In Vitro Activity of Ozenoxacin against Quinolone-Susceptible and Quinolone-Resistant Gram-Positive Bacteria


Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain; Department of Clinical Microbiology, Hospital Clinic, Faculty of Medicine, University of Barcelona, Barcelona, Spain; Department of Clinical Microbiology, Hospital Universitario Ramón y Cajal and the Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; Ferrer Laboratories, Barcelona, Spain

In vitro activity of ozenoxacin, a novel nonfluorinated topical (L. D. Saravolatz and J. Leggett, Clin. Infect. Dis. 37:1210–1215, 2003) quinolone, was compared with the activities of other quinolones against well-characterized quinolone-susceptible and quinolone-resistant Gram-positive bacteria. Ozenoxacin was 3-fold to 321-fold more active than other quinolones. Ozenoxacin could represent a first-in-class nonfluorinated quinolone for the topical treatment of a broad range of dermato logical infections.

Antibiotic resistance remains a major public health threat worldwide (1, 2). Gram-positive bacteria, particularly Gram-positive cocci, are important pathogens in both hospital and community environments. They are associated mainly with bacteremia, pneumonia, and skin and soft tissue infections (SSTIs) (3). Nowadays, the incidence of both hospital- and community-acquired methicillin-resistant Staphylococcus aureus (MRSA) presents therapeutic problems in many countries (4, 5).

Fluoroquinolones, an alternative therapeutic option, have shown good activity against Gram-positive bacteria, including methicillin-susceptible S. aureus (MSSA) and MRSA. However, the emergence of resistant strains has compromised the clinical usefulness of quinolones currently available for the treatment of staphylococcal infections (3, 5, 6). The emergence of resistance has made it necessary to design new drugs for the treatment of infections caused by resistant strains.

Ozenoxacin (Fig. 1), 1-cyclopropyl-8-methyl-7-[5-methyl-6-(methylamino)-3-pyridinyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (Ferrer Laboratories), belongs to a new generation of nonfluorinated quinolone antibacterials, having a pyridinyl group at C-7. Developed for topical use, it has recently successfully completed a phase III clinical trial with adult and pediatric patients and patients with impetigo. Ozenoxacin has demonstrated excellent in vitro activity against MRSA and methicillin-resistant Staphylococcus epidermidis (MRSE), including quinolone-resistant strains (7, 8).

In this study, the in vitro activity of ozenoxacin was compared to those of moxifloxacin, levofloxacin, and ciprofloxacin against both quinolone-susceptible and quinolone-resistant Gram-positive bacteria with well-characterized resistance mechanisms.

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A total of 50 quinolone-susceptible (QS) and quinolone-resistant (QR) Gram-positive cocci strains collected between 2009 and 2010 were studied. The clinical isolates were obtained from the Clinical Microbiology Laboratory at the Hospital Clinic in Barcelona, Spain. The strains included methicillin-susceptible S. aureus (n = 9), methicillin-resistant S. aureus (n = 5), methicillin-susceptible S. epidermidis (n = 8), methicillin-resistant S. epidermidis (n = 5), Streptococcus pyogenes (n = 8), Streptococcus agalactiae (n = 8), and Enterococcus faecium (n = 6). S. aureus ATCC 25923 was used as a control microorganism. All strains were grown aerobically at 37°C in brain heart infusion broth (BHI) (Becton Dickinson Co., Sparks, MD).

Susceptibilities to ozenoxacin (OZN) (Ferrer Laboratories), moxifloxacin (MXF), levofloxacin (LVX), and ciprofloxacin (CIP) (Sigma-Aldrich, St. Louis, MO) were determined using broth microdilution according to the CLSI standard method (9). The MIC of each antimicrobial agent was determined in the presence and absence of 25 mg/liter of the efflux pump inhibitor reserpine (Sigma, St. Louis, MO). CLSI breakpoints were used to classify strains as QS or QR. If one of the strains was found to be resistant to LVX and MXF, it was allocated to the QR group, although they were susceptible to the remaining fluoroquinolones. The quinolone resistance-determining regions (QRDRs) of the gyrA, gyrB, parC, and parE genes were amplified by PCR using a set of previously described primers (10–14).

