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Ciprofloxacin and Azithromycin Susceptibility in Salmonella Causing Bloodstream Infection in a Tertiary Care Hospital in south India

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Abstract:
BACKGROUND: Enteric fever is a major health concern worldwide, especially in tropical countries. Enteric fever caused by Salmonella enterica is a gastrointestinal tract disease to begin with and later involving many systems,. After the advent of multidrug resistant Salmonellae, ciprofloxacin became the focus of its treatment. Azithromycin is widely being used for the prevention and treatment of Enteric fever. Of late, there are reports in literature to support that Salmonella have regained susceptibility to Ampicillin, Cotrimaxazole and Chloramphenicol. Therefore, this study undertaken aims to find out the antimicrobial susceptibility of Salmonella isolates to conventional drugs like Ampicillin, Cotrimaxazole and Chloramphenicol and find out susceptibility pattern of Ciprofloxacin that is over used for treatment of enteric fever and Azithromycin, most often used in children. As there is no disc diffusion method as per CLSI 2019 guidelines to detect the susceptibility to ciprofloxacin, ciprofloxacin resistance was determined by minimum inhibitory concentration(MIC)by E-test.Kirby-Bauer disk diffusion technique was used to monitor Azithromycin resistance among Salmonella entericaserovarTyphi as per CLSI 2019 guidelines.
METHODS: The Prospective study was conducted over a period of 8 months (May 2019- December 2019) with the clinical samples yielding the growth of Salmonella from blood. These strains were screened for Ciprofloxacin susceptibility by E-test. Azithromycin,Ampicillin, Co-Trimaxazole and Chloramphenicol susceptibility were detected by Kirby-Bauer disc diffusion technique.
RESULTS: Of the 80 isolates that were screened, 52 (65%) were resistant, 24 (30%) were intermediate susceptible and remaining 4(5%) were sensitive to ciprofloxacin by MIC detection. 69 (86.25%) of the isolates were sensitive and 11 (13.75%) were resistant to Azithromycin by disc diffusion technique. All isolates (100%)were found to be susceptible to Ampicillin, Co-Trimaxazole and Chloramphenicol
CONCLUSION: The current study shows a high prevalence of resistance to ciprofloxacin in Salmonella species isolated in and around Mysuru district in southern India. Detection of ciprofloxacin resistance by the Kirby Bauer disk diffusion method is less effective, so it is advised that MIC of ciprofloxacin must be regularly done in the diagnostic laboratories for all strains. The findings from our study also showed low level resistance (13.75%) to Azithromycin. It is advised that Azithromycin can be used as a therapeutic agent only if it is reported to be sensitive by the susceptibility test. Conventional agents

like Ampicillin, Co-Trimaxazole and Chloramphenicol can be reconsidered for treatment of Enteric fever.

KEY WORDS: *Salmonella enterica*, Ciprofloxacin, Azithromycin, Minimum Inhibitory Concentration (MIC), Ampicillin, Co-Trimaxazole, Chloramphenicol, Antibiotic recycling.

INTRODUTION

Enteric fever caused by Salmonella enterica species, a common cause of undifferentiated fever, is a global health problem due to poor sanitation and poor personal hygiene, especially in endemic regions such as India. It is an infectious disease with significant incidences of fluctuation. [4] It is one of the main differential diagnoses for the undifferentiated fever and a major cause of global morbidity and mortality.

Globally, the World Health Organization (WHO) reported that 21.7 million outbreaks (217,000 deaths) were induced by typhoid fever annually and 5.4 million of these cases were triggered by paratyphoid fever. The highest incidence of enteric fever (> 100 cases per 100,000 people per year) is observed in South Central Asia and Southeast Asia. In India, a Delhi survey reported the occurrence of enteric fever to be 9.8 cases in 1,000 person/year. Salmonella Entericaserovar Typhi and Paratyphi A, are the most common serovar types in India. [4]

Worldwide, the highest incidence of Salmonella infections exists in Asia, particularly in South and Southeast Asia, where high levels of antibiotic resistance are found. Anti microbial resistance is more recognized among *S.Typhi* lineages spreading from South Asia to Africa with resistance to first-line antibiotics (Cotrimoxazole, Ampicillin and Chloramphenicol) and fluoroquinolones.[3]. Fluoroquinolones are reported to be the drug of choice for the treatment of invasive infections of Salmonella. But decreased susceptibility to ciprofloxacin is rapidly increasing worldwide. In case of reduced susceptibility to ciprofloxacin, azithromycin and ceftriaxone are recommended as treatment alternatives for enteric fever [1]. Anti Microbial Resistance in typhoid is not globally standardized and in various endemic areas, it has developed resistance at different rates. [3]. Ciprofloxacin resistance has been reported in Salmonella since the last decade and increasing minimum inhibitory concentration (MIC) among other fluoroquinolones have also been observed.

