Future MICROBIOLOGY

Research Article

Nisin Z and lacticin 3147 improve efficacy of antibiotics against clinically significant bacteria

Jenna-Claire Ellis[1], Reynolds P Ross[1,2] & Colin Hill*[,1,2]
[1]School of Microbiology, University College Cork, T12 K8AF, Ireland
[2]APC Microbiome Ireland, University College Cork, T12 YT20, Ireland
*Author for correspondence: c.hill@ucc.ie

Aim: To determine if bacteriocins improve antibiotic efficacy. **Materials & methods:** Deferred antagonism assays identified bacteriocins with activity. Growth curves and time kill assays demonstrated bactericidal activity of antimicrobial combinations, and checkerboard assays confirmed synergy. Methicillin-resistant *Staphylococcus aureus*-infected porcine skin model determined *ex vivo* efficacy. **Results:** Subinhibitory concentrations of lacticin with penicillin or vancomycin resulted in complete growth inhibition of strains and the improved inhibitory effect was apparent after 1 h. Nisin with methicillin proved more effective against methicillin-resistant *Staphylococcus aureus* than either antimicrobial alone, revealing partial synergy and significantly reduced pathogen numbers on porcine skin after 3 h compared with minimal inhibition for either antimicrobial alone. **Conclusion:** Nisin Z and lacticin 3147 may support the use of certain antibiotics and revive ineffective antibiotics.

First draft submitted: 21 May 2019; Accepted for publication: 9 December 2019; Published online: 5 February 2020

Keywords: antibiotics • antimicrobial • bacteriocins • lacticin 3147 • methicillin • nisin z • pathogen • penicillin • synergy • vancomycin

Antibiotic resistance is a global crisis. It has become an international priority to reduce the emergence of resistant bacteria, prevent the spread of resistance and accelerate the development of new antimicrobial therapies [1–3]. In addition, the impact of antibiotic administration on the human commensal microbiota has led researchers to investigate alternative antimicrobial therapies that exert minimal, if any, impact on the microbiome [4–6]. Bacteriocins are bacterially produced, ribosomally synthesized, often post-translationally modified antimicrobial peptides, having either a broad or narrow spectrum of antimicrobial activity [7]. Their activity against numerous clinically significant pathogens, including various strains of methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis, Salmonella enterica* and *Clostridium difficile,* has heightened interest in their potential as therapeutics [7–12]. Other notable attributes of bacteriocins are their activity at nanomolar concentrations, low toxicity and the possibility of *in situ* production by probiotics [8]. Indeed, studies examining the potential use of bacteriocin-producing probiotic bacteria, rather than purified bacteriocins, may also aid in making the use of bacteriocins more cost-effective [13].

Cutibacterium acnes, originally classed as *Corynebacterium parvum* and more recently *Propionibacterium acnes*, is a Gram-positive, aerotolerant, commensal human skin bacterium capable of producing biofilms that contribute to the development of acne [14]. Recent studies have reported the increasing emergence of *C. acnes* resistant to various antibiotics, including fluoroquinolones, macrolides, erythromycin, clindamycin, tetracycline and trimethoprim-sulfamethoxazole [15–17]. *Corynebacterium xerosis* is a commensal bacterium found on human skin and mucous membranes and rarely causes infections of any clinical relevance; however, there have been reports that the microbe contributes to strong underarm odors [18–20]. *Enterococcus faecium* nosocomial infections have emerged with growing frequency, and include strains resistant to aminoglycosides, ampicillin and vancomycin (vancomycin-resistant *Enterococcus*; VRE) [21,22]. Enterococci can also be intrinsically resistant to a variety of antibiotics such as clindamycin, penicillin and cephalosporins, making their treatment more difficult. MRSA is a Gram-positive

Table 1. Bacteriocin producers and growth media used in this study.

Bacterial strain	Bacteriocin	Growth medium	Incubation temp. (°C)	Aerobic/anaerobic	Ref.
Lactococcus lactis MG1363 (pMRCO1, pOMO2)	Lacticin 3147	GM17	30	Aerobic	[46]
L. lactis DPC 5552	Lacticin 481	GM17	30	Aerobic	[47]
L. lactis NZ9700	Nisin A	GM17	30	Aerobic	[48]
L. lactis NZ9800 (pCI372*nisF*)	Nisin F	GM17	30	Aerobic	[49]
L. lactis NZ9800 (pCI372*nisQ*)	Nisin Q	GM17	30	Aerobic	[49]
L. lactis NZ9800 (pCI372*nisZ*)	Nisin Z	GM17	30	Aerobic	[49]
Streptococcus uberis 42	Nisin U	BHI	37	Aerobic	[50]
S. uberis D536	Nisin U2	BHI	37	Aerobic	[49,50]
Staphylococcus aureus C55	Staphylococcin C55	BHI	37	Aerobic	[51]
S. aureus TY4	Staphylococcin TY4	BHI	37	Aerobic	[52]
Streptococcus salivarius DPC 6490	Salivaricin A5	BHI	37	Anaerobic	[53]

BHI: Brain heart infusion medium.

