Indian Journal of Microbiology Research 6 (2019) 198–201

Content available at: iponlinejournal.com

Indian Journal of Microbiology Research

Journal homepage: www.innovativepublication.com

Original Research Article

Pefloxacin susceptibility as a surrogate test to detect ciprofloxacin-resistance in typhoidal Salmonella

Arunava Kali[1,*], Pravin M. V Charles[1], Sreenivasan Srirangaraj[1], K.S Seetha[1]

[1] *Dept. of Microbiology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth University, Pondicherry, India*

ARTICLE INFO

Article history:
Received 10-06-2019
Accepted 11-07-2019
Available online 09-09-2019

Keywords:
Quinolone resistance
Pefloxacin
Typhoidal salmonella

ABSTRACT

Introduction: Quinolones have been widely prescribed antibiotics worldwide for treating enteric fever. Emergence of resistance to quinolones has limited its therapeutic reliability. Low-level quinolone resistance frequently escapes detection by routine disc diffusion method of antibiotic susceptibility testing and results in treatment failure. Currently, pefloxacin disc diffusion test has been advocated as a surrogate for to differentiate ciprofloxacin -resistant *Salmonella* strains.
Objective: To determine effectiveness of pefloxacin disc as marker for ciprofloxacin resistance.
Materials and Methods: A total of 14 *S.enterica* serovar Typhi and 4 *S. enterica* serovar Paratyphi A were tested by E-test for ciprofloxacin and disc diffusion test for nalidixic acid, ciprofloxacin and pefloxacin.
Results: All 18 of our isolates had resistance to nalidixic acid and pefloxacin. Ciprofloxacin E-test detected 16 intermediate and two resistant strains, while 14 intermediate and four resistant strains were found by disc diffusion test.
Conclusions: Ciprofloxacin non-susceptibility was common in typhoidal *Salmonella* isolates in our hospital. Susceptibility of nalidixic acid and pefloxacin by disc diffusion correctly revealed isolates which had increased ciprofloxacin minimum inhibitory concentration (MIC).

1. Introduction

Infections caused by *Salmonella* species is a serious health-care problem in developing countries all over the world. While *Salmonella enteric*a serovar Typhi (*S*.Typhi) and *Salmonella enteric*a serovar Paratyphi A, B & C (*S*.Paratyphi) cause enteric fever, non-typoidal *Salmonella* species are associated with gastroenteritis, bacteraemia and invasive infections. As per the WHO estimate, typhoidal *Salmonella* accounts for about 11 to 21 million cases and 128000 to 161000 deaths every year worldwide.[1] Despite the introduction of newer antibiotics and community health measures, enteric fever continues to be a significant burden in India.[2] National Health Profile 2016 data show about 18,45997 cases and 393 deaths were attributed to Enteric fever in 2015 in India.[3]

Chloramphenicol, cotrimoxazole and amino- penicillins were primarily prescribed drugs for definite treatment of enteric fever.[4] In view of rapid widespread resistance to these antibiotics, fluoroquinolones such as ciprofloxacin, levofloxacin and ofloxacin became the preferred treatment. These fluoroquinolones have bactericidal action and are available in oral preparations which are inexpensive and well tolerated.[5] However, these fluoroquinolones have been found to lose their therapeutic utility gradually with the emergence of quinolone-resistant and multidrug resistant strains. Several cases of suboptimal or delayed response and treatment failure with fluoroquinolones have been reported.[6,7] Typhoidal *Salmonella* isolates with reduced susceptibility (increased MIC) to ciprofloxacin and nalidixic acid resistance have appeared in increased frequency.[7] Detection of these resistant strains has critical role in guiding antibiotic therapy and for preventing development of multidrug-resistance. In 2015, pefloxacin disc diffusion has been recommended by CLSI as a surrogate test for

* *Corresponding author*.
E-mail address: ak.arunava@gmail.com (A. Kali).

https://doi.org/10.18231/j.ijmr.2019.044
2394-546X/© 2019 Published by Innovative Publication.

fluoroquinolone resistance.[8] In this study, we evaluated the utility of pefloxacin in comparison to nalidixic acid and ciprofloxacin.