Antimicrobial resistance is a major problem in enteric fever management. Chloramphenicol tolerance had increased in *S. Typhi*in the early 1970s accompanied by Ampicillin and Cotrimoxazole resistance subsequently. Since the 1990s, S.Typhihas occurred all over the world, particularly in Southeast Asia. Resistance to these three agents has culminated in widespread use of fluoroquinolones (FQ) in enteric fever care worldwide. [5]

The production of quinolone-resistant strains with decreased sensitivity to ciprofloxacin (MIC 0.125-1µg / mL) has further aggravated the situation. Many reports have documented a steady increase in MIC of ciprofloxacin that correlated with medication failure or chronic ciprofloxacin defervescence. High-level resistance to ciprofloxacin has been reported across India since 2004. Third-generation cephalosporins and Azithromycin are solutions to fluoroquinolonesresistant enteric fever, but there has also been a rise in MICs of Ciprofloxacin in these Salmonella strains. [5] Epidemiological monitoring of bacterial populations has led to the recommendation that an azithromycin MIC of approximately 16µg / ml should be considered susceptible to Salmonella isolates [6].

There has been no systematic review of chronological trends in AMR among Salmonella Typhi and S Paratyphi A isolates in India. The Expert Committee of WHO Strategic Group, which makes global vaccine policy recommendations, highlighted the need for countries to strengthen typhoid fever surveillance and monitor the occurrence of AMR strains before and after the typhoid conjugate vaccines (TCVs) have been implemented. India has a unique advantage that the TCVs have already been approved and the nation has already sold over five million doses. However, it is still to be used programmatically [2].

In addition, large-scale randomized control trials with follow-up and laboratory correlation are required for the use of azithromycin in Southeast Asian countries and restraining from blindly following Western prescribing practices as typhoid is not primarily an issue for developed countries. Hence, we need to generate real data where typhoid and antibiotic resistance areendemic problems.

METHODOLOGY:

Type of study: Hospital based prospective study.

Study Setting: This study was conducted in the department of Microbiology, in a tertiary care Hospital, Mysore, South India.

Method:

Salmonella enterica isolates were collected from blood cultures received during 8 months in the microbiology laboratory (May 2019- December 2019). This prospective study was conducted in Mysuru, Karnataka, in a 1,800-bed tertiary care hospital. During the period of study, a total of 414 blood samples were received for culture. Blood culture was performed using the five-day incubation of blood culture in BACT / ALERT ® 3D (BIOMERIEUX India Private Limited, New Delhi, INDIA). Following positive signals from the blood culture bottles, samples were subcultured on blood agar and Mac Conkey agar and were aerobically incubated at 37 ° C for 24 hours. The non-lactose fermenting colonies were further processed for identification and antimicrobial susceptibility.

These isolates were routinely subjected to identification (ID) and antimicrobial susceptibility testing (AST) by VITEK 2 Compact for ID and AST panels (BIOMERIEUX India Private Limited, New Delhi, INDIA) as per manufacturer instructions. Isolates identified as *Salmonella* were confirmed manually by standard biochemical tests and confirmed by serotyping using antisera for *Salmonella* (polyvalent O, O2, O9 from CRI Kasauli).Manual antimicrobial susceptibility testing was also performed simultaneously to Ciprofloxacin (5 µg), Azithromycin (15 µg), Ampicillin (10µg),Co-Trimaxazole (25µg) and Chloramphenicol (30µg) disc (Hi-Media Laboratories, Mumbai, India) by the standard Kirby Bauer's disc diffusion method, with zone sizes measured and recorded. A standard strain of *E. coli* ATCC 25922 was included as quality control to check the efficacy of the above discs. Results were interpreted as per the clinical laboratory standards institute (CLSI) guidelines. [15]

