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Original Article

HIGH LEVEL CIPROFLOXACIN RESISTANCE IN SALMONELLA ENTERICA ISOLATED FROM BLOOD

R Raveendran, *C Wattal, A Sharma, JK Oberoi, KJ Prasad, S Datta

Abstract

Purpose: Over the last few years, resistance to ciprofloxacin in *Salmonella enterica* has become a global concern. The present study was undertaken to find out the susceptibility pattern of *Salmonella enterica* isolates in our hospital. **Methods:** Blood cultures were done using BacT/ALERT 3D system. The antimicrobial susceptibility testing was carried out by the Kirby-Bauer disc diffusion method using CLSI breakpoints. Minimum inhibitory concentration was determined for ciprofloxacin-resistant strains using E-test and Vitek-1 automated system. **Results:** A total of 25,953 samples of blood culture yielded 431 *Salmonella enterica* serotype Typhi and 198 serotype Paratyphi A isolates. Twenty-two isolates of serotype Typhi were resistant to ciprofloxacin resistance is 5.6% (24 isolates) among *Salmonella enterica* serotype Typhi appears to have decreased to 14.9% (64/431) in comparison to the 27% (55/205) during 2003. All isolates were sensitive to ceftriaxone. **Conclusions:** Ciprofloxacin can no longer be considered as the drug of choice in treating *Salmonella* infections. While first-line antimicrobials may still have a role to play in the treatment of enteric fever, ceftriaxone remains the sole defence against ciprofloxacin-resistant *Salmonella* infections.

Key words: Ciprofloxacin resistance, enteric fever, MIC, Salmonella enterica

Enteric fever is a major public health problem in the developing world. It affects 6 million people worldwide with more than 600,000 deaths a year. Almost 80% of the cases and deaths are in Asia and the rest occur mostly in Africa and Latin America.^[1]

Enteric fever is endemic in many developing countries, including India and, if not treated appropriately, has a mortality rate of 30%. Appropriate treatment reduces the mortality rate to as low as 0.5%.^[2]

Chloramphenicol was introduced in 1948 as the first effective antibiotic in the treatment of typhoid fever. Even though resistance started to develop within two years of its introduction, it did not emerge as a major problem until 1972.^[3] Chloramphenicol resistance was associated with high molecular weight, self-transferable, Inc HI plasmids. Amoxicillin and co-trimoxazole were the effective alternatives till the development of 'multidrug resistant (MDR) strains' (resistant to ampicillin, chloramphenicol and co-trimoxazole - ACCo) towards the end of 1980s and 1990s.^[3] These MDR strains also carry the Inc HI plasmids that encoded the resistant genes. MDR *Salmonella enterica* serotype Typhi are still common in many areas, although in some regions fully sensitive strains have re-emerged.

The emergence of MDR *Salmonella enterica* isolates led to the use of fluoroquinolones (ciprofloxacin and ofloxacin) as the first-line drugs for its treatment. Fluoroquinolones have good *in vitro* and clinical activity against salmonellae and became the treatment of choice in cases of MDR salmonellosis.^[4] Isolates with low-level resistance (MIC $\geq 0.25 \ \mu\text{g/mL}$ but <4 $\mu\text{g/mL}$) to fluoroquinolones appeared within a few years of this change.^[5-8] Quinolone resistance is frequently mediated by single-point mutations in the quinolone resistance determining region of the gyr A gene, characteristically occurring at position 83 of the DNA gyrase enzyme (changing serine to phenylalanine) and position 87 (changing aspartate to tyrosine or glycine).^[7]

Until recently, quinolone resistance was believed to arise solely from chromosomal mutations in genes encoding target enzymes or due to decreased accumulation of the drug inside the bacteria. In 1998, mobile elements with the potential for horizontal transfer of quinolone resistance genes were described.^[9] The locus responsible for this plasmidmediated quinolone resistance, designated qnr A, qnr B and qnr S, has been identified in Enterobacteriaceae species.^[10] The qnr A gene confers nalidixic acid (NA) and low-level fluoroquinolone resistance and its presence has been shown to facilitate selection of chromosomal mutations that confer higher levels of resistance.^[9] This plasmid-mediated quinolone resistance was unknown in Salmonella enterica until recently. There is a report of plasmid-mediated quinolone resistance in non-Typhi serotypes of Salmonella enterica carrying either gnr B or gnr S from United States.[11] Plasmid-mediated quinolone resistance in Salmonella is of great concern, since

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horizontal transfer of quinolone resistance would facilitate rapid dissemination of the quinolone resistance genes, further compromising the use of these antimicrobial agents.

