Study of azithromycin sensitivity in enteric fever in pediatric population

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ABSTRACT

Background: Enteric fever is a major human infectious disease in Southeast Asia. It is exacerbated by a high level of resistance some isolates display to the drugs routinely used in treatment. Azithromycin may be a treatment option for such isolates. **Objective:** The objective of this study was to analyze the azithromycin susceptibility in culture positive enteric fever and to evaluate the relationship between ciprofloxacin and azithromycin sensitivity and resistance patterns. **Methods:** This retrospective study was conducted at a tertiary care hospital, Bengaluru, India, between June 2012 and June 2016. Case records of 363 children in the age group of 0–18 years diagnosed with proven enteric fever were reviewed. Susceptibility to antimicrobial drugs was tested by the disc diffusion according to Kirby-Bauer method. They were interpreted based on Clinical and Laboratory Standards Institute guidelines 2016. **Results:** There were 280 *Salmonella enterica* serovar *typhi* (77.13%) and 83 serovar *paratyphi* A strains (22.86%) among the 363 enteric fever children. The susceptibility to azithromycin and third-generation cephalosporins has been 100% among these isolates. Azithromycin minimum inhibitory concentrations (MICs) were 0.064–12 μg/ml among the 363 isolates, and there is no increase in resistance in the past 4 years. Only 5 (18.5%) of the isolates which were resistant to ciprofloxacin had MIC >4 to azithromycin, but none were resistant to azithromycin. **Conclusion:** *Salmonella typhi* continues to remain susceptible to azithromycin and third-generation cephalosporins. There is no increasing trend in resistance to azithromycin over the years in the area of study. Azithromycin can be used in the isolates resistant to ciprofloxacin as there is no correlation between their resistances, thus making it a safe alternative for oral therapy of enteric fever in children.

Key words: *Antimicrobial resistance, Azithromycin, Ciprofloxacin, Enteric fever, Salmonella enterica*

Enteric fever is *a* major human infectious disease since centuries, surviving in conditions of poor sanitation, crowding, and social chaos [1]. It is caused by *Salmonella typhi* and *Salmonella paratyphi* A, B, and C. The crude incidence of typhoid fever in Southeast Asia is 110/1 lakh persons per year. Thus, India is among the high incidence countries for typhoid fever [2]. India and Pakistan account for a very high incidence of typhoid fever compared to other Southeast Asian countries such as Vietnam, Indonesia, and China [3].

Chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole were initially used for the management of typhoid fever. In the late 1980s, there was widespread emergence of multidrug-resistant (MDR) S. typhi to all three drugs [4]. Later in the 1990s, high-level ciprofloxacin-resistant enteric fever evolved in Asian countries, including India [1,2,5-10]. In recent years, the third-generation cephalosporins have been used in fluoroquinolone-resistant areas, but resistance is also emerging to extended-spectrum cephalosporin [7,11]. An Indian study reported that 2% of *Salmonella enterica* isolates were resistant to the third-generation cephalosporins [12]. Until now, there are a few reports of extended-spectrum beta-lactamase (ESBL) producers in S. *typhi* and S. *paratyphi* A. This type of resistance being transferable, the major risk would be its transfer to S. *typhi* and S. p*aratyphi* A. Their overuse in outpatient settings can induce and select strains with ESBLs, early reports of which are emerging from the Indian subcontinent [10,13,14].

Azithromycin, a broad spectrum azilide, has shown promise in the treatment of typhoid fever. It is an attractive alternative to the cephalosporin used in view of its single daily dosing, oral route, possibility of use in β-lactam allergic patients, and lower cost. *In vitro*, azithromycin has a minimum inhibitory concentration (MIC) range of 4–16 μg/ml against S. typhi, suggesting that the drug has limited utility for the treatment of typhoid fever [15]. A rise in MIC over the years has been attributed to irrational prescription for minor community-acquired upper respiratory, ear, and sinus infections [4,5,16-18]. In enteric fever, its role needs to be appreciated, as it is very effective in removing intracellular salmonellae, defervescence is rapid, gastrointestinal carriage is eradicated and, in particular, it represents a potential alternative in the pediatric population for whom quinolones are contraindicated [4].

