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EVALUATION OF MINIMUM INHIBITORY CONCENTRATION OF CHLORAMPHENICOL FOR *SALMONELLA* SPP. ISOLATED FROM ENTERIC FEVER CASES IN A TERTIARY HOSPITAL IN IMPHAL

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ABSTRACT: Chloramphenicol was introduced as the effective antibiotic in the treatment of typhoid fever. Resistance started to develop within two years of its introduction. The emergence of antibiotic resistant strains of bacteria is closely linked to the irrational use of antibiotics. The sensitivity/resistance pattern of *Salmonella* spp. had been varying with time and geographical locations. Changing trends in antibiotic resistance patterns especially of chloramphenicol have been recorded in different parts of India. So the study was done to evaluate the minimum inhibitory concentration of chloramphenicol. Two hundred clinically suspected cases of enteric fever were included in the studies during the period of one year and nine months. Positive blood culture for *Salmonella* spp. was 8%. Isolates of *Salmonella* Typhi were 100% sensitive to cotrimoxazole, chloramphenicol, and cefotaxime. 12 (75%) were sensitive to ampicillin, 7 (43.7%) to azithromycin, 14 (87.50%) to ceftriaxone and 10 (62.5%) were sensitive to cefixime. The isolates show 100% resistance to ciprofloxacin and nalidixic acid. The minimum inhibitory concentration (MIC) for all the chloramphenicol sensitive isolates ranged in between 1-4µg/ml.

INTRODUCTION: Antimicrobial drugs are the greatest contribution of the twentieth century to therapeutics. Their importance is magnified in the developing countries, where infective diseases predominate. As a class, they are one of the most frequently used as well as misused drugs [1].

Enteric fever (Typhoid fever) continues to be a global health problem with an estimated 12-33 million cases [2] and 6 lakhs death occurring worldwide [3].

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It is endemic in the Indian subcontinent [4]. Antibiotic therapy constitutes an integral part of the management of enteric fever since mortality without treatment can be as high as 30%.This can be reduced to <1% by appropriate treatment [5]. Chloramphenicol was introduced in 1948 as the effective antibiotic in the treatment of typhoid fever. Compared with other antibiotics, it was the "gold standard" therapy [6].

Even though resistance started to develop within two years of its introduction; it did not emerge as a major problem until 1972 [5, 7]. Resistance developed to chloramphenicol is caused by plasmid encoded acetyltransferase that inactivates the drug. Resistance can also result from decreased permeability into the resistant bacteria cells and from ribosomal mutation.

The acetylated derivatives of chloramphenicol fail to bind to bacterial ribosomes [8]. Cotrimoxazole and ampicillin were also used for treatment of enteric fever but problem arose with the development of resistance. The emergence of multidrug resistant strains (resistant to chloramphenicol, ampicillin and cotrimoxazole) led to the use of first generation fluoroquinolones. With the development of ciprofloxacin resistance among MDR *Salmonella* Typhi, third generation cephalosporin have been recommended as an alternative to quinolones [9].

No doubt the discovery of antibiotics has revolutionized the management of infectious diseases [10] but changing trends in antibiotic resistance patterns especially with the development of reemergence of sensitivity to chloramphenicol have been reported from different parts of India. So the present study was undertaken to know the antimicrobial susceptibility pattern of *Salmonella* spp. isolates to chloramphenicol in RIMS Hospital, Imphal, Manipur by determining its minimum inhibitory concentration (MIC).

MATERIALS AND METHODS:

Type of study: It is a cross sectional study.

The present study 'Evaluation of minimum inhibitory concentration (MIC) of chloramphenicol for *Salmonella* spp. isolated from enteric fever cases in a tertiary hospital' was carried out in the Department of Pharmacology, RIMS, Imphal, Manipur in collaboration with the department of Microbiology, RIMS for a period of one and half years from October 2011 to March 2013 in a total of 200 cases taken from outdoor and inpatient Department of Medicine, Department of Paediatrics, RIMS.

Ethical issues:
The study was carried out only after obtaining approval from Institutional Ethical Committee (IEC), RIMS. Consent from the participating individuals was obtained

Criteria for selection of cases:
Inclusion criteria:

- All subjects with clinical suspicion of enteric fever. Patients above three years of age, both male and female were included in the study.

Exclusion criteria:

- Diagnosed case of fever

Procedure:
Venous blood of 10ml from an adult and 2ml from children was collected aseptically by venipuncture from each patient.

Culture:

1. Venous blood was inoculated in BHI biphasic media and 0.5% bile broth. It was incubated for 24hrs at 37˚C.

2. After overnight incubation, subculture was made on MacConkey's agar. The agar was prepared as per standard recommendation in the Department of Microbiology [11].

3. After inoculation, the agar plates were then incubated aerobically at 37^{o} C overnight [12].

4. For subculture in BHI biphasic media it is sufficient if the bottle is tilted so that the broth flows over the surface of the agar slant as both liquid and solid media are available in the same bottle. Colonies will appear on the slant if the culture is positive.