Table 2. Skin and gut relevant pathogens used in this study.

Bacterial strain	Medium	Incubation temp. (°C)	Aerobic/anaerobic
Cutibacterium acnes LMG16711	RCM	37	Anaerobic
Corynebacterium xerosis DPC 5629	BHI	37	Aerobic
Enterococcus faecium UCC1	BHI	37	Aerobic
E. faecium UCC2	BHI	37	Aerobic
Methicillin resistant *Staphylococcus aureus* ST291	BHI	37	Aerobic

BHI: Brain heart infusion medium; RCM: Reinforced clostridial medium.

coccus and has become one of the most worrying pathogens due to its highly mutable nature. Reports have shown more recent cases of resistance to other antibiotics such as erythromycin, fluoroquinolones, tetracycline and clindamycin [23–25]. Vancomycin is now the drug of choice to treat an MRSA infection, but a decreased susceptibility to this drug is also on the rise (known as vancomycin-resistant *Staphylococcus aureus*; VRSA), with a minimum inhibitory concentration (MIC) of $\geq$32 μg/ml rendering it completely resistant [25]. MRSA is also frequently associated with wound infections, including surgical site infections, and upon entering the bloodstream through cuts or lesions can lead to internal infections [26–28].

The aim of this study was to evaluate the impact of using bacteriocins in combination with different antibiotics for the treatment of drug-resistant bacterial strains. We determined the MIC of nisin Z, lacticin 3147 and selected antibiotics against the above pathogens and the checkerboard method was used to identify synergy between bacteriocin and antibiotic. Growth curves and time kill assays were used to demonstrate bactericidal activity of the combinations versus each antimicrobial alone. Finally, we determined if the benefits of combining bacteriocin and antibiotic extended to an *ex vivo* porcine skin model infected with MRSA.

Materials & methods

Bacterial strains & growth conditions

All bacteriocin producers and pathogen strains used in this study are listed in Tables 1 and 2 and were acquired from the University College Cork culture collection (University College Cork, Ireland). *Lactococcus lactis* strains were grown in M17 broth or agar (1.5% agar) (Oxoid Ltd, Basingstoke, UK) supplemented with 0.5% glucose (GM17) and incubated at 30°C (Table 1). All *Streptococcus uberis*, *S. salivarius*, *S. aureus*, *E. faecium* and *C. xerosis* strains were grown in brain heart infusion (BHI) broth or agar (1.5% agar; Oxoid) and incubated at 37°C (Tables 1 & 2). *C. xerosis* DPC 5629 was supplemented with 0.5% v/v Tween 80 (Sigma-Aldrich, Arklow, Ireland) and *C. acnes* LMG 16711 was grown in reinforced clostridial medium (RCM; Oxoid) and both were incubated at 37°C (Table 2).

Deferred antagonism assays

Following overnight incubation, the bacteriocin producer (Table 1) was spotted onto the appropriate agar-containing medium (Table 1) and incubated for 18 h. Following incubation, the producer strain plates were UV treated for 30 min. Indicator strains (*C. acnes* LMG 16711*, C. xerosis* DPC 5629*, E. faecium* UCC1, *E. faecium* UCC2 and MRSA ST291) were grown overnight and 50 μl was subsequently inoculated into 50 ml of the appropriate medium (0.75% agar; Table 2). This was then overlaid onto the plates containing bacteriocin producer strains. The overlaid plates were incubated overnight at the appropriate temperature and aerobic/anaerobic conditions for the indicator strains (Table 2). The results were represented as the diameter of the zone of inhibition minus the diameter of the bacteriocin producer spot and were measured using digital callipers. This experiment was performed in triplicate.

Purification of lacticin 3147

Following overnight incubation, 10 ml of lacticin 3147 producer, *L. lactis* MG1363 (pMRCO1, pOMO2) was added to 3 l of Tryptone Yeast broth. A 20% glucose in H_2O solution (5%) and 20% β-glycerophosphate in H_2O solution (5%) were also added and together incubated at 30°C for 16 h. The culture was subsequently centrifuged for 15 min at 7000×*g*. The pelleted cells were resuspended in 300 ml of 70% isopropanol (IPA) containing 0.1% v/v trifluoroacetic acid (TFA) and stirred at 4°C for 4 h before centrifugation at 7000 rpm for 15 min. The volume of the supernatant was subsequently reduced by 70% using rotary evaporation (Buchi; 40°C at 50 mbar), in order to remove the IPA giving a final volume of approximately 60 ml. The concentrated supernatant was subjected to solid-phase extraction using a 10 g/60 ml Strata C-18E Giga-Tube (Phenomenex, Cheshire, UK) postequilibration with 60-ml methanol followed by 60-ml sterile, deionized H_2O. The column was subsequently washed with 120-ml 30% ethanol and the peptide was eluted from the column with 60-ml 70% IPA (pH 2).

