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Letter to Editor

Microbiology Section

Pefloxacin as a Surrogate Marker for Fluoroquinolone Susceptibility for *Salmonella* typhi: Problems and Prospects

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Sir,

The rise of MDR *Salmonella* typhi promoted the use of ciprofloxacin as the first line therapy since 2000 for enteric fever [1,2]. Due to selective pressure by extensive usage, there had been an emergence of resistance to ciprofloxacin. As per Clinical and Laboratory Standards Institute (CLSI), strains of *Salmonella* that test non-susceptible (intermediate), especially to ciprofloxacin, levofloxacin, ofloxacin, pefloxacin, or nalidixic acid may be associated with clinical failure or delayed response in fluoroquinolone-treated patients with salmonellosis [3]. Tests with nalidixic acid 30 µg and ciprofloxacin 5 µg disc will not reliably detect low-level resistance in *Salmonella* spp. Recently in 2015, CLSI recommended the use of 5 µg pefloxacin disc diffusion as a surrogate marker for identification of fluoroquinolone resistance in *S.*typhi [3]. This study was undertaken to evaluate the effectiveness of pefloxacin disc diffusion with ciprofloxacin disc diffusion and MIC breakpoints and highlights the problem and prospects of pefloxacin as surrogate marker.

Prospects of Pefloxacin

Earlier in 2012 the interpretative breakpoints for ciprofloxacin had been revised, where the susceptibility cut off using disc diffusion was raised from 21 to 31 mm and the MIC value was lowered from 1 to 0.06 µg/mL. In 2013, the disc diffusion interpretative criterion of levofloxacin and ofloxacin for *S.*typhi was removed. Meanwhile, the MIC interpretative criteria for levofloxacin and ofloxacin have been lowered to ≤0.12 µg/mL susceptible, 0.25-1 µg/mL intermediate and ≥2 µg/mL resistant. It is noteworthy that the interpretative criteria for ciprofloxacin, levofloxacin and ofloxacin have been changed only for typhoidal *Salmonella* in the *Enterobacteriaceae* family [4].

Recently (in 2015), CLSI and The European Committee on Anti-microbial Susceptibility Testing (EUCAST) have recommended the use of 5 µg pefloxacin disc diffusion test as reliable surrogate marker to identify the fluoroquinolone susceptibility to *S.*typhi [3,5]. Pefloxacin is understood to identify chromosomal (*gyr*A, *gyr*B, *par*C and *par*E); plasmid (*qnr*A, *qnr*B, *qnr*S and *aac(6')-Ib-cr*) mediated fluoroquinolone resistance better than nalidixic acid and ciprofloxacin (see [Table/Fig-1]). In addition, using pefloxacin can avoid the testing of ciprofloxacin and nalidixic acid by disc diffusion and MIC determination of levofloxacin, ofloxacin and ciprofloxacin [3].

Pefloxacin, in Our Observation

Overall, 282 *S.*typhi isolates from community acquired blood stream infection were collected from January 2012 to December 2014 at Christian Medical College, Vellore, Tamil Nadu, India. All the isolates were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method using ciprofloxacin (5 µg) and pefloxacin (5 µg), and E-test for ciprofloxacin. The results were interpreted according to the CLSI 2015 breakpoints and analysed.

Among the total 282, 4.3% (n = 12), 80.5% (n = 227) and 15.2% (n = 43) isolates were susceptible, intermediate and resistant respectively to ciprofloxacin by disc diffusion method. Similarly, 4.3% (n = 12), 80.5% (n = 227) and 15.2% (n = 43) isolates were susceptible, intermediate and resistant respectively to ciprofloxacin MIC breakpoints. Interestingly, 4.6% (n = 13) and 95.4% (n = 271) isolates were susceptible and resistant respectively to pefloxacin by disc diffusion.

According to CLSI interpretative criteria 80% (*n* = 225) of the isolates, intermediate to ciprofloxacin MIC fell under the category of resistant as per pefloxacin disc diffusion test. This observation perfectly matches with EUCAST ciprofloxacin MIC breakpoints as well. Further, a representative of 25 pefloxacin resistant isolates {ciprofloxacin MIC resistant (*n* = 14) and ciprofloxacin MIC intermediate (*n* = 11)} and two pefloxacin susceptible isolates were tested for gene mutations in *gyr*A, *gyr*B and *par*C genes. All the tested pefloxacin resistant isolates (*n* = 25) were observed to harbour *gyrA* and *parC* mutations (unpublished data).

