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Chapter

Potency of Netilmicin against Staphylococci Compared to Other Ophthalmic Antibiotics

Andrea Sudano Roccaro, Carmela Giovanna Spoto, Luca Rosario La Rosa, Claudine Civiale, Manuela Santonocito, Santa Viola, Cristina Zappulla, Maria Cristina Curatolo and Maria Grazia Mazzone

Abstract

Netilmicin is a potent and safe antibiotic with a very low incidence of resistance used as a topical ophthalmic medication in bacterial ocular infections. The aim of this study was to compare netilmicin’s Quotient of Inhibitions (QIs) and killing kinetics vs Staphylococci with other ophthalmic antimicrobials. Conjunctival and corneal QIs of netilmicin formulations, in single and multiple doses of administration, were compared with those of tobramycin, ofloxacin, levofloxacin and azithromycin preparations. The same analysis was performed in human tears, comparing netilmicin eye drops solution with tobramycin ofloxacin and levofloxacin. Furthermore, killing kinetics against Staphylococci (ATCC strains and ocular isolates) of the above-cited antibiotics, as well as chloramphenicol, were compared at different time points. QI results showed that in the conjunctiva, netilmicin, in both single and multiple doses of administration, is highly effective against all staphylococcal strains tested, while in the cornea it was particularly active against methicillin-resistant Staphylococci strains. Moreover, in human tears, netilmicin eye drops solution showed a more favourable QI against Staphylococci than tobramycin, ofloxacin and levofloxacin all in single-dose administration regimen. Killing kinetic results showed that netilmicin has a great bactericidal activity vs all the microbe strains tested as netilmicin showed to be almost the most active antibiotic. Results suggest that netilmicin has one of the most favourable killing kinetic and tissue inhibitory effects against Staphylococci than the principal ophthalmic antibiotics on the market.

Keywords: netilmicin, quotient of inhibition, killing curves, Staphylococci, methicillin resistance
1. Introduction

Netilmicin is a third-generation aminoglycoside antibiotic considered a first-line therapy for the treatment of acute bacterial conjunctivitis. It is a semisynthetic 1-N-ethyl derivative of sisomycin, endowed with an excellent \textit{in vitro} and \textit{in vivo} activity against a wide range of ocular pathogens both Gram-positive and Gram-negative bacteria including \textit{Staphylococcus aureus} (\textit{S. aureus}), \textit{S. epidermidis} (\textit{S. epidermidis}) and others \textit{Staphylococcus} Coagulase negative, \textit{Acinetobacter} spp., \textit{Pseudomonas} spp. and \textit{Haemophilus influenzae} [1–3].

The most common causative agents for external ocular infections are \textit{S. aureus} and \textit{S. epidermidis} although the specific causal organisms are frequently unknown [2]. Of particular concern among ocular isolates is the increasing frequency of methicillin-resistant \textit{S. aureus} (MRSA) and methicillin-resistant \textit{S. epidermidis} (MRSE) [4].

Various classes of topical antibacterial products have been used in the treatment of bacterial conjunctivitis. These include aminoglycosides (e.g. tobramycin, gentamicin and netilmicin), macrolides (e.g. azithromycin), chloramphenicol and, most recently, fluoroquinolones (e.g. ofloxacin and levofloxacin) [4].

Nonetheless, successful therapy for bacterial conjunctivitis continues to be limited by several factors. A primary concern is the development of bacterial resistance that may be impacted not only by widespread antibiotic use but also by antibacterial pharmacokinetics, such as maintenance of insufficient bactericidal concentrations at the site of infection [2, 4].

Importantly, netilmicin is active against strains and resistant to other aminoglycosides, such as tobramycin or gentamicin [2], including methicillin-resistant strains [1, 2, 5, 6]. Indeed, being an antibiotic almost totally dedicated to ophthalmology and not used for systemic route, netilmicin has maintained unchanged its susceptibility and resistance profile over the last 20 years towards the major strains responsible for eye infections [7, 8].

