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Use of pefloxacin as a surrogate marker to detect ciprofloxacin susceptibility in Salmonella enterica serotypes typhi and paratyphi A

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Abstract

Objective: To determine the use of pefloxacin as a surrogate marker to detect fluoroquinolone (ciprofloxacin) susceptibility against Salmonella enterica serotypes Typhi and Paratyphi A.

Methods: The prospective, descriptive cross-sectional study was conducted at the Aga Khan University Hospital, Karachi, from September 2016 to March 2018, and comprised Salmonella Typhi and Paratyphi A isolates of blood cultures. Disk susceptibility tests and broth microdilution to test minimum inhibitory concentration were performed as per standard guidelines. Data was analysed using SPSS 21.

Results: Of the 138 isolates, 91(66%) were intermediate resistant to ciprofloxacin but were resistant to pefloxacin, 42(30%) were resistant to both ciprofloxacin and pefloxacin, and 5(4%) were susceptible to both ciprofloxacin and pefloxacin. Of the isolates that were intermediate resistant to ciprofloxacin, 85(93%) had minimum inhibitory concentration range0.12-0.5mg\L, while 6(7%) had MIC >1mg\L ($p<0.0001$).

Conclusion: Pefloxacin disk diffusion test was found to be reliable in detecting fluoroquinolone resistance among enteric fever causing Salmonella.

Keywords: Enteric fever causing Salmonella, Ciprofloxacin, Pefloxacin, Fluoroquinolones.

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Introduction

Salmonella spp. cause significant morbidity and mortality in the developing world, especially when they cause enteric fever.[1] *Salmonella* Typhi and Paratyphi (A, B, C) are notorious pathogens to humans and cause 13.5 million annual episodes of enteric fever along with its complications, especially in the low and middle income countries (LMICs).[2] Prompt and appropriate therapeutic interventions affect the prognosis of the patient.[3]

Due to good cellular uptake in macrophages and subsequent high concentration in bile, fluoroquinolones, especially ciprofloxacin, remains a preferred therapeutic option to treat enteric fever in the fluoroquinolone-susceptible strains.[4,5] However, the concern about its resistance in *Salmonella* Typhi and Paratyphi still exists. Fluoroquinolone resistance is mainly caused by chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of the topoisomerase genes *gyrA, gyrB, parC* and *parE*.[6,7] These mutations usually confer stepwise resistance; a single mutation is associated with a ciprofloxacin minimum inhibitory concentration (MIC) of 0.12-0.5 mg\L, whereas two or more mutations result in higher MIC values.[1] For many years nalidixic acid disc (30 µg) was used for the screening of fluoroquinolone resistance in *Salmonella* Typhi and Paratyphi.[8,9] Topoisomerase mutations are associated with resistance to the nalidixic acid (MIC >32 mg\L).[4,10] In addition to the QRDR topoisomerase mutations, a number of plasmid-mediated quinolone resistance (PMQR) mechanisms, including qnr variants, *aac (6')-Ib-cr, qepA,* and *oqx AB* have been described.[1] These demonstrate low-level resistance to ciprofloxacin (MIC 0.12-1.0 mg\L) but only a modest or no increase in nalidixic acid susceptibility (MIC 8-32 mg\L).[11] Therefore, low-level quinolone resistance is not detected by using nalidixic acid disc.

Although the PMQR mechanisms confer only a moderate increase in fluoroquinolone MICs, they are clinically relevant. Patients infected with *Salmonella* Typhi and Paratyphi with ciprofloxacin MICs of 0.12-1.0 mg\L have

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more treatment failures and longer times to fever clearance than patients with isolates fully susceptible to ciprofloxacin (MICs ≤0.06 mg\L).[2]

Since, suboptimal response of treatment has been noticed in infections due to isolates having low-level fluoroquinolone resistance, it is important that laboratories should report correct antimicrobial susceptibility results.[1] Recently, pefloxacin has been introduced as a surrogate marker to detect ciprofloxacin and other fluoroquinolones susceptibility.[12] Therefore, the current study was planned to check the susceptibility of pefloxacin along with ciprofloxacin in local isolates.

Materials and Methods

The prospective, descriptive cross-sectional study was conducted at the Department of Pathology and Laboratory Medicine, Microbiology section, Aga Khan University Hospital (AKUH), Karachi, from September 2016 to March 2018.

