**Short Communication**

# Comparative activity of ozenoxacin and other quinolones in Staphylococcus aureus strains overexpressing the efflux pumps encoding genes *mepA* and *norA*.

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**Abstract**

**Background:** To evaluate the activity of ozenoxacin in *Staphylococcus aureus* strains with overexpression of *mepA* and *norA* genes, which encode efflux pumps.

**Methods:** *S. aureus* NCTC-8325-1, *S. aureus* NCTC 8225-2 (overexpressing mepA), *S. aureus* SA 1199 and *S. aureus* SA 1199B (overexpressing norA) were used. The minimal inhibitory concentration (MIC) of ozenoxacin (OZN), moxifloxacin (MOX), levofloxacin (LVX), ciprofloxacin (CIP) and norfloxacin (NOR) in the presence/absence of reserpine (20 mg/L) were performed by microdilution method.

**Results:** The MIC of OZN was lower in all evaluated strains in comparison with the other studied quinolones and independent of the pump that is being overexpressed. MIC values of OZN ranged from 0.0039 to 0.0078 mg/L. Similar results were observed with MOX with MIC values between 0.016 and 0.0312 mg/L, without variations in presence of reserpine. In the case of LEV, the MIC values were between 0.125 and 1 mg/L with a slight increase (8-fold) in MIC observed in strains overexpressing the *mepA* or *norA* genes (from 0.125 to 1 mg/L). The overproduction of the efflux pump MepA did not affect CIP whereas it increased 8-fold the MIC of NOR. Finally, the overproduction of NorA affects by a 4-fold and 64-fold increase the MICs of CIP and NOR, respectively, resulting in a high-level of resistance to these antibiotics in comparison with OZN (0.0078 mg/L).

**Conclusion:** OZN does not seem to be a substrate for the efflux pumps MepA and NorA, commonly found in Gram-positive bacteria and that affect other quinolones.

**keywords:** Ozenoxacin, quinolone, reserpine, efflux pump, Gram-positive bacteria.

## 1. Introduction

*Staphylococcus aureus* it is the most common bacterial agent associated with skin infections, such as folliculitis and impetigo, affecting mainly children more than adults worldwide [1]. The treatment for these infections includes topical antimicrobial agents such as mupirocin, fusidic acid and retapamulin [2]. Unfortunately, an increasing number of Gram-positive pathogens, especially methicillin-resistant *S. aureus* (MRSA), have developed resistance to topical antimicrobial agents typically used in clinical practice, potentially limiting its overall efficacy [3].

Nowadays, ozenoxacin (OZN) is the most recent topical option for the treatment of skin infections; it belongs to a new generation of non-fluorinated quinolones and has shown great clinical benefit in two recent Phase III trials [4]. Ozenoxacin has, demonstrated excellent antibacterial activity against Gram-positive bacteria including resistant strains to other quinolones and low capacity to select resistant mutant strains [5–7].

In Gram-positive bacteria, resistance to quinolones typically arises as a result of amino acid substitutions in the target enzymes DNA gyrase and topoisomerase IV (both of which are involved in bacterial DNA synthesis). In addition, changes in drug efflux associated with the overexpression of genes encoding efflux pumps play a role as a complementary mechanism of resistance. Specifically, the development of quinolone resistance is caused by one or a combination of mutations in different amino acid codons in a specific region [Quinolone Resistance-Determining Regions (QRDR)] of the *gyrA* gene, encoding the A subunit of DNA gyrase and *parC (grlA* in *S. aureus)* encoding the A subunit of topoisomerase IV genes [8]. However, OZN showed strong activity against MRSA with multiple mutations in QRDR. On the other hand, the efflux pump are transport proteins involved in the extrusion of toxic substrates from the interior of bacterial cells to the

external environment, including practically all classes of clinically relevant antibiotics including quinolones [9]. In addition, reserpine is a known inhibitor of efflux pumps in Gram-positive bacteria, including norA and mepA genes [10,11]. This alkaloid can block the efflux pumps, increasing the intracellular concentration of quinolones or other antimicrobials, thus potentially lowering MICs. For this reason, the reserpine-based screening procedure is often used as the benchmark to detected strains with overexpression at least one efflux pump gene. In summary, the expression of these mechanisms of active expulsion does not generally provide a high level of clinically significant resistance, however, when it acts in conjunction with mutations in the QRDR, these results in highly resistant strains, difficult to treat in the clinical setting [12].