Netilmicin is available on the market in three different topical ophthalmic dosage forms: solution, gel and ointment. Those products are indicated for the treatment of bacterial ocular infections of the anterior segment of the eye and ocular adnexa [2]. Netilmicin ophthalmic formulation is able to cross the cornea of rabbits, reaching aqueous humor levels comparable with the minimal inhibitory concentration (MIC) for usual ocular pathogens [9]. Netilmicin has no toxic effect \textit{in vitro} on human corneal epithelial cells (HCE-T) and human conjunctival epithelial cells (Wong-Kilbourne derivative of Chang conjunctiva) [10]. Bonfiglio \textit{et al.} evaluated the \textit{in vitro} activity of netilmicin of other antibiotics used in ophthalmology against gram-positive and gram-negative microorganisms isolated from ocular infections. The results of this study showed that the activity of netilmicin was superior to that of ofloxacin against both groups of bacteria, with more than 90% of the isolated strains being susceptible to netilmicin [3]. Additionally, in a study carried out on 1333 ocular clinical isolates, netilmicin was revealed to have the best susceptibility profile among aminoglycosides showing the lowest minimum inhibitory concentration (MIC) values against all isolates. Specifically, netilmicin superiority was pronounced against \textit{S. aureus} and coagulase-negative \textit{staphylococci} (CoNS) [11]. These two \textit{Staphylococci} strains represent the most diffused clusters of bacteria in ocular infections ranging from 65 to 76.2% of the total isolates under investigation [11, 12].

Moreover, Blanco \textit{et al.} evidenced that netilmicin was effective as vancomycin against both methicillin-resistant \textit{Staphylococcus aureus} (MRSA) and
methicillin-resistant *Staphylococcus epidermidis* (MRSE) clinical-ocular isolates [13].

This result is worthy of note considering that an increasing incidence (55% from 2002 to 2008) of *MRSA* and *MRSE* strains was registered in ophthalmology with few antibiotics capable of being efficacious against these pathogens [14]. Considering this evidence, the aim of the present study was to, directly, compare, within the same experimental setting, anti-staphylococcal activity of netilmicin to those of the main ophthalmic antimicrobials on the market. To this end, two of the most important pharmacological indices for antibiotics, that is, the quotient of inhibition (QI), corresponding to the C\textsubscript{max}/MIC\textsubscript{90} ratio [15], and the antibiotic kinetic of killing was investigated. Antibiotic QIs were calculated by mining respective maximum concentration (C\textsubscript{max}) and MIC\textsubscript{90} data from peer-reviewed literature and from internal pharmacokinetic studies in rabbits carried out according to Good Laboratory Practice (GLP). The QIs of two netilmicin formulations, eye drops solution and gel, in single and multiple dose administrations (both in cornea and conjunctiva), were compared to those of tobramycin, ofloxacin, levofloxacin and azithromycin. The QI of chloramphenicol was not calculated since no data on C\textsubscript{max} in the target tissues (cornea and conjunctiva) were found in literature. Moreover, the same analysis was performed in human tears, comparing netilmicin eye drops solution with tobramycin ofloxacin and levofloxacin, all in single-dose administration. Concerning the killing kinetics against two ATCC *Staphylococci* strains and two *Staphylococci* methicillin-resistant ocular isolates, netilmicin activity was directly compared to those of the above-cited antibiotics and to chloramphenicol as well.

### 2. Materials and methods

#### 2.1 QIs determination

Meta-analysis for QIs determination was carried out by scanning scientific literature in search of *in vitro* studies, reporting levofloxacin, ofloxacin, netilmicin, tobramycin and azithromycin MIC\textsubscript{90} values against the most diffused bacterial species in ocular infections (*MSSA*, *MRSA*, *MSCoNS*, *MRCoNS*), and pre-clinical *in vivo* studies in rabbits, reporting corneal and conjunctival C\textsubscript{max} values of ophthalmic formulations containing the above antibiotics. In detail, as described in Tables 1 and 2, the following eye drops formulations were taken into account: (i) netilmicin 0.3% eye drops solution (Nettacin® eye drops); (ii) Netilmicin 0.3% gel formulation (Xanternet®); (iii) tobramycin 0.3% (Tobrex®); (iv) levofloxacin 1.5% (Iquix® and Cravit®); (v) ofloxacin 0.3% (Tarivid® and OcuFloX®); and (vi) azithromycin 1% (Azasite® and Azimycin®).