Salmonella Typhi and Paratyphi A blood isolates regardless of gender and age were included. Non-lactose fermenter, gram-negative bacilli were identified by using either conventional tests or API-20E (Bio Murex, France) sticks. Serological confirmation was done by slide and tube agglutination method using type-specific antisera (Difco Laboratories).

Disk susceptibility testing was performed by Kirby-Bauer method as per Clinical Laboratory Scientific Institute (CLSI) guidelines.[13] Briefly, a suspension of test organism equivalent to a 0.5 McFarland standard was prepared in saline from isolated colonies grown overnight on a sheep blood agar plate (SBA) (Oxoid Thermo Fisher, Switzerland). Pefloxacin (5µg) and ciprofloxacin (5µg) disks (Oxoid Thermo Fisher, Reinach, Switzerland) were tested on Mueller Hinton Agar (Becton Dickinson MD). Plates were incubated at 35±2^{0}C, in ambient air, for 18-24 hours. Zone sizes were interpreted according to CLSI.[13]

An isolate was defined as ciprofloxacin-resistant (R) with a zone of inhibition of ≤20mm (MIC ≥1mg/L); as susceptible (S) with a zone of inhibition of ≥31mm (MIC ≤0.06 mg\L); and as intermediate resistant (IR) with a zone of inhibition of 21-30mm (MIC 0.12 - 0.5 mg\L). An isolate was defined as pefloxacin-resistant with a zone of inhibition of ≤23mm and susceptible with a zone of inhibition ≥24mm.[13]

For ciprofloxacin IR isolates, MIC was checked by the broth microdilution method as per CLSI guidelines.[13] Briefly, a suspension of the test organism equivalent to a 0.5 McFarland turbidity standard was prepared in saline from well-isolated colonies from an overnight growth on SBA plate. Ninety-six well U-bottom micro-titer plates (Costar, Corning Incorporated) were used, 50µl brain heart infusion broth was added to all the wells except the antibiotic control wells. Further, 100µl of 16µg/ml ciprofloxacin (Sigma Aldrich, MERCK) working solution was dispensed in the antibiotic control wells. Ciprofloxacin concentrations ranged from 0.007 to 16mg\L, and 50µl of organism inoculum were added to all the wells except the antibiotic control wells. Plates were incubated at 35±2^{0}C in ambient air for 18-24 hours. MIC was interpreted as per CLSI guidelines.[13]

American Type Culture Collection (ATCC) strains used as controls in the susceptibility testing were Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853. The quality control results of both, MIC and disk diffusion tests were within acceptable quality control ranges.[13] Ethical exemption was obtained from the institutional ethics review committee.

Data was analyzed using SPSS 21. Descriptive statistics were calculated. Shapiro wilk test was applied to check the normality of the variable. Mean ± standard deviation (SD) or median (interquartile range [IQR]) was calculated for quantitative variables. Frequencies and percentages of qualitative variable were calculated. For 95% confidence interval)CI), p≤0.05 was taken as significant.

Results

Of the 138 isolates, 96(69.5%) were *Salmonella* Typhi and 42(30.4%) were Paratyphi A. Of the total, 91(66%) isolates were IR to ciprofloxacin but R to pefloxacin; 42(30.4%) were R to both drugs; and 5(4%) were S to both ciprofloxacin and pefloxacin. In 85(93%) IR isolates, MIC of ciprofloxacin ranged 0.12-0.5 mg\L(IR) while 6(7%) had MIC ≥1mg\L (R to ciprofloxacin) (Figure) (p<0.0001).

Discussion

Detection of low-level fluoroquinolone resistance in *Salmonella* enterica serotypes Typhi and Paratyphi has always remained challenging.[1] For the past many years nalidixic acid was being used to detect low-level fluoroquinolone resistance among these isolates. Unfortunately, it does not detect plasmid-mediated fluoroquinolone resistance, as isolates appear susceptible

