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An *in vitro* evaluation of the efficacy of tedizolid: implications for the treatment of skin and soft tissue infections

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Abstract

Skin and soft tissue infections (SSTI) are among the most commonly occurring infections and evidence suggests that these are increasing world-wide. The aetiology is diverse, but *Staphylococcus aureus* predominate and these are often resistant to antimicrobials that were previously effective. Tedizolid is a new oxazolidinone-class antibacterial indicated for the treatment of adults with SSTI caused by Gram-positive pathogens, including *S. aureus*.

The aim of this study was to evaluate the *in vitro* efficacy of tedizolid in comparison to other clinically used antibacterials against antibiotic sensitive- and resistant-staphylococci, grown in planktonic cultures and as biofilms reflecting the growth of the microorganism during episodes of SSTI.

Against a panel of 66 clinical staphylococci, sensitivity testing revealed that a lower concentration of tedizolid was required to inhibit the growth of staphylococci compared to linezolid, vancomycin and daptomycin; with the tedizolid MIC_{50} being 8-fold (*S. aureus*) or 4-fold (*S. epidermidis*) below that obtained for linezolid. In addition, *cfr*+ linezolid-resistant strains remained fully susceptible to tedizolid. Against *S. aureus* biofilms, 10×MIC tedizolid was superior or comparable with 10×MIC comparator agents in activity, and superior to 10×MIC linezolid against those formed by *S. epidermidis* (65 vs. 33% reduction, respectively). Under flow-conditions both oxazolidinones at 10×MIC statistically out-performed vancomycin in their ability to reduce the viable cell count within a *S. aureus* biofilm with fewer the 12% of cells surviving compared to 63% of cells.

In conclusion, tedizolid offers a realistic lower-dose alternative agent to treat staphylococcal SSTI, including infections caused by multi-drug resistant strains.

Keywords: skin and soft tissue infections, tedizolid, linezolid, staphylococcus, biofilm, minimum inhibitory concentration

Abbreviations:

CFU, colony forming unit

DAP, daptomycin

DMSO, dimethyl sulfoxide

EUCAST, European Committee on Antimicrobial Susceptibility Testing

GMO, genetically modified organism

LZD, linezolid

MRSA, methicillin resistant *Staphylococcus aureus*

MSSA, methicillin sensitive *Staphylococcus aureus*

MIC, minimum inhibitory concentration

MHB, Mueller-Hinton broth

PBS, phosphate buffered saline

SSTI, skin and soft tissue infection

TZD, tedizolid

VAN, vancomycin

VISA, vancomycin intermediate susceptibility *S. aureus*

1. Introduction

Skin and soft tissue infections (SSTIs) are common within both hospitalised patients and individuals within the community, yet providing a suitable treatment remains a clinical challenge. Published national and international guidelines for the treatment of SSTIs broadly agree [1]. The United Kingdom's National Institute for Clinical Excellence (NICE) guidelines, for example, emphasise the importance of using empirical treatment effective against methicillin resistant *Staphylococcus aureus* (MRSA) [1]. With the subsequent knowledge of bacterial cultures, treatment can be de-escalated to a narrow spectrum agent, preferably with oral administration allowing treatment to continue in the community. In reality, a microbiological diagnosis may not be available and initial therapy inadequate leading to clinical failure, recurrence of infection and readmission to hospital increasing the overall length of patient stay. Complicating therapy further, resistant *Staphylococcus aureus* can be responsible for in the region of half of complicated SSTIs, yet empirical therapy is often not appropriate for these microorganisms [2].

Currently vancomycin, linezolid, daptomycin, ceftaroline and telavancin are among those antibacterials recommended for the treatment of severe SSTIs with other agents in reserve for milder infections [3]. Newer agents are becoming available, including tedizolid, dalbavancin and oritavancin, but clinical evidence for the role of these agents is limited and needs to be provided if future guidelines are to be established [4].

Tedizolid phosphate (Sivextro®) is a next-generation oxazolidinone antibacterial approved for the treatment of adults with acute SSTIs caused by susceptible Gram–positive microorganisms, including staphylococci [5]. The spectrum of activity is similar to linezolid,

92 though activity is retained against some strains that are resistant to linezolid [6]. Similar in
93 mode of action to other oxazolidinones, antibacterial activity is mediated by inhibiting
94 protein synthesis [7].
95 Tedizolid is a new drug approved for the treatment of SSTIs in a number of countries,
96 including the United States, Cananda and the European Union [4]. The aim of this study was
97 to evaluate the *in vitro* efficacy of tedizolid in comparison to other clinically used
98 antibacterials against antibiotic sensitive and resistant staphylococci, grown in planktonic
99 cultures and as biofilms reflecting the growth of the microorganism during episodes of SSTI.
100

2. Material and Methods

2.1. Strains, culture conditions and preparation of antibiotics

The study included 66 clinical staphylococcal isolates: 27 methicillin sensitive *S. aureus* (MSSA) (including two linezolid-resistant), 27 MRSA (including two linezolid-resistant) and 12 *Staphylococcus epidermidis* (including two linezolid-resistant). Except the six linezolid-resistant strains (provided by J. Mingorance, Madrid), all strains were supplied by the Scottish MRSA Reference Laboratory, Glasgow (Supplementary Table 1).