As to the MIC\textsubscript{90}, the selected references adhered to the criteria of encompassing large epidemiological/surveillance studies and including the above-cited groups of bacterial strains. As to the second parameter (C\textsubscript{max}), only those studies comprising the same animal species and the same regimen of ocular drug administration (single or multiple doses) were considered as eligible and comparable to each other. Then MIC\textsubscript{90} and C\textsubscript{max} data were put into ratio and the QI values for the two ocular tissues and regimens were calculated.

Moreover, the same analysis was performed in human tears, comparing netilmicin 0.3% eye drops solution with tobramycin 0.3%, ofloxacin 0.3% and levofloxacin 0.5%, all in single-dose administration.
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2.2 Bacterial strains and killing curves determination

Two eye swabs of bacterial isolates *S. aureus* 801 CT (MRSA) and *S. epidermidis* 829 CT (MRSE), belonging to SIFI’s private collection were used throughout this study. These strains collection comprises different microbial eye isolates collected from 1998 to 2014 from patients with community-acquired ocular infections coming from three different geographical sites (Messina, Catania and Rome (Italy)). The above microorganisms, and their antibiotic resistance patterns, were previously identified by standard laboratory methodologies. Genetic profiles of all *S. aureus* and *S. epidermidis* isolates of the collection were previously determined (data not shown) with the aim of grouping all isolates in ‘strain types’. The two methicillin-resistant microorganisms under study were each representative of the most diffused ‘strain type’ within the collection with regards to species and the geographical provenience. In addition, *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228 strains (purchased from Thermo Fisher Scientific, Lenexa, KS, USA) were also included in the experiment. Time kill determination was performed as previously described by Bonfiglio et al. [3]. Briefly, microorganisms were grown overnight to reach a density of about 10^8 colony-forming unit (cfu)/ml

<table>
<thead>
<tr>
<th>References for C_{max} values</th>
<th>C_{max} (μg/g) single dose</th>
<th>Antibiotic formulations</th>
<th>References for MIC_{90} values</th>
<th>MIC_{90} μg/ml (strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxxone M.G., unpublished data</td>
<td>0.35</td>
<td>0.81</td>
<td>NETTACIN EYE DROPS (netilmicin 0.3%)</td>
<td>Sanfilippo CM et al. 2016 [11]</td>
</tr>
<tr>
<td></td>
<td>0.98</td>
<td>3.75</td>
<td>NETLMICIN GEL (netilmicin 0.3%)</td>
<td></td>
</tr>
<tr>
<td>Gilbert ML et al. 1987 [16]; Owen GR et al. 2007 [17]</td>
<td>0</td>
<td>3.1</td>
<td>TOBREX (tobramycin 0.3%)</td>
<td></td>
</tr>
<tr>
<td>Chung JI, et al. 2013 [18]</td>
<td>10.67</td>
<td>1.99</td>
<td>IQUIX (levofloxacin 1.5%)</td>
<td>Ashell PA et al. 2020 [12]</td>
</tr>
<tr>
<td>Sakai T et al. 2019 [19]</td>
<td>2.21</td>
<td>20.4</td>
<td>TARIVID (ofloxacin 0.3%)</td>
<td></td>
</tr>
<tr>
<td>Product Monograph (revision 2013) [20]</td>
<td>3.32</td>
<td>2.95</td>
<td>OCUFLOX (ofloxacin 0.3%)</td>
<td></td>
</tr>
<tr>
<td>Akpek EK et al. 2009 [21]</td>
<td>40.4</td>
<td>108</td>
<td>AZASITE (azithromycin 1%)</td>
<td></td>
</tr>
<tr>
<td>Sakai T et al. 2019 [19]</td>
<td>79.9</td>
<td>44.2</td>
<td>AZIMYCYN (azithromycin 1%)</td>
<td></td>
</tr>
</tbody>
</table>

*referring to cornea.
**referring to conjunctiva.

