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Original Article

Minimum inhibitory concentration (MIC) of Ceftriaxone and Azithromycin for blood culture isolates of *Salmonella enterica* spp.

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Abstract

Introduction: Enteric fever caused by *Salmonella enterica* continues to be a major public health problem worldwide. In the last decade, ceftriaxone and azithromycin have become the drugs of choice for treating enteric fever caused by Nalidixic acid resistant *Salmonella* (NARS) *enterica*. This has led to reports of drug resistance to both drugs. Since enteric fever is endemic in India, accurate drug susceptibility surveillance is crucial to ensure empiric management of enteric fever is appropriate. The aim of this study is to evaluate the minimum inhibitory concentration (MIC) of ceftriaxone and azithromycin for blood culture isolates of NARS isolated at our centre.

Methodology: This is a retrospective study conducted in a tertiary care center in Mumbai for blood culture isolates of NARS from 2016 to 2018. Isolates were tested for antimicrobial susceptibility testing (AST) against ceftriaxone and azithromycin using a manual broth microdilution method (BMD).

Results: Of 155 blood culture isolates of NARS: *S.* Typhi (n = 112) and *S.* Paratyphi A (n = 43) were included in the study. 81.9% (127 / 155) isolates were susceptible, 6.4% (10 / 155) isolates were intermediate while 11.6% (18 / 155) isolates were resistant to ceftriaxone. 100% susceptibility of NARS was observed to azithromycin.

Conclusions: This study documents an alarming increase in resistance to ceftriaxone among NARS in Mumbai while azithromycin continues to be susceptible in vitro. It is essential to know MICs to understand epidemiological trends and choose appropriate treatment regimens for treating enteric fever.

Key words: NARS; MIC; ceftriaxone; azithromycin.

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Introduction

Enteric fever is caused mainly by *Salmonella enterica* serovars Typhi and Paratyphi A. It is the most common cause of community acquired bacteraemia in India and a significant public health problem in many low and middle income countries (LMICs) countries, mainly due to inadequate access to safe water and sanitation. Globally, the disease accounts for 25 million cases of febrile illness, with children being affected the most [1]. In the year 2010, the global burden of typhoid fever was estimated to be 12 million cases and 130,000 deaths. It exceeded 100 cases/100,000 people per year in South East Asian countries with enteric fever being endemic in India [2]. Globally, a 44.6% decline in enteric fever cases have been reported since 1990 which is mainly attributed to improvements in water and sanitation and better uptake of the typhoid vaccine especially among children.[3] In India, prevalence of laboratory corroborated enteric fever cases caused by *S.* Typhi and *S.* Paratyphi is estimated to be 9.7% and 0.9% respectively [2]. However, high morbidity and increase in antimicrobial resistance still remain important concerns as millions of people are exposed to these pathogens globally.[3]

Traditionally ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol have been used as the first line of treatment for typhoid fever. In 1970s and early 1980s multidrug resistant (MDR) *S.* Typhi strains which were resistant to these three drugs emerged. The first outbreak of chloramphenicol resistant *S.* Typhi in India, was reported in 1972 [4]. During 1990-1992, approximately 65% MDR *S.* Typhi strains were reported from all over India; 71% in central India and 55% in southern parts of India [5]. Because of the high prevalence of MDR strains among *Salmonella* species, the first line of drugs became ineffective, thus reducing their usage, making fluoroquinolones (FQ) (ciprofloxacin and ofloxacin) the preferred treatment option for enteric fever [6,7]. The next few decades saw the emergence and

dissemination of strains with decreased susceptibility to fluoroquinolones [2]. This led to a reduction in fluoroquinolone usage and shifted attention towards the third generation cephalosporins like Cefixime, Ceftriaxone (CRO) and macrolides like Azithromycin (AZM) for the primary management of enteric fever [2]. AZM is increasingly used as an empiric treatment option for treating uncomplicated enteric fever especially in areas where NARS and MDR infections are prevalent [8,9]. Resistance to AZM is uncommon but reported sporadically. The first case of AZM treatment failure in a patient with invasive salmonellosis caused by *S.* Paratyphi A was reported in 2010 [10]. Cefixime and CRO have been reliably effective; however there have been increasing reports since 2010 of extended-spectrum cephalosporin-resistant strains in Asia and Africa [10]. Resistance to cephalosporins is mediated through production of acquired AmpC β-lactamases or Extended-spectrum β-lactamases (ESBLs) such as bla_{SHV} and $bla_{CTX\text{-}M\text{-}15}$ [11]. The recent outbreak of ceftriaxone-resistant typhoid in Pakistan [12] demonstrates the importance of understanding the local resistance patterns to enable the selection of appropriate antibiotics and management of enteric fever.