There are reports of 12% *Salmonella* isolates resistant to azithromycin from a multicenter trial in India [16]. Another risk

factor to add on is that the isolates from Southern Asia showed increased MICs for ciprofloxacin and azithromycin in 21.4% of the isolates, which stresses the fact that azithromycin has to be used with caution in areas with high resistance and decreased susceptibility to ciprofloxacin [19]. Hence, the aim of this study was to determine the *in vitro* MIC patterns of azithromycin for the treatment of enteric fever in an endemic region reporting an increase in ciprofloxacin resistance and a raising ceftriaxone resistance. We also planned to determine if there is a correlation between ciprofloxacin and azithromycin susceptibility pattern among the study population.

MATERIALS AND METHODS

This retrospective study was conducted at a tertiary care hospital, Bengaluru, India, between June 2012 and June 2016. Case records of 363 children in the age group of 0–18 years diagnosed with proven enteric fever were reviewed. The list of children whose blood serology, i.e., Widal test and blood cultures were positive for *S. typhi* or *S. paratyphi* was retrieved from the microbiology laboratory records. Their respective case records were retrieved for verifying the clinical symptoms and diagnosis. Children with clinical symptoms and signs of fever, anorexia, lethargy, abdominal discomfort, nausea, vomiting, hepatomegaly, splenomegaly, and loose stools/constipation, which are compatible with enteric fever and isolation of *S. typhi* or *S. paratyphi* A, B, or C from blood culture were included in the study. Those children who were diagnosed based only on above clinical symptoms and signs with positive serologic tests such as Widal and typhidot/typhidot M along with negative blood cultures for *S. typhi* or *S. paratyphi* were excluded from the study.

Blood culture was done by BacT/Alert 3D system and serotypes were identified by biochemical tests after performing a Gram stain and subculture on blood agar and MacConkey agar. They were also identified by Vitek method. The sensitivity to ampicillin, cotrimoxazole, chloramphenicol, nalidixic acid, and ceftriaxone was interpreted by Kirby-Bauer method using Clinical and Laboratory Standards Institute (CLSI) guidelines 2016 [20]. The disc strength of 10 µg, 1.25/23.7 µg, 30 µg, 30 µg, and 30 µg was used with inhibition zone diameter of ≤13, ≤10, ≤12, ≤13, and ≤19 was considered as sensitive for the above antibiotics, respectively.

The MIC was determined against, azithromycin and ciprofloxacin by Etest. MIC of ≤0.06, 0.12–0.5, and ≥1 to ciprofloxacin disc of 5 µg/ml was considered sensitive, intermediate sensitive, and resistant, respectively, according to the CLSI 2016. However, ciprofloxacin (cipro) MIC of ≤1 and ≥4 was considered as sensitive and resistant, respectively, according to the CLSI guidelines 2011; till, the new guidelines 2016 were available. MIC of ≤16 and ≥32 was considered sensitive and resistant, respectively, for azithromycin disc of 15 µg/ml. Azithromycin MIC ≥16 was considered resistant according to BSAC guidelines when CLSI guidelines were not available for azithromycin before 2016.

The data were analyzed by descriptive univariate analysis. Azithromycin sensitivity was analyzed using time-trend analysis.

RESULTS

Of the 363 blood isolates of *Salmonella*, 280 were *S. enterica* serovar *typhi* (77.14%) and 83 were serovar paratyphi A strains (22.86%). Among these, 361 (99.44%), 357 (98.34%), and 360 (99.17%) were sensitive to first line antityphoid drugs, i.e., ampicillin, cotrimoxazole, and chloramphenicol, respectively. 343 (94.49%) were nalidixic acid-resistant (NAR) *Salmonella* and remaining 20 (5.51%) were nalidixic acid-sensitive (NAS). Of the 272 (74.93%) isolates which showed reduced susceptibility to ciprofloxacin, 29 (7.98%) were resistant and 243 (66.66%) were intermediately sensitive to ciprofloxacin which is less than the NAR *Salmonella* (94.49%). As per definition, only one (0.27%) of the *Salmonella* isolates was MDR, i.e. resistant to ampicillin, chloramphenicol, and cotrimoxazole. The MDR isolate was *S. enterica* serotype typhi and it was also NAR *Salmonella*. 82 (98.7%) strains of serotype Paratyphi A were also NAR *Salmonella,* only one was NAS. All the 363 isolates had maintained 100% sensitivity to ceftriaxone (Fig. 1).