All experiments were performed in Mueller-Hinton broth (MHB, Oxoid); for testing with daptomycin the medium was supplemented with 50 mg/L Ca^{2+} [8].

Tedizolid and linezolid were gifted by MSD and Pfizer, respectively (MSD, Hertfordshire, UK; Pfizer Ltd, Surrey, UK). Vancomycin and daptomycin were purchased from Sigma-Aldrich (Dorset, UK). Stock solutions of tedizolid were prepared in dimethyl sulfoxide (DMSO, 1,600 mg/L) prior to 2-fold dilutions in DMSO as per the supplier's guidelines. Other antibiotic stocks of 10,000 mg/L (except linezolid 1,000 mg/L) were prepared using distilled water and used or stored at -20°C for a maximum of two weeks.

2.2. Antibiotic susceptibility of staphylococcal planktonic cultures

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated for vancomycin, daptomycin and linezolid according to EUCAST guidelines [8]. For tedizolid, using 96-well plates, 2 µL of the relevant 50× tedizolid was combined with 98 µL of an overnight culture adjusted to 5×10^5 cfu/mL. *S. aureus* ATCC

29213 was included as a control strain; all results were within guideline limits. MIC/MBCs were repeated independently at least three times.

2.3 Time-kill assays

The time-kill kinetics were determined for two MRSA isolates; one linezolid-sensitive and one linezolid-resistant. Overnight cultures were diluted to a final concentration of 1×10^6 cfu/mL in 50 mL fresh MHB (antibiotic-free control) and MHB supplemented with each antibiotic (tedizolid, linezolid and vancomycin) at a concentration of 0.25×, 1× and 10×MIC and then incubated at 37°C with aeration at 200rpm for 24 h. Aliquots of 1 mL were removed at time zero and then every 30 minutes for the first 6 h and finally 24 h post-inoculation and viable counts obtained. Experiments were performed in triplicate.

2.4. Antibiotic susceptibility of biofilms

Twenty robust biofilm forming strains, selected using the crystal violet staining technique (data not shown), were evaluated for antibacterial susceptibility whilst in a biofilm mode of growth; 5 each MRSA, MSSA, *S. epidermidis* and linezolid-resistant *Staphylococcus* strains. Overnight cultures adjusted to 1×10^6 cfu/mL were inoculated into 96-well plates and incubated for 24 h at 37°C on a rocking platform (60 oscillations/min). Then, supernatants were removed, biofilms washed three times with phosphate buffered saline (PBS, Oxoid) and 150 µL of antibiotic supplemented MHB added at concentrations of 0.25×, 1×, 10× or 100×MIC (except where 100×MIC exceeded C_{max}). Antibiotic-free controls were included. After 24 h antibiotic exposure at 37°C, 0.001% (v/v) resazurin (Sigma) in PBS was added to each washed biofilm and incubated at 37°C in the dark for 2 h, then fluorescence measured (EM_{590nm}/EX_{540nm}) using a plate reader (FLUOstar Optima, BMG Labtech, Germany),

148 providing an indirect measure of the viable cells. The experiment was repeated on two
149 further occasions. Any significant outliers among technical replicates were determined using
150 Grubbs' test (p-value <0.05) and excluded from further analysis. Using the fluorescence
151 readings, the percentage of cells surviving within an antibiotic-treated biofilm was
152 determined by comparison with the untreated control. Statistical difference between
153 treated and untreated biofilms was determined using Student's t-test and GraphPad Prism 7
154 Software.
155
156 *2.5. Tedizolid susceptibility of biofilms under flow-conditions*
157 A flow-cell system was used to evaluate susceptibility under conditions replicating the *in*
158 *vivo* environment. Three silicone coupons were place in each of two chambers of a FC 275
159 flow-cell (BioSurface Technologies Corporation, Montana, USA) and MHB introduced into
160 the system via two reservoirs. Using overnight cultures of MSSA31, the coupons were
161 inoculated with 1×10^6 cfu/mL and maintained under static conditions for 1 h at 37°C to aid
162 attachment, and then media flow (1 mL/minute) continued for 3 days during biofilm
163 formation. Subsequently, one reservoir was replaced with fresh MHB (antibiotic-free
164 control) and the second with MHB supplemented with 10×MIC tedizolid, linezolid or
165 vancomycin and flow resumed for a further 24 h at 37°C. Finally, the coupons were
166 removed, rinsed and individually sonicated 3×5 minutes in PBS using a sonicating waterbath
167 and viable counts determined. Each experiment was performed either in duplicate or
168 triplicate. Percentage cell survival was calculated (section 2.4) and statistical difference
169 between treated and untreated biofilms determined using Student's t-test.
170