Table 1. C_{max}, MIC_{90} values and relative bibliographic references as to the single dose of antibiotics administration.
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on Mueller Hinton (MH) broth (purchased from Biogenerica, CT, Italy) and then diluted to about $1 \times 10^6$ cfu/ml in the same pre-warmed medium containing netilmicin (32 $\mu$g/ml; purchased from Zhejiang Zhenyuan Pharmaceutical Co., Ltd, Shaoxing, China), tobramycin (16 $\mu$g/ml; purchased from Sigma Aldrich, MI, Italy), ofloxacin, levofloxacin (4 $\mu$g/ml; purchased from Sigma Aldrich, MI, Italy), azithromycin (8 $\mu$g/ml, purchased from Sigma Aldrich, MI, Italy) or chloramphenicol (32 $\mu$g/ml, Chemo, Spain). These antibiotics concentrations corresponded to the resistance breakpoints values according to CLSI’s (2015) interpretative criteria. An antibiotic-free control was similarly inoculated. At 0, 1, 2, 6 and 24 hours after drug exposure, 0.1 ml of the culture was collected, diluted in phosphate buffered saline, inoculated (by inclusion) onto MH agar plates and incubated at 37° C for 24 hours to determine viable cfu/ml. All the experiments were performed in triplicate for three experimental days. Killing curves were constructed by plotting the log$_{10}$ of cfu/ml versus the established time points (i.e 0, 1, 2, 6 and 24 h). Bactericidal activity was defined as a >3 log$_{10}$ decrease of the initial inoculum size. A statistical analysis of the area under the curve (AUC) was conducted by the one-way ANOVA statistical test (GraphPad Prism 6; CA, USA).

3. Results

3.1 QIs determination

Corneal and conjunctival $C_{\text{max}}$ values for netilmicin, tobramycin, levofloxacin, ofloxacin and azithromycin formulations, in both single and multiple doses.
administration, were gathered from bibliographic \textit{in vivo} pharmacokinetic studies [16–19, 21, 23] and, as to netilmicin, from two internal GLP studies (M.G. Mazzone and C. Civiale unpublished data). All these studies have been carried out on rabbit and included the same regimen of drug administration. The QI of chloramphenicol was not calculated since no data on $C_{\text{max}}$ in the target tissues (cornea and conjunctiva) were found in literature. The values of MIC$_{90}$ for the five antibiotics under investigation, were retrieved from two studies [11, 12] that fully match the fixed bibliography selection criteria. In the study selected for quinolones (i.e. levofloxacin, ofloxacin) and macrolide (i.e azithromycin) antibiotics, MIC$_{90}$ values were calculated from a total of 6091 eye isolates while the second study, for the aminoglycoside antibiotics, considered a total of 734 ocular isolates. $C_{\text{max}},$ MIC$_{90}$ values and their relative bibliographic references were reported in Tables 1 and 2. $C_{\text{max}}$ values were put into ratio with the above MICs$_{90}$ and the resulting QIs were reported as graphs. Analysis of the QI values showed that, as to the single dose, Nettacin\textsuperscript{®} eye drops (netilmicin 0.3\% eye drops solution) were revealed to be the best performing formulation in conjunctiva against MRSA, MRCoNS and MSCoNS (Figure 1). Interestingly, under the same conditions, netilmicin 0.3\% gel formulation (Xanternet) allowed a general sensible increase of the QIs to make this formulation the best performing one among those studied, also against the MSSA (Figure 2). In cornea, netilmicin 0.3\% eye drops solution showed higher QIs with respect to other formulations vs MRSA and MRCoNS strains. IQUIX\textsuperscript{®} (levofloxacin 1.5\%), due to the fortified formulation, overcame all the other ones in the MSCoNS category (Figure 3). Also in this case, under the same conditions, the netilmicin 0.3\% gel formulation allowed a general sensible increase of the QIs so that netilmicin gel was revealed to be the best performing formulation against the MSCoNS and the second best performing one against the MSSA category (Figure 4). As to the multiple doses of administration, only Nettacin\textsuperscript{®} eye drops were eligible to be included in the analysis. This netilmicin

![Figure 1](image_url)

\textit{Figure 1.} Netilmicin eye drops (Nettacin eye drops) conjunctival QIs in a single dose against (A) MSSA; (B) MRSA; (C) MSCoNS; and (D) MRCoNS.
formulation in the conjunctiva was the best performing one among the three antibiotic formulations considered against MRCoNS and MSCoNS and the second best performing one against the other two strain categories (Figure 5). In the cornea, netilmicin in multiple-dose presentation, revealed to be the best performing formulation against MRCoNS and MRSA (Figure 6).
Moreover, QI determination was evaluated in human tear, comparing netilmicin eye drops solution with tobramycin and fluoroquinolones (i.e. ofloxacin and levofloxacin). QIs were established, using MIC_{90} and C_{max} values retrieved from peer-reviewed literature [11, 24–28]. Results demonstrated that in human tear,
Netilmicin eye drops solution showed a more favourable QI against Staphylococci than tobramycin, ofloxacin and levofloxacin, all evaluated in single-dose administration regimen (Figure 7).