Susceptibility was determined by measuring the zone of inhibition around Azithromycin (15mg), Ampicillin (10µg),Co-Trimaxazole (25µg) and Chloramphenicol (30µg)discs. The isolates were labeled as Azithromycin susceptible (Zone Diameter of Inhibition ≥13mm) and resistant (ZDI≤12mm), Ampicillin susceptible (ZDI ≥17mm) and resistant (ZDI≤13mm), Co-Trimaxazolesusceptible (ZDI ≥16mm) and resistant (ZDI≤12mm) and Chloramphenicol susceptible (ZDI ≥18mm) and resistant (ZDI≤12mm), according to the CLSI guidelines 2019.

Further ciprofloxacin susceptibility was determined by Minimum inhibitory concentration (MIC) for all the isolates using E-test strips obtained from Hi Media Laboratories, Mumbai, India, and interpreted according to the CLSI guidelines 2019.[15] Ciprofloxacin MIC was interpreted as (susceptible ≤0.06 µg/ml, intermediate 0.12-0.5 µg/ml, resistant ≥1 µg/ml). *Escherichia coli* ATCC 25922 strains was used for quality control.

RESULTS:

A total of 80 Salmonella EntericaserovarTyphi isolates were recovered from samples of blood culture. The isolation rate was higher in pediatric patients (92.5%- 74 out of 80), while 6 (7.5%) were from adults obtained from emergency ward. All the positive blood cultures yielded the growth of Salmonella EntericaserovarTyphi.

For Azithromycin, 69 (86.25%) isolates were sensitive and 11 (13.75%) were resistant. The susceptibility is based on measuring the inhibition zone diameter around 15 µg Azithromycin disk. More than or equal to 13mm is considered susceptible and <13mm is considered resistant according to the CLSI guidelines 2019. For Ampicillin (10µg), Co-Trimaxazole (25µg) and Chloramphenicol (30µg), all 80 (100%) isolates were sensitive. The susceptibility is based on measuring the inhibition zone diameter around the disk. (Table-1) (Figure-1)

For Ciprofloxacin, 52(65%) isolates were resistant, 24 (30%) were intermediate and 4(5%) were sensitive. The susceptibility was determined by the E-test. More than or equal to 0.06µg/ml was considered susceptible, more than or equal to 0.12-0.5µg/ml was considered intermediate and more than or equal to 1µg/ml was considered resistant according to the CLSI guidelines 2019.(Figure-2)

Table-1: Showing the susceptibility pattern of different antimicrobial agents

Antibiotic	Sensitive	Intermediate	Resistant
Azithromycin disc (15µg)	69 (86.25%)	0	11 (13.75 %)
Ampicillin disc (10µg)	80 (100%)	0	0
Co-Trimaxazole disc (25µg)	80 (100%)	0	0
Chloramphenicol disc (30µg)	80 (100%)	0	0
Ciprofloxacin MIC (µg/ml)	4(5%)	24 (30%)	52 (65%)

Figure-1: The Graph showing the susceptibility pattern of different antimicrobial agents