For reverse-phase HPLC (RP-HPLC), 10-ml aliquots were concentrated through rotary-evaporation to approximately 2 ml. Injection volumes of 1800 μl were applied to a Phenomenex C_{12} RP-HPLC column (Jupiter 4 μ 90 A, 250 × 10.0 mm, 4 μm) previously equilibrated with 25% IPA containing 0.1% TFA. The column was set up in a gradient of 30% IPA containing 0.1% TFA to 60% IPA containing 0.1% TFA in 4–40 min at a flow rate of 1.2 ml/min (Shimazdu, OR, USA; FMG Inc.). Fractions that generated a peak were pooled seperately and rotary-evaporated to remove approximately 40% of the IPA solvent before freeze-drying. Final lyophilized peptide was subject to MALDI-TOF MS analysis to validate its purity.

MIC assays

MIC assays were performed in triplicate on nisin Z and lacticin 3147 using target strains *C. xerosis* DPC 5629, *C. acnes* LMG 16711, *E. faecium* UCC1, *E. faecium* UCC2 and MRSA ST291 (Table 2). 96-well microtiter plates (Sarstedt, Numbrecht, Germany) were initially treated with bovine serum albumin (Sigma-Aldrich) by adding 200 μl of a 1% bovine serum albumin and 5% phosphate-buffered saline (PBS) in sterile-deionized H_2O solution to each well, incubating at 37°C for 30 min before decanting and washing with a 5% PBS in sterile-deionized H_2O. Peptides and antibiotics were adjusted to a 5-μM starting concentration (16.67 μg/ml nisin Z, 30.75 μg/ml lacticin 3147, 1.78 μg/ml penicillin G and 7.245 μg/ml vancomycin) and a twofold serial dilution was performed on each peptide. The target strains were subcultured from overnight cultures and incubated until they reached an optical density (OD) OD_{600} of 0.5. The strains were diluted in appropriate liquid medium (Table 2) to a final concentration of 10^5 colony forming units (cfu)/ml and 200 μl was added to each well. The plate was incubated for 18 h followed by MIC determination.

Growth curve assays

Following overnight incubation, bacterial strains (Table 2) were transferred into the appropriate liquid medium (Table 2). Upon reaching an OD_{600} reading of 0.5, the cultures were supplemented with purified peptide (nisin Z/lacticin3147) and antibiotic (penicillin G/vancomycin; concentrations outlined in Figure 1), and 0.2 ml was subsequently transferred to a 96-well microtiter plate (Sarstedt) in triplicate. Growth curves were measured spectrophotometrically, using a SpectraMax MP3 spectrophotometer and were plotted with incubation time as the abscissa and absorbance as the vertical axis.

Time-kill assays

Following overnight incubation, cultures were transferred into the respective liquid medium. Upon reaching an OD_{600} reading of 0.5, 20 μl was transferred into 1 ml of appropriate broth (Table 2) containing purified peptide