5. Identification: Identification of the isolates was carried out by standard recommended methods[13]. A host of biochemical and other tests were carried out towards the end.

When no growth was detected, repeated subculture was done every alternate day up to the 7th day before declaring the culture as negative [14].

The isolates were confirmed for Salmonella isolates by slide agglutination test with specific antisera (Bio-Rad laboratories India Pvt. Ltd) [13].

Antibiotic sensitivity test:
Antibiotic sensitivity test was carried out against *Salmonella* Typhi isolates. Standard discs purchased from Hi-media laboratories were used

and tested by Kirby Bauer Disk-diffusion method following guidelines laid down by CLSI [15]. ATTC strain, *Escherichia coli* 25922 available in Microbiology Department, RIMS were put to use for quality control purpose. Antibiotics panels which were put up include:

Ampicillin (10µg/ml), Azithromycin (15µg/ml), Ciprofloxacin (5µg/ml), Nalidixic acid (30µg/ml), Cotrimoxazole (1.25/23.75µg/ml), Ceftriaxone (30µg/ml), Cefixime (5µg/ml). For sensitivity testing Mueller-Hinton agar was used [11].

Minimum inhibitory concentration chloramphenicol (0.016-256µg/ml) was determined using E-Test strip (Hi-media Laboratories, Mumbai) placed on inoculated Mueller-Hinton agar followed by overnight incubation[11].

Statistical analysis:

The data obtained were compiled and master chart was prepared. The data so collected was processed by using SPSS 16.0 and the required calculation, analysis, interpretation and conclusions were made.

RESULTS:

Two hundred clinically suspected cases of enteric fever were included in the studies during the period one year and nine months. Positive blood culture for *Salmonella* spp. was 8%.

TABLE 1: ANTIBIOTIC SENSITIVITY PATTERN OF *SALMONELLA TYPHI*

Antibiotics	*Salmonella* Typhi , (n=16)			
	Sensitive	Percentage	Resistance	Percentage
Ampicillin	12	75%	4	25%
Azithromycin	7	43.70%	9	56.20%
Ciprofloxacin	-	-	16	100%
Nalidixic acid	-	-	16	100%
Co-trimoxazole	16	100%	-	-
Chloramphenicol	16	100%	-	-
Ceftriaxone	14	87.50%	2	12.5%
Cefixime	10	62.50%	6	37.50%