Nevertheless, CRO or AZM remains the treatment of choice for treating *S.* Typhi in developing countries. CRO is administered intravenously, adult dosage/day: 1-2g bid, dosage: 50-75mg/kg 1-2 doses/day for 7-10 days [13]. AZM has a half-life of 2 to 3 days and can achieve intracellular concentrations 50 to 100 times greater than serum levels [9] and can be used in children and in pregnant or nursing mothers [13]. It is administered orally: 20 mg/kg per day for 6 days [2]. Treatment courses of 500 mg per day [10 mg (kg body weight) per day] for 7 days and 1g per day (20 mg/kg per day) for 5 days have resulted in successful outcomes for both adults and children including cases with MDR/nalidixic-acid-resistant infections [8]. A combination treatment course of ceftriaxone (IV) – 2 g daily for 14 days with Azithromycin – 500 mg daily for the first 7 days have resulted in successful outcomes [14].

The above doses of CRO and AZM used for the treatment of enteric fever are higher than the routine dose used for treating other infections with these antibiotics. Minimum inhibitory concentration (MIC) is an important pharmacokinetic parameter which allows dose modifications to be done to achieve clinical success. This study was performed to understand the variations in MIC of isolates of *Salmonella enterica* spp using the broth microdilution method (BMD) which will further help clinicians to decide upon the antibiotic, dosage and combined therapy required to treat enteric fever.

Methodology

Bacterial isolates

Of the 155 Nalidixic acid resistant *Salmonella enterica* (NARS) isolates obtained from bloodstream infections, from a 220 bed tertiary care hospital in Mumbai (January 2016 - December 2018), 112 were *S.* Typhi and 43 were *S.* Paratyphi A. Identification of the isolates was carried out using Vitek 2 Compact gram negative identification cards (bioMérieux, Durham, USA) and confirmation for the same was done by serotyping with Polyvalent O, serotype 2, 4 and 9 (Remel™, India).

Antimicrobial agents

Disodium salts of Ceftriaxone (CRO) and Azithromycin (AZM) dihydrate used in the study were procured from Sigma Aldrich Chemicals Pvt Ltd, Bangalore, India.

Antimicrobial susceptibility testing (AST): The in-vitro susceptibility of the test isolates to AZM and CRO were determined using Broth Microdilution method (BMD) described by Clinical and Laboratory Standard Institute [15]. The BMD method was carried out on sterile 96 well polystyrene round bottom micro titre plates (Tarsons India Pvt. Ltd, Kolkata, India) using two-fold dilutions ranging from 0.03 μg/mL to 16 μg/mL, prepared in Cation Adjusted Mueller Hinton Broth (CAMHB) (BBL Becton, Dickinson & Company, Sparks, MD21152, USA) The tests were performed in duplicates to ensure repeatability. Control wells were maintained in each row for growth control and media control. The quality of every batch was assessed using the standard strains of *Escherichia coli* ATCC 25922 *& Klebsiella pneumoniae* ATCC 700603 (an ESBL producer).

Interpretation of results

A definite turbidity or button formation in the growth well is considered as positive. MIC was recorded as the lowest concentration at which the isolate was completely inhibited (absence of visible bacterial growth). The CLSI recommended MIC breakpoints for CRO (susceptible ≤ 1 μg/mL, intermediate 2 μg/mL and resistant ≥ 4 μg/mL) and AZM (susceptible ≤ 16 μg/mL and resistant ≥ 32 μg/mL) were used for result analysis [16].