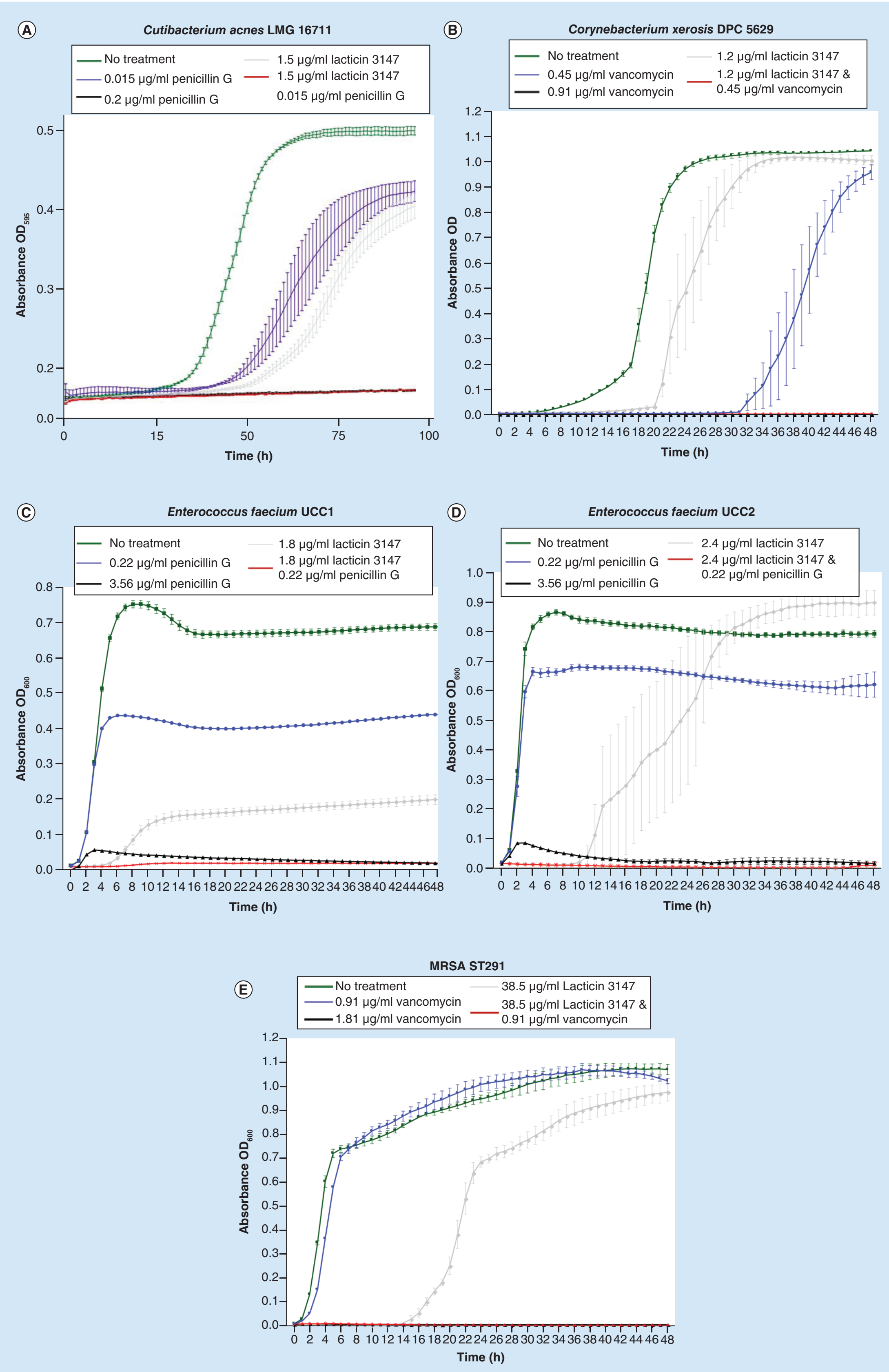

Figure 1. Effect of bacteriocins and antibiotics against pathogenic bacteria. OD growth curve results of **(A)** penicillin G and lacticin 3147 against *Cutibacterium acnes* LMG 16711, **(B)** vancomycin and lacticin 3147 against *Corynebacterium xerosis* DPC 5629, **(C)** penicillin G and lacticin 3147 against *Enterococcus faecium* UCC1, **(D)** penicillin G and lacticin 3147 against *E. faecium* UCC2 and **(E)** vancomycin and lacticin 3147 against MRSA ST291.
MRSA: Methicillin-resistant *Staphylococcus aureus*; OD: Optical density.

(nisin Z/lacticin 3147) alone and in combination with antibiotic (penicillin G/vancomycin/methicillin; concentrations provided in Figures 2 & 3). The samples were incubated for 1 and 3 h (Figure 2) or 3, 6, 9, 12 and 24 h (Figure 3) at applicable temperatures (Table 2). Cell growth was measured by performing a tenfold serial dilution of cultures in a quarter-strength Ringers solution (Sigma-Aldrich) and plating and enumerating viable cell counts on the appropriate agar medium (Table 2) [29]. This experiment was performed in triplicate.

FIC/checkerboard assays

For FIC experiments, eight-times the MIC of two antimicrobials (nisin Z and methicillin) were transferred separately into two 96-well microtiter plates upon which a serial dilution was then performed horizontally across each plate in broth (50 μl). 50 μl from plate 1 was added vertically to plate 2. The overnight culture (MRSA ST291), having been transferred into BHI medium and grown until an OD_{600} reading of 0.5, was diluted to a final concentration of 10^5 cfu/ml and added to each well [30].

The FIC is characterized by the following equation:

$$\text{FIC} = \text{FICX} + \text{FICY} = (\text{X/MICX}) + (\text{Y/MICY})$$

Where ‘X’ is the minimum amount of antimicrobial X used in combination with another that achieved an inhibitory effect and the MICX is the MIC of the same antimicrobial that produces an inhibitory effect on its own. FIC index results are as follows: FIC $\leq$0.5 is synergy, 0.5$<$ FIC $\leq$0.75 is partial synergy, 0.75$<$ FIC $\leq$1.0 is additive, FIC $>$1.0 is indifferent and FIC $>$4 is antagonistic [30,31].

Ex vivo porcine skin model

Approximately 2 cm by 2 cm squares of pig skin samples were cut with a scalpel and stored at -20°C. Samples were sterilized by soaking in 80% IPA for 10 min before washing with deionized sterile H_2O and subsequently exposing to UV light for 15 min. Samples were inoculated with OD_{600} 0.5 MRSA ST291 ($\sim10^7$ cfu/ml) and incubated at 37°C for 1 h. 50 μl of either 1 μg/ml nisin Z and/or 32 μg/ml methicillin was pipetted onto the skin and spread over the surface using an inoculating loop before incubating at 37°C for 3 h. Enumeration was carried out by homogenizing the skin in a stomacher (Masticator Basic, IUL Microbiology Instruments, Barcelona, Spain) with 10-ml PBS for 15 s and performing plate counts on De Mann Rogosa Sharpe (MRS) agar (Oxoid) incubated at 37°C for 18 h. This experiment was performed in triplicate.

