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Short Communication

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 disc diffusion 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 (99.04%) 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

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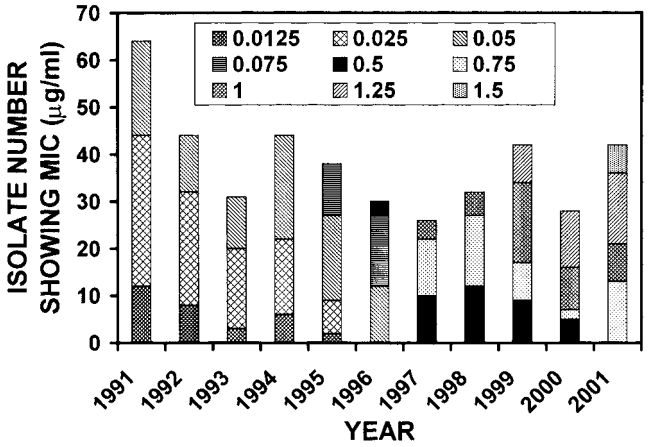

Fig. 1. Minimum inhibitory concentration (MIC) values of ofloxacin (OFX) for 421 *Salmonella enterica* serovar Typhi isolates (1991-2001).

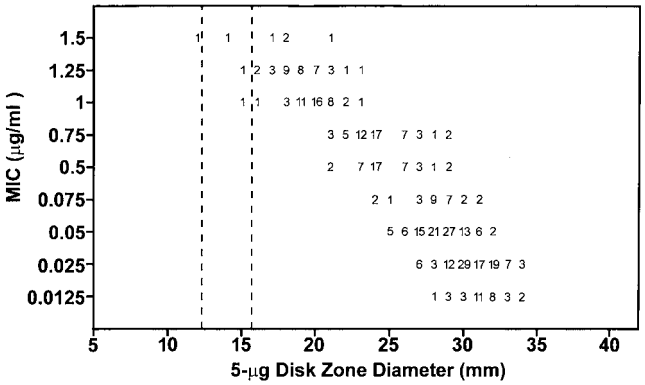

Fig. 2. Scattergram for 421 *S. enterica* serovar Typhi isolates; the minimum inhibitory concentration (MIC) values of ofloxacin (OFX) were compared with the zone diameters of inhibition around 5-μg OFX discs processed by NCCLS methods. The broken vertical lines represent the interpretive breakpoint suggested for Enterobacteriaceae (susceptible at ≧16 mm, resistant at ≦12 mm). The numbers within the graph indicate the number of *S. enterica* serovar Typhi isolates.

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 intermediately-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 μg/ml). The isolates among those with OFX MICs of ≦0.075 μg/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 μg/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 ≧0.5 μg/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 ≧8 and ≦2 μg/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 ≧0.5 μg/ml. In this study, among the 173 isolates showing MICs of ≧0.5 μg/ml, the majority (169) remained within the sensitive range, according to the disc diffusion method showing inhibitory zone diameters of ≧16 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 sudies 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-μg discs versus analysis of the MICs can be applied to detect reduced susceptibility to OFX (Fig. 2.): the zone diameters of inhibition around 5-μg discs for all 173 isolates with OFX MICs of ≧0.5 μg/ml were ≦29 mm. In addition, 121 of 248 isolates with MICs of ≦0.075 μg/ml were also included in this category, whereas for 127 of the isolates with a zone diameter of inhibition of >29 mm, the MICs were ≦0.075 μg/ml. Thus, when a MIC of ≧0.5 μg/ml was used as the breakpoint, an OFX inhibitory zone diameter of ≦29 mm resulted in 100% sensitivity and 51.2% specificity in screening for decreased OFX susceptibility. Further screening with 30-μg 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.

REFERENCES

1. Mandal, S., Deb Mandal, M. and Pal, N. K. (2002): Antibiotic resistance pattern of *Salmonella typhi* isolates in Kolkata, India during 1991-2001: a retrospective study. Jpn. J. Infect. Dis., 55, 58-59.
2. Hirose, K., Tamura, K., Sagara, H. and Watanabe, H. (2001): Antibiotic susceptibilities of *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A isolated from patients in Japan. Antimicrob. Agents