Results

A retrospective study was at a 220-bed tertiary care hospital in Mumbai, India. It was conducted for a period of 3 years from January 2016 to December 2018. Total 155 blood culture positive NARS isolates were included in the study.

Of 155 tested NARS, 81.9% were susceptible, 6.4 % were intermediate and 11.6% were resistant to CRO whereas for AZM 100% susceptibility was observed. Antimicrobial susceptibility pattern results of the NARS isolates to CRO and AZM are presented in Table 1.

From the table it is observed that there was 12.5% and 9.3% resistance to CRO in NAR *S.* Typhi and NAR *S.* Paratyphi A respectively.

The distribution of MIC trend against CRO and AZM in 155 NARS isolates is shown in Figure 1 and 2 respectively. The MIC_{50} and MIC_{90} values of CRO for *S.* Typhi were 0.125 µg/mL and 8 µg/mL, respectively. For the *S.* Paratyphi A isolates, MIC_{50} and MIC_{90} values of CRO were 0.125 µg/mL and 2 µg mL, respectively.

Table 1. Antimicrobial susceptibility patterns of NARS isolates to CRO and AZM.

Organisms	No of strains tested (n)	Ceftriaxone (CRO)			Azithromycin (AZM)	
		Susceptible	Intermediate	Resistant	Susceptible	Resistant
		% (n)	% (n)	% (n)	% (n)	% (n)
S. Typhi	112	84.8 (95)	2.6 (3)	12.5 (14)	100 (112)	0
S. Paratyphi A	43	74.4 (32)	16.2 (7)	9.3 (4)	100 (43)	0
Total	155	81.9 (127)	6.4 (10)	11.6 (18)	100 (155)	0

Figure 1. Distribution of MIC trend for Ceftriaxone (CRO) to NARS isolates.

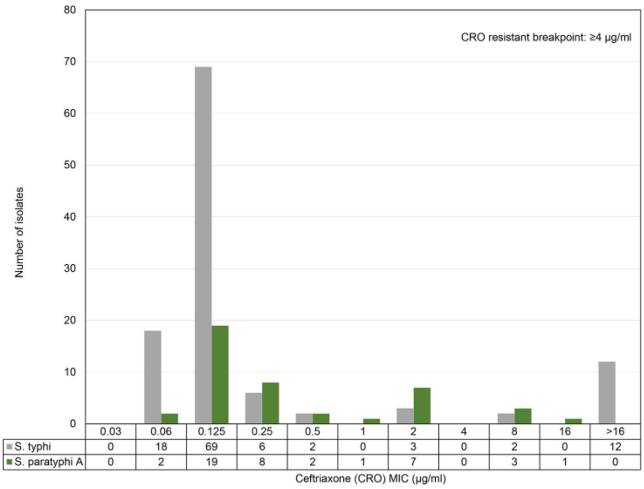

Figure 2. Distribution of MIC trend for Azithromycin (AZM) to NARS isolates.

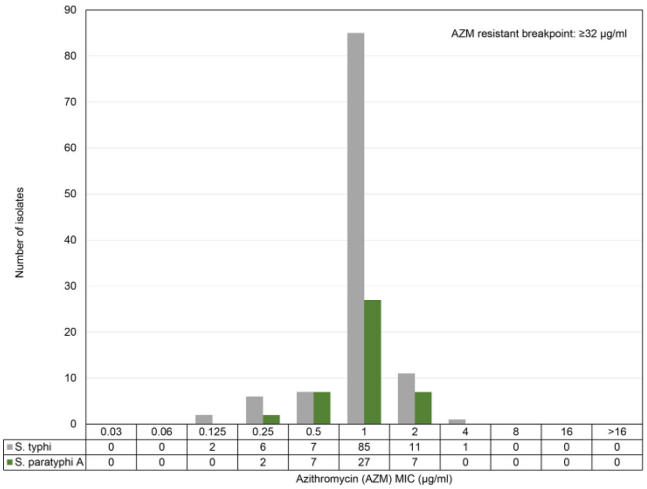

Table 2. Prevalence of NARS in India.