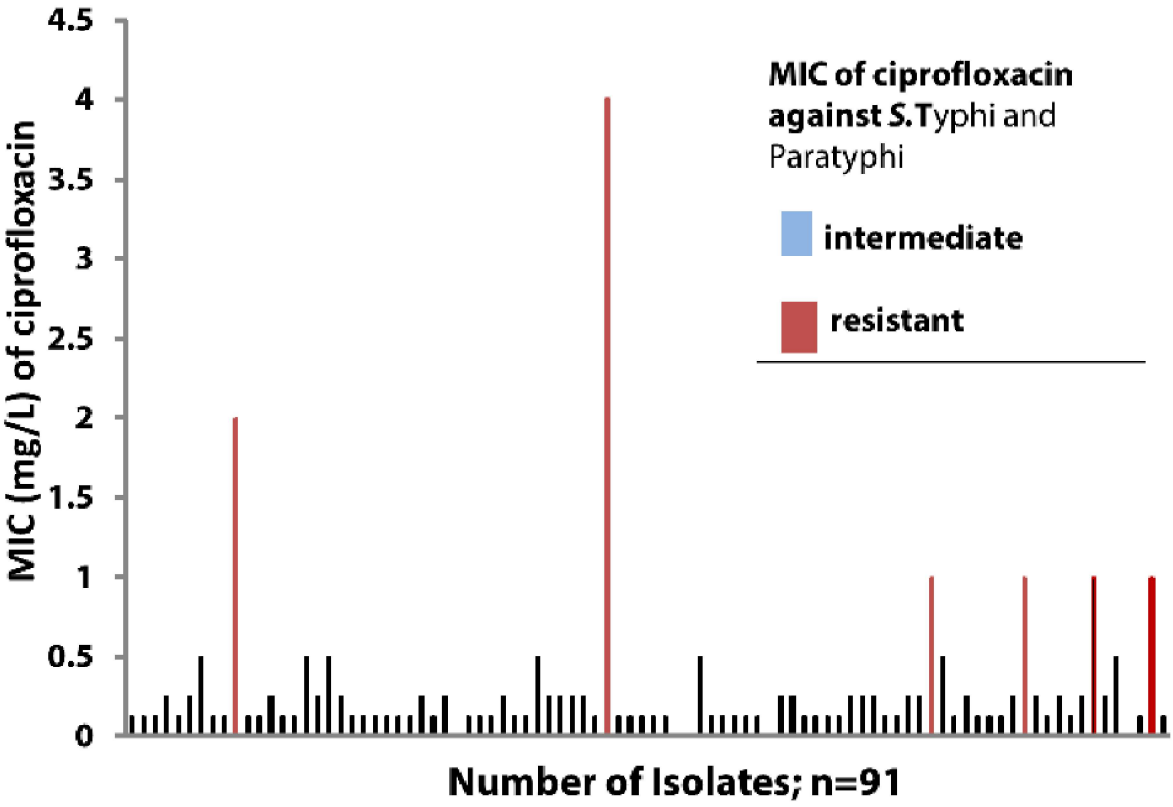

Figure: Minimum inhibitory concentration (MIC) ranges of isolates reported as intermediate to ciprofloxacin by the disk diffusion method. (R: ? 1 mg/ L, IR: between 0.12 mg/ L - 0.5 mg/ L, S ? 0.06 mg/ L).

to nalidixic acid in disc diffusion test but in reality, they are resistant to fluoroquinolones. In many resource-limited settings, MIC tests cannot be performed in the majority of clinical laboratories due to cost constrains and dearth of skilled staff. Pefloxacin has recently been identified as a surrogate marker to detect low-level fluoroquinolone resistance in such *Salmonella spp*. For this purpose, pefloxacin disk assay was recently approved and recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI in 2015.[13,14] The findings of the current study are consistent with those of other studies.[1,4,15,16] Pefloxacin disc diffusion test was able to detect low-level fluoroquinolone resistance in local clinical isolates.

However, pefloxacin disc testing has some limitations. It does not detect all fluoroquinolone resistance mechanisms, such as resistance mediated by the aac(6')-Ib-cr gene.[1,4,17,18] Ciprofloxacin and norfloxacin are the only fluoroquinolones which possess the piperazinyl amide side chain which is the target for the enzyme encoded by aac(6')Ib-cr which acetylates the amino nitrogen on the R7 piperazinyl substituent.[18] For example, isolates that acquire aac(6')-Ib-cr as the sole fluoroquinolone resistance determinant will test susceptible to pefloxacin.[19] Fortunately, this resistance mechanism remains rare.[20]

We recommend the use of pefloxacin as a surrogate marker in resource-limited laboratories to detect ciprofloxacin resistance among enteric *Salmonella* Typhi and Paratyphi.

Conclusion

Pefloxacin disk diffusion assay was found to be a convenient, reliable and cost-effective test to determine fluoroquinolone susceptibility among enteric fever causing *Salmonella*.

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Conflict of interest: None.

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