Altogether, QI results showed that netilmicin in the conjunctiva, in both single and multiple doses, is a highly effective antibiotic against all staphylococcal categories, while in the cornea it was revealed to be particularly active in single and multiple
doses, against methicillin-resistant Staphylococci strains. Importantly, QI data on human tears supports what was found in the cornea and conjunctiva of rabbits, thus confirming the better efficacy of netilmicin with respect to other antibiotics.

3.2 Killing curves

Data showed that netilmicin has a pronounced bactericidal activity against all the four Staphylococci tested strains. For these Staphylococci, netilmicin was revealed to be one of the most active antibiotics achieving, at 24 hours, a > 3 \( \log_{10} \) reduction of the initial inoculum (Figures 8–11).

In detail, netilmicin, together with tobramycin, showed a bactericidal effect against \textit{S. aureus} 6538 (ATCC strain) with a higher efficacy at 24h (>4 and 6 \( \log_{10} \) decrease, respectively) compared to levofloxacin, ofloxacin, azithromycin and chloramphenicol (Figure 8).

The same activity was found against \textit{S. aureus} 801 MRSA (ocular isolate), where netilmicin, together with tobramycin, showed the best performance reaching the total eradication of the initial inoculum at 24h, (both 6 \( \log_{10} \) decreases at 24h) (Figure 9). Importantly, this effect is better than levofloxacin, ofloxacin, azithromycin and chloramphenicol that failed to induce a > 3 \( \log_{10} \) reduction of the initial inoculum at all time points, with the exception of levofloxacin and ofloxacin that induce a reduction > 3 \( \log_{10} \) at 24h (Figure 9).

Moreover, netilmicin reduced bacterial growth of \textit{S. epidermidis} 12228 (ATCC strain) with a killing activity from 2h to 24h similar to that of tobramycin (6 \( \log_{10} \) decrease, Figure 10). Except for levofloxacin which is effective only at 24h (6 \( \log_{10} \) decrease), this effect is better than ofloxacin, azithromycin and chloramphenicol that failed to produce a > 3 \( \log_{10} \) reduction of the initial inoculum (Figure 10).

Surprisingly, it was found that netilmicin was the only antibiotic able to reach the total eradication of the bacterial inoculum of \textit{S. epidermidis} 829 MRSE (ocular

![S. aureus 6538 (ATCC)](image)

**Figure 8.**

Time-killing curves of netilmicin (32 \( \mu \)g/ml), tobramycin (16 \( \mu \)g/ml), levofloxacin (4 \( \mu \)g/ml), ofloxacin (4 \( \mu \)g/ml), azithromycin (8 \( \mu \)g/ml) and chloramphenicol against \textit{S. aureus} (ATCC 6538 strain) exposed for 0, 1, 2, 6 and 24 h. Data represent mean ± S.E.M. of \( \log_{10} \) cfu/ml of three replicates.
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**S. aureus 801 MRSA**

![Figure 9.](image1)

*Time-killing curves of netilmicin (32 μg/ml), tobramycin (16 μg/ml), levofloxacin (4 μg/ml), ofloxacin (4 μg/ml), azithromycin (8 μg/ml) and chloramphenicol against S. aureus 801 isolate (MRSA) exposed for 0, 1, 2, 6 and 24 h. Data represent mean ± S.E.M. of log<sub>10</sub> cfu/ml of three replicates.*

**S. epidermidis 12228 (ATCC)**

![Figure 10.](image2)

*Time-killing curves of netilmicin (32 μg/ml), tobramycin (16 μg/ml), levofloxacin (4 μg/ml), ofloxacin (4 μg/ml), azithromycin (8 μg/ml) and chloramphenicol against S. epidermidis (ATCC 12228 strain) exposed for 0, 1, 2, 6 and 24 h. Data represent mean ± S.E.M. of log<sub>10</sub> cfu/ml of three replicates.*

Isolate) at 24h (6 log<sub>10</sub> decrease, **Figure 11**) with respect to all other tested antibiotics. Moreover, levofloxacin and ofloxacin showed a bactericidal activity at 24h (3 log<sub>10</sub> decrease, **Figure 11**) but this effect is lower than netilmicin. Importantly, tobramycin failed to produce a bactericidal effect against MRSE ocular isolate (**Figure 11**).
Moreover, in order to better understand and compare the efficacy of the different antibiotics tested, area under the curve (AUC) was calculated for each killing curve obtained and compared to netilmicin AUC for each strain tested (Table 3).