2. Materials and Methods

A total of 14 *Salmonella enteric*a serovar Typhi (*S*.Typhi) and four *Salmonella enteric*a serovar Paratyphi A (*S*.Paratyphi A) isolated from blood, urine and stool samples during March 2013 to December 2014 were included in this study. Among the 14 *S*.Typhi isolates, 11 were from blood and three from urine samples, while two *S*.Paratyphi A were from blood and one each from urine and stool samples.

The isolates were identified by colony characteristics, biochemical reactions and sero -grouping with specific antisera. Disc diffusion test was done in Kirby-Bauer method with 30 μg nalidixic acid, 5 μg ciprofloxacin and 5 μg pefloxacin discs (Himedia, Mumbai, India) Figure 1. Ciprofloxacin MIC of all strains for were estimated by E-test (Himedia, Mumbai, India). E.coli ATCC 25922 stain was used for quality control of antibiotic susceptibility tests. The disc diffusion and E-test break points were interpreted as per CLSI 2015 guidelines.[8] The data was tabulated and analysed in Microsoft excel.

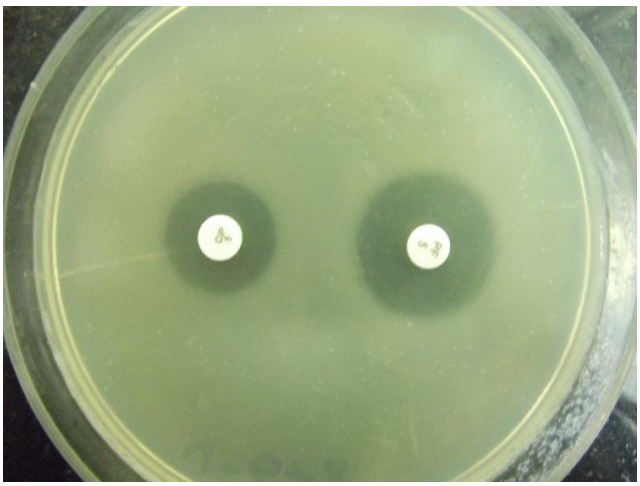

Fig. 1: Ciprofloxacin and pefloxacin disc diffusion test

3. Results

We carried out disc diffusion for nalidixic acid, ciprofloxacin and pefloxacin and E-test for ciprofloxacin. The result is detailed in table 1.

4. Discussion

Antibiotic therapy is the most decisive component of enteric fever management. While untreated cases of enteric fever remains a life threatening acute febrile illness with a case fatality rate of 10-30%, appropriate antibiotic therapy not only brings down the fatality to 1-4%, but also reduces the duration of fever and complications.[1] Although, quinolones were preferred for enteric fever treatment by clinicians, currently quinolones are losing therapeutic utility with rapid emergence and spread of quinolone-resistant typhoidal *Salmonella* strains.[9] Fluoroquinolone resistance is attributed to mechanisms such as mutation of chromosomal genes such as quinolone resistance-determining regions (QRDRs) of gyrase and topoisomerase genes, procurement of plasmid mediated quinolone resistance genes (PMQR) and overexpression of drug efflux pumps in *Salmonella* species.[10–12] Firstly, mutations in topoisomerase genes such as *gyrA* , *gyrB, parC,* and *parE* results in increase in MIC for fluoroquinolones as well as nalidixic acid. Mutations in *gyrA* is more common. While single mutation marginally increase the ciprofloxacin MIC above the wild type (MIC $>$ 0.06 μg /ml), subsequent mutations results in high-level resistance in a stepwise manner.[12] Although less common, plasmid borne various genes such as *qnr* or *aac(6=)-Ib-cr, qepA*, and *oqxAB* confer quinolone resistance in *Salmonella species*.[13] A moderate elevation of ciprofloxacin MICs (0.125 to 1.0 μg /ml) without reciprocal resistance to nalidixic acid is a unique feature of PMQR.[11] Unlike the QRDR mechanism, this resistance is low-level and escapes detection by nalidixic acid disc diffusion test, which was used as a surrogate test for fluoroquinolone resistance by CLSI before 2015. This low-level resistance by PMQR genes is more significant as it is more frequently associated with treatment failures and delayed fever clearance.[6,11] Continued treatment with quinolones for these strains may lead to development of high-level resistant and multi-resistant strains.[11] In 2015, CLSI endorsed pefloxacin disc diffusion as a better marker to represent fluoroquinolone resistance.

