Antimicrobial susceptibility to azithromycin among *Salmonella enterica* Typhi and Paratyphi A isolates from India

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Decreased ciprofloxacin susceptibility (DCS) and multidrug resistance in typhoidal *Salmonella* isolates in areas of endemicity are significant therapeutic problems. Guidelines for azithromycin disc diffusion and MIC interpretive criteria for *Salmonella enterica* serovar Typhi were published recently by the Clinical and Laboratory Standards Institute in 2015. We investigated the antimicrobial susceptibility pattern of azithromycin in 100 isolates of *Salmonella* Typhi (*n* = 80), Paratyphi A (*n* = 18) and B (*n* = 2) recovered from bloodstream infections from January 2013 to December 2015. Zone sizes were extrapolated against MIC values, and a scatter plot was constructed. The azithromycin MICs by Etest ranged from 2 to 16 µg ml⁻¹, while the disc diffusion diameters were from 13 to 22 mm. We observed that the margin of the zone of inhibition around the azithromycin disc may not be very clear and therefore difficult to interpret and that there was wide variation in the zone sizes for the same MIC value in both serovars. DCS was observed in 85% of *Salmonella* Typhi recovered (68/80) and in 15/18 (83.3%) Paratyphi A isolates. Judicious use of azithromycin is advocated as an alternative oral agent in endemic areas where DCS is common.

**INTRODUCTION**

Enteric fever caused by *Salmonella enterica* serovars Typhi, Paratyphi A, B and sometimes C is a systemic infection endemic to low-resource countries of the world (Ochiai et al., 2008). Fluoroquinolones have become the drugs of choice for the treatment of typhoid fever after the emergence of multidrug-resistant (MDR) *Salmonella* Typhi isolates (Parry et al., 2013). However, decreased ciprofloxacin susceptibility (DCS) and fluoroquinolone resistance have developed in association with chromosomal mutations in the quinolone-resistance-determining regions of genes encoding DNA gyrase and topoisomerase IV and also by plasmid-mediated resistance mechanisms (Chau et al., 2007). As a consequence, ceftriaxone for intravenous treatment and azithromycin as an oral agent are increasingly being used for the empirical treatment of uncomplicated enteric fever (Crump et al., 2015). Currently, azithromycin is recommended for the treatment of both shigellosis and invasive salmonellosis by the World Health Organization and the American Academy of Pediatrics. Several randomized controlled trials too have confirmed azithromycin to be equally efficacious or superior to chloramphenicol, fluoroquinolones and extended-spectrum cephalosporins for the successful management of typhoid fever with a low prevalence of relapse and convalescent faecal carriage (Frenck et al., 2000, 2004). The reason is that azithromycin has a half-life of 2 to 3 days and can achieve intracellular concentrations 50 to 100 times greater than serum levels (Frenck et al., 2004).

Guidelines for azithromycin disc diffusion and MIC interpretive criteria for *S. enterica* serovar Typhi were published only recently in the Clinical and Laboratory Standards Institute (CLSI) document M100-S24 (CLSI, 2015). In addition, most studies on the susceptibility pattern of azithromycin are from the developed world and on non-typhoidal *Salmonella* isolates (Sjölund-Karlsson et al., 2011).

We investigated the antimicrobial susceptibility pattern of azithromycin in 100 isolates of *S. enterica* serotypes Typhi, Paratyphi A and B isolates recovered from bloodstream infections in cases of suspected enteric fever at a tertiary care centre in North India. We additionally aimed to study the relationship between azithromycin MIC distribution and disc inhibition zone size in these isolates.

**METHODS**

**Bacterial strains.** This study was conducted at the Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India, from
January 2013 to December 2015. A total of 100 S. enterica serotypes Typhi (n=80), Paratyphi A (n=18) and B (n=2) recovered from blood cultures in suspected cases of enteric fever were included in the study. Isolates were identified by standard biochemical reactions and confirmed by slide agglutination using specific antisera (Denka Seiken).

**Antimicrobial susceptibility testing.** It was performed by Kirby–Bauer disc diffusion (15 µg) as well as by Etest (bioMérieux) to azithromycin as well as ciprofloxacin. Results for azithromycin were interpreted as per the CLSI (2015) guidelines: sensitive ≥13 mm and ≤16 µg ml⁻¹; resistant ≤12 mm and ≥32 µg ml⁻¹ an MIC histogram was constructed and the MIC₅₀ and MIC₉₀ values were calculated. Zone sizes were extrapolated against MIC values, and a scatter plot was constructed. The control strains used for all susceptibility tests were Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923.

**RESULTS**

A total of 100 isolates of S. enterica serovars Typhi (n=80), Paratyphi A (n=18) and B (n=2), respectively, were recovered during the study period. Twenty-eight, thirty-five and thirty-seven isolates were collected during the years 2013, 2014 and 2015, respectively. None of the isolates tested had a zone of inhibition ≤13 mm or an MIC value ≥32 µg ml⁻¹. The distribution of MICs against azithromycin in 100 Salmonella isolates is shown in Fig. 1.