An analysis of the relationship between the MICs of the different quinolones tested and mutations in the QRDRs found in all of the strains studied is shown in Tables 1 and 2.

Overall, OZN showed the highest antibacterial activities against clinical isolates of Gram-positive microorganisms, with
MICs ranging from 0.008 to 4 mg/liter. The good activities of OZN against strains of MRSA, MSSA, MSSE, and MRSE with 2, 3, or 4 mutations in the \( \text{gyrA} \) and \( \text{grlA} \) (\( \text{parC} \)) genes is worthy of note.

Two groups of MSSA strains were established: (i) MSSA strains with a mutation in the amino acid codon Ser84 of the \( \text{gyrA} \) gene and MICs of CIP, MXF, and LVX of between 0.25 and 2 mg/liter (the MIC of OZN was 0.008 mg/liter) and (ii) MSSA strains with the same \( \text{gyrA} \) mutation but MICs of CIP, MXF, and LVX ranging from 4 to 128 mg/liter. The MICs of the above-mentioned fluoroquinolones against strains with MICs of \( \geq 4 \) mg/liter remained the same in the presence of reserpine. This may be due to the overexpression of an unknown reserpine-inhibited efflux pump. The MIC of OZN for MRSA strains with a mutation at the amino acid codon Ser84 was between 0.06 and 0.12 mg/liter, whereas the range of MICs of the other fluoroquinolones was between 2 and 256 mg/liter. It should be noted that the MIC of OZN of a MRSA strain with four mutations, two in the \( \text{gyrA} \) gene and two in the \( \text{parC} \) genes (\( \text{Ser84} \rightarrow \text{Tyr/} \text{Glu88} \rightarrow \text{Lys} \) and \( \text{Ser80} \rightarrow \text{Phe/} \text{Glu84} \rightarrow \text{Val} \)), was 2 mg/liter. The MICs of MXF, CIP, and LVX were 32 mg/liter, 256 mg/liter, and 512 mg/liter, respectively.

Our results are similar to those described by Yamakawa et al. (15), who showed that the MIC of T-3912 (now OZN) against quinolone-resistant MSSA with 2 or 3 mutations in these genes was 0.06 or 0.25 mg/liter, respectively, compared with the MICs of between 0.12 and 2 mg/liter found in the present study. The effects of the different mutations in the \( \text{gyrA} \) and \( \text{grlA} \) genes on the MIC of OZN were similar for MRSA and MRSE.

In vitro activities of nemonoxacin, another nonfluorinated quinolone, was tested against clinical isolates of \( \text{S. aureus} \), enterococci, and \( \text{Streptococcus pneumoniae} \) by Chen et al. (16). These authors found that nemonoxacin had a potent activity against MSSA and CIP-susceptible MRSA (MIC\(_{90}\) of \( \leq 0.03 \) mg/liter),

### TABLE 1  Distribution of different fluoroquinolone MICs in staphylococcus strains and QDRD amino acid substitutions responsible for elevated fluoroquinolone MICs

<table>
<thead>
<tr>
<th>Organism and isolate no.</th>
<th>MIC (mg/liter)</th>
<th>Amino acid substitution(s)</th>
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</thead>
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<tr>
<td></td>
<td>OZN</td>
<td>MXF</td>
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<td>MSSA-QS</td>
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<tr>
<td>361</td>
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\( a \) R, reserpine; QS, quinolone susceptible; QR, quinolone resistant; NM, no mutations in the \( \text{gyrA} \) and \( \text{parC} \) genes.
whereas activity was limited against CIP-resistant MRSA (MIC$_{90}$ of 1 mg/liter). No reference was made, however, to mutations in the $gyrA$ and/or $gtrA$ genes in these last strains.