In our study, we compared pefloxacin disc diffusion results with ciprofloxacin disc diffusion and E-test results. None of the 18 *Salmonella* strains were sensitive to pefloxacin and nalidixic acid. All had pefloxacin zones and nalidixic acid zones in resistant range, ciprofloxacin intermediate susceptibility and resistance were seen in 4 and 1 4 isolates by disc diffusion and in 2 and 16 isolates by E-test respectively. C iprofloxacin MIC of our strains varied from 0.25 to 32 μg /ml. Lo w-level resistance (0.125 to 1.0 μg /ml) was found in 16(12 *S.* Typhi and 4 *S.* Paratyphi A) isolates and high-level resistance ($\geq$ 1 μg /ml) in two *S.* Typhi isolates (MIC of 32 and 8 μg /ml respectively). Smaller pefloxacin zones of inhibition (15 mm and 19 mm respectively) were obtained in these two *S.* Typhi in comparison to other isolates. Since no ciprofloxacin intermediate / resistant strain were nalidixic acid sensitive, PMQR mechanism is unlikely in our isolates. Our result suggests that quinolone resistance in our isolates might be due to QRDR mutation. The predominance of low-level ciprofloxacin resistance may

Table 1: Susceptibility pattern of S. Typhi and S. Paratyphi A isolates to ciprofloxacin, pefloxacin and nalidixic acid

	S. Typhi (n=14)			*S.* Paratyphi A (n=4)		
	Sensitive	**Intermediate**	**Resistant**	**Sensitive**	**Intermediate**	**Resistant**
Ciprofloxacin E-test	0	12(85.7%)	2(14.2%)	0	4(100%)	0
Ciprofloxacin disc diffusion test	0	10(71.4%)	4(28.5%)	0	4(100%)	0
Pefloxacin disc diffusion test	0	0	14(100%)	0	0	4(100%)
Nalidixic acid disc diffusion test	0	0	14(100%)	0	0	4(100%)

represent more prevalence of strains with single mutations in topoisomerase genes. However, these results has not been validated by molecular test. In a study from India, Joshi et al reported that out of 50 typhoidal *Salmonella* isolates 76% were nalidixic acid resistant with QRDR mutation and 14% were nalidixic acid sensitive as well as intermediate to ciprofloxacin.[14] Although we could not find any difference in pefloxacin zones and nalidixic acid results due to low number of isolates tested, the superiority of pefloxacin has been reported by several authors. Sharma *et al* identified pefloxacin disc diffusion as an effective surrogate test for ciprofloxacin MIC having 100% sensitivity, 99.5% specificity and 94.4% positive predictive value.[5] Similar findings were observed in studies from various parts of the world.[10–12,15] However, narrow interpretative breakpoints for pefloxacin delineated by CLSI and EUCAST guideline increases the possibilities for error.[15] Slight variation of pefloxacin zone of inhibition has been noted with discs from various manufacturers.[10] Furthermore, strains with *aac(6=)-Ib-cr* gene which confers resistance to fluoroquinolones with piperazinyl substituent such as ciprofloxacin, norfloxacin, and enoxacin, cannot be identified by pefloxacin disc.[11]

