Supplement

Future MICROBIOLOGY

Comparative *in vitro* antibacterial activity of ozenoxacin against Gram-positive clinical isolates

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Aim: To compare the *in vitro* activity of the anti-impetigo agent, ozenoxacin, and other antimicrobial agents against Gram-positive clinical isolates from skin and soft tissue infections. **Materials & methods:** Isolates were collected in two studies: 1097 isolates from 49 centers during 2009–2010 and 1031 isolates from ten centers during 2014. Minimum inhibitory concentrations were determined for 18 and 11 antimicrobials in these studies, respectively, using standard broth microdilution methods. Isolates were stratified by species and methicillin susceptibility/resistance and/or levofloxacin susceptibility/nonsusceptibility status. **Results:** Ozenoxacin exhibited high *in vitro* activity against *Staphylococcus aureus* and coagulase-negative staphylococci isolates in both studies. Ozenoxacin was also highly active against *Streptococcus pyogenes* and *Streptococcus agalactiae* isolates. **Conclusion:** Ozenoxacin is a potent antimicrobial agent against staphylococci and streptococci.

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Impetigo is a common bacterial skin infection affecting both children and adults although it is more prevalent in children. Infection with *Staphylococcus aureus* or *Streptococcus pyogenes* causes the nonbullous type of impetigo which occurs in around 70% of cases, whereas *S. aureus* exclusively causes bullous impetigo, with the production of exfoliative toxins [1–3].

Topically administered antibacterial agents, mupirocin and fusidic acid, are commonly used to treat impetigo, although retapamulin is a more recent alternative [4,5]. The most recent topical option and the only compound with bactericidal properties is the nonfluorinated quinolone, ozenoxacin [6], which demonstrated clinical benefit in a recent Phase III trial [7]. The results of a second Phase III trial which is currently being published showed similar clinical and microbiological results (ClinicalTrials.gov identifier: NCT02090764) [8].

During the development of ozenoxacin, surveillance studies addressing its antimicrobial activity in comparison with other antimicrobials were conducted. In this article, the *in vitro* activity of ozenoxacin against Gram-positive clinical isolates recovered from skin and soft-tissue infections (SSTIs) is compared with a panel of antibacterial agents. More than 2000 isolates were collected in two worldwide studies from 2009 to 2010, and during 2014. The isolates include the causative microorganisms of impetigo, *S. aureus* and *S. pyogenes,* and coagulase-negative staphylococci which are the most common microorganisms on normal skin flora, with *Staphylococcus epidermidis* being the predominant species [3]. Comparisons with a wide range of antimicrobial agents were made using the minimum inhibitory concentration (MIC) value, which is also useful for assessing antimicrobial phenotypic resistance [9].

Future Medicine

Materials & methods

Study 1: evaluation of the *in vitro* activity of ozenoxacin & comparative antimicrobial agents against Gram-positive clinical isolates collected during 2009 & 2010

The *in vitro* activity of ozenoxacin was evaluated against Gram-positive isolates collected from 49 centers in the Czech Republic (n = 3), Germany (n = 3), The Netherlands (n = 3), Romania (n = 3), South Africa (n = 3), Spain (n = 4) and the USA (n = 30). Organisms were obtained randomly from uncomplicated SSTIs (uSSTIs) and/or complicated SSTIs (cSSTIs) during 2009 and 2010. A total of 1097 isolates were collected from participating sites, with 50.0% originating from inpatients (n = 548) and 49.9% (n = 547) from outpatients; the origin of two isolates was not recorded. Isolates were classified as *S. aureus* (n = 486), *S. epidermidis* (n = 190), other coagulase-negative *Staphylococcus* (CNS) species (n = 37), *S. pyogenes* (n = 217), *Streptococcus agalactiae* (n = 37), *Streptococcus pneumoniae* (n = 29) and *Corynebacterium* spp. (n = 52). Other isolates were *Micrococcus* spp. (n = 7), *Lactobacillus* spp. (n = 7), Group G *Streptococcus* (n = 19), Group C *Streptococcus* (n = 9), and *Brevibacterium* spp. (n = 3); plus *Enterococcus faecalis* (n = 2), *Kocuria kristinae* (n = 1) and *Rothia mucilaginosa* (n = 1).

S. aureus was identified by Gram stain, catalase and DNAse production, and the staphylococcal latex agglutination test. In addition, cefoxitin susceptibility was performed to determine methicillin resistance. Identification of coagulase-negative staphylococci was performed using Gram stain, catalase and DNAse production, the staphylococcal latex agglutination test, API Identification Systems or mass spectrometry (matrix assisted laser desorption/ionization–time of flight mass spectrometry [MALDI–TOF MS], Bruker Daltonics, Bremen, Germany). Cefoxitin susceptibility was also performed. Groups A, B, C and G streptococci were identified using Gram stain, catalase production and Lancefield grouping. Identification of *S. pneumoniae* used Gram stain, optochin susceptibility and bile solubility. Identification of *Corynebacterium* spp., *Micrococcus* spp. and *Lactobacillus* spp. used Gram stain, catalase production and identification by API Identification Systems or MALDI-TOF MS.

MIC was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [10]. MIC_{50} and MIC_{90} values were calculated for each antimicrobial agent.

For determination of MICs, the same range of concentrations for each antimicrobial were tested against species of *Staphylococcus*, *Corynebacterium*, *Micrococcus* and *Lactobacillus* (group 1 species); with the tested MIC range often differing for *Streptococcus* species. Respective tested MIC ranges for group 1 species and *Streptococcus* species were: ozenoxacin (0.001–2; 0.001–2 mg/l), amoxicillin-clavulanate (0.03–32; 0.008–16 mg/l), ceftriaxone (0.03–64; 0.03–64 mg/l), cefuroxime (0.03–64; 0.015–16 mg/l), ciprofloxacin (0.002–4; 0.03–32 mg/l), clindamycin (0.03–64; 0.015–32 mg/l), daptomycin (0.015–32; 0.015–32 mg/l), erythromycin (0.03–64; 0.015–32 mg/l), fusidic acid (0.03–64; 0.03–64 mg/l), gentamycin (0.03–32; 0.03–64 mg/l), levofloxacin (0.004–8; 0.03–64 mg/l), linezolid (0.015–32; 0.015–32 mg/l), mupirocin (0.03–64; 0.03–64 mg/l), neomycin (0.03–64; 0.03–64 mg/l), penicillin G (0.03–64; 0.008–16 mg/l), retapamulin (0.008–16; 0.008–16 mg/l), tetracycline (0.03–64; 0.03–64 mg/l) and vancomycin (0.03–32; 0.03–32 mg/l). Two quality-control strains (*S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619) were also included in the study.

Susceptibility results were interpreted using breakpoints set by the CLSI [10,11]. Nevertheless, European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12] or British Society for Antimicrobial Chemotherapy [13] breakpoints were used for different antimicrobials: staphylococci and fusidic acid [13]; *S. pneumoniae* and ciprofloxacin [12]; staphylococci and mupirocin [13]; and *Corynebacterium* species for amoxicillin-clavulanate, cefuroxime and levofloxacin [12]. No breakpoints are yet defined by the CLSI or EUCAST for ozenoxacin.

Study 2: evaluation of the *in vitro* activity of ozenoxacin & comparative antimicrobials against relevant clinical isolates collected during 2014

A total of 1031 clinical isolates of *S. aureus* (n = 504), coagulase-negative staphylococci (n = 315), *S. pyogenes* (n = 124) and *S. agalactiae* (n = 88) were collected from January to December 2014 at ten participating centers located in Argentina, Brazil, Colombia, Germany, Romania, South Africa, Spain, Sweden and at two sites in the USA. Species in the coagulase-negative staphylococci group were *Staphylococcus capitis* (n = 17), *Staphylococcus caprae* (n = 4), *Staphylococcus cohni* (n = 1), *S. epidermidis* (n = 195), *Staphylococcus haemolyticus* (n = 27), *Staphylococcus hominis* (n = 12), *Staphylococcus intermedious* (n = 3), *Staphylococcus lugdunensis* (n = 42), *Staphylococcus saprophyticus* (n = 2), *Staphylococcus simulans* (n = 4) and *Staphylococcus warneri* (n = 8). Two quality-control strains (*S. aureus* ATCC 29213 and *S. pneumoniae* ATCC 49619) were also included in the study.