Results of the analysis showed that netilmicin has a better activity with respect to tobramycin against MRSE strain ($p \leq 0.0001$, Table 3).

Moreover, netilmicin is better than azithromycin for three of the total four strains tested, specifically *S. aureus* 6538 and MRSA with $p \leq 0.0001$ and *S. epidermidis* 12228.

**Table 3.**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Strains/ocular isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus 6538 (ATCC)</td>
</tr>
<tr>
<td></td>
<td>Netilmicin</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>ns</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>ns</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>ns</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>ns</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>ns</td>
</tr>
</tbody>
</table>

$p \leq 0.05$.  
$p \leq 0.01$.  
$p \leq 0.001$.  
$p \leq 0.0001$ vs netilmicin.  
One-way ANOVA followed by Dunnett’s post-hoc test; ns = no statically significant differences.

Figure 11.  
Time-killing curves of netilmicin (32 μg/ml), tobramycin (16 μg/ml), levofloxacin (4 μg/ml), ofloxacin (4 μg/ml), azithromycin (8 μg/ml) and chloramphenicol against *S. epidermidis* 829 isolate (MRSE) exposed for 0, 1, 2, 6 and 24 h. Data represent mean ± S.E.M. of log$_{10}$ cfu/ml of three replicates.
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Finally, it was found that netilmicin is better than chloramphenicol for all tested strains (p ≤ 0.01 for *S. epidermidis* 12228; p ≤ 0.001 for MRSE; p ≤ 0.0001 for *S. aureus* 6538 and MRSA) (Table 3).

4. Discussion

Bacterial conjunctivitis affects many people and imposes economic and social burdens [29]. Clinical studies of ocular infections have documented a gradual decline in the effectiveness of many commonly used topical antibacterial agents, creating a continuous demand for newer, effective treatments and better strategies to minimize resistance [4]. Netilmicin is a broad-spectrum, semisynthetic and water-soluble antibiotic of the aminoglycoside group. Compared to gentamicin and tobramycin, netilmicin has a good activity against gentamicin and tobramycin resistant strains, this depending on the N-ethyl substitution of the 2-deoxystreptamine ring [1–3]. Interestingly, netilmicin might also be considered an antibiotic of choice in the preoperative setting thanks to the high *in vitro* susceptibility (>90%) to this antibiotic of those isolates belonging to the normal ocular flora (including also the multi-resistant coagulase-negative Staphylococci) [30].

As to the arising of resistant strains, this antibiotic, when compared to other aminoglycosides and fluoroquinolones, has one of the lowest incidences of resistance among both gram-positive and negative organisms (2% and 3%, respectively) [31]. To truly understand the pharmacology of antimicrobial agents, it’s better to go beyond MICs, using metrics that account for the rate of bacterial killing and effects of different dosing regimens and formulations on accumulation in tissues. Appreciation of the pharmacokinetic properties of an antibiotic and its pharmacodynamic measures of efficacy can maximise the utility of these sight-saving drugs.

To better understand the antibacterial activity of netilmicin and compare it with that of other ophthalmic antibiotics, two key pharmacological indices, QI and killing kinetic, useful to facilitate this comparison were investigated [15]. QI results showed that netilmicin has more affinity to cornea and conjunctiva with respect to the other molecules studied ensuring, in these tissues, amounts of drug suitable to eradicate Staphylococci (including methicillin-resistant strains). Interestingly, netilmicin 0.3% tested in both solution and gel formulation form, selectively guarantee at the main ocular sites high antibiotic concentrations without neglecting patient’s compliance [32]. Particularly, the present study demonstrates that the gel formulation of netilmicin greatly amplifies the potency of the molecule by concurring with the increase of its QIs within target tissues. In fact, the gel formulation of netilmicin contains 1% xanthan gum which affects the retention time of the antibiotic on the ocular surface and, consequently, on the tissues’ *C*<sub>max</sub> values [24].

