Ofloxacin Minimum Inhibitory Concentration versus Disk Diffusion Zone Diameter for Salmonella enterica Serovar Typhi Isolates: Problems in the Detection of Ofloxacin Resistance

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SUMMARY: Using the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS), a total of 421 blood culture isolates of Salmonella enterica serovar Typhi obtained during 1991-2001 were tested for susceptibility to ofloxacin (OFX) by the agar dilution method, and for the determination of minimum inhibitory concentration (MIC) values of OFX by the agar dilution method. Among 421 isolates, 248 were fully OFX-sensitive showing MICs of 0.0125 - 0.075 μg/ml and inhibitory zone diameters of ≥24 mm. The remaining 173 isolates (MICs of 0.5 - 1.5 μg/ml) that were treated with OFX did not respond to the therapy. However, 169 (97.69%) of the 173 isolates were determined to be susceptible (zone diameter ≥16 mm) by the disc diffusion method, whereas only 3 were intermediately-susceptible (zone diameter 13 - 15 mm) and the final isolate showed OFX-resistance (zone diameter 12 mm). Thus, following the NCCLS guidelines, OFX-resistance in S. enterica serovar Typhi was not detected by the disc diffusion test. The present data suggest a revision of the NCCLS breakpoints in selecting OFX as the preferred treatment regimen for S. enterica serovar Typhi.

Enteric fever caused by the multidrug-resistant (MDR) Salmonella enterica serovar Typhi is a global problem (1-4). MDR S. enterica serovar Typhi isolates have emerged in different parts of the world, and these isolates are resistant to ampicillin, chloramphenicol, and cotrimoxazole. Thus, ciprofloxacin (CPFX), a fluoroquinolone, was introduced for the treatment of typhoid fever (5). Physicians have also suggested another fluoroquinolone, ofloxacin (OFX), for the treatment of MDR S. enterica serovar Typhi infection (6). Both drugs were found to be effective against MDR S. enterica serovar Typhi. However, several treatment failures with CPFX and OFX have been reported (7-13), due to the development of fluoroquinolone resistance in S. enterica serovar Typhi. Nonetheless, according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS), these infections were due to fluoroquinolone-sensitive S. enterica serovar Typhi (14). Therefore, the present study was undertaken to determine the OFX minimum inhibitory concentrations (MICs) and zone diameters of inhibition around 5-μg OFX discs in order to evaluate OFX-resistance among a large collection of S. enterica serovar Typhi isolates.

A total of 421 S. enterica serovar Typhi isolates studied were obtained at the Calcutta School of Tropical Medicine during 1991-2001 from blood samples of enteric fever patients from Kolkata and its suburbs. Escherichia coli ATCC 25922 was used as the control strain.

The OFX-susceptibility test for the S. enterica serovar Typhi isolates was done by disc diffusion method (15) using 5-μg OFX discs (Hi-Media, Mumbai, India), which were placed on Mueller-Hinton agar (MHA) plates swabbed with the test organisms. The inhibitory zone diameters obtained around the OFX discs were measured after incubation for 24 h at 37°C.

MIC values of OFX were determined by the agar dilution method (16) using OFX at concentrations ranging from 0.005-2 μg/ml in MHA. The details of this method have been described in a previous publication from our group (17).

The zone diameters of inhibition obtained around 5-μg OFX discs and MICs of OFX were compared by scattergram analysis based on the criteria of the NCCLS (14).

According to the inhibitory zone diameters obtained around the OFX discs, the isolates were classified following the NCCLS criteria as susceptible, intermediately-susceptible, or resistant. Among 421 S. enterica serovar Typhi isolates tested for OFX susceptibility, 417 (97.69%) were susceptible, showing zone diameters of ≥16 mm. The remaining three isolates (0.72%) were intermediately-susceptible, having zone diameters 13 - 15 mm; only one isolate (0.24%) had a zone diameter of 12 mm and was determined to be OFX resistant.

The MIC values of OFX for the S. enterica serovar Typhi isolates (1991-2001) are given in Fig. 1. The MICs ranged from 0.0125 - 0.05 μg/ml during 1991-1994. The values were 0.0125 - 0.075 and 0.05 - 0.5 μg/ml in 1995 and 1996, respectively. During 1997-2000, the MICs increased from 0.5 - 1.25, and in the year 2001, the range was 0.75 - 1.5 μg/ml. Thus there was a definite upward shifting of the MICs of OFX for S. enterica serovar Typhi isolates obtained during 1991-2001.

Figure 2 shows the scattergram of OFX MIC test values with zone diameters of inhibition for 421 tested S. enterica serovar Typhi isolates. Among the total 421 isolates, 248 (59%) had inhibitory zone diameters of ≥24 mm and MICs of ≤0.075 μg/ml. Of the other 173 isolates showing OFX MICs of 0.5 - 1.5 μg/ml, 169 (97.69%) had zone diameters of ≥16 mm, three (1.73%) had zone diameters of 13 - 15 mm, and one (0.58%) had a zone diameter of 12 mm.