Chemother., 45, 956-958.
3. Alsoub, H., Uwaydah, A. K., Matar, I., Zebeib, M. and Elhag, K. M. (1997): A clinical comparison of typhoid fever caused by susceptible and multidrug-resistant strains of *Salmonella typhi*. Br. J. Clin. Pract., 51, 8-10.
4. Ackers, M. L., Puhr, N. D., Tauxe, R. V. and Mintz, E. D. (2000): Laboratory based surveillance of *Salmonella* serotype Typhi infections in the United States. Antimicrobial resistance on the rise. JAMA, 283, 2668-2673.
5. Ramirez, C. A., Bran, J. L., Meija, C. R. and Gracia, J. F. (1985): Open prospective study of the clinical efficacy of ciprofloxacin. Antimicrob. Agents Chemother., 28, 128-132.
6. Laudico, A. V., Cortez, E. R., Quebral, J. D., Crisostomo, A. C. and Pena, A. C. (1995): Complicated typhoid ileitis. Phil. J. Surg. Spec., 50, 13-15.
7. Jesudasan, M. V., Malathy, B. and John, T. J. (1996): Trend of increasing levels of Minimum inhibitory concentration of ciprofloxacin to *Salmonella typhi*. Indian J. Med. Res., 103, 247-49.
8. Rowe, B., Ward, L. R. and Threlfall, E. J. (1995): Ciprofloxacin resistant *Salmonella typhi* in the United Kingdom. Lancet, 346, 1302.
9. Choudhari, A. and Bansal, M. (1997): Multidrug resistant *Salmonella typhi* in Aurangabad. Indian J. Med. Microbiol., 15, 155.
10. Wain, J., Hoa, N. T., Chinh, N. T., Vinh, H., Everett, M. J., Diep, T. S., Day, N. P., Solomon, T., White, N. J., Piddock, L. J. and Parry, C. M. (1997): Quinolone-resistant *Salmonella typhi* in Viet Nam: molecular basis of resistance and clinical response to treatment. Clin. Infect. Dis., 25, 1404-1410.
11. Kapil, A., Das, R. and Das, B. (2002): Nalidixic acid susceptibility test to screen ciprofloxacin resistance in *Salmonella typhi*. Indian J. Med. Res., 115, 49-54.
12. Hakanen, A., Kotilainen, P., Jalava, J., Shtonen, A. and Huovinen, P. (1999): Detection of decreased fluoroquinolone susceptibility in salmonellas and validation of nalidixic acid screening test. J. Clin. Micrbiol., 37, 3572-3577.
13. Launay, O., Van, J. C. N., Buu-Hoi, A. and Acar, J. F. (1997): Typhoid fever due to a *Salmonella typhi* strain of reduced susceptibility to fluoroquinolones. Clin. Microbiol. Infect., 3, 541-543.
14. National Committee for Clinical Laboratory Standards (1994): Table 2. Zone diameter interpretive standards and equivalent minimum inhibitory concentration breakpoints for organisms other than *Haemophilus* sp., *N. gonorrheae* and *S. pneumoniae*. Vol. 114, No. 16: 8. National Committee for Clinical Laboratory Standards. Wayne, Pa.
15. National Committee for Clinical Laboratory Standards (1997): Performance standards for antimicrobial disk susceptibility tests. Approved standard. 6th ed. M2-A6. National Committee for Clinical Laboratory Standards. Wayne, Pa.
16. National Committee for Clinical Laboratory Standards (1997): Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard, 4th ed. M7-A4. National Committee for Clinical Laboratory Standards. Wayne, Pa.
17. Mandal, S., Deb Mandal, M. and Pal, N. K. (2003): R-factor in *Salmonella enterica* serovar Typhi: transfer to and acquisition from *Escherichia coli*. Jpn. J. Infect. Dis., 56, 65-67.
18. Kumar, R., Aneja, K. R., Punia, A. K., Roy, P., Sharma, M., Gupta, R. and Ram, S. (2001): Changing pattern of biotypes, phage types and drug resistance of *Salmonella typhi* in Ludhiana during 1980-1999. Indian J. Med. Res., 113, 175-180.
19. Rodrigues, C., Mehta, A. and Joshi, V. R. (1998): Quinolone-resistant enteric fever–problem and remedies. J. Assoc. Physicians India, 46, 751-752.
20. Murdoch, D. H., Banatvala, N. A., Bone, A., Shoismatulloev, B. I., Ward, L. R. and Threlfall, E. J. (1998): Epidemic ciprofloxacin resistant *Salmonella typhi* in Tajikistan. Lancet, 351, 339.