All isolates were tested for susceptibility to amoxicillin-clavulanate, ciprofloxacin, clindamycin, erythromycin, fusidic acid, mupirocin, levofloxacin, ozenoxacin, penicillin and vancomycin using prepared dry panels (Sensititre™,

Thermo Fisher Scientific). They were prepared using the broth microdilution method as recommended by the CLSI [10]. The range of concentrations tested were: ozenoxacin (0.001–16 mg/l), amoxicillin-clavulanate (4/2–8/2 mg/l), ciprofloxacin (0.015–16 mg/l), clindamycin (0.015–16 mg/l), erythromycin (0.015–16 mg/l), fusidic acid (0.015–16 mg/l), levofloxacin (0.015–16 mg/l), mupirocin (0.015–256 mg/l), penicillin G (0.12–0.25 mg/l), retapamulin (0.015–1 mg/l) and vancomycin (0.5–2 mg/l). Susceptibility results were interpreted using an approach that was similar to the previous study but using CLSI and EUCAST documents from 2014.

Results

Source of isolates & quality control results

Isolates in study 1 were recovered from skin (n = 541; 49.3%), wounds (n = 300; 27.3%), abscesses (n = 98; 8.9%), tissue (n = 45; 4.1%), blood (n = 40; 3.6%) or other (n = 73; 6.7%). SSTIs were further categorized as cSSTI, uSSTI or unknown. The proportion of skin isolates (expressed as a percentage of the total sample) categorized as cSSTI, uSSTI and unknown were 18.3% (n = 201), 23.4% (n = 257) and 7.6% (n = 83), respectively. Respective proportions by site of recovery were, for wound: 10.8% (n = 118), 8.2% (n = 92) and 8.4% (n = 90); for abscess: 2.6% (n = 28), 1.9% (n = 49) and 4.5% (n = 21); for tissue: 2.5% (n = 27), 1.5% (n = 2) and 0.2% (n = 16); for blood: 1.3% (n = 14; 1.7% (n = 7) and 0.6% (n = 19); and for other: 2.2% (n = 24), 1.5% (n = 33) and 3.0% (n = 16).

Isolates in study 2 were recovered from wounds (n = 410; 39.8%), abscesses (n = 163; 15.8%), skin (n = 20; 1.9%), acne (n = 11; 1.1%), tissues (n = 8; 0.8%) and nonspecified SSTIs (n = 419; 40.6%).

All quality control results were within the quality control ranges specified by CLSI Documents M100-S20 (2011) [14] and M100-S24 (2014) [15].

S. aureus

Study 1

Table 1 shows MIC_{50} and MIC_{90} values for ozenoxacin and 18 comparator antimicrobial agents against *S. aureus* isolates, which were also stratified by methicillin (-susceptible *S. aureus* [MSSA]; -resistant *S. aureus* [MRSA]) and levofloxacin susceptibility. Ozenoxacin was highly active against the 486 *S. aureus* isolates tested, with a MIC_{50} of 0.004 mg/l and a MIC_{90} of 0.25 mg/l. For levofloxacin-nonsusceptible *S. aureus* isolates (n = 168; levofloxacin MIC_{50} = 8 mg/l and MIC_{90} = 16 mg/l), the ozenoxacin MIC_{50} was 0.12 mg/l and the MIC_{90} was 0.5 mg/l. Ozenoxacin had lower MIC values (MIC_{50} = 0.004 mg/l and MIC_{90} = 0.004 mg/l) against levofloxacin-susceptible *S. aureus* isolates (n = 312; levofloxacin MIC_{50} = 0.25 mg/l and MIC_{90} = 0.25 mg/l; Table 1). The MIC_{90} of ozenoxacin (0.25 mg/l) was comparable to those of fusidic acid, mupirocin and retapamulin (all 0.25 mg/l) against all *S. aureus* isolates tested. In contrast, ciprofloxacin (8 mg/l) and levofloxacin (16 mg/l) had much higher MIC_{90} values.

MSSA isolates

Ozenoxacin showed excellent activity (range ≤0.001–1 mg/l) against MSSA isolates (n = 247), with a MIC_{50} and MIC_{90} of 0.004 mg/l. Only two isolates had a MIC for ozenoxacin above 0.25 mg/l (MIC of 1 mg/l). 14 MSSA isolates were levofloxacin nonsusceptible (levofloxacin MIC_{50} = 4 mg/l and MIC_{90} ≥16 mg/l): the MIC_{50} and MIC_{90} values for ozenoxacin against levofloxacin nonsusceptible MSSA isolates were 0.12 and 1 mg/l, respectively. Lower MIC_{50} and MIC_{90} values (both 0.004 mg/l) were found for ozenoxacin against the 231 levofloxacin-susceptible MSSA isolates (levofloxacin MIC_{50} = 0.25 mg/l and MIC_{90} = 0.25 mg/l; Table 1). Comparative MIC data showed that ozenoxacin (MIC_{90} = 0.004 mg/l) was more active than all reference compounds against MSSA isolates. The next most active compound was clindamycin (MIC_{90} = 0.12 mg/l) which was 30-fold less active than ozenoxacin; followed by fusidic acid, mupirocin and retapamulin (MIC_{90} = 0.25 mg/l); daptomycin, gentamicin, levofloxacin (MIC_{90} = 0.5 mg/l); ciprofloxacin, amoxicillin-clavulanate, tetracycline, vancomycin (MIC_{90} = 1 mg/l); cefuroxime and linezolid (MIC_{90} = 2 mg/l); neomycin and ceftriaxone (MIC_{90} = 4 mg/l); penicillin (MIC_{90} = 16 mg/l); and erythromycin (MIC_{90} = 64 mg/l; Table 1).

MRSA isolates

Ozenoxacin was highly active against MRSA isolates (n = 239), with a MIC_{50} of 0.12 mg/l and a MIC_{90} of 0.25 mg/l. One isolate had an ozenoxacin MIC of ≥4 mg/l, and five isolates from four different sites had an ozenoxacin MIC of 2 mg/l. Eight strains of MRSA from seven sites had an ozenoxacin MIC of 1 mg/l. Ozenoxacin

Table 1. Study 1: MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against *Staphylococcus aureus* stratified by methicillin and levofloxacin susceptibility.

	MIC (mg/l)	OZE	MUP	FUS	RET	LVX	CIP	AMC	CRO	CXM	CLI	DAP	ERY	GEN	LZD	NEO	PEN	TET	VAN
S. aureus (n = 486; all)	MIC_{50}	0.004	0.12	0.12	0.12	0.25	0.5	2	8	2	0.12	0.5	2	0.25	2	1	16	0.25	1
	MIC_{90}	0.25	0.25	0.25	0.25	16	8	16	≥128	≥128	≥128	0.5	≥128	1	2	64	32	4	1
MSSA (n = 247)	MIC_{50}	0.004	0.12	0.12	0.12	0.25	0.5	1	4	1	0.12	0.25	0.25	0.25	2	0.5	2	0.25	1
	MIC_{90}	0.004	0.25	0.25	0.25	0.5	1	1	4	2	0.12	0.5	64	0.5	2	4	16	1	1
MRSA (n = 239)	MIC_{50}	0.12	0.12	0.12	0.12	4	≥8	8	32	16	0.12	0.5	32	0.25	2	4	32	0.25	1
	MIC_{90}	0.25	8	0.25	0.12	≥16	≥8	32	≥128	≥128	≥128	0.5	≥128	1	2	≥128	64	32	1
Levofloxacin-susceptible *S. aureus* (n = 312)	MIC_{50}	0.004	0.12	0.12	0.12	0.25	0.5	1	4	2	0.12	0.5	0.25	0.25	2	0.5	4	0.25	1
	MIC_{90}	0.004	0.25	0.25	0.25	0.25	1	8	32	16	0.12	0.5	64	0.5	2	16	32	2	1
Levofloxacin-nonsusceptible *S. aureus* (n = 168)	MIC_{50}	0.12	0.12	0.12	0.12	8	8	8	32	32	0.12	0.5	64	0.25	2	8	32	0.25	1
	MIC_{90}	0.5	16	0.25	0.12	16	8	32	≥128	≥128	≥128	0.5	≥128	1	2	≥128	64	2	1
Levofloxacin-susceptible, MSSA (n = 231)	MIC_{50}	0.004	0.12	0.12	0.12	0.25	0.5	1	4	1	0.12	0.25	0.25	0.25	2	0.5	2	0.25	1
	MIC_{90}	0.004	0.25	0.25	0.25	0.25	1	1	4	2	0.12	0.5	64	0.5	2	2	16	1	1
Levofloxacin-nonsusceptible, MSSA (n = 14)	MIC_{50}	0.12	0.12	0.12	0.12	4	≥8	0.5	4	1	0.12	0.25	64	0.25	2	0.5	2	0.25	1
	MIC_{90}	1	1	0.25	0.12	≥16	≥8	1	8	2	≥128	0.5	≥128	16	2	≥128	16	64	1
Levofloxacin-susceptible, MRSA (n = 81)	MIC_{50}	0.004	0.12	0.12	0.12	0.25	0.5	8	32	16	0.12	0.5	32	0.5	2	2	32	0.25	1
	MIC_{90}	0.008	0.25	0.12	0.12	0.5	1	16	64	32	16	0.5	≥128	1	2	64	32	64	1
Levofloxacin-nonsusceptible, MRSA (n = 154)	MIC_{50}	0.12	0.12	0.12	0.12	8	≥8	16	32	32	0.12	0.5	64	0.25	2	8	32	0.25	1
	MIC_{90}	0.25	16	0.25	0.12	≥16	≥8	32	≥128	≥128	≥128	0.5	≥128	1	2	≥128	64	2	1