Given the variation in the susceptibility patterns reported for *Salmonella enterica*, it is important to constantly monitor its susceptibility so as to provide suitable guidelines for treatment. The present study was undertaken to find out the susceptibility pattern of *Salmonella enterica* isolates in a tertiary health care facility in Delhi, India.

Materials and Methods

A total of 25,953 blood culture samples collected in BacT/ALERT 3D culture bottles (bioMéuriex, France) were processed in the Department of Clinical Microbiology, Sir Ganga Ram Hospital, New Delhi over a period of 20 months (1 January 2005-31 August 2006). Positive blood cultures (signalled by the BacT/ALERT 3D machine) were processed; and the isolates were identified as *Salmonella enterica* serotype Typhi and Paratyphi A by standard biochemical methods^[12] and confirmed by slide agglutination with specific antisera (Salmonella agglutinating serum, Remel, Europe) The antimicrobial susceptibility testing was carried out by the Kirby-Bauer disc diffusion method^[13] using CLSI breakpoints. The antimicrobial agents tested were ampicillin (10 μ g), chloramphenicol (30 μ g), co-trimoxazole (25 μ g), NA (30 μ g), ceftriaxone (30 μ g), ciprofloxacin (5 μ g) and

Table 1: Combination antimicrobial resistance inSalmonella enterica serotype Typhi and Paratyphi A						
Pattern of antimicrobial	Number of isolates (%)					
resistance	Typhi	Paratyphi A				
Susceptible to all the six antimicrobials	35 (8.1%)	5 (2.5%)				
Resistant to nalidixic acid Resistant to A. C. Co*	396 (91.1%) 64 (14.9%)	193 (97.5%) Nil				
Resistant to A & Co*	2 (0.9%)	Nil				
Resistant to A alone* Resistant to C alone*	Nil Nil	2 (1%) Nil				
Resistant to Co alone* Resistant to ciprofloxacin	72 (16.7%) 24 (5.6%)	Nil 2 (1%)				

*with or without NA resistance A, C, Co – Ampicillin, Chloramphenicol, Co-trimoxazole

cefixime (5 µg) (Hi-Media Laboratories, Mumbai). Minimum inhibitory concentration (MIC) was determined for the ciprofloxacin-resistant strains by E-test (AB Biodisk, Sweden) and Vitek-1 automated system (bioMéuriex, France), as per the manufacturers' specifications.

Results

Of the 25,953 blood culture samples, a total of 632 strains of *Salmonella enterica* were isolated. Of these, 431 were *Salmonella enterica* serotype Typhi, 198 were *Salmonella enterica* serotype Paratyphi A, two were *Salmonella enterica* serotype Enteritidis and one was *Salmonella enterica* serotype Typhimurium. The pattern of antimicrobial resistance of the 431 *Salmonella enterica* serotype Typhi and 198 serotype Paratyphi A isolates is shown in Table 1. Among the NAresistant strains, 22 isolates of serotype Typhi were showing high-level resistance, two isolates of serotype Typhi and two isolates of serotype Paratyphi A were intermediate susceptible to ciprofloxacin (defined as MIC >1 µg/mL but <4 µg/mL) and the rest were sensitive. All NA-sensitive strains of serotype Typhi as well as Paratyphi A were sensitive to ciprofloxacin (MIC <0.25 µg/mL).

Antibiogram of *Salmonella enterica* serotype Typhi and Paratyphi A is shown in Table 2. All isolates were sensitive to ceftriaxone. Sensitivity testing for cefixime was started recently and all 45 isolates of serotype Typhi and 13 isolates of serotype Paratyphi A tested were found to be sensitive.

The Fig. 1 shows the distribution of MIC of ciprofloxacin among serotype Typhi and Paratyphi A isolates that were resistant to ciprofloxacin. Twenty (83.3%) of the 24 Typhi isolates had an MIC of \geq 24 µg/mL while both isolates of Paratyphi A had an MIC of 1.5 µg/mL. All the ciprofloxacinresistant isolates were sensitive to ampicillin, chloramphenicol and ceftriaxone but 13 serotype Typhi isolates were resistant to co-trimoxazole.