Year	NARS %	Location	References
1994	2%	Mumbai	[30]
1996	7%	Mumbai	[30]
1998	67%	Mumbai	[30]
2000	82%	Mumbai	[30]
2002 - 2003	83.43%	Pondicherry	[31]
2006 - 2007	64%	Chennai	[32]
2006 - 2007	31.57%	Gulbarga	[33]
2006 – 2007	>90%	Delhi	[34]
2008	96%	Maharashtra	[35]
2011 - 2012	99.1%	Chandigarh	[20]
2013	100%	India	[36]
2008 - 2014	98%	India	[37]
2000 - 2016	100%	Hyderabad	[21]

The MIC_{50} and MIC_{90} value for AZM was 1 µg/ mL for both *S.* Typhi and *S.* Paratyphi A.

Discussion

Enteric fever is a significant global problem. Drug resistance in *Salmonella* spp causing enteric fever is considered as one of the important factors in the morbidity of the disease. Especially, emergence of NARS holds extreme importance in the treatment of enteric fever in the travelers visiting endemic areas. There is a necessity to record the burden of antimicrobial resistance pattern in *Salmonella* spp to guide treatment options. Decreased cephalosporin susceptibility in *Salmonella* are major therapeutic obstacles [9]. Table 2 shows an increased prevalence in NARS strains in India from 2% in 1994 to almost 100% in 2016.

Ceftriaxone and Azithromycin are the drugs of choice (DOC) for treating enteric fever caused by NARS strains [17,10]. Emerging resistance to extended spectrum cephalosporins like Ceftriaxone is quite concerning. A north Indian study did not report any CRO resistant strains in their study but stated that MICs of *S.* Typhi and *S.* Paratyphi A are creeping towards resistance [18]. While no CRO resistance was reported, MIC_{50} and MIC_{90} for *S.* Typhi was reported to be 0.064 µg/mL and 0.19 µg/mL respectively and MIC_{50} and MIC_{90} for *S.* Paratyphi A were 0.04 µg/mL and 0.19 µg/mL respectively [18]. In addition, there are some sporadic reports from different parts of India reporting CRO resistance in *S.* Typhi due to *CTX-M-15* and *SHV-12* extended spectrum β-lactamases [11,19]. In our study, we observed 11.6% resistance to CRO, with MIC_{50} and MIC_{90} of 0.125 µg/mL and 8 µg/mL respectively for *S.* Typhi and 0.125 µg/mL and 2 µg/mL respectively for *S.* Paratyphi A. These results were consistent with MIC_{50} of 0.125 µg/mL for *S.* Typhi and *S.* Paratyphi A by agar dilution method as reported by Behl et al [20]. Iyer et al reported complete CRO susceptibility to *S.* Typhi and *S.* Paratyphi A with MIC_{50} 0.064 µg/mL and 0.094 µg/mL respectively [21]. This suggests that there is considerable geographic variation in susceptibilities. An outbreak of XDR (extensively drug resistant) *S.* Typhi (resistant to ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones and 3rd generation cephalosporins), enteric fever cases were reported in Hyderabad, Pakistan in 2016 [12]. Sporadic cases of high-level resistance to ceftriaxone in typhoidal salmonellae due to CTX-M and SHV ESBLs have been reported from Bangladesh, Nepal, Philippines, Kuwait, United Arab Emirates, Germany, and Guatemala [21].