AMC: Amoxicillin-clavulanate; CRO: Ceftriaxone; CXM: Cefuroxime; CIP: Ciprofloxacin; CLI: Clindamycin; DAP: Daptomycin; ERY: Erythromycin; FUS: Fusidic acid; GEN: Gentamicin; LZD: Linezolid; LVX: Levofloxacin; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-susceptible *S. aureus*; MUP: Mupirocin; NEO: Neomycin; OZE: Ozenoxacin; PEN: Penicillin; RET: Retapamulin; TET: Tetracycline; VAN: Vancomycin.

had a MIC_{50} of 0.004 mg/l and a MIC_{90} of 0.008 mg/l for levofloxacin-susceptible MRSA isolates (n = 81; levofloxacin MIC_{50} = 0.25 mg/l and MIC_{90} = 0.5 mg/l). A total of 154 MRSA isolates were also levofloxacin-nonsusceptible (levofloxacin MIC_{50} = 8 mg/l and MIC_{90} ≥16 mg/l). Ozenoxacin had a MIC_{50} and a MIC_{90} of 0.12 and 0.25 mg/l, respectively, against levofloxacin-nonsusceptible MRSA isolates (Table 1). Comparative MIC_{90} data for all MRSA isolates tested showed that retapamulin (MIC_{90} = 0.12 mg/l) was the most active compound, followed by ozenoxacin and fusidic acid (MIC_{90} = 0.25 mg/l); daptomycin (MIC_{90} = 0.5 mg/l); gentamicin and vancomycin (MIC_{90} = 1 mg/l); linezolid (MIC_{90} = 2 mg/l); mupirocin (MIC_{90} = 8 mg/l); ciprofloxacin (MIC_{90} ≥8 mg/l); levofloxacin (MIC_{90} ≥16 mg/l); amoxicillin-clavulanate and tetracycline (MIC_{90} = 32 mg/l); penicillin (MIC_{90} = 64 mg/l); and ceftriaxone, cefuroxime, clindamycin, erythromycin and neomycin (MIC_{90} ≥128 mg/l; Table 1). Ozenoxacin was significantly more potent (MIC_{90} = 0.008 mg/l) than all comparative compounds for levofloxacin-susceptible MRSA isolates. It was 15-fold more active than retapamulin and fusidic acid (MIC_{90} = 0.12 mg/l), with the rank order of other compounds being: mupirocin (MIC_{90} = 0.25 mg/l); daptomycin and levofloxacin (MIC_{90} 0.5 mg/l); ciprofloxacin; gentamicin and vancomycin (MIC_{90} = 1 mg/l); linezolid (MIC_{90} = 2 mg/l); clindamycin and amoxicillin-clavulanate (MIC_{90} = 16 mg/l); cefuroxime and penicillin (MIC_{90} = 32 mg/l); ceftriaxone, neomycin and tetracycline (MIC_{90} = 64 mg/l); and erythromycin (MIC_{90} ≥128 mg/l; Table 1). Against MRSA isolates (n = 239), including those which were levofloxacin susceptible (n = 81) and nonsusceptible (n = 154), ozenoxacin (MIC_{90} = 0.25 ug/ml) was 32-fold more active than mupirocin (MIC_{90} = 8 μg/ml; Table 1).

Study 2

The antibacterial activity of ozenoxacin and comparators against *S. aureus*, stratified by methicillin and levofloxacin susceptibility, is summarized in Table 2. Of the 504 *S. aureus* isolates included in the study, 225 (44.6%) were MRSA and 279 (55.4%) were MSSA. Almost half (45.7%) of the MRSA isolates were also nonsusceptible to levofloxacin, whereas only 6.5% of MSSA isolates were levofloxacin nonsusceptible. Ozenoxacin demonstrated excellent overall activity (MIC_{50} = 0.002 mg/l, MIC_{90} = 0.06 mg/l) against all 504 *S. aureus* isolates, inhibiting 99.4% at a MIC of ≤0.5 mg/l. The activity of ozenoxacin was higher against levofloxacin-susceptible *S. aureus* isolates (MIC_{50} and MIC_{90} = 0.002 mg/l) compared with levofloxacin nonsusceptible isolates (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.5 mg/l; Table 2). MIC values to ozenoxacin in MRSA isolates (MIC_{50} = 0.004 mg/l; MIC_{90} = 0.12 mg/l) were slightly higher than those found with all *S. aureus* isolates.

Ozenoxacin was the most potent agent against all *S. aureus* isolates tested. Comparing MIC_{50} values, ozenoxacin (MIC_{50} = 0.002 mg/l) had 32-fold greater activity than clindamycin (MIC_{50} = 0.06 mg/l); 64-fold greater activity than retapamulin or fusidic acid (MIC_{50} = 0.12 mg/l); and 128-fold greater activity than erythromycin, mupirocin, ciprofloxacin or levofloxacin (MIC_{50} = 0.25 mg/l). At the MIC_{90} level, ozenoxacin (MIC_{90} = 0.06 mg/l) was twofold more potent than retapamulin (MIC_{90} = 0.12 mg/l); fourfold more potent than fusidic acid (MIC_{90} = 0.25 mg/l); eightfold more active than mupirocin (MIC_{90} = 0.5 mg/l); and at least 256-fold more potent than erythromycin, clindamycin, ciprofloxacin or levofloxacin (MIC_{90} ≥16 mg/l; Table 2).

Against MRSA, ozenoxacin (MIC_{50} = 0.004 mg/l; MIC_{90} = 0.12 mg/l) had greater activity than mupirocin (MIC_{50} = 0.25 mg/l; MIC_{90} = 0.5 mg/l) and fusidic acid (MIC_{50} = 0.12 mg/l; MIC_{90} = 0.25 mg/l), and using MIC_{50} values, was more potent than retapamulin (MIC_{50} = 0.12 mg/l; MIC_{90} = 0.12 mg/l). Against levofloxacin nonsusceptible *S. aureus,* ozenoxacin (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.5 mg/l) had greater activity than mupirocin (MIC_{50} = 0.25 mg/l; MIC_{90} = 2 mg/l) and fusidic acid (MIC_{50} = 0.12 mg/l; MIC_{90} = 0.25 mg/l), and was similar to retapamulin using MIC_{50} values (Table 2). Whereas MIC_{50} values for clindamycin and ozenoxacin were both 0.06 mg/l, MIC_{90} values indicated that ozenoxacin (MIC_{90} = 0.5 mg/l) was at least 64-fold more potent than clindamycin (MIC_{90} > 16 mg/l). The remaining agents had higher MIC_{50} and MIC_{90} values than ozenoxacin (Table 2).

Table 3 shows the proportion of *S. aureus*, MSSA, MRSA and levofloxacin susceptible or resistant *S. aureus* isolates, which were susceptible, resistant or had intermediate status to 11 antimicrobials, as assessed using EUCAST and CLSI criteria. Up to approximately 7% of *S. aureus*, MRSA, and levofloxacin susceptible or resistant *S. aureus* isolates were resistant to mupirocin and fusidic acid.

Table 2. Study 2: MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against *Staphylococcus aureus* stratified by methicillin and levofloxacin susceptibility.