Susceptibility to OFX according to the disc diffusion method for S. enterica serovar Typhi isolates has previously been reported by several authors in India (9,18), who showed...
showed OFX resistance due to MICs that were reported to be within the range above. Hakanen et al. (12) found only three isolates that were intermediately-susceptible (susceptible at $0.075 \text{ g/ml}$, the majority ($169$) remained within the sensitive range, according to the disc diffusion method showing inhibitory zone diameters of $\geq 16 \text{ mm}$. Thus, the OFX-resistance of $S. enterica$ serovar Typhi was not detected by either the MIC breakpoints or the equivalent zone diameters suggested by the NCCLS.

Several previous studies have observed clinical failure in response to CPFX therapy due to infection with nalidixic acid (Nx)-resistant $S. enterica$ serovar Typhi (11-13,19,20), and many studies have considered an Nx-susceptibility test as a surrogate marker for decreased susceptibility to CPFX among $S. enterica$ serovar Typhi (11,12). Wain et al. (10) reported similar observations regarding OFX susceptibility to $S. enterica$ serovar Typhi. However, an Nx screening test alone cannot reflect the actual MICs of fluoroquinolones for the isolates. Moreover, since Nx is not administered for the treatment of $S. enterica$ serovar Typhi infection, most laboratories, even those in developing countries, use fluoroquinolones, including OFX, in susceptibility tests along with chloramphenicol, ampicillin, cotrimoxazole, and expanded-spectrum cephalosporins. Thus, this approach of including an additional Nx disc to all susceptibility tests may not be cost-effective. Furthermore, the results of the present study suggest that the scattergram analysis of zone diameters around $5-\mu g$ discs versus analysis of the MICs can be applied to detect reduced susceptibility to OFX (Fig. 2): the zone diameters of inhibition around $5-\mu g$ discs for all 173 isolates with OFX MICs of $\geq 0.5 \mu g/ml$ were $\geq 29 \text{ mm}$. In addition, 121 of 248 isolates with MICs of $\leq 0.075 \mu g/ml$ were also included in this category, whereas for 127 of the isolates with a zone diameter of inhibition of $>29 \text{ mm}$, the MICs were $\leq 0.075 \mu g/ml$. Thus, when a MIC of $\geq 0.5 \mu g/ml$ was used as the breakpoint, an OFX inhibitory zone diameter of $\leq 29 \text{ mm}$ resulted in $100\%$ sensitivity and $51.2\%$ specificity in screening for decreased OFX susceptibility. Further screening with $30-\mu g$ Nx discs could be used to decrease the number of isolates referred to full-range OFX MIC determination. Still, universally observed guidelines for breakpoints of susceptibility and resistance are required for the worldwide surveillance of the emergence and spread of fluoroquinolone-resistant $S. enterica$ serovar Typhi isolates. Therefore, re-evaluation of the NCCLS breakpoints for OFX by the determination of disc diffusion zone diameters and MIC values is imperative for detecting OFX-resistance among current $S. enterica$ serovar Typhi isolates. In addition, other studies (12,13) have already suggested the need to revise the NCCLS breakpoints of fluoroquinolones for $S. enterica$ serovar Typhi.

that most of the $S. enterica$ serovar Typhi isolates were OFX-sensitive, and OFX-resistance was found to be negligible. We found only three isolates that were intermediate-susceptible to OFX, and only one that showed OFX resistance by the disc diffusion method. However, we encountered problems in treating enteric fever with OFX therapy during and after 1996 due to infection with isolates that had been determined to be within the sensitive range by the disc diffusion method. Obviously, the isolates showed high MICs of OFX (0.5 - 1.5 $\mu g/ml$). The isolates among those with OFX MICs of $\leq 0.075 \mu g/ml$ during or before 1996 responded to the OFX therapy. However, as we did not have access to isolates with OFX MICs of $>0.075$ to $<0.5 \mu g/ml$, we cannot be certain that the failure of treatment of typhoid fever was not due to OFX MICs within the range above. Hakanen et al. (12) reported that most of the $Salmonella$ isolates in their study showed OFX resistance due to MICs that were $\geq 0.5 \mu g/ml$ of OFX.

Previous studies (9,18) have not reported the MIC values of OFX for $S. enterica$ serovar Typhi isolates that would indicate OFX resistance. This communication reports for the first time the MICs of a large collection of $S. enterica$ serovar Typhi isolates from Kolkata, India, which may be of help in detecting OFX-resistance among $S. enterica$ serovar Typhi. According to the NCCLS recommendation, the MIC breakpoints of OFX are $\geq 8$ and $\leq 2 \mu g/ml$ for resistance and susceptibility, respectively (14). In our cases, treatment failure occurred due to infection with $S. enterica$ serovar Typhi isolates with OFX MICs $\geq 0.5 \mu g/ml$. In this study, among the 173 isolates showing MICs of $\geq 0.5 \mu g/ml$, the majority (169) remained within the sensitive range, according to the disc diffusion method showing inhibitory zone diameters of $\geq 16 \text{ mm}$. Thus, the OFX-resistance of $S. enterica$ serovar Typhi was not detected by either the MIC breakpoints or the equivalent zone diameters suggested by the NCCLS.

REFERENCES