3. Results

3.1. Antibiotic susceptibility of planktonic cultures

The MICs and MBCs were determined against the 66 staphylococci (Supplemental Data Table 1). There was no evidence of resistance to any of the antibiotics tested, except for the Spanish strains that were resistant to linezolid, and all the MIC ranges and MIC_{50} values for linezolid, vancomycin and daptomycin were as expected being within one-dilution of the EUCAST published data [8] (Supplemental Data Table 1). All the linezolid-sensitive strains were highly susceptible to tedizolid with MICs within the narrow range of 0.125-0.5 mg/L; a median MIC value 8-fold below that of linezolid. The tedizolid sensitivity of linezolid-resistant strains varied with the resistance mechanism; those *cfr*+ had a tedizolid MIC of 0.25-0.5 mg/L (versus 8 mg/L linezolid), those that possessed the G2576T mutation had tedizolid MIC values of 2-4 mg/L (versus 16-64 mg/L linezolid), whilst the strain exhibiting both linezolid-resistance mechanisms had MIC values of 4 mg/L tedizolid and 512 mg/L linezolid.

Vancomycin and daptomycin were shown to be bactericidal, with only 16% and <1% of isolates presenting with a MBC:MIC ratio $\geq$8. By contrast linezolid and tedizolid were bacteriostatic (Supplemental Data Table 1).

3.2 Time-kill kinetics

Time-kill kinetics were determined for tedizolid, linezolid and vancomycin for two MRSA strains; one sensitive (MRSA23) and one resistant (*cfr*$^{+}$ JM02) to linezolid (Fig.1). Sub-MIC antibiotic exerted minimal effect on the growth of the organisms with viable bacterial cell concentrations remaining similar to the untreated control. At 1× and 10×MIC tedizolid was

bacteriostatic against both isolates with activity against the linezolid-resistant strain comparable to that exerted against the sensitive strain (Fig.1). Despite initially impeding growth, 1×MIC linezolid failed to inhibit growth of *cfr*$^{+}$ JM02 with a 2-log increase in bacterial cell number compared to the initial inoculum after 24 h exposure to the agent (10×MIC exceeded the therapeutically achievable concentration and was not tested). Conversely, a >3-log reduction in viable cell number in comparison to the initial inoculum was attained after 24 h exposure to 1× and 10×MIC vancomycin confirming the bactericidal nature of the agent.

3.3. Antibiotic susceptibility of biofilms

A dose-dependent response was noted for biofilms challenged with each antibacterial. At a concentration of 1×MIC, no agent was able to reduce the proportion of viable cells within the biofilm to 60% or fewer of untreated control biofilm; vancomycin in particular had little if any impact (Fig.2a). The mean level of activity exerted against *S. epidermidis* isolates (77-107% mean survival) by each antibacterial was inferior to that exhibited against the *S. aureus* isolates (64-103% mean survival), an effect that was of particular note with linezolid (103% vs 64% mean survival, *S. epidermidis* and *S. aureus* respectively).

When challenged with 10×MIC antibacterial there was a marked reduction in the proportion of cells within the biofilm remaining viable (28-77% mean cells remaining viable) (Fig.2b). Against *S. aureus* all of the agents tested reduced the biofilm to below a 50% mean of the untreated control (28-45% mean cell survival) compared to *S. epidermidis* where only vancomycin and tedizolid attained a comparable reduction (29 and 35% mean cell survival, respectively); linezolid and daptomycin achieved only a 33% and 47% mean decrease in

viable cells, respectively, with the majority of cells remaining viable after treatment. In addition to the greater level of tolerance exhibited by *S. epidermidis* isolates, there was also greater variation in susceptibility between the strains. At least one *S. epidermidis* isolate was unaffected by 10×MIC of any agent tested (97-103% cell viability), the exception being tedizolid which retained a good level of activity against all the *S. epidermidis* strains tested, including the linezolid-resistant strains.