	MIC (mg/l)	Ozenoxacin	Mupirocin	Fusidic acid	Retapamulin	Levofloxacin	Ciprofloxacin	Amoxicillin-clavulanate	Clindamycin	Erythromycin	Penicillin	Vancomycin
S. aureus (n = 504; all)	MIC_{50}	0.002	0.25	0.12	0.12	0.25	0.25	≤4 / 2	0.06	0.25	>0.25	≤0.5
	MIC_{90}	0.06	0.5	0.25	0.12	16	>16	>8 / 4	>16	>16	>0.25	1
MSSA (n = 279)	MIC_{50}	0.002	0.25	0.12	0.12	0.12	0.25	≤4 / 2	0.06	0.25	>0.25	≤0.5
	MIC_{90}	0.004	0.5	0.25	0.25	0.25	1	≤4 / 2	0.12	>16	>0.25	1
MRSA (n = 225)	MIC_{50}	0.004	0.25	0.12	0.12	0.25	0.5	>8 / 4	0.06	>16	>0.25	≤0.5
	MIC_{90}	0.12	0.5	0.25	0.12	>16	>16	>8 / 4	>16	>16	>0.25	1
Levofloxacin susceptible *S. aureus* (n = 383)	MIC_{50}	0.002	0.25	0.12	0.12	0.12	0.25	≤4 / 2	0.06	0.25	>0.25	≤0.5
	MIC_{90}	0.002	0.25	0.25	0.25	0.25	0.5	>8 / 4	0.12	>16	>0.25	1
Levofloxacin non-susceptible *S. aureus* (n = 121)	MIC_{50}	0.06	0.25	0.12	0.12	8	>16	>8 / 4	0.06	>16	>0.25	≤0.5
	MIC_{90}	0.5	2	0.25	0.12	>16	>16	>8 / 4	>16	>16	>0.25	1

MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-susceptible *S. aureus*.

Table 3. Proportion of *Staphylococcus aureus*, MSSA, MRSA and levofloxacin susceptible or resistant *S. aureus* isolates showing susceptibility and resistance to antimicrobials as assessed using EUCAST and CLSI criteria.

Antimicrobial†	EUCAST			CLSI			EUCAST			CLSI			EUCAST			CLSI			EUCAST			CLSI			EUCAST			CLSI		
% of isolates	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
	S. aureus (n = 504)						MSSA (n = 279)						MRSA (n = 225)						Levofloxacin susceptible *S. aureus* (n = 383)						Levofloxacin nonsusceptible *S. aureus* (n = 121)					
Ozenoxacin‡	–*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mupirocin	94	2.6	3.4	–	–	–	95	2.2	2.9	–	92.9	3.1	4	–	–	–	95.6	2.3	2.1	–	–	–	89.3	3.3	7.4	–	–	–		
Fusidic acid	95.6	–	4.4	–	–	–	97.5	–	2.5	–	93.3	–	6.7	–	–	–	95.6	–	4.4	–	–	–	95.9	–	4.1	–	–	–		
Retapamulin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Levofloxacin	76	1	23	76	1	23	93.5	1.1	5.4	93.5	54.2	0.9	44.9	54.2	0.9	44.9	100	0	0	100	0	0	0	4.1	95.9	0	4.1	95.9		
Ciprofloxacin	75	–	25	75	0.8	24.2	92.8	–	7.2	92.8	52.9	–	47.1	52.9	0.9	46.2	98.7	–	1.3	98.7	1	0.3	0	–	100	0	0	100		
Amoxicillin-clavulanate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Clindamycin	87.7	0	12.3	87.7	0.4	11.9	96.4	0	3.6	96.4	76.9	0	23.1	76.9	0.4	22.7	95.6	–	4.4	95.6	0.5	3.9	62.8	–	37.2	62.8	0	37.2		
Erythromycin	60.7	0.4	38.9	59.7	2.2	38.1	82.1	0	17.9	80.7	34.2	0.9	64.9	33.8	3.1	63.1	73.4	0.3	26.3	72.3	2.4	25.3	20.7	0.8	78.5	19.8	1.7	78.5		
Penicillin	8.3	–	91.7	8.3	–	91.7	15.1	–	84.9	15.1	0	–	100	0	–	100	9.7	–	90.3	9.7	–	90.3	4.1	–	95.9	4.1	–	95.9		
Vancomycin	100	–	0	100	0	0	100	–	0	100	100	–	0	100	0	0	100	–	0	100	0	0	100	–	0	100	0	0		

†Resistance phenotypes were defined according to CLSI nonsusceptible and resistant breakpoints.
‡Not published; interpretative criteria.
CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; I: Intermediate; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin susceptible *S. aureus*; R: Resistant; S: Susceptible.

Streptococcus species

S. pyogenes study 1

Ozenoxacin was highly active (range: 0.008–0.06 mg/l) against 217 *S. pyogenes* isolates, with a MIC_{50} of 0.03 mg/l and a MIC_{90} of 0.06 mg/l. The most active compounds against *S. pyogenes* were cefuroxime (MIC_{90} ≤0.015 mg/l); penicillin (MIC_{90} = 0.015 mg/l); ceftriaxone and amoxicillin-clavulanate (MIC_{90} = 0.03 mg/l); ozenoxacin, clindamycin, daptomycin, erythromycin and retapamulin (MIC_{90} = 0.06 mg/l); and mupirocin (MIC_{90} = 0.12 mg/l). *S. pyogenes* isolates were less susceptible to ciprofloxacin and vancomycin (MIC_{90} = 0.5 mg/l); levofloxacin and linezolid (MIC_{90} = 1 mg/l); fusidic acid and gentamicin (MIC_{90} = 8 mg/l); tetracycline (MIC_{90} = 32 mg/l); and neomycin (MIC_{90} = 64 mg/l; Table 4). Thus, against *S. pyogenes* isolates ozenoxacin was equipotent to retapamulin, had twofold higher activity than mupirocin and 13-fold higher activity than fusidic acid.

S. agalactiae study 1

Ozenoxacin was highly active (range: 0.008–0.06 mg/l) against the 37 *S. agalactiae* isolates tested, with a MIC_{50} of 0.03 mg/l and a MIC_{90} of 0.06 mg/l. Ozenoxacin, together with penicillin and retapamulin (MIC_{90} = 0.06 mg/l), were the most active compounds against *S. agalactiae*. The rank order of the remaining compounds was: ceftriaxone, cefuroxime and amoxicillin-clavulanate (MIC_{90} = 0.12 mg/l); daptomycin (MIC_{90} = 0.25 mg/l); vancomycin (MIC_{90} = 0.5 mg/l); ciprofloxacin, levofloxacin and mupirocin (MIC_{90} = 1 mg/l); linezolid (MIC_{90} = 2 mg/l); fusidic acid (MIC_{90} = 16 mg/l); gentamicin (MIC_{90} = 32 mg/l); tetracycline (MIC_{90} = 64 mg/l); clindamycin and erythromycin (MIC_{90} ≥64 mg/l); and neomycin (MIC_{90} ≥128 mg/l; Table 4).

S. pneumoniae study 1

Ozenoxacin was highly active (range: 0.015–0.06 mg/l) against *S. pneumoniae* isolates (n = 29), with a MIC_{50} and MIC_{90} of 0.03 and 0.06 mg/l, respectively. In comparison to the reference compounds, ozenoxacin (MIC_{90} = 0.06 mg/l) was significantly the most active. The rank order of the reference compounds was: daptomycin (MIC_{90} = 0.25 mg/l); retapamulin and vancomycin (MIC_{90} = 0.5 mg/l); levofloxacin (MIC_{90} = 1 mg/l); ceftriaxone, ciprofloxacin, linezolid and mupirocin (MIC_{90} = 2 mg/l); penicillin (MIC_{90} = 4 mg/l); amoxicillin-clavulanate and gentamicin (MIC_{90} = 8 mg/l); cefuroxime (MIC_{90} = 16 mg/l); fusidic acid and tetracycline (MIC_{90} = 32 mg/l); neomycin (MIC_{90} = 64 mg/l); and clindamycin and erythromycin (MIC_{90} ≥64 mg/l; Table 4).

S. pyogenes & S. agalactiae study 2

The antibacterial activity of ozenoxacin and comparators against *S. pyogenes* (n = 124) and *S. agalactiae* (n = 88) is summarized in Table 4. All isolates were susceptible to penicillin (MIC ≤0.12 mg/l), amoxicillin-clavulanate (MIC ≤4/2 mg/l) and vancomycin (MIC ≤0.5 mg/l). All *S. pyogenes* isolates and 95.5% (84/88) of *S. agalactiae* isolates were susceptible to levofloxacin by CLSI interpretative criteria (Table 4). Ozenoxacin was highly active against both *S. pyogenes* and *S. agalactiae.* MIC_{50} and MIC_{90} values were 0.008 and 0.015 mg/l, respectively, against *S. pyogenes* and were 0.015 and 0.03 mg/l, respectively, against *S. agalactiae* (Table 4).