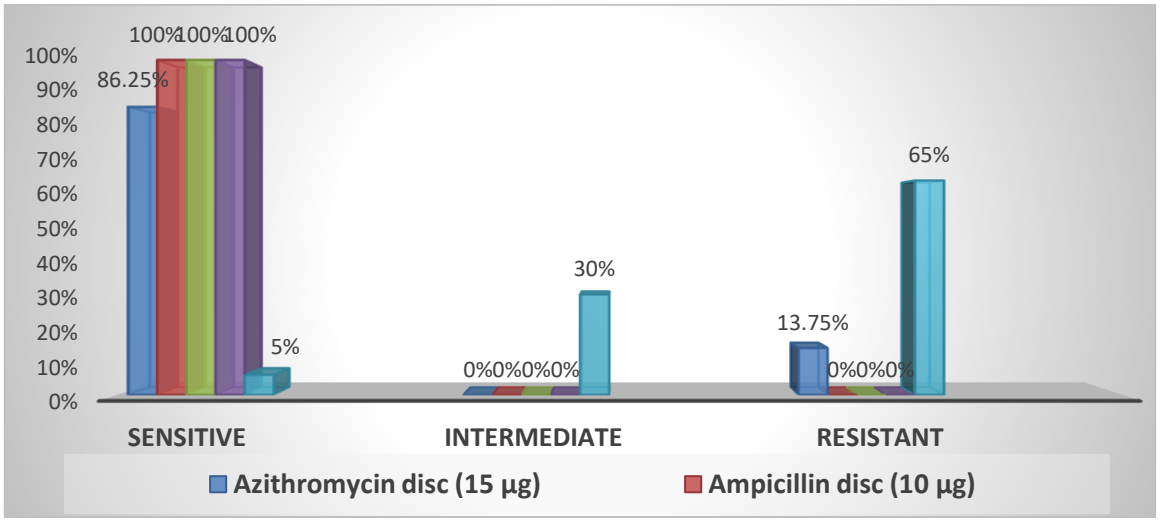
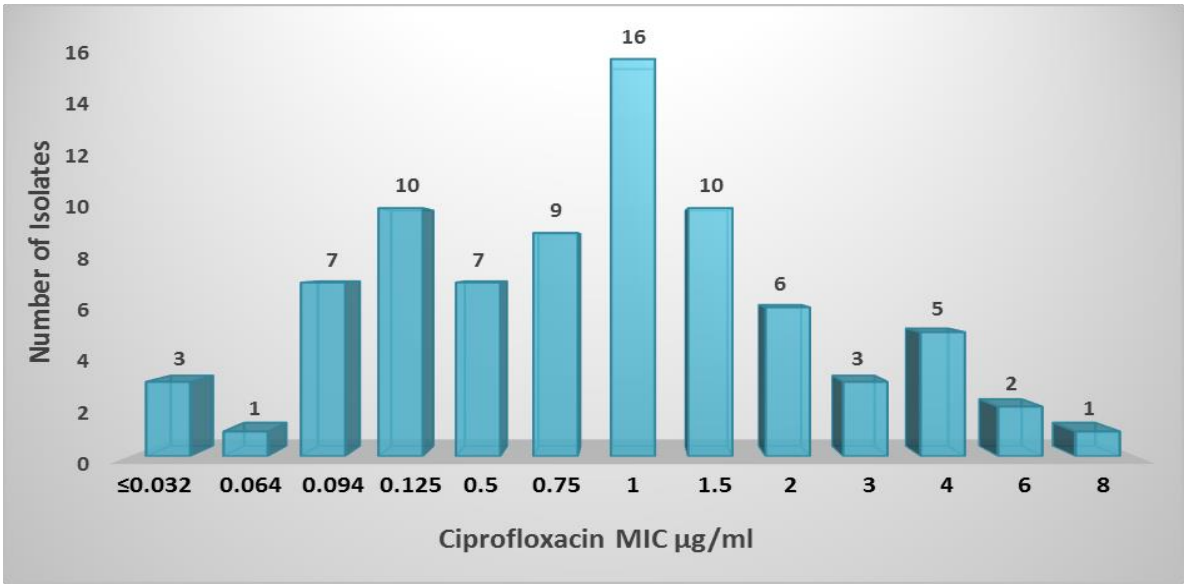


Figure-2: The distribution of Ciprofloxacin MIC:



The CLSI (2019) interpretive criteria for sensitive, intermediate and resistant strains, respectively in µg/ml are: ciprofloxacin MIC (susceptible ≤0.06 µg/ml, intermediate 0.12-0.5 µg/ml, and resistant ≥1 µg/ml).

(Among the common antibiotics used for enteric fever treatment, it was found that, 13.75% of the isolates were resistant to Azithromycin while 65% of the isolates were resistant to ciprofloxacin.)

DISCUSSION

Enteric fever is more common in the tropics and is a major cause of morbidity and mortality, more often affecting the younger age group. This preponderance may be explained by the consumption of contaminated food and water by children. Similar findings were found in this study; mean age was less than 14 years among pediatric patients. This study found that there was a higher isolation rate for Salmonella entericaserovar Typhi and no isolate of either Salmonella entericaserovar Paratyphi A or Salmonella entericaserovarParatyphi B were found during the study period.