Due to the important role that efflux mechanisms play in the acquisition of quinolone resistance, the aim of this study was to evaluate the antimicrobial activity of OZN in different strains of staphylococci with overexpression of genes encoding efflux pumps that affect other quinolones.

## 2. Materials and method

### *2.1 Bacterial strains*

Four strains with different active efflux system kindly donated from different researchers were analyzed in this study (Table1). Species identification was confirmed by MALDI-TOF mass spectrometry (BRUKER Daltonics GmbH, Bremen, Germany) and stored in skim milk (BD) at −80 ◦C.

### *2.2 Antimicrobial susceptibility testing*

The minimum inhibitory concentration (MIC) of ozenoxacin (OZN), moxifloxacin (MOX), levofloxacin (LVX), ciprofloxacin (CIP) and norfloxacin (NOR) was determined by the broth microdilution method according to CLSI recommendations [13]. The MIC

was determined in presence and absence of 20 mg/L of reserpine. A four-fold reduction in the MIC in the presence of reserpine was considered a positive screen. As control, the *S. aureus* ATCC 29213 collection strain was used. All experiments were carried out in triplicates.

Minimum

## 3. Results

The MIC values of quinolones for strains with two different efflux pumps are shown in Table 2.

The MIC of OZN was considerably lower in all evaluated strains in comparison to MOX, LVX, CIP and NOR and independent of the pump that is being overproduced, observing MIC values of OZN between 0.0039 and 0.0078 mg/L. Similar results were observed with MOX, the MIC values were between 0.016 and 0.0312 mg/L, and change was not observed in the MIC in presence of reserpine. In the case of LVX, the MIC values were higher in comparison with OZN and MOX in both strains with overexpression of efflux pump (1 mg/L), however, they were below the cut-off points recommended by the EUCAST (≤1 - >2 mg/L), but is 128-fold higher compared with OZN. A slight increase (8-fold) in the MIC of LVX was observed in strains than overproduced MepA or NorA (from 0.125 to 1 mg/L). Moreover, the MIC of LVX in strains with overproduction of either MepA or NorA decreased from 1 to 0.5 mg/L in the presence of reserpine.

On the other hand, the results with CIP as substrate showed a MIC 1000-fold higher than OZN in the strain with overexpression of NorA (affected by reserpine, from 8 to 1 mg/L) and 64-fold higher in the strain with overexpression of mepA (MIC of 0.5 mg/L). Noteworthy that these values were highest with NOR as substrate, being more than 8000-

fold the MIC with respect to OZN in the strains with NorA (MIC of 64 mg/L) and 512-fold higher (MIC of 4 mg/L) in the strains with MepA (both strains affected by reserpine).

## 4. Discussion and conclusion

In this study, we evaluated the effect of two important fluoroquinolone-efflux systems in *S. aureus*, such as NorA, MepA, on the MIC of OZN, MOX, LVX, CIP and NOR in the presence and absence of reserpine.

The result of this study shows that the MIC of OZN was considerably lower in all evaluated strains in comparison with MOX, LVX, CIP and NOR and independent of the efflux pump that is being overexpressed.

These difference of MICs between quinolones studied is probably due to hydrophobicity and the bulkiness of C-7 and C-8 substituents of the antibiotic, which elicits to act as a weak or strong substrate for the efflux pump. Several reports about efflux of quinolones by transporters such as norA and mepA have observed that hydrophobic quinolones are less affected by efflux mechanisms, in this case, OZN, MOX and LEV are hydrophobic compounds and CIP and NOR are considered more hydrophilic and a strong substrate for NorA but weak for MepA, which could explain our results [14,15]. Thus, OZN could interact with NorA in the way hydrophilic quinolones do, without being transported through them, shown to be a weak substrate for NorA and MepA. On the other hand, it has been published that, the bulkiness of the C-7 and bulkiness and hydrophobicity of C-8, could be responsible for low activity of efflux systems [16,17]. In this sense, it is interesting to see that OZN and MOX, which are the least effluxed from the inside of the cell, possess both substituents, which are absent in CIP and NOR. However, LVX that showed a lower MIC value than CIP and NOR but higher that OZN and MOX does not possess a bulky substituent in C-7 but has a C-8 substituent [17].