Ozenoxacin was the most potent (MIC_{50} = 0.008 mg/l; MIC_{90} = 0.015 mg/l) agent tested against all *S. pyogenes* isolates, inhibiting 98.3% at a MIC of ≤0.03 mg/l. Ozenoxacin was fourfold more active than erythromycin, clindamycin or retapamulin (MIC_{50} = 0.03 mg/l; MIC_{90} = 0.06 mg/l), at least eightfold more active than mupirocin (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.25 mg/l), 64-fold more active than ciprofloxacin or levofloxacin (MIC_{50} = 0.5 mg/l; MIC_{90} = 1 mg/l) and at least 256-fold more active than fusidic acid (MIC_{50} and MIC_{90} = 4 mg/l; Table 4).

Ozenoxacin was also the most potent (MIC_{50} = 0.015 mg/l; MIC_{90} = 0.03 mg/l) agent tested against *S. agalactiae* isolates, inhibiting 95.5% of isolates at a MIC of ≤0.03 mg/l. Ozenoxacin was at least twofold more active than erythromycin or clindamycin (MIC_{50} = 0.03 mg/l; MIC_{90} >16 mg/l); fourfold more active than retapamulin (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.12 mg/l); 32-fold more active than ciprofloxacin or levofloxacin (MIC_{50} = mg/l; MIC_{90} = 0.5/1 mg/l); at least 32-fold more active than mupirocin (MIC_{50} and MIC_{90} = 1 mg/l); and 512-fold more active than fusidic acid (MIC_{50} = 8 mg/l; MIC_{90} = 16 mg/l; Table 4).

S. epidermidis study 1

MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobial agents against *S. epidermidis* isolates, which were also stratified by methicillin and levofloxacin susceptibility, are shown in Table 5. Ozenoxacin was highly active against *S. epidermidis* isolates (n = 190), with a MIC_{50} of 0.06 mg/l and a MIC_{90} of 1 mg/l. Two isolates from different sites had an ozenoxacin MIC of ≥4 mg/l and 12 isolates from nine sites had an ozenoxacin MIC of 2 mg/l.

Table 4. MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against *Streptococcus* species in studies 1 and 2.

	MIC (mg/l)	OZE	MUP	FUS	RET	LVX	CIP	AMC	CRO	CXM	CLI	DAP	ERY	GEN	LZD	NEO	PEN	TET	VAN
Streptococcus pyogenes (n = 217; study 1)	MIC_{50}	0.03	0.06	8	0.03	0.5	0.5	0.015	0.03	≤0.015	0.03	0.03	0.03	8	1	32	≤0.008	0.12	0.5
	MIC_{90}	0.06	0.12	8	0.06	1	0.5	0.03	0.03	≤0.015	0.06	0.06	0.06	8	1	64	0.015	32	0.5
Streptococcus agalactiae (n = 37; study 1)	MIC_{50}	0.03	0.5	16	0.06	1	0.5	0.06	0.06	0.06	0.06	0.12	0.06	32	1	≥128	0.06	32	0.5
	MIC_{90}	0.06	1	16	0.06	1	1	0.12	0.12	0.12	≥64	0.25	≥64	32	2	≥128	0.06	64	0.5
Streptococcus pneumoniae (n = 29; study 1)	MIC_{50}	0.03	0.5	16	0.25	1	0.5	0.03	0.03	0.06	0.06	0.12	0.12	8	1	64	0.03	0.25	0.5
	MIC_{90}	0.06	2	32	0.5	1	2	8	2	16	≥64	0.25	≥64	8	2	64	4	32	0.5
S. pyogenes (n = 124; study 2)	MIC_{50}	0.008	0.06	4	0.03	0.5	0.5	≤4 / 2			0.03		0.03				≤0.12		≤0.5
	MIC_{90}	0.015	0.25	4	0.06	1	1	≤4 / 2			0.06		0.06				≤0.12		≤0.5
S. agalactiae (n = 88; study 2)	MIC_{50}	0.015	1	8	0.06	0.5	0.5	≤4 / 2			0.03		0.03				≤0.12		≤0.5
	MIC_{90}	0.03	1	16	0.12	1	1	≤4 / 2			>16		>16				≤0.12		≤0.5

AMC: Amoxicillin-clavulanate; CRO: Ceftriaxone; CXM: Cefuroxime; CIP: Ciprofloxacin; CLI: Clindamycin; DAP: Daptomycin; ERY: Erythromycin; FUS: Fusidic acid; GEN: Gentamicin; LZD: Linezolid; LVX: Levofloxacin; MIC: Minimum inhibitory concentration; MUP: Mupirocin; NEO: Neomycin; OZE: Ozenoxacin; PEN: Penicillin; RET: Retapamulin; TET: Tetracycline; VAN: Vancomycin.

Table 5. Study 1: MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against *Staphylococcus epidermidis* stratified by methicillin and levofloxacin susceptibility.

	MIC (mg/l)	OZE	MUP	FUS	RET	LVX	CIP	AMC	CRO	CXM	CLI	DAP	ERY	GEN	LZD	NEO	PEN	TET	VAN
S. epidermidis (n = 190; all)	MIC_{50}	0.06	0.12	0.12	0.12	4	4	1	16	4	0.12	0.5	16	0.06	1	0.12	4	1	2
	MIC_{90}	1	≥1024	4	0.12	≥16	≥8	16	≥128	64	≥128	0.5	≥128	4	2	4	32	8	2
MSSE (n = 64)	MIC_{50}	0.008	0.12	0.12	0.12	0.25	0.25	0.25	2	0.25	0.12	0.5	0.25	0.06	1	0.12	0.5	0.25	2
	MIC_{90}	0.12	≥1024	4	0.12	8	≥8	0.5	4	1	32	1	≥128	0.5	2	2	4	4	2
MRSE (n = 126)	MIC_{50}	0.12	0.25	0.12	0.12	8	≥8	2	32	8	0.12	0.5	32	0.12	1	0.5	8	1	2
	MIC_{90}	2	≥1024	4	0.12	≥16	≥8	16	≥128	≥128	≥128	0.5	≥128	8	2	8	32	8	2
Levofloxacin-susceptible *S. epidermidis* (n = 92)	MIC_{50}	0.008	0.12	0.12	0.12	0.25	0.25	0.25	2	0.5	0.12	0.5	1	0.06	1	0.12	1	0.5	2
	MIC_{90}	0.015	≥1024	2	0.12	0.25	0.5	2	32	8	≥128	0.5	≥128	1	2	8	16	8	2
Levofloxacin-nonsusceptible *S. epidermidis* (n = 97)	MIC_{50}	0.12	8	0.12	0.06	≥16	≥8	4	32	8	0.5	0.5	32	0.5	1	0.25	8	1	2
	MIC_{90}	2	≥1024	8	0.12	≥16	≥8	16	≥128	≥128	≥128	1	≥128	8	2	4	32	4	2
Levofloxacin-susceptible MSSE (n = 51)	MIC_{50}	0.008	0.12	0.12	0.12	0.25	0.25	0.25	1	0.25	0.12	0.5	0.25	0.06	1	0.12	0.5	0.25	2
	MIC_{90}	0.015	256	4	0.12	0.5	0.5	0.5	2	0.5	2	0.5	64	0.12	2	1	4	2	2
Levofloxacin-nonsusceptible MSSE (n = 12)	MIC_{50}	0.12	8	0.12	0.06	8	≥8	0.25	2	0.5	0.12	0.5	8	0.06	1	0.12	1	1	2
	MIC_{90}	1	≥1024	0.25	0.12	≥16	≥8	0.5	4	2	≥128	1	≥128	4	2	2	4	64	4
Levofloxacin-susceptible MRSE (n = 41)	MIC_{50}	0.008	0.12	0.12	0.12	0.25	0.25	1	8	4	0.12	0.5	16	0.06	1	0.5	4	2	2
	MIC_{90}	0.015	≥1024	0.25	0.25	0.25	0.5	4	32	8	≥128	0.5	≥128	4	2	8	32	32	2
Levofloxacin-nonsusceptible MRSE (n = 85)	MIC_{50}	0.12	0.5	0.12	0.06	≥16	≥8	4	32	16	8	0.5	64	0.5	1	0.25	8	1	2
	MIC_{90}	2	≥1024	8	0.12	≥16	≥8	32	≥128	≥128	≥128	0.5	≥128	16	2	4	32	2	2

AMC: Amoxicillin-clavulanate; CRO: Ceftriaxone; CXM: Cefuroxime; CIP: Ciprofloxacin; CLI: Clindamycin; DAP: Daptomycin; ERY: Erythromycin; FUS: Fusidic acid; GEN: Gentamicin; LZD: Linezolid; LVX: Levofloxacin; MIC: Minimum inhibitory concentration; MRSE: Methicillin-resistant*S. epidermidis*; MSSE: Methicillin-susceptible *S. epidermidis*; MUP: Mupirocin; NEO: Neomycin; OZE: Ozenoxacin; PEN: Penicillin; RET: Retapamulin; TET: Tetracycline; VAN: Vancomycin.

