25 (12.5)
IMIPENEM	IPM	200	171 85.5)	5 (2.5)	24 (12)
Ofloxacin	OF	200	153 (76.5)	15 (17.5)	34 (17)
NALIDIXIC ACID	NA	200	110 (55)	26 (13)	64 (32)
CIPROFLOXACIN	CIP	200	163 (81.5)	7 (3.5)	30 (15)
PEFLOXACIN	PF	200	165 (82.5)	4 (2)	31 (15.5)
CEFEPIME	CPM	200	136 (68)	18 (9)	46 (23)

Table 2: Minimum inhibitory concentration of different antibiotics against Salmonella isolates.

Drugs	MIC Breakpoint of Ciprofloxacin n (%) in µg									
	0.032		0.064		0.125		0.5		1	
	Sen n(%)	Res n(%)	Sen n(%)	Res n(%)	Sen n(%)	Res n(%)	Sen n(%)	Res n(%)	Sen n(%)	Res n(%)
CIPROFLOXACIN	91(45)	109(55)	97(48)	103(52)	128(64)	72(36)	139(69)	61(31)	155(77)	45(23)
Tetracycline	44(22)	156(78)	56(28)	144(72)	87(43)	113(57)	108(54)	92(46)	155(77)	45(23)
AMPICILLIN	54(27)	145(73)	92(46)	108(54)	119(59)	81(41)	134(67)	66(33)	158(77)	45(23)
CHLORAMPHENICOL	9(5)	190(95)	15(8)	184(92)	47(24)	152(76)	65(33)	134(67)	114(57)	85(43)
NALIDIXIC ACID	6(3)	193(97)	12(6)	188(94)	62(31)	138(69)	101(50)	99(50)	115(57)	85(43)
TRIMETHOPRIME	33(16)	167(84)	41(20)	159(80)	72(36)	128(64)	121(60)	79(40)	139(69)	61(31)

For the drug tetracycline and Ampicillin susceptibility was increased from 0.032 to 1 µgas 22% - 77% and 27% -77%. For the drug Chloremphenicol and nalidic acid, the resistance was in increased percentage to 0.32(95%) and 43% for 1 µg, for nalidic acid,193(97%) for 0.032 µg and for 1 µg 85(43%) resistance was observed. Similarly, the resistance pattern was high in 0.032 (84%) and for 1 µg it was 31%.

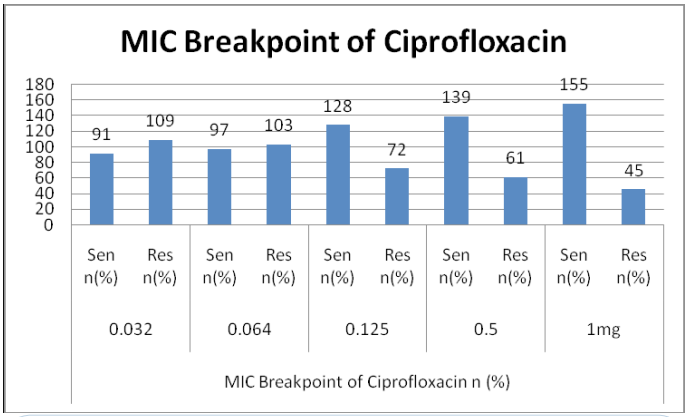

Figure 3: MIC to tetracycline.

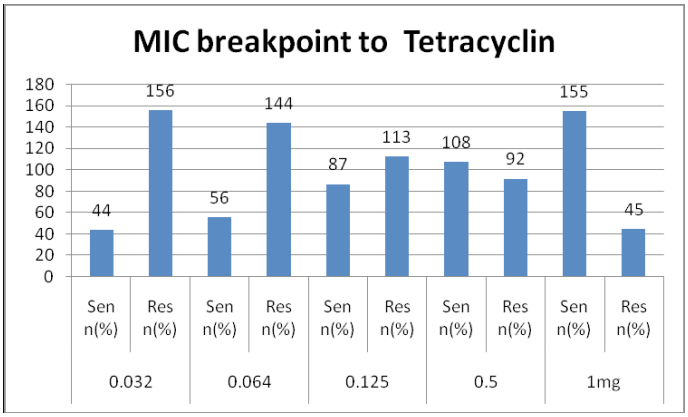

Figure 4: MIC to tetracycline.

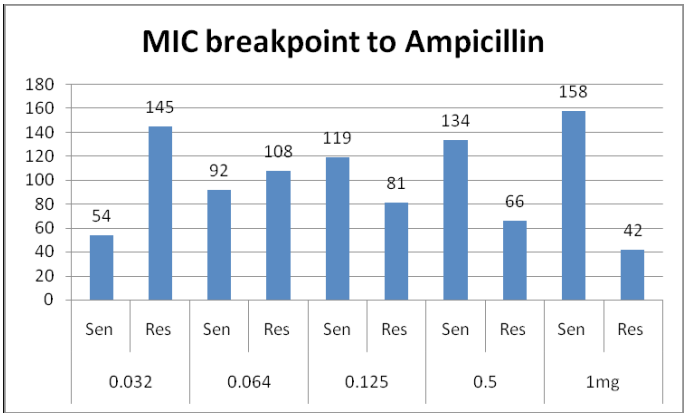

Figure 5: MIC breakpoint to Ampicillin.