Under flow-conditions tedizolid was superior to vancomycin and comparable to linezolid in the ability to reduce the proportion of viable biofilm-associated cells remaining on the silicone coupons (Fig.3). Exposure to 10xMIC tedizolid or linezolid led to a statistically significant reduction in the proportion of viable cells remaining within the biofilm (8%, $p < 0.05$ or 12%, $p < 0.005$ cell survival compared to the untreated control, respectively) while vancomycin did not achieve a statistically significant reduction in viable cells (63%, $p=0.08$).

4. Discussion and Conclusion

Ranging from mild to life-threatening, SSTI are among the most commonly occurring infections and evidence suggests that these are increasing. From 1993-2005, the number of emergency department visits in the USA by patients with these presentations increased from 1.2 million visits to 3.4 million [9]. Whilst SSTIs are diverse in aetiology, *S. aureus* are consistently predominating world-wide with multi-drug resistant strains increasingly being reported [10]. In the USA, one study reported that 81% of culture-positive SSTIs were caused by *S. aureus*, with almost half (46%) of those strains recovered being resistant to methicillin [11]. The high prevalence of the USA300 MRSA strain may account in part for these figures. In Europe however, where USA300 remains typically rare, a similar profile is seen. Morrissey *et al.* (2012) reported that approximately one half of SSTIs caused by *S. aureus* were MRSA [12]. As such, SSTIs pose an immense, and increasing, physical and economic burden to healthcare providers.

Achieving an effective treatment combining surgical debridement or drainage with empirical antibiotic therapy is not without its challenges. From the microbiological prospective, the agent is often unknown, multidrug-resistance is prevalent and a biofilm mode of growth complicates therapy. Though more prevalent in chronic wounds with 60% of samples being positive, biofilms have also been detected in 6% of acute wounds [13]. In this study, the increased activity of tedizolid compared to linezolid and other anti-staphylococcal agents was achieved typically using lower concentrations against both planktonic and biofilm-associated cells, including *cfr*+ multidrug-resistant strains. Against *S. aureus* biofilms, tedizolid was superior or comparable with comparator agents in activity, and typically superior against those formed by *S. epidermidis* strains. Under flow-conditions mimicking

257 the *in vivo* environment of a SSTI, for example infection related to an indwelling-device, the
258 bacteriostatic oxazolidinones both out-performed vancomycin.
259
260 Vancomycin has been a mainstay of treatment for staphylococcal SSTI. However, the
261 decreased susceptibility observed in vancomycin intermediate susceptibility *S. aureus* (VISA)
262 and strains displaying heteroresistance, the need for intravenous slow-infusion and to
263 monitor serum levels, and the potential for toxicity have led to moves towards other
264 antibacterial agents. The alternatives currently available include linezolid, telavancin and
265 daptomycin. Linezolid has been shown to be more effective at treating SSTIs than
266 vancomycin with fewer complications being reported and patient discharge occurring
267 sooner [14]. Whilst linezolid retains a good level of activity against staphylococci [15], the
268 emergence of linezolid resistance in staphylococci is a concern [16]. Tedizolid has however,
269 been demonstrated by this study to retain activity against *cfr*+ staphylococci. It has
270 previously been reported that the sterically compact nature of the hydroxymethyl group of
271 tedizolid greatly improves activity against strains possessing the *cfr* gene [6]. In addition,
272 tedizolid was reported by Russo *et al.* (2016) to be statistically non-inferior to linezolid in
273 patients with SSTI for an early clinical response evaluated 48–72 h after beginning therapy
274 [17]. Other factors favouring the use of tedizolid over contemporary agents in the treatment
275 of SSTI include the long half-life (double linezolid) allowing once a day dosing, short course
276 duration and an easy switch from intravenous to oral administration. It is recognised that
277 these studies are undertaken *in vitro* and as such cannot infer *in vivo* activity, however, the
278 data generated suggest that tedizolid offers an additional drug choice for the treatment of
279 SSTIs.
280

In conclusion, in this *in vitro* study the anti-staphylococcal activity of tedizolid has been shown to be at least comparable and often superior to comparator agents that are routinely prescribed in the treatment of SSTIs. Taken with the drive to de-escalate SSTI treatment sooner, switching early to a short-course oral agent allowing early discharge, tedizolid offers a realistic lower dose alternative agent in the treatment of staphylococcal SSTI, including those where biofilms are present.

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Declarations

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Competing Interests: None

Ethical Approval: Work involving the transconjugate strain *Staphylococcus aureus* ATCC 29213 *cfr*$^{+}$ was performed under *The Genetically Modified Organisms (Contained Use) Regulation 2014* of the Health and Safety Executive.