Table 6. Study 2: MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against *Staphylococcus epidermidis* stratified by methicillin and levofloxacin susceptibility.

	MIC (mg/l)	OZE	MUP	FUS	RET	LVX	CIP	AMC	CLI	ERY	PEN	VAN
S. epidermidis (n = 195; all)	MIC_{50}	0.008	0.25	0.12	0.06	0.25	0.5	≤4/2	0.06	>16	>0.25	1
	MIC_{90}	0.25	>256	8	0.25	>16	>16	>8/4	>16	>16	>0.25	2
MSSE (n = 86)	MIC_{50}	0.004	0.25	0.12	0.06	0.25	0.25	≤4/2	0.06	>16	>0.25	1
	MIC_{90}	0.03	256	8	0.25	4	4	≤4/2	0.12	>16	>0.25	2
MRSE (n = 109)	MIC_{50}	0.06	0.25	0.12	0.06	4	8	≤4/2	0.25	>16	>0.25	1
	MIC_{90}	0.5	>256	16	0.12	>16	>16	>8/4	>16	>16	>0.25	2
Levofloxacin susceptible *S. epidermidis* (n = 105)	MIC_{50}	0.004	0.25	0.12	0.06	0.25	0.25	≤4/2	0.06	>16	>0.25	1
	MIC_{90}	0.008	>256	4	0.25	0.25	0.5	≤4/2	0.12	>16	>0.25	2
Levofloxacin nonsusceptible *S. epidermidis* (n = 90)	MIC_{50}	0.06	0.25	0.12	0.06	8	>16	≤4/2	>16	>16	>0.25	1
	MIC_{90}	1	>256	16	0.12	>16	>16	>8/4	>16	>16	>0.25	2

AMC: Amoxicillin-clavulanate; CIP: Ciprofloxacin; CLI: Clindamycin; ERY: Erythromycin; FUS: Fusidic acid; LVX: Levofloxacin; MIC: Minimum inhibitory concentration; MRSE: Methicillin-resistant *S. epidermidis*; MSSE: Methicillin-susceptible *S. epidermidis*; MUP: Mupirocin; OZE: Ozenoxacin; PEN: Penicillin; RET: Retapamulin; VAN: Vancomycin.

13 strains of *S. epidermidis* from ten sites had an ozenoxacin MIC of 1 mg/l (Table 5). Against all *S. epidermidis* isolates tested (n = 190), retapamulin (MIC_{90} = 0.12 mg/l) was the most active compound. Ozenoxacin (MIC_{90} = 1 mg/l) was fourfold more active than fusidic acid (MIC_{90} = 4 mg/l) and more than 1000-fold more active than mupirocin (MIC_{90} ≥1024 mg/l). Daptomycin (MIC_{90} = 0.5 mg/l) was the second most active compound tested (Table 5).

Ozenoxacin was highly active against methicillin-susceptible *S. epidermidis* (MSSE) isolates (n = 64) with a MIC_{50} of 0.008 mg/l and a MIC_{90} of 0.12 mg/l. 12 MSSE isolates were levofloxacin nonsusceptible (levofloxacin MIC_{50} = 8 mg/l and MIC_{90} ≥ 16 mg/l): the ozenoxacin MIC_{50} and MIC_{90} were 0.12 and 1 mg/l, respectively. An ozenoxacin MIC_{50} of 0.008 mg/l and a MIC_{90} of 0.015 mg/l were found for levofloxacin-susceptible MSSE isolates (n = 51; levofloxacin MIC_{50} = 0.25 mg/l and MIC_{90} = 0.5 mg/l; Table 5).

Ozenoxacin was highly active against methicillin-resistant *S. epidermidis* (MRSE) isolates (n = 126), with a MIC_{50} of 0.12 mg/l and a MIC_{90} of 2 mg/l. For levofloxacin nonsusceptible *S. epidermidis* isolates (n = 97; levofloxacin MIC_{50} ≥16 mg/l and MIC_{90} ≥16 mg/l) the ozenoxacin MIC_{50} was 0.12 mg/l and the MIC_{90} was 2 mg/l. Ozenoxacin was much more active against levofloxacin-susceptible *S. epidermidis* isolates (n = 92; levofloxacin MIC_{50} = 0.25 mg/l and MIC_{90} = 0.25 mg/l), with MIC_{50} and MIC_{90} values of 0.008 and 0.015 mg/l, respectively (Table 5). Against levofloxacin susceptible *S. epidermidis* (n = 92), including MSSE (n = 51) and MRSE (n = 41), ozenoxacin (MIC_{90} = 0.015 mg/l against all isolates) was at least eightfold more active than retapamulin (MIC_{90} = 0.12, 0.12 and 0.25 mg/l, respectively) and at least 128-fold more active than fusidic acid (MIC_{90} = 2, 4 and 0.25 mg/l, respectively). The respective MIC_{90} values of mupirocin against this group of isolates were ≥1024, 256 and ≥1024 mg/l (Table 5).

S. epidermidis study 2

The antibacterial activity of ozenoxacin and comparators against *S. epidermidis*, stratified by methicillin and levofloxacin susceptibility, is shown in Table 6. Of the 195 *S. epidermidis* isolates included in the study, 109 (55.9%) were methicillin-resistant and 86 (44.1%) were methicillin-susceptible. Levofloxacin resistance among MRSE and MSSE isolates was 72.5 and 12.8%, respectively (Table 6). Ozenoxacin was the most potent agent tested against *S. epidermidis* (MIC_{50} = 0.008; MIC_{90} = 0.25 mg/l), in common with data for all coagulase-negative staphylococci isolates. The activity of ozenoxacin was higher against MSSE isolates (n = 86; MIC_{50} = 0.004; MIC_{90} = 0.03) than against MRSE isolates (n = 109; MIC_{50} = 0.06; MIC_{90} = 0.5). The activity of ozenoxacin was also higher against levofloxacin-susceptible *S. epidermidis* isolates (n = 105; MIC_{50} = 0.004; MIC_{90} = 0.008) than against levofloxacin nonsusceptible *S. epidermidis* isolates (n = 90; MIC_{50} = 0.06; MIC_{90} = 1) regardless of the methicillin resistance status (Table 6).

Comparative analyses of MIC_{50} values against *S. epidermidis* showed that ozenoxacin (MIC_{50} = 0.008 mg/l) had eightfold greater activity than clindamycin and retapamulin (MIC_{50} = 0.06 mg/l); 16-fold greater activity than fusidic acid (MIC_{50} = 0.12 mg/l); 32-fold greater activity than mupirocin or levofloxacin (MIC_{50} = 0.25 mg/l);

64-fold greater activity than ciprofloxacin (MIC_{50} = 0.5 mg/l); and at least greater than 256-fold greater activity than erythromycin (MIC_{50} >16 mg/l; Table 6).

Against levofloxacin nonsusceptible *S. epidermidis* isolates, retapamulin (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.12 mg/l) was the most active antimicrobial agent tested, with ozenoxacin (MIC_{50} = 0.06 mg/l; MIC_{90} = 1 mg/l) ranked second in potency of all tested compounds (Table 6).

Ozenoxacin had higher activity against MRSE (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.5 mg/l) than mupirocin (MIC_{50} = 0.25 mg/l; MIC_{90} >256 mg/l) and fusidic acid (MIC_{50} = 0.12 mg/l; MIC_{90} = 16 mg/l). Against levofloxacin nonsusceptible *S. epidermidis*, ozenoxacin (MIC_{50} = 0.06 mg/l; MIC_{90} = 1 mg/l) was more potent than mupirocin (MIC_{50} = 0.25 mg/l; MIC_{90} >256 mg/l) and fusidic acid (MIC_{50} = 0.12 mg/l; MIC_{90} = 16 mg/l; Table 6).

CNS species other than *S. epidermidis*: study 1

For CNS spp. which were not strains of *S. epidermidis* (n = 37), oxenoxacin was more active than all reference compounds with an MIC_{50} of 0.008 mg/l and a MIC_{90} of 0.12 mg/l. In comparison, the rank order of reference compounds was daptomycin (MIC_{90} = 0.5 mg/l); linezolid (MIC_{90} = 1 mg/l); neomycin, retapamulin and vancomycin (MIC_{90} = 2 mg/l); gentamicin and tetracycline (MIC_{90} = 4 mg/l); ciprofloxacin, amoxicillin-clavulanate and levofloxacin (MIC_{90} = 8 mg/l); fusidic acid and penicillin (MIC_{90} = 16 mg/l); ceftriaxone and cefuroxime (MIC_{90} = 64 mg/l); clindamycin and erythromycin (MIC_{90} ≥128 mg/l); and mupirocin (MIC_{90} ≥1024 mg/l; **Table 7**).

