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Is nalidixic acid screening still valid for the detection of reduced susceptibility of fluoroquinolone with salmonella typhi?

Seema Irfan
Aga Khan University

Kauser Jabeen
Aga Khan University, kausar.jabeen@aku.edu

Umair Anwar Jilani
Aga Khan University

Bushra Ahmed
Aga Khan University

Jaishri Mehraj
Aga Khan University

See next page for additional authors

Authors
Seema Irfan, Kauser Jabeen, Umair Anwar Jilani, Bushra Ahmed, Jaishri Mehraj, Tanwir Ahsan, and Afia Zafar

Original Article

Is nalidixic acid screening still valid for the detection of reduced susceptibility of fluoroquinolone with *Salmonella*Typhi?

Seema Irfan[1], Kauser Jabeen[1], Umair Anwar Jilani[2], Bushra Ahmed[1], Jaishri Mehraj[1], Tanwir Ahsan[1], Afia Zafar[1]

[1]*Department of Pathology Microbiology, Aga Khan University, Karachi, Pakistan*

Abstract

Introduction: Considering the limitations of screening with nalidixic acid to detect reduced susceptibility to fluoroquinolones of *Salmonella enterica* serovar Typhi (*S.*Typhi) strains, we evaluated the use of a 30 µg nalidixic acid disc screening method in Pakistan.

Methodology: Non duplicate nalidixic acid susceptible *S.* Typhi isolates (246) from 2003-2008 were retrieved from the *Salmonella* strain bank. Minimum inhibitory concentrations of ciprofloxacin for all strains were determined by agar dilution and further rechecked by ciprofloxacin E-tests.*E. coli* ATCC 25922 was used as the control strain. The MIC data for ciprofloxacin were compared with nalidixic acid disk (30µg) zone diameters.

Results: Repeat testing of all *S.* Typhi isolates with a nalidixic acid (30µg) disk showed 100% susceptibility with an average zone diameter of 26 mm. Agar dilution testing revealed reduced susceptibility to ciprofloxacin, with MICs of 0.125 µg /ml for three (1.2%) isolates only. Zone sizes of strains with higher MICs were significantly lower than the strains with lower MICs (20 versus 26 mm) (p value <0.001).

Conclusion: Estimation of fluoroquinolone MICs on every nalidixic acid susceptible *S.* Typhi strain is not cost effective in our setting; the proportion of strains with high fluoroquinolone MICs was found to be very low. We recommend periodic fluoroquinolone MIC determination to include all isolates with a nalidixic acid borderline zone (size 20-22 mm).

Key words: enteric fever; antimicrobial resistance; nalidixic acid susceptible; fluoroquinolone resistant

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Introduction

Fluoroquinolones have been recommended by World Health Organization (WHO) as empirical therapy for the management of multidrug resistant enteric fever [1]. Globally the spread of *Salmonella enterica* serovar Typhi (*S.* Typhi) strains with fluoroquinolone reduced susceptibility has become a major problem [2,3]. Current Clinical Laboratory Standards Institute (CLSI) break points for reporting fluoroquinolone susceptibility in *Enterobacteriaceae* do not detect these strains [4]. By using a disk diffusion method, usually these strains appear resistant when tested with a nalidixic acid disk (30 µg) and therefore screening with it is a sensitive, specific and cost-effective approach, used by many laboratories in resource-limited countries [5].

Pakistan is a high burden country for typhoid fever with an annual incidence of 412.9/100,000 person years [6]. Optimal detection of endemic strains with reduced susceptibility to fluoroquinolones, for example, ciprofloxacin MIC ≥ 0.125 or ofloxacin [6], is crucial as this drug is frequently used by family physicians for the management of typhoid. Emergence of strains with reduced susceptibility to fluoroquinolone has led to treatment failure, as previously reported in regional and local studies [7,8)]. The method of screening with nalidixic acid has been validated and reported to be efficient for endogenous isolates and is currently in use by many laboratories in Pakistan [9,10]. However the recent emergence of nalidixic acid susceptible *S.* Typhi strains with high fluoroquinolone minimum inhibitory concentration (MIC) is of great concern [11-14]. According to recent publications, the majority of reported nalidixic acid susceptible and fluoroquinolone resistant strains originated from India, Pakistan or Bangladesh [12]. This finding is alarming as it questions the validity of the nalidixic acid screening test and could lead to underreporting of fluoroquinolone resistance, resulting in clinical failure. In view of reports of emergence of these discordant strains, we reevaluated the fluoroquinolone MICs of nalidixic acid susceptible local *S.* Typhi strains with an aim to assess the current

utilization of the nalidixic acid screening method in our setup.

Methodology

This study was conducted in a clinical microbiology laboratory of the Aga Khan University, Karachi, Pakistan (AKU). The university and its laboratory have Joint Commission International accreditation (JCIA). The laboratory routinely participates in external quality control surveys with the College of American Pathologists (CAP). The clinical microbiology laboratory of AKU receives on the whole 400,000 specimens/year from both inpatients and outpatients from clinics and hospitals within the city as well as from all over the country via laboratory collection points in 50 major cities and towns of Pakistan.