Statistical analysis

Statistical analysis was performed using the Data analysis tool in Excel 2016 (Microsoft, WA, USA). Bacterial counts were transformed to $\log_{10}$ values to normalize the data and the student’s t-test was used to compare bacterial survival between treated and untreated samples. Graphs were generated using GraphPad Prism software.

Results

In this study, we investigated the antimicrobial activity of a number of bacteriocins including lacticin 3147, lacticin 481, six nisin variants (A, F, Q, Z, U and U2), staphylococcin C55, staphylococcin TY4 and salivaricin A5 against three pathogens for which antibiotic resistance has been reported (*C. acnes*, *E. faecium* and MRSA) as well as the skin commensal, *C. xerosis*. The most potent bacteriocins, lacticin 3147 and nisin Z, were selected for use in combination with both currently used (vancomycin and penicillin) and a less effective antibiotic (methicillin) in an effort to improve or restore antimicrobial efficacy.

Deferred antagonism assays

An initial screening using deferred antagonism agar assays was performed to determine if selected bacteriocins (Table 1) displayed antimicrobial activity against five pathogens. Each bacteriocin producer tested produced an antimicrobial effect against at least one of the five pathogens but none produced a zone against the strain MRSA ST291. Nisin Z and lacticin 3147 produced zones of clearing greater in size against four of the pathogens than the other bacteriocins tested and were therefore selected for further investigation (Figure 4A–D).

MICs of lacticin 3147, nisin Z, penicillin G & vancomycin

The MIC was visually determined as the lowest concentration of peptide (lacticin 3147 and nisin Z) or antibiotic (penicillin G and vancomycin) that resulted in the absence of growth of the target strain after 18 h of incubation