CNS species other than *S. epidermidis*: study 2

Table 7 summarizes the antibacterial activity of ozenoxacin and comparators against CNS, stratified by methicillin and levofloxacin susceptibility. Of the 315 CNS isolates included in the study, 146 (46.3%) were methicillin-resistant (MR-CNS) and 169 (53.7%) were methicillin-susceptible (MS-CNS). Most of the MR-CNS isolates (72.6%) were nonsusceptible to levofloxacin, compared with only 8.3% of the MS-CNS isolates. Ozenoxacin was highly active against all CNS isolates (MIC_{50} = 0.008 mg/l; MIC_{90} = 0.12 mg/l) inhibiting 96.8% of isolates at a MIC of ≤0.5 mg/l. Ozenoxacin tested against levofloxacin-nonsusceptible CNS isolates (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.5 mg/l) showed MIC values at least 16-fold higher than isolates with levofloxacin susceptibility (MIC_{50} = 0.004 mg/l; MIC_{90} = 0.008 mg/l). Similar results were obtained for MR-CNS (MIC_{50} = 0.06 mg/l; MIC_{90} = 0.5 mg/l) compared with MS-CNS (MIC_{50} = 0.004 mg/l; MIC_{90} = 0.015 mg/l; Table 7). These differences were due to quinolone cross-resistance rather than methicillin-susceptibility, because levofloxacin-susceptible MR-CNS isolates had lower MIC_{90} values (MIC_{50} = 0.004 mg/l; MIC_{90} = 0.008 mg/l; Table 7).

Ozenoxacin had higher activity against methicillin-resistant CNS isolates and levofloxacin nonsusceptible CNS isolates (MIC_{50} = 0.06 mg/l and MIC_{90} = 0.5 mg/l against both) than mupirocin (MIC_{50} = 0.25 mg/l and MIC_{90} >256 mg/l against both) and fusidic acid (MIC_{50} = 0.12 mg/l and MIC_{90} = 0.16 mg/l against both; Table 7).

Corynebacterium species (study 1)

The range of ozenoxacin against *Corynebacterium* species was 0.008 to ≥4 mg/l, with a MIC_{50} of 1 mg/l. This activity was considerably greater than that of ciprofloxacin (MIC_{50} = 8 mg/l) and levofloxacin (MIC_{50} = 16 mg/l). Ozenoxacin (MIC_{90} of ≥4 mg/l) was less active than daptomycin and vancomycin (MIC_{90} = 0.5 mg/l), and fusidic acid, linezolid and retapamulin (MIC_{90} = 1 mg/l), against *Corynebacterium* species.

Ozenoxacin was significantly more potent (MIC_{90} = 0.06 mg/l) than all reference compounds for levofloxacin-susceptible isolates of *Corynebacterium* species. By comparison, the rank order for reference compounds was gentamicin (MIC_{90} = 0.25 mg/l); daptomycin and fusidic acid (MIC_{90} = 0.5 mg/l); ciprofloxacin, levofloxacin, linezolid, neomycin, retapamulin and vancomycin (MIC_{90} = 1 mg/l); cefuroxime and amoxicillin-clavulanate (MIC_{90} = 8 mg/l); ceftriaxone and erythromycin (MIC_{90} = 16 mg/l); penicillin and tetracycline (MIC_{90} = 32 mg/l); mupirocin (MIC_{90} = 128 mg/l) and clindamycin (MIC_{90} ≥128 mg/l).

Other isolates including *Micrococcus* & *Lactobacillus* species (study 1)

A small number of other strains were evaluated for ozenoxacin susceptibility in study 1. The MIC range of ozenoxacin was 0.004–0.06 mg/l for *Micrococcus* spp. (n = 7); 0.008–0.25 mg/l for Group G *Streptococcus* spp. (n = 19); 0.008–0.03 mg/l for Group C *Streptococcus* spp. (n = 9); 0.03–1 mg/l for *Brevibacterium* spp. (n = 3);

Table 7. MIC_{50} and MIC_{90} values for ozenoxacin and comparator antimicrobials against coagulase-negative *Staphylococcus* species stratified by methicillin and levofloxacin susceptibility.

	MIC (mg/l)	OZE	MUP	FUS	RET	LVX	CIP	AMC	CRO	CXM	CLI	DAP	ERY	GEN	LZD	NEO	PEN	TET	VAN
CNS (n = 37; study 1)	MIC_{50}	0.008	0.12	0.12	0.06	0.25	0.25	0.25	4	1	0.06	0.25	0.25	≤0.03	1	0.06	0.25	0.25	1
	MIC_{90}	0.12	>1024	16	2	8	8	8	64	64	≥128	0.5	≥128	4	1	2	16	4	2
CNS (n = 315; all; study 2)	MIC_{50}	0.008	0.25	0.12	0.06	0.25	0.25	≤4/2			0.06		>16				>0.25		1
	MIC_{90}	0.12	>256	8	0.25	16	>16	>8/4			>16		>16				>0.25		2
Methicillin-susceptible CNS (n = 169)	MIC_{50}	0.004	0.25	0.12	0.06	0.25	0.25	≤4/2			0.06		0.12				>0.25		1
	MIC_{90}	0.015	8	4	0.25	2	0.5	≤4/2			0.25		>16				>0.25		2
Methicillin-resistant CNS (n = 146)	MIC_{50}	0.06	0.25	0.12	0.06	4	8	≤4/2			0.5		>16				>0.25		1
	MIC_{90}	0.5	>256	16	0.12	>16	>16	>8/4			>16		>16				>0.25		2
Levofloxacin susceptible (n = 195)	MIC_{50}	0.004	0.25	0.12	0.06	0.25	0.25	≤4/2			0.06		0.25				0.5		1
	MIC_{90}	0.008	64	4	0.25	0.25	0.5	≤4/2			0.25		>16				0.5		2
Levofloxacin nonsusceptible (n = 120)	MIC_{50}	0.06	0.25	0.12	0.06	8	>16	≤4/2			>16		>16				>0.25		1
	MIC_{90}	0.5	>256	16	0.12	>16	>16	>8/4			>16		>16				>0.25		2

AMC: Amoxicillin-clavulanate; CNS: Coagulase-negative *Staphylococcus* species; CRO: Ceftriaxone; CXM: Cefuroxime; CIP: Ciprofloxacin; CLI: Clindamycin; DAP: Daptomycin; ERY: Erythromycin; FUS: Fusidic acid; GEN: Gentamicin; LZD: Linezolid; LVX: Levofloxacin; MIC: Minimum inhibitory concentration; MUP: Mupirocin; NEO: Neomycin; OZE: Ozenoxacin; PEN: Penicillin; RET: Retapamulin; TET: Tetracycline; VAN: Vancomycin.

Table 8. Comparison of ozenoxacin MIC_{50} and MIC_{90} values in studies 1 and 2.

Microorganism isolate	Study 1			Study 2		
	n	MIC_{50}	MIC_{90}	n	MIC_{50}	MIC_{90}
S. aureus (all)	486	0.004	0.25	504	0.002	0.06
MSSA	247	0.004	0.004	279	0.002	0.004
MRSA	239	0.12	0.25	225	0.004	0.12
Levofloxacin-susceptible *S. aureus*	312	0.004	0.004	383	0.002	0.002
Levofloxacin-nonsusceptible *S. aureus*	168	0.12	0.5	121	0.06	0.5
Levofloxacin-nonsusceptible MSSA	14	0.12	1	18	0.06	0.12
Levofloxacin-susceptible MSSA	231	0.004	0.004	261	0.002	0.002
Levofloxacin-susceptible MRSA	81	0.004	0.008	122	0.002	0.002
Levofloxacin-nonsusceptible MRSA	154	0.12	0.25	103	0.06	0.5
Streptococcus pyogenes	217	0.03	0.06	124	0.008	0.015
Streptococcus agalactiae	37	0.03	0.06	88	0.015	0.03
Streptococcus pneumoniae	29	0.03	0.06			
S. epidermidis (all)	190	0.06	1	195	0.008	0.25
MSSE	64	0.008	0.12	86	0.004	0.03
MRSE	126	0.12	2	109	0.06	0.5
Levofloxacin-susceptible *S. epidermidis*	92	0.008	0.015	105	0.004	0.008
Levofloxacin-nonsusceptible *S. epidermidis*	97	0.12	2	90	0.06	1
Levofloxacin-nonsusceptible, MSSE	12	0.12	1	11	0.06	0.5
Levofloxacin-nonsusceptible, MRSE	85	0.12	2	79	0.06	1
Levofloxacin-susceptible, MRSE	41	0.008	0.015	30	0.004	0.008
Levofloxacin-susceptible, MSSE	51	0.008	0.015	75	0.004	0.004
CNS (all)	37	0.008	0.12	315	0.008	0.12
Methicillin-susceptible CNS				169	0.004	0.015
Methicillin-resistant CNS				146	0.06	0.5
Levofloxacin-susceptible CNS				195	0.004	0.008
Levofloxacin-nonsusceptible CNS				120	0.06	0.5
Corynebacterium species (all)	52	1	≥4			
Levofloxacin-resistant *Corynebacterium* species	35	2	≥4			
Levofloxacin-susceptible *Corynebacterium* species	17	0.015	0.06			