For the current study a total of 246, non-duplicate, nalidixic acid susceptible bacteremic isolates of *S.* Typhi were selected by convenience sampling and retrieved from the *Salmonella* strain bank and saved at -80°C. The laboratory *Salmonella* bank is comprised of both nalidicic acid disk susceptible and resistant isolates. Out of those, only nalidixic acid disk susceptible isolates were chosen. All isolates were previously reported as fluoroquinolone susceptible based on nalidixic acid disk (30µg) screening. Out of these 246 isolates, 26 were retrieved from year 2003, 164 from 2004, 27 from 2005, 7 from 2006, 12 from 2007, and 10 from 2008. These strains were first cultured on MacConkey's agar. The repeat identification and serotyping were done using standard procedures [15]. The susceptibility of all isolates to nalidixic acid was reevaluated using a 30 µg nalidixic acid disk [4] and zone diameters of ≤ 13 were considered as sensitive,, 14-18 were considered as intermediate, and ≥ 19 mm were considered as resistant [3]. The MIC of ciprofloxacin was determined using the agar dilution method. Isolates with ciprofloxacin MIC of ≤ 0.06 µg /ml were considered as susceptible while isolates with MIC of ≥ 0.125 µg /ml were considered as having reduced susceptibility to fluoroquinolones [16]. Isolates with MIC of ≥ 0.125 µg /ml were further confirmed by using Etest (AB Bio disk). *E. coli* ATCC 25922 was used as the control strain.

Results

Repeat testing of 246 *S.* Typhi isolates using a nalidixic acid (30 µg) disk showed nalidixic acid susceptibility of all strains with an average zone diameter of 26 mm (ranged between 20-33 mm). A total of 175 isolates (71%) showed a ciprofloxacin MIC of ≤ 0.03µg/ml and an average zone diameter of 27 mm with a nalidixic acid (30 µg) disk. Sixty-eight *S.* Typhi isolates (27.6%) showed MIC of 0.06 µg /ml with an average zone size of 25 mm, and only three isolates (1.2%) had MIC considered to represent reduced susceptibility (0.125 µg /ml and an average zone size of 20 mm). As shown in the Figure, two of

Figure 1.

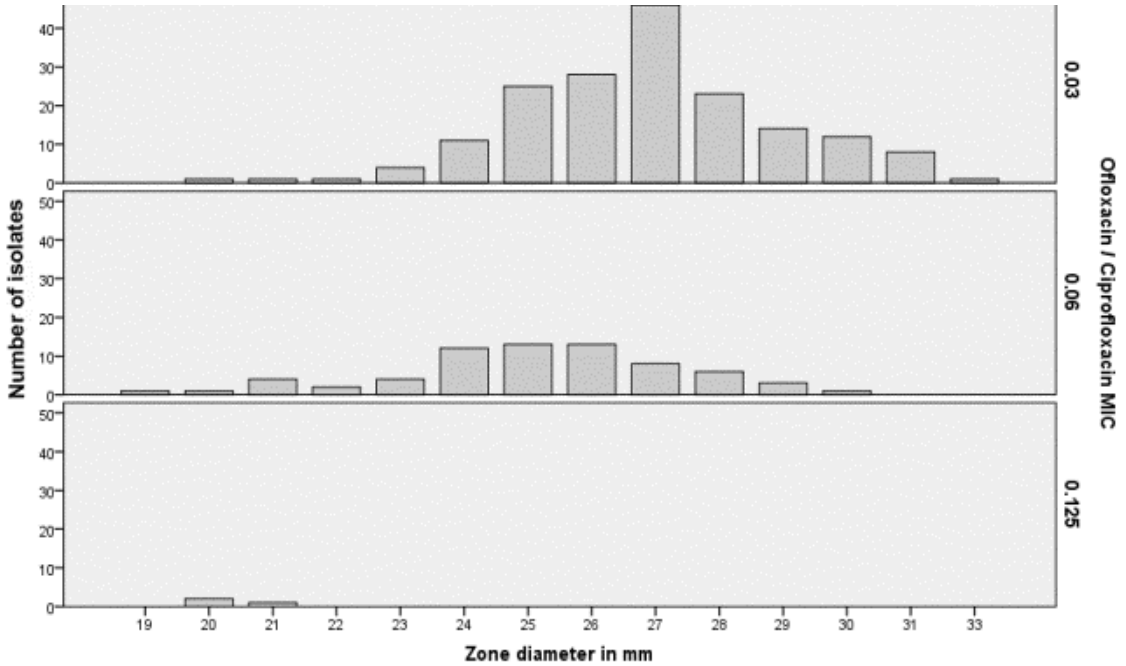

Table. Ciprofloxacin MIC results and their correlation with nalidixic acid zone sizes in *S.* Typhi isolates (N = 246).