5. Conclusions

Preponderance of typhoidal *Salmonella* strains with ciprofloxacin MIC (0.125 to 1.0 μg /ml) indicating low-level resistance was observed in our study. Nalidixic acid and pefloxacin by disc diffusion results were in agreement with ciprofloxacin MIC. Pefloxacin disc diffusion is an effective and economical alternative to ciprofloxacin MIC testing. It has essential role in identifying quinolone-resistant *Salmonella* strains in resource poor setups where facilities of determination of MIC is not available.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. World Health Organization. *Vaccine-Preventable Diseases Surveillance Standards: Typhoid and other invasive salmonellosis*. 2018;.
2. John J, Aart CV, Grassly NC. The Burden of Typhoid and Paratyphoid in India: Systematic Review and Meta-analysis. *PLoS Negl Trop Dis*. 2016;10:4616–4616.
3. C N. Enteric Fever (Typhoid) - Prevalence & Deaths in India ; 2017,.
4. BN H, Menezes GA, K S, SC P. A case report and review of the literature: ciprofloxacin resistant Salmonella enterica serovar Typhi in India. *J Infect Dev Ctries*. 2008;2:324–331.
5. Sharma P, Dahiya S, Kumari B, Balaji V, Sood S, et al. Pefloxacin as a surrogate marker for quinolone susceptibility in Salmonella enterica serovars Typhi & Paratyphi A in India. *Indian J Med Res*. 2017;145:687–92.
6. Slinger R, Desjardins M, Mccarthy AE, Ramotar K, P J, et al. Suboptimal clinical response to ciprofloxacin in patients with enteric fever due to Salmonella spp. with reduced fluoroquinolone susceptibility: a case series. *BMC Infect Dis*. 2004;4:36–36.
7. Rattanaumpawan P, Nachamkin I, Bilker WB, Roy JA, Metlay JP, et al. High fluoroquinolone MIC is associated with fluoroquinolone treatment failure in urinary tract infections caused by fluoroquinolone susceptible Escherichia coli. *Ann Clin Microbiol Antimicrob*. 2017;16:25–25.
8. Performance standards for antimicrobial susceptibility test. Approved Standard. CLSI Document M100-S25. Wayne, Pennysylvani ; 2015,. 25th ed.
9. Sharma P, Dahiya S, Manral N, Kumari B, Kumar S, et al. Changing trends of culture-positive typhoid fever and antimicrobial susceptibility in a tertiary care North Indian Hospital over the last decade. *Indian J Med Microbiol*. 2018;36:70–76.
10. Deak E, Skov R, Hindler JA, Humphries RM. Evaluation of Surrogate Disk Tests for Detection of Ciprofloxacin and Levofloxacin Resistance in Clinical Isolates of Salmonella enterica. *J Clin Microbiol*. 2015;53:3405–3415.
11. FC F. Fluoroquinolone Resistance in Salmonella and the Utility of Pefloxacin Disk Diffusion. *J Clin Microbiol*. 2015;53:3401–3404.
12. Skov R, Matuschek E, Sjolund-Karlsson M, Ahman J, Petersen A, et al. Development of a Pefloxacin Disk Diffusion Method for Detection of Fluoroquinolone-Resistant Salmonella enterica. *J Clin Microbiol*. 2015;53:3411–3417.
13. Veeraraghavan B, Anandan S, DP MS, N P, K W, et al. Molecular Characterization of Intermediate Susceptible Typhoidal Salmonella to Ciprofloxacin, and its Impact. *Mol Diagn Ther*. 2016;20:213–219.
14. Joshi S, Amarnath SK. Fluoroquinolone resistance in Salmonella typhi and S. paratyphi A in Bangalore. *India Transactions Royal Soc Tropical Med Hygiene*. 2007;101:308–318.
15. Veeraraghavan B, Anandan S, Sethuvel DP, Ragupathi NK. Pefloxacin as a Surrogate Marker for Fluoroquinolone Susceptibility for Salmonella typhi: Problems and Prospects. *J Clin Diagn Res*. 2016;10:1–1.

Author biography

Arunava Kali Associate Professor

Pravin M. V Charles Associate Professor

Sreenivasan Srirangaraj Professor

K.S Seetha Professor

Cite this article: Kali A, Charles PMV, Srirangaraj S, Seetha KS. Pefloxacin susceptibility as a surrogate test to detect ciprofloxacin-resistance in typhoidal Salmonella. *Indian J Microbiol Res* 2019;6(3):198-201.