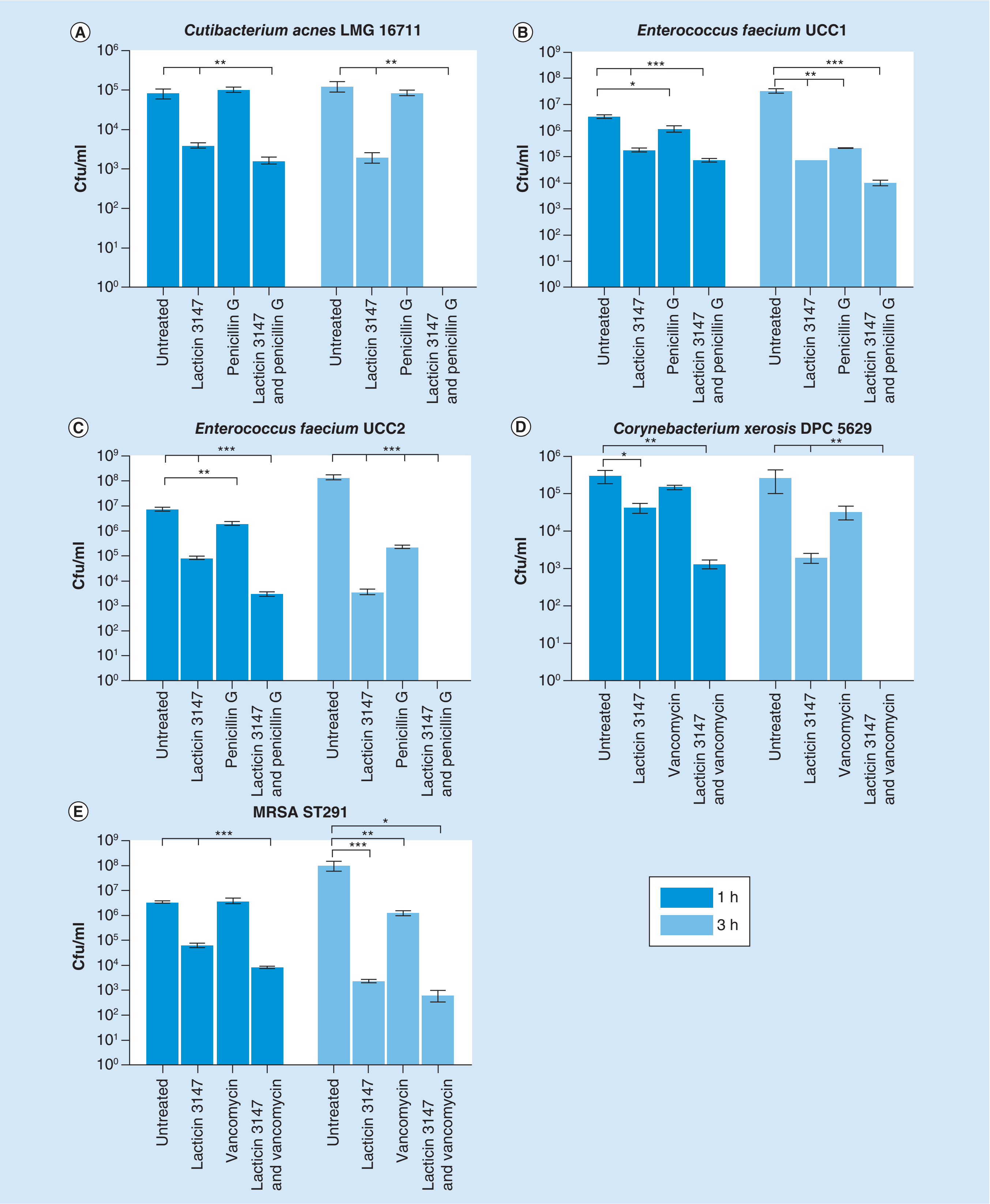

Figure 2. Combinations of bacteriocins and antibiotics against selected pathogens. Time-kill results of **(A)** penicillin G (0.176 μg/ml) and lacticin 3147 (7.5 μg/ml) against *Cutibacterium acnes* LMG 16711, **(B)** penicillin G (3.56 μg/ml) and lacticin 3147 (1.80 μg/ml) against *Enterococcus faecium* UCC1, **(C)** penicillin G (3.56 μg/ml) and lacticin 3147 (1.44 μg/ml) against *E. faecium* UCC2, **(D)** vancomycin (0.91 μg/ml) and lacticin 3147 (1.2 μg/ml) against *Corynebacterium xerosis* DPC 5629 and **(E)** vancomycin (0.91 μg/ml) and lacticin 3147 (11.55 μg/ml) against MRSA ST291.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
MRSA: Methicillin-resistant *Staphylococcus aureus*.

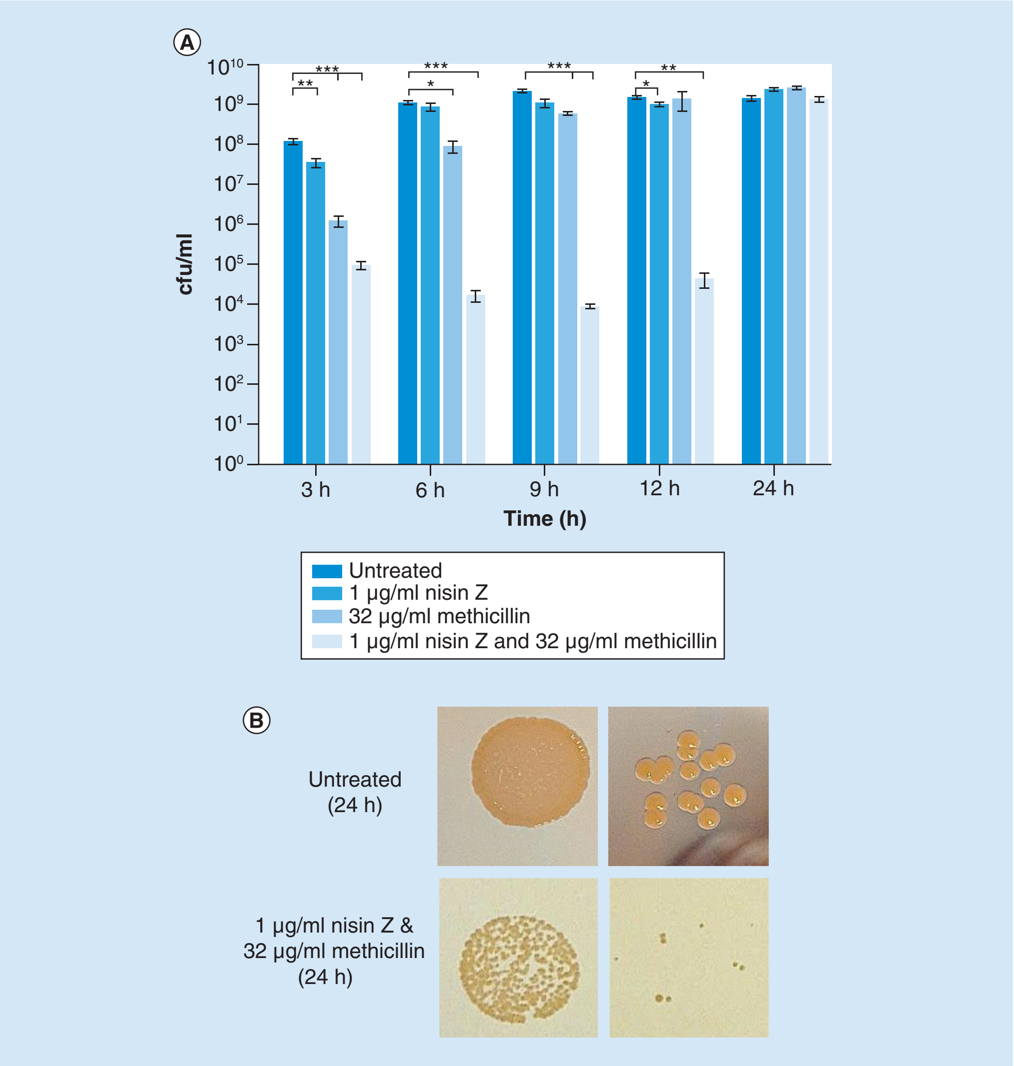

Figure 3. Effect of nisin and methicillin against methicillin-resistant *Staphylococcus aureus*. (A) Time-kill plate counts of methicillin-resistant *Staphylococcus aureus* ST291 after 3, 6, 9, 12 and 24 h of treatment with nisin Z (1 μg/ml) and methicillin (32 μg/ml). **(B)** Examination of methicillin-resistant *Staphylococcus aureus* ST291 colony morphology after 24 h of no treatment and after 24 h of treatment with nisin Z and methicillin.
$^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