Discussion

The resistance pattern of *Salmonella enterica* had been varying with time and geographical locations. In our hospital, since April 2003, after noticing inadequate response to treatment with quinolones, NA susceptibility testing was started routinely for all *Salmonella* isolates. NA-resistant

Table 2: Antimicrobial resistance among Salmonella enterica serotype Typhi and Paratyphi A						
Antibiotic	Serotype Typhi (431 isolates)		Serotype Paratyphi A (198 isolates)			
	Number	% Resistance	Number	% Resistance		
Ampicillin	64	14.9	2	1.0		
Chloramphenicol	64	14.9	0	0		
Co-trimoxazole	102	23.7	0	0		
Nalidixic acid	396	91.9	193	97.5		
Ciprofloxacin	24	5.6	2	1.0		
Ceftriaxone	0	0	0	0		





Figure 1: MIC distribution of ciprofloxacin-resistant isolates (24 isolates of serotype Typhi and 2 isolates of Paratyphi A)

Salmonella isolates were found to have almost tenfold higher MIC to ciprofloxacin.^[8] The first high-level ciprofloxacinresistant (defined as MIC $\geq 4 \mu g/mL$) strain was isolated in our hospital in July 2005 with an MIC of >32 μ g/mL. Following that, 21 more high-level ciprofloxacin-resistant and two intermediately susceptible Salmonella enterica serotype Typhi strains and two intermediately susceptible strains of serotype Paratyphi A were isolated. Highlevel ciprofloxacin resistance increased to 6.78% in 2006 (16 Salmonella enterica serotype Typhi and no Paratyphi A) from 1.52% (6 Salmonella enterica serotype Typhi and no Paratyphi A) in 2005. This increase in high-level ciprofloxacin resistance probably reflects the overuse or irrational use of ciprofloxacin in the treatment of typhoid as well as in other unrelated infections. Incomplete treatment may be another factor contributing to development of resistance.

There are several reports of therapeutic failure of fluoroquinolones in patients with enteric fever.^[14,15] Although reported as susceptible by disc diffusion assay using recommended breakpoints to fluoroquinolones, these isolates have smaller zones of inhibition to fluoroquinolones by Kirby-Bauer disc diffusion method and MIC is almost tenfold higher than fully susceptible strains.^[6,8] In recent years, there are some sporadic reports of high-level ciprofloxacin resistance in Salmonella enterica.^[5,16] Renuka et al. reported isolation of Salmonella enterica serotype Typhi strains showing highlevel resistance to ciprofloxacin.^[17] The exact mechanism of fluoroquinolone resistance in Salmonella enterica serotype Typhi and Salmonella enterica serotype Paratyphi A is not fully understood. Various studies have found that a single mutation in the gyr A gene is sufficient to confer resistance to NA and reduced susceptibility to fluoroquinolones, and a second mutation leads to high-level fluoroquinolone resistance.^[17]

In view of the above case reports of therapeutic failure of ciprofloxacin in NA-resistant cases (NA resistance was >90% in the present study) and the recent emergence of high-level ciprofloxacin resistance, ciprofloxacin can not be considered the first choice of antimicrobial for empiric treatment of enteric fever.

All the isolates in our study were sensitive to ceftriaxone in contrast to some studies that recorded resistance to ceftriaxone.^[18,19] Therefore, ceftriaxone is advised to be a reserve drug for treating MDR and ciprofloxacin-resistant cases.

An interesting observation in our study is that multidrug resistance in *Salmonella enterica* serotype Typhi has come down to 14.9% in comparison to the 27% during 2003 (55/205).^[8,20] Moreover, ACCo resistance was not observed in serotype Paratyphi A. One recent study published from North India also shows the same observation;^[21] this trend encourages the use of first-line antibiotics in sensitive cases.

The current study suggests that ciprofloxacin can no longer be considered the drug of choice in treating Salmonella infections due to its high-level resistance. The resistance to first-line antimicrobials (ACCo) appears to be waning in serotype Typhi and not yet appeared in serotype Paratyphi A. Based on our experience of in vitro susceptibility pattern, ACCo may be considered in the empiric therapy of enteric fever. If susceptibility shows otherwise, the therapy can be switched to ceftriaxone. No ceftriaxone resistance was observed in our study. While first-line antimicrobials may still have a role to play in the treatment of enteric fever, ceftriaxone remains the sole defence against ciprofloxacinresistant Salmonella enterica serotype Typhi and Paratyphi A. The usage of this drug in the empiric therapy should be discouraged. Considering the rapid emergence of highlevel ciprofloxacin resistance, is it time to re-think about ciprofloxacin breakpoints or an alternate therapy for enteric fever?

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