CNS: Coagulase-negative *Staphylococcus* species; MRSA: Methicillin-resistant *S. aureus*; MRSE: Methicillin-resistant *S. epidermidis*; MSSA: Methicillin-susceptible *S. aureus*; MSSE: Methicillin-susceptible *S. epidermidis*.

0.03–2 mg/l for *Enterococcus faecalis* (n = 2); 0.03–2 mg/l for *Kocuria kristinae* (n = 1); and 0.015 mg/l for *R. mucilaginosa* (n = 1).

Comparison between study 1 & study 2

An overall summary comparing ozenoxacin MIC_{50} and MIC_{90} values in *S. aureus*, *Streptococcus* spp., *S. epidermidis*, coagulase-negative staphylococci and *Corynebacterium* spp. in both studies (Table 8) showed good general agreement.

Discussion

Clinical isolates were collected and analyzed for susceptibility or resistance to a panel of antimicrobial agents in two *in vitro* studies. The first collection of 1097 isolates from 49 centers was made during 2009–2010, and a later collection of 1031 clinical isolates at ten centers was made during 2014. *S. aureus* isolates predominated in both collections accounting for 44 and 49% of all isolates, respectively. *S. aureus* and *S. epidermidis* isolates were stratified by methicillin and levofloxacin resistance/susceptibility status. The antibacterial effects of ozenoxacin determined using MIC_{50} and MIC_{90} values were compared with 17 and ten antimicrobial agents, respectively. These included the topical agents mupirocin, fusidic acid and retapamulin, and also other antimicrobials for comparative activity against resistant and susceptible strains.

Ozenoxacin was the most potent agent tested against all *S. aureus* isolates. At the MIC_{90} level, ozenoxacin (MIC_{90} = 0.06 mg/l) was twofold more potent than retapamulin (MIC_{90} = 0.12 mg/l), fourfold more potent than fusidic acid (MIC_{90} = 0.25 mg/l), eightfold more active than mupirocin (MIC_{90} = 0.5 mg/l) and at least 256-fold more potent than erythromycin, clindamycin, ciprofloxacin or levofloxacin ($MIC_{90} \geq 16$ mg/l for all; study 2 data).

Tested against levofloxacin nonsusceptible *S. aureus* isolates, ozenoxacin ($MIC_{50/90}$, 0.06/0.5 mg/l) was also the most potent compound. Only clindamycin had an MIC_{50} (0.06 mg/l) equal to that of ozenoxacin. The remaining agents had higher MIC_{50} and MIC_{90} values than ozenoxacin (study 2 data).

Methicillin-resistant staphylococci had raised MICs to ozenoxacin, but this was due to quinolone cross-resistance rather than methicillin susceptibility, as levofloxacin-susceptible but methicillin-resistant staphylococci had an ozenoxacin MIC_{90} of 0.002 against *S. aureus* and 0.008 mg/l against coagulase-negative staphylococci (study 2 data). Compared with staphylococcal isolates with susceptibility to levofloxacin, ozenoxacin had lower activity when tested against levofloxacin nonsusceptible staphylococci with MIC_{50} and MIC_{90} values of 0.06 and 0.5 mg/l, respectively (study 2 data). This clearly high intrinsic activity in levofloxacin nonsusceptible isolates was also addressed in a previous study in which the presence of GyrA and ParC amino acid substitutions were characterized [16]. Interestingly, only isolates with double mutations in both GyrA and ParC had ozenoxacin MIC values higher than 0.5 mg/l. The percentage of *S. aureus* displaying MIC values more than 0.5 mg/l in study 2 was only 0.6%.

Comparison of ozenoxacin MIC_{50} and MIC_{90} values for isolates collected in the two studies showed good general agreement and differences between study 1 and study 2 may reflect differences in the diversity of clinical isolates.

A previous study of ozenoxacin susceptibility performed in Japan reported MIC_{90} values for ozenoxacin against MSSA, MRSA and *S. pyogenes* isolates obtained from clinical cutaneous specimens of $\leq$0.06, 4 and $\leq$0.06 mg/l, respectively. There was no difference between ozenoxacin MIC_{90} values for MSSA and *S. pyogenes* isolates obtained from adults and children, but the ozenoxacin MIC_{90} (0.12 μg/ml) against pediatric MRSA isolates was 32-fold lower than that found for adult isolates [17]. This could be due to the impact of fluoroquinolone use in adults. In comparison, in the present studies, ozenoxacin MIC_{90} values were 0.004 mg/l in both studies 1 and 2 against MSSA; 0.25 and 0.12 mg/l in study 1 and study 2, respectively, against MRSA; and 0.06 and 0.015 mg/l in study 1 and study 2, respectively, against *S. pyogenes*.

A second Japanese study of the antimicrobial activity of ozenoxacin against isolates from patients with acne vulgaris reported MIC_{90} values of ozenoxacin against *S. aureus*, *S. epidermidis* and other coagulase-negative staphylococci of $\leq$0.06, 0.125 and $\leq$0.06 mg/l, respectively [18]. In comparison, in the present studies, ozenoxacin MIC_{90} values were 0.25 and 0.06 mg/l in study 1 and study 2, respectively, against *S. aureus*; 1 and 0. 25 mg/l in study 1 and study 2, respectively, against *S. epidermidis*; and 0.12 mg/l in both studies 1 and 2 against coagulase-negative staphylococci. The MIC_{90} of ozenoxacin against *Propionibacterium acnes* was $\leq$0.06 mg/l in the Japanese study [18], but strains of this species were not included in the present study.

Conclusion

Ozenoxacin is a potent antimicrobial agent against both staphylococci and streptococci, irrespective of levofloxacin susceptibility status.

Future perspective

The *in vitro* spectrum of activity of ozenoxacin against staphylococci and streptococci, irrespective of methicillin or levofloxacin susceptibility, is mirrored by the efficacy of ozenoxacin in a clinical setting for the treatment of impetigo. Phase III trials showed that ozenoxacin produced a statistically significant superior microbiological response compared with placebo [7,8], and had comparable efficacy to retapamulin but with a higher microbiological clearance rate [7]. These results support future ozenoxacin use and inclusion in impetigo guidelines.

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Executive summary

- The *in vitro* activity of the anti-impetigo agent, ozenoxacin, and other antimicrobial agents against Gram-positive clinical isolates obtained from skin and soft tissue infections were compared.
- Isolates were collected in two studies: 1097 isolates from 49 centers during 2009–2010 in study 1 and 1031 isolates from ten centers during 2014 in study 2. The antibacterial effects of ozenoxacin were compared with 17 and ten antimicrobial agents in studies 1 and 2, respectively, by using MICs. Isolates were stratified by species and methicillin susceptibility/resistance and/or levofloxacin susceptibility/nonsusceptibility status.
- Comparison of ozenoxacin MIC_{50} and MIC_{90} values for isolates collected in both studies showed good general agreement. Overall, ozenoxacin was the most potent antimicrobial agent tested against staphylococci and streptococci. Ozenoxacin exhibited high *in vitro* activity against *Staphylococcus aureus* (MIC_{90} = 0.06 mg/l; n = 504) and coagulase-negative staphylococci isolates in both studies (study 2 data: MIC_{90} = 0.12 mg/l; n = 315). Ozenoxacin was also highly active against *Streptococcus pyogenes* (MIC_{90} = 0.015 mg/l; n = 217) and *Streptococcus agalactiae* (MIC_{90} = 0.03 mg/l; n = 88) isolates.
- In conclusion, ozenoxacin is a potent antimicrobial agent against staphylococci and streptococci, major pathogens involved in impetigo.

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