Moreover, in human tears, netilmicin eye drops, in single dose, showed to have a more favourable QI against Staphylococci than tobramycin and fluoroquinolones (i.e. ofloxacin and levofloxacin). Also, in this case, to calculate the QIs, MIC<sub>90</sub> and *C*<sub>max</sub> values were retrieved from peer-reviewed literature as above [11, 25–28].

Results of the present study highlight that netilmicin has also a faster killing activity and a more potent antimicrobial effect, against most of the staphylococcal strains tested, including *MRSA* and *MRSE*, than the main ophthalmic antibiotics.

Results demonstrated that netilmicin and tobramycin, both included in the aminoglycosides class, are effective against *S. aureus*, *MRSA* and *S. epidermidis* with a greater effect compared to the other tested antibiotics, that is, azithromycin, ofloxacin, levofloxacin and chloramphenicol. Surprisingly, netilmicin is the only antibiotic...
able to inhibit the growth of MRSE compared to all other molecules including tobramycin although belonging to the same class. Importantly, tobramycin was found to be unable to inhibit the growth of MRSE strains, indeed bacterial growth increases as the control (condition without treatment).

These findings are of particular interest considering that among ocular isolates, the frequency of MRSE and MRSA in bacterial conjunctivitis is increasing [4].

Moreover, the aforementioned susceptibility profile of netilmicin against multidrug resistant organism (MDRO) like CoNS, including *S. epidermidis* [30], guarantees that its use is beneficial and warranted also to restore the balance of the eye microbiota frequently disrupted in patients suffering from recurrent eye infections who are subjected to repeated, and sometimes empirical, antibiotic treatments.

In fact, normal ocular microbiota could be considered a reservoir of antibiotic resistance and some bacterial strains have the capacity of centralizing and carrying antibiotic resistance genes (belonging to other organisms) so to become MDRO.

It was already proven that empirical antibiotic treatment in eye infections, such as keratitis, leads to an increase in the antibiotic resistome profile of the ocular surface microbiota [33].

As an example of antibiotics leading to multi-drug resistance patterns, it is known that the repeated use of topical antibiotics, e.g. azithromycin or fluoroquinolones, could significantly alter the microbiota composition by increasing the percentage of *S. epidermidis* at the expense of other strains populating the commensal flora. *S. epidermidis* could rapidly emerge after antibiotic exposure acquiring both co-resistance to several classes of antibiotics and alterations in their biofilm formation capacity so to produce considerable clinical implications, such as conjunctivitis, keratitis and endophthalmitis [34].

In this sense, the use of netilmicin in patients where a chronic disruption of the normal balance of the ocular flora occurs as a consequence of the emergence of pathogens like MDRO CoNS should be recommended in order to reconstitute a normal eye surface homeostasis and, therefore, to resolve the aforementioned pathologies.

Moreover, it is worthy of note that in literature there is evidence showing that bactericidal action of netilmicin is maintained for up to 4 hours by its post-antibiotic effect [3]. This evidence could be useful for reducing the antibiotic administration regimen in vivo.

5. Conclusions

Netilmicin exhibits superior activity against the most common ocular bacterial strains, with faster and greater inhibitory potency in target tissues than other ophthalmic antibiotics. QI data on cornea and conjunctiva in rabbits demonstrated that netilmicin 0.3% is better than the other products when tested in both dosage forms, that is, eye drops solution and gel. Importantly, netilmicin 0.3% gel, due to the presence of 1% xanthan gum prolongs the retention time on the ocular surface and, consequently, increases the $C_{\text{max}}$. Therefore, based on the severity of the pathology and any other concerns (e.g. corneal healing) associated with bacterial conjunctivitis, it is possible to use netilmicin 0.3% eye drops solution or gel formulation.

Moreover, netilmicin offers an excellent broad-spectrum coverage against all tested isolates, including MRSA and MRSE, compared with other ophthalmic antibiotics. These findings are of particular interest considering that among ocular isolates, the frequency of MRSE and MRSA in bacterial conjunctivitis is increasing. Overall,
these data support the use of netilmicin as a first-line agent in the treatment of such bacterial ocular infections, due to ocular safety, potency and low resistance.

Conflict of interest

All the authors declare not to have any conflict of interest.
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