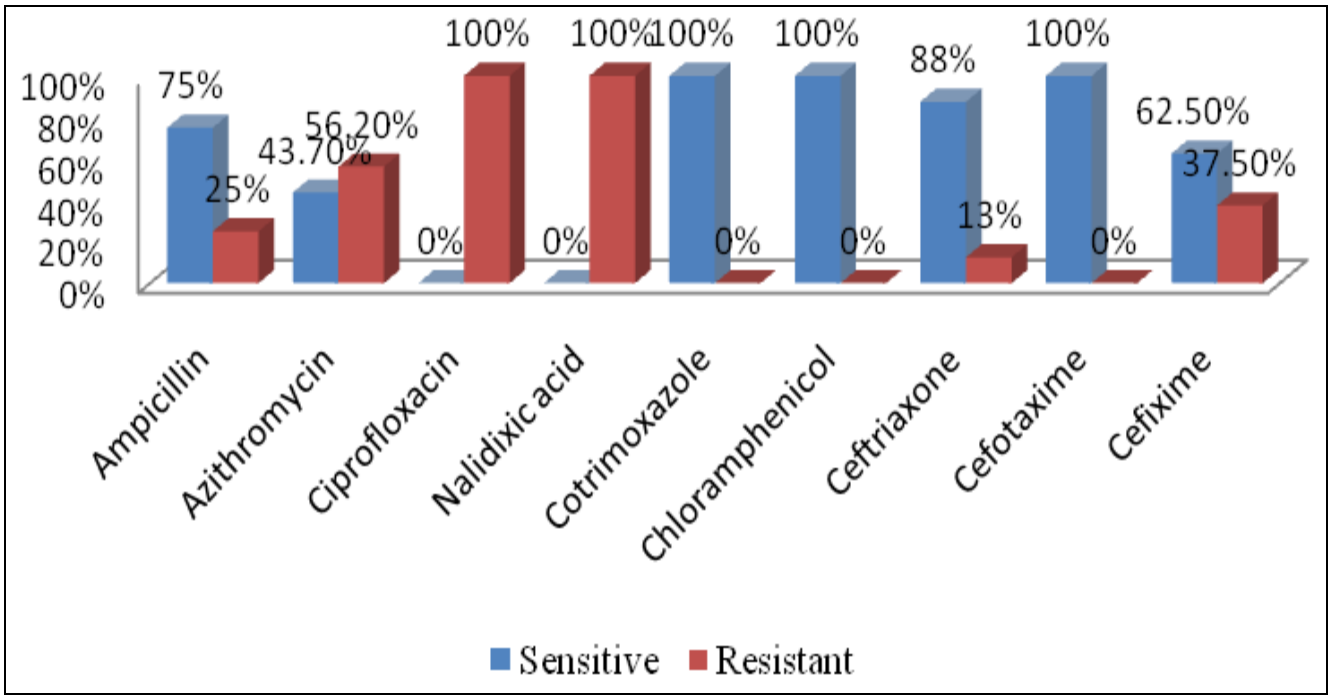

FIG.1: ANTIBIOGRAM OF *SALMONELLA* TYPHI

Isolates of *Salmonella* Typhi were 100% sensitive to cotrimoxazole, chloramphenicol, and cefotaxime. 12 (75%) were sensitive to ampicillin, 7 (43.7%) to azithromycin, 14 (87.50%) to ceftriaxone and 10 (62.5%) were sensitive to cefixime. The isolates show 100% resistance to ciprofloxacin and nalidixic acid.

TABLE 2: MIC OF CHLORAMPHENICOL

MIC (µg/ml)	Number of isolates (%)
<8	100%
>8	0%

MIC of chloramphenicol of all the isolates was in the susceptible range of less than 8µg/ml. In the present study the minimum inhibitory concentration (MIC) for all the chloramphenicol sensitive isolates ranged in between 1-4µg/ml, the maximum being 4µg/ml and the minimum range being 1µg/ml.

DISCUSSION: In the present study, changing trends in the antibiotic sensitivity pattern of *Salmonella Typhi* can be seen. Strains showing intermediate sensitivity were included in the resistant category. The antimicrobial sensitivity against various drugs tested showed sensitivity as follows-ampicillin (75%), azithromycin (43.7%), ceftriaxone (93.75%), cefixime (62.5%), cotrimoxazole (100%), chloramphenicol (100%). The strains exhibited 100% resistance to ciprofloxacin and nalidixic acid.

High-level ciprofloxacin resistance in Salmonella enterica serotype typhi in India was recorded by Sanghavi et al [16] and Renuka K et al [17]. Biswal N et al [18] also reported ciprofloxacin resistance in pediatrics patients. From the present study, it can also be seen that multi-drug resistance isolates are decreasing as all the isolates were sensitive to chloramphenicol, and cotrimoxazole. 75% of the isolates were sensitive to ampicillin.

The high degree of chloramphenicol susceptibility of *S.enterica* serovar Typhi isolates has also been reported from many parts of India. Bhattacharya et al [19] isolated *S.enterica* serovar Typhi strains from Orissa of which 87.46% were chloramphenicol sensitive. Kumar et al [20] reported from Ludhiana that there was an increase of chloramphenicol susceptibility from 43% (19195) to 93% (1999) among *S.enterica* serovar Typhi strains. Goutam et al [21] reported from Rohtak (Haryana) about reemergence of chloramphenicol sensitivity in 90% *S.enterica* serovar Typhi isolates.

In response to the development of ciprofloxacin resistance among MDR *S.enterica* serovar Typhi, a number of studies have investigated the efficacies of expanded spectrum cephalosporins. In the present study resistance of 37.50% to cefixime was noted. Ceftriaxone recorded a sensitivity of 93.75%. Presently, resistance to third generation cephalosporin has also been reported by Bhutta ZA et al [22].

Minimum inhibitory concentration (MIC):
In the present study the minimum inhibitory concentration (MIC) for all the chloramphenicol sensitive isolates ranged in between 1-4µg/ml, the maximum being 4µg/ml and the minimum range being 1µg/ml. This is in concordance with the study conducted by Chande et al [23] and Goutam et al [21] who recorded MIC of 4µg/ml for chloramphenicol sensitive strain.

CONCLUSION: The sensitivity/resistance pattern of *Salmonella* spp. had been varying with time and geographical locations. The emergence of antibiotic resistant strains of bacteria is closely linked to the irrational use of antibiotics in treating human infections. But with time, due to relieve of selection pressure following discontinuation of the resistant drug, re-emergence of antibiotic sensitivity to previously resistant drug was reported.

In the present study, changing trends in the antibiotic sensitivity pattern of *Salmonella* Typhi can be seen. Strains showing intermediate sensitivity were included in the resistant category. The antimicrobial sensitivity against various drugs tested showed sensitivity as follows-ampicillin (75%), azithromycin (43.7%), ceftriaxone (93.75%), cefixime (62.5%), cotrimoxazole (100%), chloramphenicol (100%). The strains exhibited 100% resistance to ciprofloxacin and nalidixic acid. The emergence of antibiotic resistant strain is closely linked to irrational use of antibotics and the reemergence of antibiotic susceptibility is due to removal of selective pressure on a large bacterial population.

CONFLICT OF INTEREST: None.

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