at 37°C (Table 3). It was apparent that for *C. acnes* LMG 16711 both antibiotics had a greater antimicrobial effect than either bacteriocin (MIC penicillin G: 0.06 μg/ml; MIC vancomycin: 1.25 μg/ml) with nisin Z (MIC: 1.88 μg/ml) being the more effective of the two bacteriocins (MIC lacticin 3147: 2.5 μg/ml) (Table 3). In the case of *E. faecium* UCC1 and UCC2 both nisin Z and lacticin 3147 produced greater antimicrobial effects than either antibiotic, with lacticin 3147 giving the lowest MIC values of 0.12 and 0.48 μg/ml, respectively (Table 3). Lacticin 3147 produced the lowest MIC value against *C. xerosis* DPC 5629 (MIC: 0.24 μg/ml) and had a lower MIC value than both nisin Z and penicillin G when tested against MRSA ST291 (MIC lacticin 3147: 3.85 μg/ml). However, vancomycin remained the most effective antimicrobial for MRSA ST291 (MIC: 0.91 μg/ml) (Table 3).

Growth curve assays & time-kill assays with lacticin 3147, penicillin G & vancomycin

From the MIC results, lacticin 3147 was selected for further analysis by growth curve assays and time-kill assays conducted in combination with penicillin G and vancomycin against the strains *C. acnes* LMG 16711, *E. faecium* UCC1, *E. faecium* UCC2, *C. xerosis* DPC 5629 and MRSA ST291 (Figures 1 & 2). In the case of the growth curves (Figure 1A–E), when the peptide and antibiotic were used singly at sublethal concentrations, there was an