In our study, all the 363 *Salmonella* isolates were sensitive to azithromycin (MIC breakpoint ≤16 µg/ml). Azithromycin MICs were in the range of 0.064–12 µg/ml among the 363 isolates (Fig. 2). The distribution of azithromycin MICs of *S. enterica typhi* and *paratyphi* A peaked at 1, 2, and 1.5 µg, respectively. Trend analysis showed no increasing MIC over time for all isolates or for *S. enterica* serovar *typhi* or *S. paratyphi*, individually. Only 5 (18.5%) of the isolates which were resistant to ciprofloxacin had MIC >4 µg/ml to azithromycin, and none were resistant to azithromycin (Fig. 3).

DISCUSSION

We found that *S. enterica* isolates at the place of study were 100% sensitive to azithromycin. Thus, azithromycin can still be used as a safe alternative for oral therapy of enteric fever in the area of study as opposed to few reports of increasing azithromycin resistance from India. Furthermore, there has been no increasing

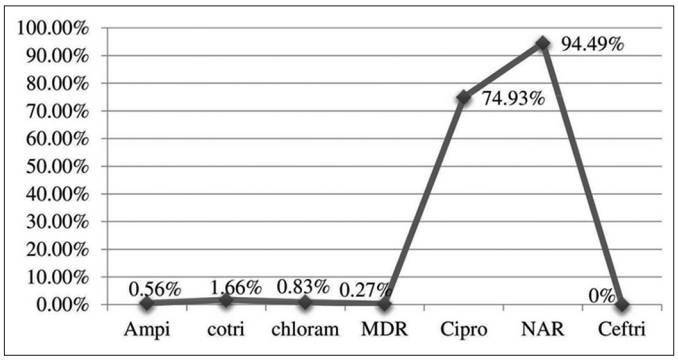

Figure 1: Resistance pattern among *Salmonella typhi* and *Salmonella paratyphi* isolates. Ampi: Ampicillin, Cotri: Cotrimoxazole, Chloram: Chloramphenicol, MDR: Multidrug resistance, Cipro: Ciprofloxacin, NAR: Nalidixic acid-resistant, Ceftri: Ceftriaxone

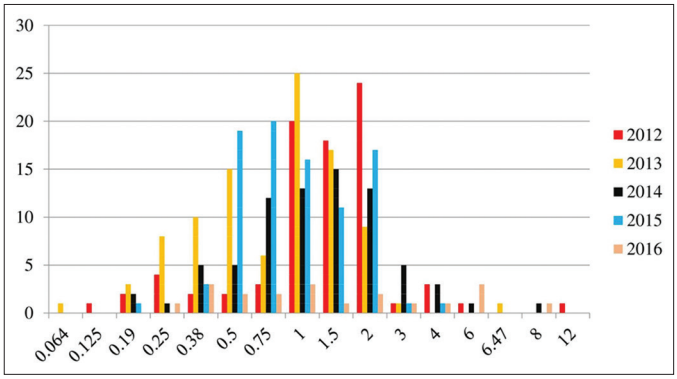

Figure 2: Distribution of azithromycin minimum inhibitory concentrations from 2012–2016. In this bar diagram, X- and Y-axis represents the MIC for azithromycin and number of *Salmonella* isolates, respectively

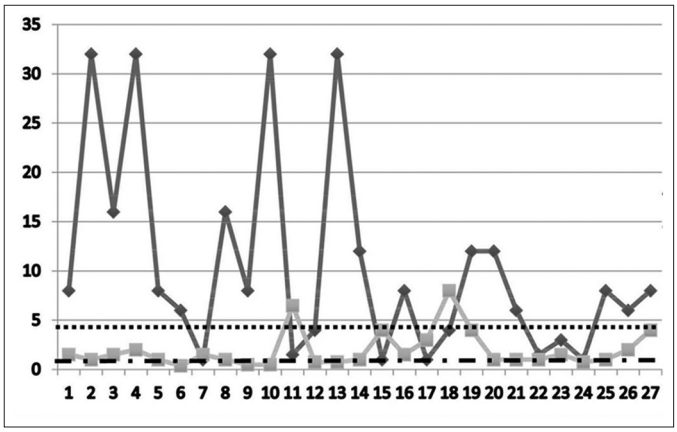

Figure 3: Comparison of azithromycin minimum inhibitory concentrations (MICs) with ciprofloxacin MIC. –––: Azithromycin MIC ≥4 µg/ml, – . – . –: Ciprofloxacin MIC ≥1 µg/ml. The Y-axis represents the MIC and X-axis is the 27 isolates of *Salmonella enterica* which are resistant to ciprofloxacin

trend of resistance for azithromycin in the past 4 years. These results were consistent with the results from study at Pakistan by Butt *et al.*, in 2011, and from Egypt by Girgis *et al.*, in 1999 [4,21].