MIC ranges for Ciprofloxacin (µg/mL)	Number of organisms (%)	Zone size (mm) (Nalidixic acid disk 30µg) Mean (±SD)
≤0.03	175 (71.1)	27 (2.0)
0.06	68 (27.6)	25(2.2)
0.125	3 (1.2)	20 (0.5)*
0.25	0	0 (0)

Total number of isolates = 246

*p<0.001 when compared isolates with MIC (≤0.03-0.06 µg/mL). P-value calculated through student's t-test.

the reduced susceptibility isolates belonged to year 2005, and one to year 2006. The mean zone of the strains with ciprofloxacin MIC of 0.125 µg /ml was significantly lower than the mean zone sizes of strains with low MICs (p value < 0.001) as shown in the Table.

Discussion

This study confirms the emergence of nalidixic acid susceptible *S.* Typhi isolates with fluoroquinolone reduced susceptibility in Pakistan. Studies from the United Kingdom, United States, and Kuwait [11-13] reported a higher percentage of discordant nalidixic acid and ciprofloxacin susceptibility results in their Southeast Asian immigrants or travellers (*i.e.*, 7%, 12.5%, and 13.8%). Cooke *et al*. report 5% *S.* Typhi isolates from Pakistan with discordant results [14]. An Indian study also reported detection of such isolates [17]. All of the above studies mentioned the emergence of such ciprofloxacin resistance strains during the years 2000 to 2003. Our data shows isolation of such stains in the years 2005 and 2006 and a previously published study from our centre also had demonstrated absence of these strains in the years 2002 to 2003 [9].

In the current study, isolates with reduced susceptibility to ciprofloxacin were not evaluated for underlying resistance mechanisms. However, recent studies have found that a fluoroquinolone resistance mechanism in nalidixic acid susceptible strains is plasmid mediated, which encodes transferable resistance to quinolones via the *qnr* gene. The *qnr* gene product has been demonstrated to directly protect DNA gyrase from quinolone inhibition [18].

A recent study evaluated the role of zone diameter measurement of various quinolone containing disks as a screening test for detecting this kind of resistance [18]. The results of that study showed that, with regard to newer mechanisms of ciprofloxacin resistance, the nalidixic acid zone diameter (30 µg disk) as well as MIC has poor correlation with raised ciprofloxacin MIC. However, our strains with higher MICs had significantly lower zone sizes than the strains with lower MICs, suggesting an alternative mechanism. This finding could be extremely important in guiding us to perform MICs in those *S.* Typhi isolates that are nalidixic acid susceptible but have a border line zone size (20-21 mm).

The clinical relevance of these strains was not evaluated in any of the previous studies [11-13]; however, one can assume therapeutic failure in these cases, as also seen with the nalidixic acid resistant strains with decreased ciprofloxacin susceptibility [19].

Keeping in view the low isolation rate of these strains in our study, evaluation of ciprofloxacin MICs on every nalidixic acid susceptible strain would not be cost effective in a local setup.

Recently, the use of a low concentration of ciprofloxacin disks (1 µg) has been proposed for better screening of *S.* Typhi isolates with a resistance mutation [18]. Any isolate showing nalidixic acid susceptibility with resistance by a 1 µg ciprofloxacin disk would be further checked for ciprofloxacin MIC. We did not use this tool; however, in view of the threat of further increase in discordant strains, this screening tool could possibly be used routinely in low-income countries.

Alternatively, we propose that local clinical laboratories should continue nalidixic acid screening with periodic ciprofloxacin MIC determination in nalidixic acid susceptible *S.* Typhi isolates to evaluate any changing trend. In addition, MIC analysis should also be performed for those nalidixic acid susceptible

S. Typhi isolates showing treatment failure after a sufficient period of therapy.

The strength of this report is that this is the first study determining ciprofloxacin MICs from this region on a large number of nalidixic acid susceptible isolates to detect the emergence of ciprofloxacin reduced susceptible strains. Our findings would be of interest not only at the regional level but also internationally as travellers, immigrants and refugees may acquire typhoid in Pakistan and travel to developed countries [20]. Our limitation was non-uniformity in the yearly distribution of isolates.

Conclusion

Currently, the evaluation of fluoroquinolone MICs in nalidixic acid susceptible *S.* Typhi isolates is neither required nor cost effective in our setting. It should be performed periodically and include all isolates of *S.* Typhi with a nalidixic acid zone of less than 21 mm. We also recommend the reevaluation of breakpoints for *S.* Typhi by CLSI with the aim of minimizing the cost as well as error of MIC reporting in resource-limited countries where the disease is highly endemic and availability of trained staff and money are major issues.

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Corresponding author

Dr Seema Irfan
Assistant Professor
Department of Pathology & Microbiology
The Aga Khan University Hospital
Stadium Road
PO Box 3500, Karachi 74800, Pakistan
Telephone: (9221) 486 4530, 486 1603
Fax: (9221) 493 4294, 493 2095
seemairfan.akuh@yahoo.com, seema.irfan@aku.edu

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