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362 **Figure Legends**

363 **Figure 1.** Time-kill kinetics of linezolid sensitive MRSA23 (a,c,e) and linezolid resistant MRSA
364 (cfr+) JM02 (b,d,f) challenged with (a,b) tedizolid, (c,d) linezolid and (e,f) vancomycin at
365 concentrations of 0.25xMIC (♦), 1xMIC (▲), 10xMIC (■), compared to untreated control
366 cultures (•). Error bars represent SEM between replicate samples (n = 3). Broken line (….)
367 indicates a 3-log reduction in viable cell number in comparison to the initial inoculum. cfu,
368 colony forming units.

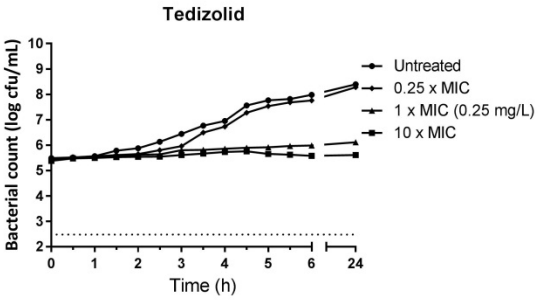
369

370 **Figure 2.** Susceptibility of biofilm-associated staphylococcal cells exposed to (a) 1×MIC or (b)
371 10×MIC antibiotic compared to untreated control cultures. Antibiotics included VAN,
372 vancomycin; DAP, daptomycin; LZD, linezolid; TZD, tedizolid. Cell survival was assessed using
373 the metabolic dye resazurin. Each experiment consisted of four replicate biofilms and was
374 repeated a further two times. Error bars represent SEM.

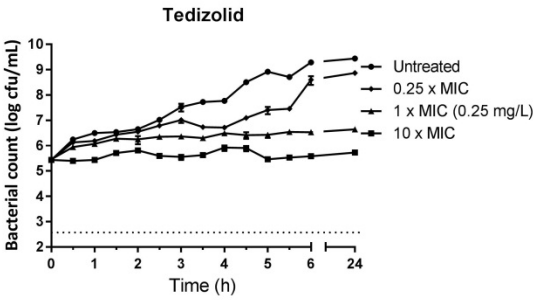
375

376 **Figure 3.** Susceptibility of MSSA31 biofilm-associated cells cultivated on silicone rubber
377 coupons and exposed to 10xMIC antibiotic under flow conditions within a BST flow-cell
378 system. Antibiotics included VAN, vancomycin; LZD, linezolid; and TZD, tedizolid. Paired t-
379 test; ** p-value < 0.05; **** p-value < 0.005; no asterisk p > 0.05. Each experiment
380 consisted of three replicate silicone rubber coupons and a minimum of two independent
381 repeats.

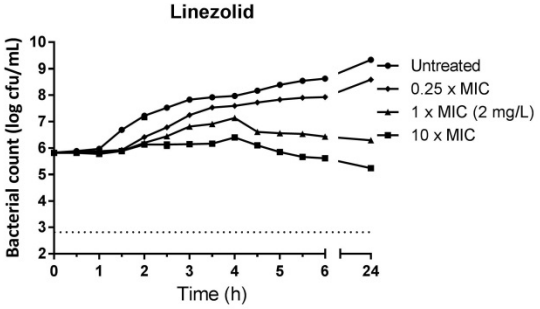
382


Tedizolid
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (0.25 mg/L)
10 x MIC

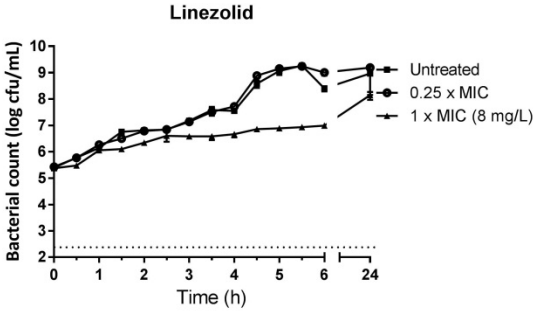
a


Tedizolid
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (0.25 mg/L)
10 x MIC

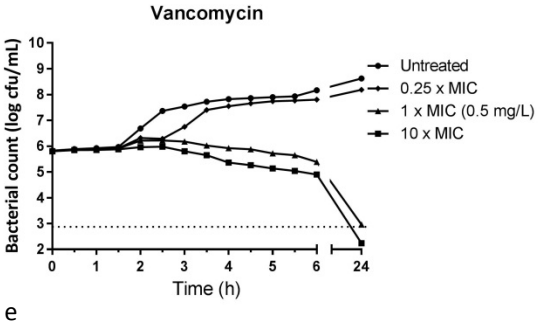
b


Linezolid
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (2 mg/L)
10 x MIC

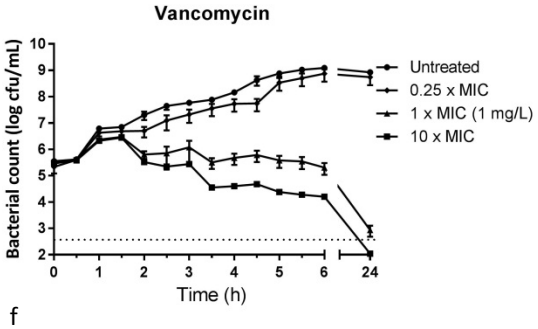
c


Linezolid
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (8 mg/L)