Crump and colleagues in 2000 from USA (Atlanta) estimated that there were 21.7 million cases of typhoid fever, out of which 5.4 million cases were of paratyphoid fever, and 2, 16,510 deaths from typhoid fever [7]. Another similar study by Buckle and colleagues estimated 26.9 million typhoid cases in 2010, which was based on low diagnostic sensitivity test. [18]. Rate of cultural positivity in our study was 19.32 %, and is similar to a study conducted in Nepal by Shrestha et al in 2016 (13.38%). [9].

Salmonella species exhibit decreased susceptibility to ciprofloxacin during therapy leading to its consequent therapeutic failure. [11, 17] A study conducted from Thakur S et al. from Shimla in 1994-2010, reported that there is an increase in resistance to ciprofloxacin from 3.67 % (1993) to 9.41 % (2000 to 2006) and currently at 47.5 %. [11, 20, 21]. In our study, only 5 % of S.Typhi were susceptible to ciprofloxacin, 30% were intermediate and remaining 65% of the isolates showed resistance to ciprofloxacin using E-test. Similarly, a study conducted by Rahman et al. at Gulf region in 2014, showed that susceptibility to Ciprofloxacin was reduced to 48 % and intermediate to 47% in S.Typhi isolates. [12]

The peak MIC found in our study was 8µg/ml in 1 (2%) isolate. Similarly, a study reported by BN Harish and GA Menezes from Pondicherry in 2011, shows that the resistance of ciprofloxacin, with MIC

ranging from 8 to 64µg/ml. [23]. Another study conducted by BalajiVeeraraghavan from Vellore in 2018, showed an increase in the MIC of fluoroquinolones against S.Typhi in South East Asia (SEA) and Africa. This was correlated with longer duration of febrile episodes. [14]

Shesh Raj Patel et al. from Varanasi in 2016 and BalajiVeeraraghavan from Vellore in 2018, reported that the presence of MDR (i.e. resistance to Ampicillin, Chloramphenicol and co-Trimaxazole) in late 1980s. For the past 15 years, the MDR rates have drastically declined and the rates have fallen down from 26% in 2004 to 1% in 2018. [9, 22] In our study, the isolates shows 100% sensitivity to Ampicillin, Co-Trimaxazole, and Chloramphenicol. KavitaNagshetty in Karnataka (Gulbarga) during 2010 reported that only 10% of S.Typhiare drug resistant. [16].This tendency reflects the drug's indiscriminate and unreasonable use in managing typhoid and other illnesses.

Our findings showed susceptibility to Azithromycin (86.25%) correlating with a study conducted by Deepak Harichandran from Kochi in 2017 (78.48%) [10].In our study Azithromycin resistance was 13.75% while NidhiSingla from Chandigarh in 2012 reported Azithromycin resistance of 1%.

For Salmonella, antibiogram keeps changing from time to time and place to place. All the studies have clearly indicated that over use of particular antibiotic results in increasing emergence of resistance to that particular drug while antibiotic recycling would reverse the resistance to particular antibiotics evidenced by emergence of susceptibility to Ampicillin, Cotrimaxazole and Chloramphenicol.

CONCLUSION:

The current study shows Salmonella entericaserovar Typhi strains with decreased ciprofloxacin susceptibility in and around Mysuru region. Resistant strains of ciprofloxacin will restrict the therapeutic use of fluoroquinolones in enteric fever. The Kirby Bauer disc diffusion method is less effective in detecting ciprofloxacin resistance, so it is recommended that MIC for ciprofloxacin should be routinely determined for all the strains of Salmonella in the diagnostic laboratories.

The findings of the current study suggest that ciprofloxacin should not be considered as the drug of choice at this high level of ciprofloxacin resistance. The need of the hour is to formulate alternative treatment guidelines to effectively and rationally manage enteric fever cases. Routine monitoring of such resistance is necessary. This study also suggests that Azithromycin should only be used as an alternative anti microbial agent only if it is reported to be sensitive by the susceptibility test.

As 100% of our isolates were sensitive to Ampicillin, Co-Trimaxazole, and Chloramphenicol, these agents can be reconsidered for treatment options as antibiotic recycling is a good method to fight against antimicrobial resistance.

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