Problems with Pefloxacin

The interpretative breakpoints defined both by CLSI and EUCAST for pefloxacin were narrow. The interpretative breakpoints for pefloxacin disc diffusion as per CLSI are ≤23 resistant and ≥24 susceptible, and <24 resistant and ≥24 susceptible by EUCAST. The chances for error are high with ± 1 mm difference in zone of inhibition by manual methods.

Since there is no gold standard to compare for pefloxacin disc diffusion testing, we have included a stringent quality control (QC)

Genotype	Phenotype		Remarks	
	CLSI		CLSI	CLSI & EUCAST
	Nalidixic acid (30 µg disc)	Ciprofloxacin (MIC - µg/ml)		Pefloxacin (5 µg disc)
Chromosomal *gyr*A	Resistant	0.12-1.0	Nalidixic acid does not detect all mechanisms of fluoroquinolone resistance	Pefloxacin surrogate marker for all mechanism 24 mm
Chromosomal *gyr*A and *gyr*B	Resistant	≥4		
Plasmid *qnr*A, *qnr*B, *qnr*S and *aac(6')-Ib-cr*	Susceptible	0.12-1.0	-	
No resistance gene	Susceptible	≤0.06	-	-

[Table/Fig-1]: Genotypic, phenotypic and quinolone correlation of resistance mechanism in *Salmonella* typhi.
Note: Limitation is that not all resistance mechanisms can be identified by a single test.

to avoid interpretation errors to a great extent. QC range for *E. coli* ATCC 25922 should be 25-31 mm, with a target of 28 mm and mean value of repeated tests for pefloxacin should be within 27-29 mm (target ± 1 mm) [6]. Also, one should be aware that the presence of inner colonies in pefloxacin disc diffusion testing suggests resistance [7]. In addition, pefloxacin cannot be used to detect the resistance mediated by *aac*(6')-*Ib-cr*, as this plasmid mediated mechanism is specific for fluoroquinolones possessing a piperazinyl secondary amine (ciprofloxacin and norfloxacin) [8].

The remarkable observation of 80% of the isolates, intermediate to ciprofloxacin MIC falls under the category of resistant as per pefloxacin disc diffusion test (confirmed by molecular characterization). This clearly indicates that the patients with intermediate ciprofloxacin MIC if treated with high dose of ciprofloxacin will lead to treatment failure or delayed response. Conversely, if we consider pefloxacin as a surrogate marker for fluoroquinolone resistance, this will lead to appropriate interpretation and therapeutic success rate.

REFERENCES

[1] Asperilla MO, Smego RA, Scott LK. Quinolone antibiotics in the treatment of *Salmonella* infections. *Rev Infect Dis*. 1990;12:873–89.
[2] Eykyn SJ, Williams H. Treatment of multiresistant *Salmonella typhi* with oral ciprofloxacin. *Lancet*. 1987; ii:1407–08.
[3] CLSI, Clinical and Laboratory Standards Institute (2015) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement M100-S25. Wayne: Clinical and Laboratory Standards Institute.
[4] Balaji V, Sharma A, Ranjan P, Kapil A. Revised ciprofloxacin breakpoints for *Salmonella* typhi: Its implications in India. *Indian J Med Microbiol*. 2014;32:161-63.
[5] EUCAST, The European Committee on Antimicrobial Susceptibility Testing (2015) Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, 2015. http://www.eucast.org.
[6] Matuschek E, Skov R, Ahman J, Karlsson M, Petersen A, Torpdahl M, et al. EUCAST disk diffusion with pefloxacin 5 µg as screen for fluoroquinolone resistance in *Salmonella* spp. Variation between media, disks and testing sites. ECCMID 2014. Barcelona, Spain. Pp. 279.
[7] Deak E, Skov R, Hindler JA, Humphries RM. Evaluation of surrogate disk tests for the detection of ciprofloxacin and levofloxacin resistance in clinical isolates of *Salmonella enterica*. *J Clin Microbiol*. 2015;53:3405–10.
[8] Fang FC. Fluoroquinolone Resistance in *Salmonella* and the Utility of Perfloxacin Disk Diffusion. *J Clin Microbiol*. 2015;53:3401–04.

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