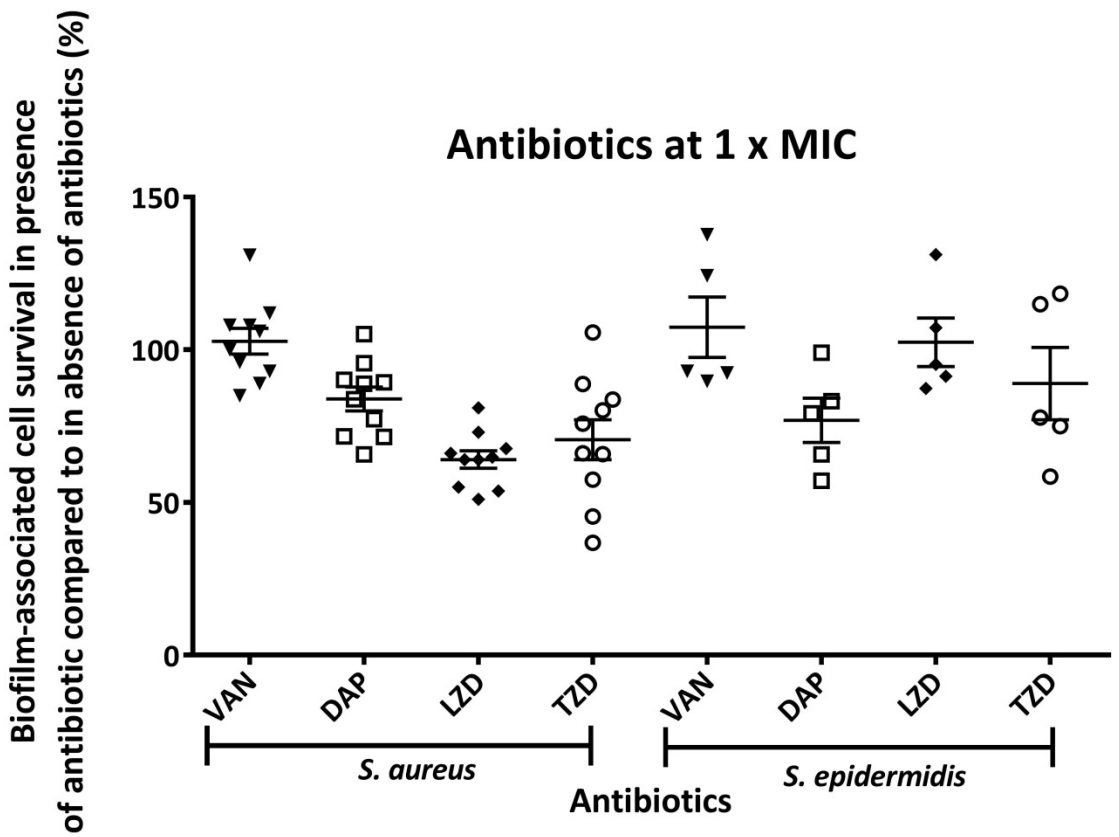
d


Vancomycin
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (0.5 mg/L)
10 x MIC