AZM is also a suitable oral alternative for managing uncomplicated enteric fever [22]. Treatment with AZM in adults and children has given successful results in treating enteric fever caused by MDR and NARS strains [8,9]. The advantage of AZM is that it has an excellent intracellular concentration [8,23]. Our study showed 100% susceptibility to AZM which is consistent with findings from a recent study from India [9]. However, their study observed high MIC_{50} (6 µg/mL) and MIC_{90} (12 µg/mL) values for *S.* Typhi. In our study the MIC_{50} and MIC_{90} was 1µg/mL. Another study from South India reported higher MIC_{50} (2 µg/mL for *S.* Typhi; 4 µg/mL for *S.* Paratyphi A) [21]. A similar study documents 100% susceptibility to AZM with MIC_{50} and MIC_{90} being 16 µg/mL and 24 µg/mL respectively for *S.* Typhi and 4 µg/mL and 24 µg/mL for *S.* Paratyphi A [23]. A study from North India reported a gradual increase in AZM MIC for *S.* Typhi from 8 µg/mL to 12 µg/mL, from 2007 to 2016. In the case of *S.* Paratyphi A, the MICs ranged between 2 - 32 µg/mL showing the higher MIC distribution than *S.* Typhi. In addition to the higher MIC values, MIC_{50} and MIC_{90} showed an increase in MICs towards resistance with time, in case of both *S.* Typhi and *S.* Paratyphi A [24]. A study from Bangladesh consisting of 1,082 typhoidal Salmonella isolates from year 2009-2016, identified 13 azithromycin-resistant isolates (12 *S.* Typhi and 1 *S.* Paratyphi A) with an MIC range from 32–64 µg/mL [25]. However, in our study we observed 100% susceptibility to AZM with lower MICs, ranging from 0.125 - 4 µg/mL and 0.25 - 2 µg/mL for *S.* Typhi and *S.* Paratyphi A respectively, MIC_{50} and MIC_{90} value was 1 µg/ mL for both the *S.* Typhi and *S.* Paratyphi A. In a study, from the Netherlands on typhoidal *Salmonella* isolates, Hassing et al. [26] reported MICs higher than 16 µg mL for AZM, with distribution of MICs peaks at 8 and 16 µg/mL, for *S.* Typhi and *S.* Paratyphi A, respectively which was very high in comparison to our study. Resistance to AZM has been reported sporadically but is not yet common. Molley et al. have reported the first case of Azithromycin treatment failure in enteric fever caused by *S.* Paratyphi A, the isolate showed MIC >64 µg/mL [27]. A recent study from India documents treatment failure where they observed in-vitro susceptibility to AZM but in-vivo the strain was found to be resistant to Azithromycin [28].

In our study, interpretations of MIC results have been based on CLSI guidelines [16]. It must be noted that the resistance data may change if another interpretative criterion like the EUCAST (European Committee for Antimicrobial susceptibility testing) is used [29]. In CLSI, the resistant breakpoint (BP) for

CRO is ≥ 4 µg/mL while for AZM it is ≥ 32 µg/mL. In EUCAST, the resistant BP for CRO is > 2 µg/mL and for AZM it is > 32 µg/mL. In our study, 14 *S.* Typhi isolates and 4 *S.* Paratyphi A isolates had MICs > 2 µg/mL for CRO and AZM MIC was < 16 µg/mL for all isolates. Based on our study results, the difference in interpretative criteria between CLSI and EUCAST did not change our resistant rates. Larger number of isolates, their MICs along with the clinical response may need to be studied to conclude if the difference in interpretive criteria between CLSI and EUCAST is significant for management of enteric fever.

Overall, India being a large country, there appears to be a lot of geographical variation in the susceptibility to CRO & AZM across the country. The increase in resistance to CRO could be due to indiscriminate, inappropriate dose and duration of therapy with Ceftriaxone. It is therefore the need of the hour that laboratories report drug susceptibility results along with MIC and interpretation to clinicians who in turn would be able to use these drugs in the appropriate dose and duration. It is also necessary that a continuous surveillance mechanism is present to understand the actual burden of the disease and also to inform clinicians for better management of enteric fever. To the best of our knowledge, our study is one among the few studies to perform manual BMD to determine the MICs of CRO and AZM to NARS isolates. The major limitations of this study are the single center nature and the small sample size. In addition, the molecular analysis for resistance determinants of CRO and AZM have not been evaluated due to lack of funding.

Conclusions

Our study provides an insight into the AST pattern of NARS to the two current therapeutic options for enteric fever i.e. CRO & AZM using the BMD method for drug susceptibility. The study showed complete susceptibility to azithromycin but an alarming 11.6% resistance to ceftriaxone. We conclude that an accurate estimation of MIC for ceftriaxone is the need of the hour to ensure appropriate management and improve clinical outcomes of enteric fever in Mumbai.

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Authors' Contributions

All authors have contributed equally in preparation of this manuscript and have reviewed and confirmed the current manuscript

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