The MICs against azithromycin in the Salmonella Typhi isolates were normally distributed and ranged from 2 to 16 µg ml⁻¹, with MIC₅₀ and MIC₉₀ values of 6 and 12 µg ml⁻¹, respectively. For the Salmonella Paratyphi A isolates, the MICs against azithromycin also ranged from 2 to 12 µg ml⁻¹, and the corresponding MIC₅₀ and MIC₉₀ values were 8 and 12 µg ml⁻¹, respectively. The two Paratyphi B isolates were susceptible to all the antimicrobial agents tested. The azithromycin MICs and disc diffusion diameters for both were 16 and 12 µg ml⁻¹ and 14 and 15 mm, respectively.

The relationship between azithromycin MIC and disc inhibition zone size for Salmonella Typhi and Salmonella Paratyphi A is shown in Fig. 2(a, b). There was substantial variation in the zone sizes in comparison to MICs. The majority of Salmonella Typhi isolates (n=47) had an MIC value of 4 or 6 µg ml⁻¹, and the corresponding zone sizes spanned 13 to 22 mm.

Eighty-five per cent (68/80) of Salmonella Typhi and 15/18 Salmonella Paratyphi A (83.3%) showed DCS.

**DISCUSSION**

Antimicrobial susceptibility data including surveillance of strains with decreased susceptibility or resistance are important not only for assisting clinicians in treating patients but also for detection of emerging resistance. DCS and MDR in typhoidal Salmonella isolates in areas of endemicity are significant therapeutic problems (Misra et al., 2015; Kariuki et al., 2010). Enteric fever due to nalidixic-acid-resistant Salmonella Typhi and/or Salmonella Paratyphi A is treated with either ceftriaxone or azithromycin (Parry et al., 2013; Crump et al., 2015). Resistance to expanded-spectrum cephalosporins is uncommon, but expense and the need for parenteral therapy limit their usefulness as first-line treatments. Azithromycin is therefore emerging as a suitable oral alternative for the management of uncomplicated enteric fever. Treatment courses of 500 mg per day [10 mg (kg body weight) per day] for 7 days and 1 g per day (20 mg kg⁻¹ per day) for 5 days have resulted in successful outcomes for both adults and children including cases with MDR/nalidixic-acid-resistant infections (Frenck et al., 2000, 2004). However, its continued use in countries like India, where indiscriminate antibiotic therapy is rampant and over-the-counter drugs are easily available, will inadvertently result in emergence of resistant isolates with subsequent treatment failure.

The azithromycin MICs of the majority of the isolates (75%) in our study were between 4 and 8 µg ml⁻¹ which is typical of strains isolated across Asia, and the same has been reported by Parry et al. (2015). More than 99% (1452/1460) of Salmonella Typhi and 86.3% of Paratyphi A (207/240) isolates in their study were susceptible to azithromycin. Contrary to the absence of resistance in our study, they reported an MIC of >16 µg ml⁻¹ in 13.8% of Paratyphi A isolates. Molloy et al. (2010) have reported the first case of clinical failure of azithromycin treatment in enteric fever caused by Salmonella Paratyphi A, in which the isolate had an azithromycin MIC of 64 µg ml⁻¹ initially, and then the MIC was 256 µg ml⁻¹ in a second blood culture. However, the specific resistance mechanism was not described. In fact, there are few reports on the mechanisms of resistance to azithromycin in Salmonella spp. A macrolide-2'-phosphotransferase encoded by mphA gene has been described in non-typhoidal Salmonella isolates. The plasmid-borne mph(A) gene that confers resistance to azithromycin and has recently emerged in Shigella sonnei is also present in MDR and non-MDR Escherichia coli isolates collected from...
four continents, and further dissemination to Salmonella species may be expected (Nguyen et al., 2009).

In a study from The Netherlands on typhoidal Salmonella isolates, Hassing et al. (2014) reported MICs of more than 16 µg ml\(^{-1}\) for azithromycin in 16.1% of 354 isolates and in 23.8% of isolates with elevated MICs for ciprofloxacin. We observed DCS in 85% of Salmonella Typhi recovered in our study (68/80) and in 83.3% (15/18) of Paratyphi A isolates. The absence of multidrug resistance in typhoidal Salmonella isolates with intermediate susceptibility to ciprofloxacin at our centre has been previously reported by the authors (Misra et al., 2015). In addition, similar to the results of Sjölund-Karlsson et al. (2011), we did not observe any discordance between the MIC distribution of Typhi and Paratyphi A as reported by other authors (Parry et al., 2015; Hassing et al., 2014; Dutta et al., 2014). CLSI is yet to assign MIC breakpoints or zone diameter criteria for azithromycin in Paratyphi A isolates. More studies on larger numbers of isolates are needed to resolve the difference of two serovars of the same species having different MIC distribution or zone sizes.

We also observed that the margin of the zone of inhibition around the azithromycin disc may not be very clear and therefore may be difficult to interpret and that there was wide variation in the zone sizes for the same MIC value in both serovars (Parry et al., 2015). The same has been represented in Fig. 2(a, b).

In conclusion, the authors acknowledge that this is a single-centre study, and they have performed Etest for MIC determination, although broth microdilution is the reference method. However, we have not come across any study that has compared the three methods for azithromycin MIC testing in S. enterica. To the best of our knowledge, our study is the first on the antimicrobial susceptibility profile of typhoidal Salmonella against azithromycin on such a large number of clinical isolates from India. The inclusion of 18 Paratyphi A isolates is significant. Since there was absence of resistance to azithromycin in all the isolates and the majority showed decreased susceptibility to ciprofloxacin, judicious use of azithromycin is advocated as an alternative oral agent in endemic areas.

**REFERENCES**

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