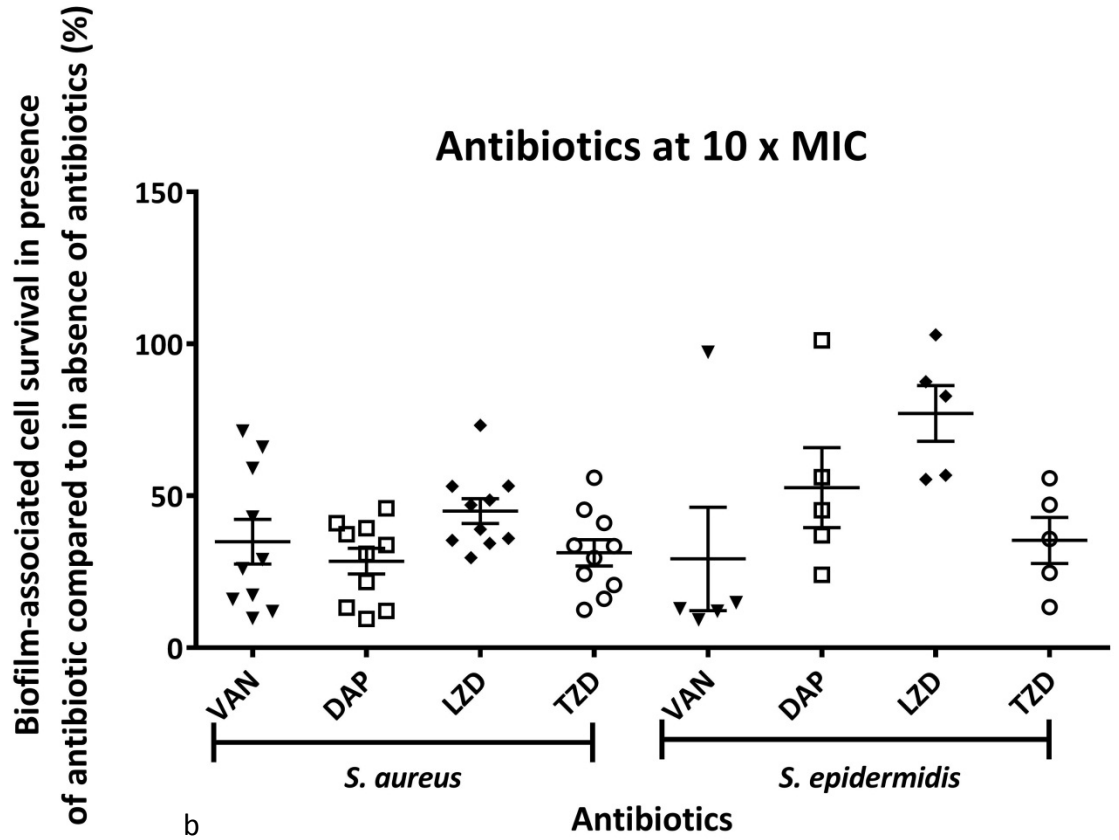
e


Vancomycin
Bacterial count (log cfu/mL)
Time (h)
Untreated
0.25 x MIC
1 x MIC (1 mg/L)
10 x MIC