Fluoroquinolone-resistant MSSE strains with a double mutation in $gyrA$ (Ser$_{84}$→Phe) and $parC$ (Ser$_{80}$→Phe) showed a MIC of OZN of 0.5 to 1 mg/liter. This was 16-fold to 64-fold lower than those of MXF, LVX, and CIP. Four groups were established when studying MRSE strains. (i) Three QS-MRSE strains had no mutation in the $gyrA$ or $parC$ genes; the MICs of OZN for these strains ranged from 0.004 to 0.06 mg/liter, whereas the MICs of the remaining quinolones ranged between 0.06 and 1 mg/liter. (ii) One QS-MRSE strain had a mutation in $gyrA$ (Ser$_{84}$→Phe), which showed a MIC of OZN of 0.03 mg/liter. (iii) One QS-MRSE strain had a double mutation (Ser$_{84}$→Phe in $gyrA$ and Ser$_{80}$→Phe in $parC$) and a MIC of OZN of 0.03 mg/liter. The MIC of OZN of the strain in group ii, however, was reduced to 0.015 mg/liter in the presence of reserpine, suggesting that this strain possesses two concomitant mechanisms of resistance, an amino acid substitution in $gyrA$ plus an overexpression of a reserpine-inhibited efflux pump. (iv) Four QR-MRSE strains that had double mutations in $gyrA$ (Ser$_{80}$→Tyr/Glu$_{88}$→Gly or Glu$_{88}$→Lys) and $parC$ (Ser$_{80}$→Phe/Asp$_{84}$→Tyr) showed a MIC of OZN of 1 to 2 mg/liter. This was at least 8-, 16-, and 64-fold higher than MICs of MXF, CIP, and LVX, which were 16 to 64 mg/liter, 32 to 128 mg/liter, and 128 to 256 mg/liter, respectively.

Although the prevalence of fluoroquinolone-resistant $S$. pyogenes and $S$. agalactiae is still low, it has been steadily increasing (13, 17). Two $S$. pyogenes strains (165 and 168) showed reduced MICs of MXF, LVX, and CIP in the presence of reserpine, whereas the MIC of OZN was not affected, thus suggesting the overexpression of a reserpine-inhibited efflux pump which does not affect OZN. As regards the $S$. pyogenes and $S$. agalactiae strains with mutations in both $gyrA$ and $parC$, the effect of these mutations on the MIC of OZN seems greater in $S$. agalactiae than in $S$. pyogenes, with MICs of 1 and 0.25 mg/liter, respectively. The effect on the fluoroquinolones tested was similar to that reported in other studies (18). Finally, the MIC values of OZN for $E$. faecium were greater than those for other microorganisms due to the high level of resistance present in $Enterococcus$ spp., which has been associated with many antibiotics with their intrinsic resistance and/or other resistance mechanisms (18). In the study described by Chen et al. (16), the MIC$_{90}$ of nemonoxacin for vancomycin-susceptible $E$. faecium was 2 mg/liter, whereas the MIC of OZN was 0.5 mg/liter. This was at least 8-, 16-, and 64-fold higher than MICs of MXF, CIP, and LVX, which were 16 to 64 mg/liter, 32 to 128 mg/liter, and 128 to 256 mg/liter, respectively.

### Table 2: Distribution of different fluoroquinolone MICs in streptococcus and enterococcus strains and amino acid substitutions responsible for elevated fluoroquinolone MICs

<table>
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<th>Organism and isolate no.</th>
<th>MIC (mg/liter)</th>
<th>Amino acid substitution(s)</th>
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<td></td>
<td>OZN</td>
<td>MXF</td>
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Note: R, reserpine; QS, quinolone susceptible; QR, quinolone resistant; NM, no mutations in the $gyrA$ and $parC$ genes.
ble and vancomycin-resistant *E. faecium* were 4 and 16 mg/liter, respectively, with the MICs ranging from 0.06 to 8 mg/liter and 0.06 to 16 mg/liter. However, those authors did not describe mutations in the strains studied. In our study, strains with double mutations presented a MIC of OZN from 2 to 4 mg/liter.

In conclusion, OZN shows excellent *in vitro* activity against the most important microorganisms isolated as etiological agents in SSTIs, even against methicillin-resistant strains and those with 2, 3, or 4 mutations in the *gyrA* and/or *parC* genes.

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**REFERENCES**