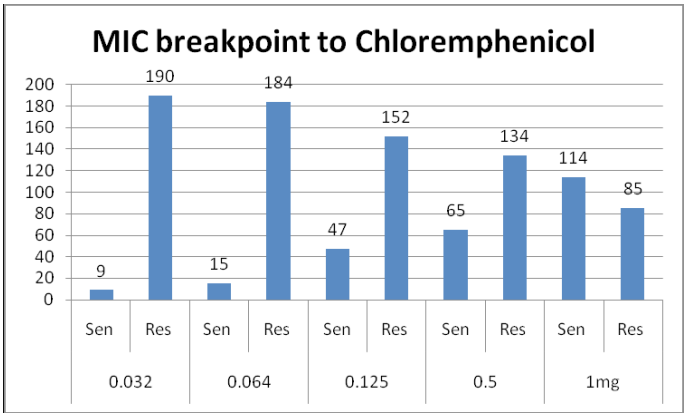

Figure 6: MIC breakpoint to Chloramphenicol.

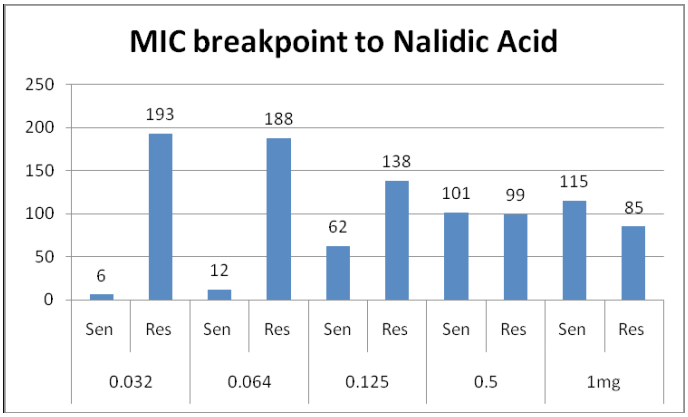

Figure 7: MIC to Chloramphenicol.

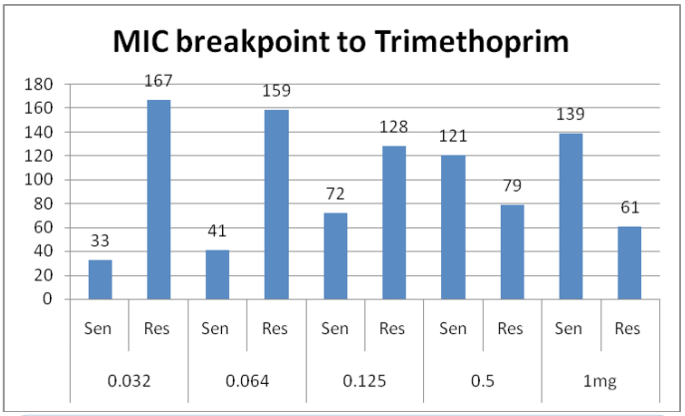

Figure 8: MIC breakpoint to Trimethoprim.

The above figures 4-8 showed the determination of MIC by agar dilution method.

Discussion

Enteric fever is becoming more and more of a problem in our nation, and the issue is being made worse by the emergence of antibiotic resistance to drugs that were once successful. S.typhi was the most common isolate in this study, followed by S. Paratyphi A, which is consistent with findings from earlier studies [14]. But contradictory, S .tophi and S.Paratyphi mixed infection was also found out. No other study had documented this mixed infection. Fluoroquinolones and third-generation cephalosporins have virtually replaced chloramphenicol as a treatment for enteric fever since multidrug-resistant strains of Salmonella have spread around the world in the past 20 years [15]. Tetracycline's effectiveness as a first-line treatment for enteric fever has, however, recently come under serious threat [16]. We observed a rise in the number of individuals with enteric fever who needed hospitalization in our research. This is explained by a corresponding rise in the frequency of strains of Salmonella Typhi and tetracyclin-resistant bacteria linked to treatment failures. Perhaps as a direct result of their widespread prescription for illnesses other than typhoid fever, multi drug resistance has increased, as seen in the current study. It is also accompanied by the concurrent re-emergence of particular drug susceptibility that is due to its restricted use, which led to the removal of selection burden. Third generation drugs have recently become more significant in the management of enteric fever. Although our investigation revealed perfect sensitivity to these medications like ceffazidine, there have been some reports of rising MICs for even third-generation drugs. These medications are not only costly for regular use in developing countries, but the selection against beta lactamases is also a problem. Additional research need to be carried out to track the increase in MICs for these drugs as well as to look for extended spectrum beta lactamases in this class of organisms.

Conclusion

In our study, we found increase of resistance to tetracyclin, ampicillin, ceffazidine and nalidic acid for salmonella isolates. The study emphasizes the requirement for constant surveillance of antibiogram of enteric fever isolates.

Acknowledgement: We thank the medical officers and lab technicians who helped us to conduct the study and at ESIC medical college and hospital.

Conflict of interest: None.

Funds: SERB funded project.

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