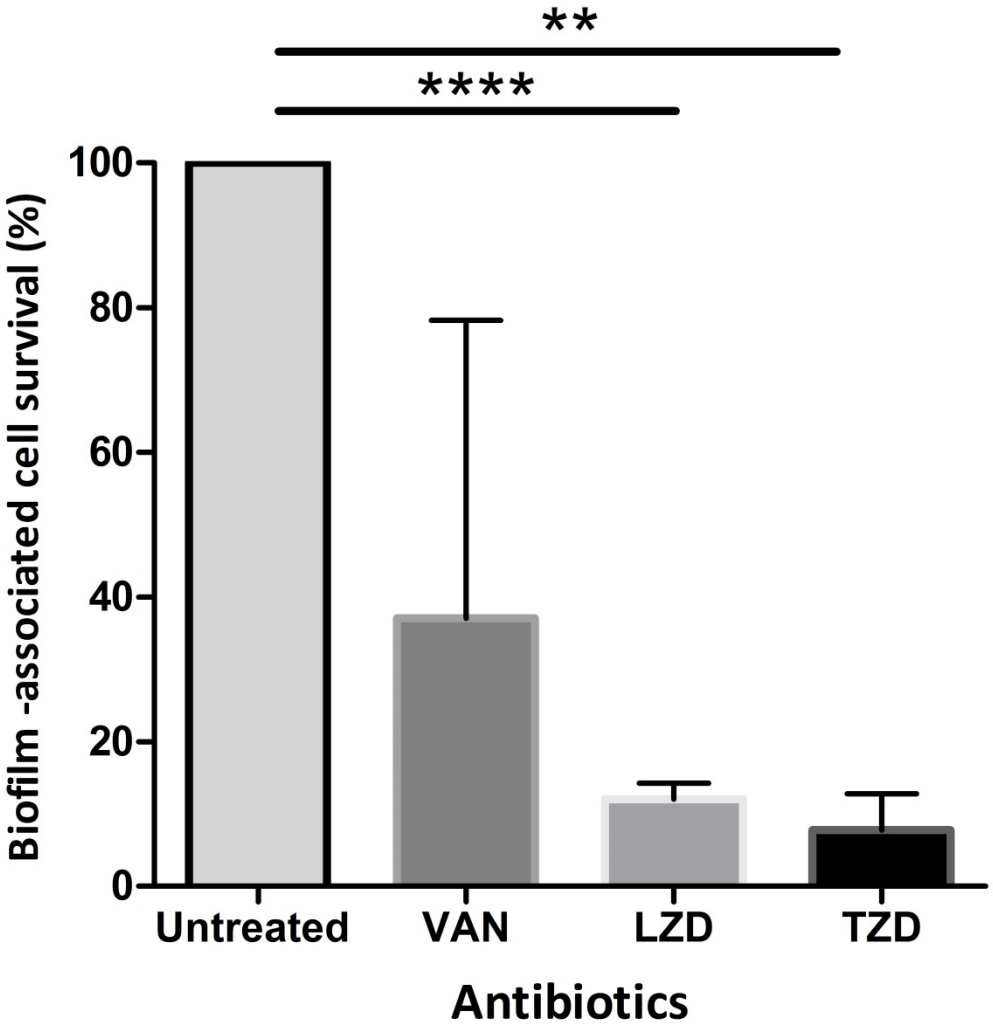
f


Antibiotics at 1 x MIC
Biofilm-associated cell survival in presence of antibiotic compared to in absence of antibiotics (%)
150
100
50
0
VAN
DAP
LZD
TZD
VAN
DAP
LZD
TZD
S. aureus
S. epidermidis
Antibiotics

a


Antibiotics at 10 x MIC
Biofilm-associated cell survival in presence of antibiotic compared to in absence of antibiotics (%)
150
100
50
0
VAN
DAP
LZD
TZD
VAN
DAP
LZD
TZD
S. aureus
S. epidermidis
Antibiotics

b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Biofilm -associated cell survival (%)
100
80
60
40
20
0
Untreated
VAN
LZD
TZD
Antibiotics

Supplementary Table 1. Origin of the strains used in this study.

***Staphylococcus* species**	**Strain**	**Sample type**	**Origin[a]**	**Isolated**	**Comments**
Methicillin resistant *S. aureus* (MRSA) n = 27	MRSA1 - 25	Blood	Scottish hospitals	2014-2015	*spa* typed[b]
	JM01	nk	Madrid, Spain	nk	Linezolid resistant (*cfr*$^+$)
	JM02	nk	Madrid, Spain	nk	Linezolid resistant (*cfr*$^+$)
Methicillin sensitive *S. aureus* (MSSA) n = 27	MSSA25 - 50	Blood	Scottish hospitals	2014-2015	*spa* typed[b]
	JM03	nk	Madrid, Spain	nk	Linezolid resistant (G2576T mutation)
	JM04	nk	Madrid, Spain	-	Linezolid resistant GMO; *cfr*$^+$ transconjugant of strain ATCC 29213
	ATCC 29213	-	Reference strain	-	Antibiotic sensitivity control strain
S. epidermidis n = 12	JM05	nk	Madrid, Spain	nk	Linezolid resistant (G2576T mutation)
	JM06	nk	Madrid, Spain	nk	Linezolid resistant (*cfr*$^+$ and G2576T mutation)
	10, 70, 93, 96, 103, 105, 117, 122, 157, 178	Various clinical isolates	Scottish hospitals	2011-13	-

[a]; Hospital Universitario La Paz, Madrid or Scottish MRSA Reference Laboratory (SMRSARL), Glasgow Royal Infirmary, Glasgow.

[b];Isolates represented 32 different spa types (1-9 representatives per *spa* type with t032 being predominant) and 12 clonal complexes (with CC22, n = 20; CC5, n = 6; and CC30, n = 5 being the principal types).

nk; not known.

Supplemental Table 2. Antibiotic susceptibility of staphylococci grown in planktonic culture.

Antibacterial	**Organism**	**Antibiotic susceptibility (mg/l)**					
		MIC range	**MIC_{50}**	**MIC_{90}**	**MBC_{50}**	**MBC_{90}**	**MBC_{50} / MIC_{50}**
Tedizolid	MSSA	0.125 - 2	0.25	0.5	4	4	16
	MRSA	0.125 - 0.5	0.25	0.5	2	4	8
	S. epidermidis	0.25 - 4	0.25	4	4	> 4	16
Linezolid	MSSA	2 - 16	2	4	16	> 16	8
	MRSA	2 - 8	2	4	16	> 16	8
	S. epidermidis	1 - 512	1	64	32	64	32
Vancomycin	MSSA	0.25 - 1	0.5	1	2	4	2
	MRSA	0.25 - 1	0.5	1	2	> 8	2
	S. epidermidis	1 - 2	1	2	2	4	2
Daptomycin	MSSA	0.25 - 1	0.5	1	1	2	2
	MRSA	0.5 - 1	0.5	1	1	2	2
	S. epidermidis	0.5 - 2	0.5	1	0.5	2	1

MSSA, n = 27 including 2 linezolid resistant strains; MRSA, n = 27 including 2 linezolid resistant strains; *S. epidermidis*, n = 12 including 2 linezolid resistant strains.