Irrational use of azithromycin in URI and LRI in the community can induce resistance to this wonder drug. Also of interest is the recent report of azithromycin treatment failure following its use in a shigellosis outbreak in Paris, which is linked to plasmid-mediated resistance to macrolides [22]. It is quite possible that such resistance could be transmitted to *Salmonella* spp. and the above scenario could facilitate this occurrence. In previous studies by Butler *et al.* from India in 1999 and Hassing *et al.* from the Netherlands during 1999–2012 reported that 12% of isolates were resistant to azithromycin and 16.1% of isolates had high MIC (≥16 µg/ml) for azithromycin, respectively [16,19]. A recent study from Delhi has also detected increasing resistance to azithromycin from 2.8% to 17.6% and from 3.6% to 25% in *S. typhi* and *S. paratyphi* A isolates [12].

However, in the murine typhoid model, azithromycin given once daily was highly effective in clearing the infection, and this activity was attributable to the remarkable property of intracellular concentration of azithromycin in macrophages (>100 times the concentrations in serum) [23]. In Bangladesh, Islam *et al.* studied *in vitro* and *in vivo* response to azithromycin and found that 97.6% of azithromycin sensitive cases showed clinical improvement and 77.8% of azithromycin-resistant cases showed improvement. Clinical improvement of patients with azithromycin in azithromycin-resistant cases raises question about the *in vitro* sensitivity of *Salmonella* to azithromycin [24]. It has also been suggested that azithromycin is better for treating typhoid in resistant cases than both fluoroquinolone and ceftriaxone. Azithromycin significantly reduces relapse rate compared with ceftriaxone [25].

Hassing *et al.* reported that percentages of elevated MICs for azithromycin (≥16 µg/ml) were highest for isolates acquired in regions that had concurrent high proportions of isolates with decreased susceptibility or resistance to ciprofloxacin. In isolates acquired in countries from Southern Asia, increased MICs for ciprofloxacin and increased MICs for azithromycin were observed in 21.4% (18/84 isolates) of the isolates [19]. In the present study, 18.5% (5/27 isolates) of the ciprofloxacin-resistant isolates had MIC ≥4 µg/ml for azithromycin but within 12 µg/ml MIC which is sensitive. Hence, there has not been any increased MIC for azithromycin in ciprofloxacin-resistant isolates. This suggests that there is no correlation between ciprofloxacin and azithromycin susceptibility and azithromycin can be used judiciously when the need arises, without fear of increased chances of resistance among ciprofloxacin-resistant isolates in the area of study.

There has been 100% sensitivity to ceftriaxone in the past 4 years. Furthermore, there is resurgence of susceptibility to the first-line antityphoid drugs to the tune of 98–99%; hence, it can be a good option for alternative drugs for enteric fever in the era of limited number of new antibiotics with increasing antimicrobial resistance. The MDR *S. typhi* has reduced drastically to 0.27% which is a good indication to recycle the old drugs. With the development of resistance reported in few studies to ceftriaxone [13,14], the first-line drugs can be considered as the treatment option. However, there was a high prevalence of NAR *Salmonella* in the study population, of which the majority was among *S. paratyphi* A. 98.2% of *S. paratyphi* isolates were NAR. This stresses that fluoroquinolone should be avoided in enteric fever in spite of it being a very effective drug.

The limitation of the study was that the study was a retrospective study; hence, the *in vivo* response to the above drugs was not assessed. Therefore, a prospective study on response to azithromycin and ciprofloxacin and other antityphoid drugs would be required to confirm the findings from the present study. Azithromycin should be used cautiously and judiciously not only in enteric fever but also in many other infections such as upper and lower respiratory infections. A regular survey of local antibiogram patterns is necessary to monitor the antibiotic susceptibility trends and thus guide in appropriate therapy and management.

CONCLUSION

S. typhi continues to remain susceptible to azithromycin and the third-generation cephalosporin. There is no increasing trend in

resistance to azithromycin over the years. Hence, azithromycin can be used safely in the area of study for enteric fever. Furthermore, azithromycin can be used safely in children with ciprofloxacin-resistant isolates as there has not been any significant correlation between ciprofloxacin resistance and high azithromycin MICs.

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