In conclusion: According to the results of this research, it seems that OZN, as it has been described for other quinolones as nemonoxacin, garenoxacin and sparfloxacin, is a poor substrate for the efflux mechanism evaluated in this investigation. Therefore it is to preview that ozenoxacin´s activity will hardly be affected in strains with active efflux systems.

## Declarations

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**Author contribution:** All authors have approved the final article and have made substantial contributions to all of the following: YL and JV conceived and designed the study; YL performed laboratory works; YL, MT, RC, DG, JV and IZ interpreted the results; YL, JV, IZ drafted the article or revised it critically for important intellectual content. All co-authors and the study group revised and approved the published version to be submitted to the journal.

**Transparency declarations:** The author Domingo Gargallo-Viola was staff member of Ferrer Laboratories at the time of this research and Ilonka Zsolt is currently member of Ferrer. All other authors: none to declare.

**Ethical Approval:** Not required

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224 Table 1. Characteristic of bacterial strains used in this study.

| Bacterial strains | Description | Donated (Year of reference) |
|---|---|---|
| *S. aureus* (NCTC 8325-1) | NCTC 8325 with ΔmepRA::erm carrying expression vector pG154. | Timothy Opperman (2010) |
| *S. aureus* (NCTC 8325-2) | Wild type NCTC8325 with (pG154-mepA) carrying expression vector pG154 with mepA under the control of the a-tet inducible promoter | |
| *S. aureus* (Sa 1199) | Wild type | Glenn Kaatz (1997) |
| *S. aureus* (Sa 1199B) | Strain with a mutation in *grlA* and overexpresses *norA* | |
| *S. aureus* ATCC 29213 | Control strain | |

225

226

**Table 2.** Average and standard deviation (SD) of MIC values to ozenoxacin and other quinolones with and without reserpine in the bacterial strains of this study.

| Bacterial strains | Reserpine | Average ± SD MIC value (µg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | OZN | MOX | LEV | CIP | NOR |
| *S. aureus* (NCTC 8325-1) | -R | 0,005 ± 0,002 | 0,031 ± 0.000 | 0,250 ± 0,217 | 0,667 ± 0,287 | 0,417 ± 0,144 |
| | +R[1] | 0,005 ± 0,002 | 0,013 ± 0,004 | 0,250 ± 0,217 | 0,500 ± 0,000 | 0,333 ± 0,144 |
| *S. aureus* (NCTC 8325-2)[2] | -R | 0,007 ± 0,002 | 0,031 ± 0,000 | 0,833 ± 0,289 | 0,417 ± 0,144 | 3,333 ± 1,154 |
| | +R | 0,007 ± 0,002 | 0,013 ± 0,005 | 0,667 ± 0,289 | 0,417 ± 0,144 | 0,333 ± 0,144 |
| *S. aureus* (Sa 1199) | -R | 0,005 ± 0,002 | 0,021 ± 0,009 | 0,167 ± 0,072 | 1,667 ± 0,577 | 1,333 ± 0,577 |
| | +R | 0,005 ± 0,002 | 0,021 ± 0,009 | 0,167 ± 0,072 | 0,250 ± 0,000 | 0,667 ± 0,289 |
| *S. aureus* (Sa 1199B)[3] | -R | 0,007 ± 0,002 | 0,031 ± 0,000 | 1,000 ± 0,000 | 8,000 ± 0,000 | 53,333 ± 18,475 |
| | +R | 0,008 ± 0,000 | 0,026 ± 0,009 | 0,833 ± 0,289 | 0,833 ± 0,289 | 0,833 ± 0,289 |
| *S. aureus* ATCC 29213 | -R | 0,005 ± 0,002 | 0,013 ± 0,005 | 0,104 ± 0,036 | 0,6667 ± 0,289 | 0,833 ± 0,289 |
| | +R | 0,005 ± 0,002 | 0,013 ± 0,005 | 0,083 ± 0,036 | 0,3750 ± 0,217 | 0,833 ± 0,289 |

[1] reserpine (20 mg/L), [2] strains with overexpression of *mepA*, [3] strains with overexpression of *norA*