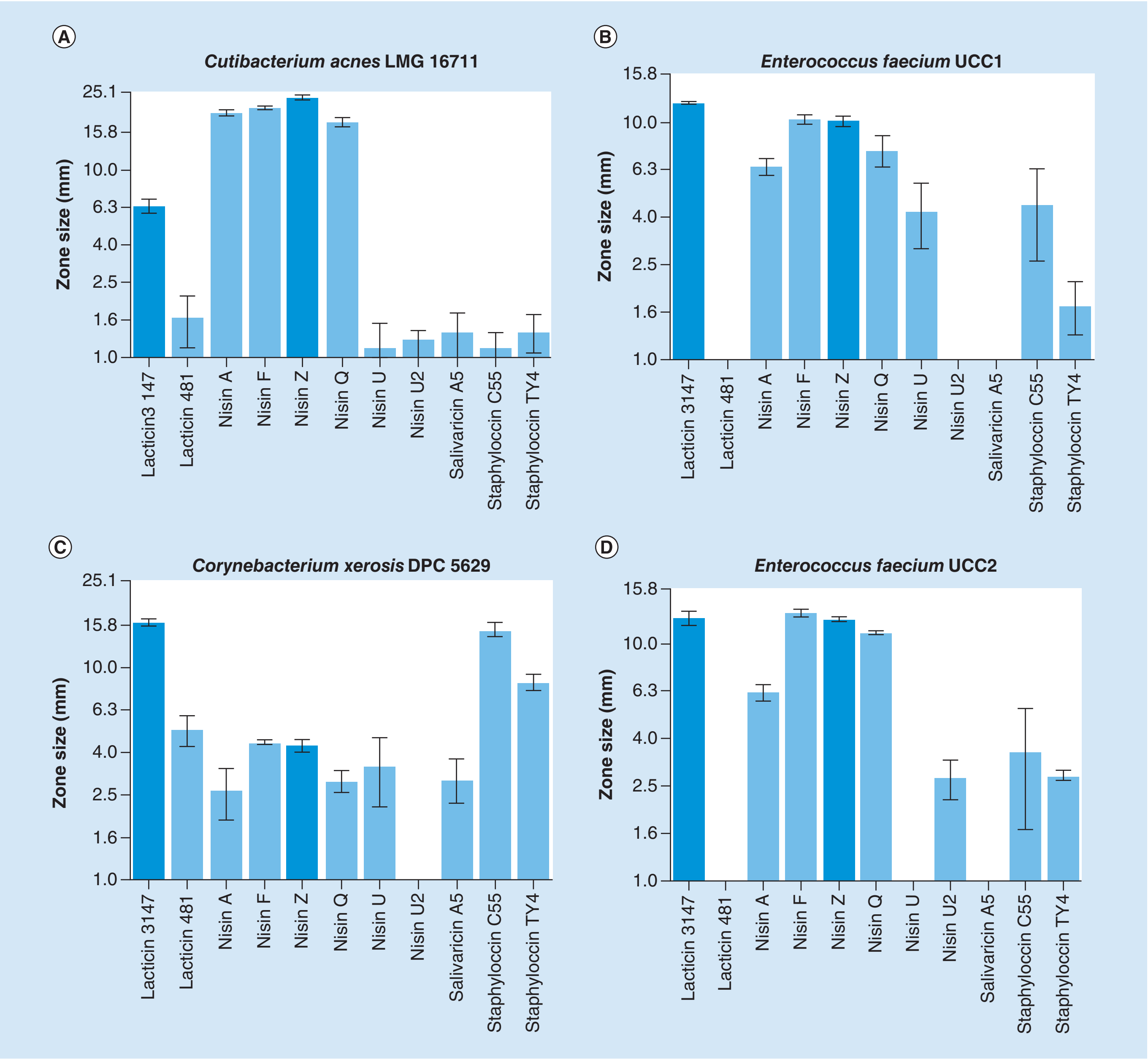

Figure 4. Effect of bacteriocin producers against selected pathogens. Deferred antagonism results of a bank of various bacteriocins producers against **(A)** *Cutibacterium acnes* LMG 16711, **(B)** *Enterococcus faecium* UCC1, **(C)** *Corynebacterium xerosis* DPC 5629 and **(D)** *E. faecium* UCC2. Deferred antagonism assays were carried out for methicillin-resistant *Staphylococcus aureus* ST291 but no zones of inhibition were observed. The zone of inhibition is expressed as the diameter of the zones minus the diameter of the 'spot' in mm. Blue bars indicate bacteriocins chosen for further analysis, lacticin 3147 and nisin Z.

increase of lag time and/or lack of full growth potential in the case of all strains tested. However, when lacticin 3147 and an antibiotic (penicillin G or vancomycin) were used in combination, compared with either compound alone, growth was completely inhibited (Figure 1A–E). This was true for all five strains tested.

Time-kill assays were performed to examine the antimicrobial effects of lacticin 3147 in combination with either penicillin G or vancomycin over a defined time period. Each test strain was exposed to different concentrations of lacticin 3147 and antibiotic (penicillin G or vancomycin) for a period of 1 or 3 h at 37°C (Figure 2A–E). These time points were selected based on previously published work [32], where time-kill assays using lacticin 3147 and similar indicator strains (*P. acnes*, MRSA and *Enterococcus*) were performed. In the case of all five test strains,

Table 3. Minimum inhibitory concentration and fractional inhibitory concentration results.

Organism	MIC (μg/ml)				FIC	
	Nisin Z	Lacticin 3147	Penicillin G	Vancomycin	Nisin Z/methicillin	Nisin Z/vancomycin
Cutibacterium acnes LMG 16711	1.88	2.50	0.06 (S)†	1.25 (S)	–	–
Enterococcus faecium UCC1	0.26	0.12	0.89 (S)	0.63 (S)	–	–
Corynebacterium xerosis DPC 5629	8.33	0.24	0.63 (R)‡	0.45 (S)	–	–
E. faecium UCC2	0.52	0.48	0.89 (S)	0.63 (S)	–	–
Methicillin-resistant *Staphylococcus aureus* ST291	4.17	3.85	20.00 (R)	0.91 (S)	0.75§	1.01¶

†Sensitive (S).
‡Resistant (R); S and R values are taken from EUCAST clinical breakpoint 2019 report [54].
§FIC results indicate partial synergy and additive.
¶Error bars or standard deviations are not included as all values would be 0 due to the nature of the experiment. MIC values presented are identical results from three independent determinations.
FIC: Fractional inhibitory concentration; MIC: Minimum inhibitory concentration.

when lacticin 3147 was used in combination with antibiotic, cell numbers decreased to a greater extent than when either antimicrobial was used alone. For *C. acnes* LMG 16711 (Figure 2A), lacticin 3147 (7.5 μg/ml) alone or in combination with 0.176 μg/ml penicillin G resulted in significant decreases in cell numbers compared with the untreated control after 1 ($p < 0.01$) and 3 h ($p < 0.01$) of treatment. Indeed, after 1 h of treatment, lacticin 3147 alone generated a 1.3 log reduction in cell numbers while the combination of lacticin 3147 and penicillin G resulted in a 1.7 log reduction but penicillin G alone did not inhibit cell growth. After 3 h of treatment with lacticin 3147 and penicillin G combined, no growth was recorded, whereas numbers reached 2.0×10^3 cfu/ml for lacticin 3147 alone and 8.67×10^4 cfu/ml for penicillin G alone, compared with 1.27×10^5 cfu/ml in the untreated sample. In the case of *E. faecium* UCC1 (Figure 2B), all treatments resulted in significant decreases in growth compared with the untreated control after 1 and 3 h of treatment, but the greatest reduction was observed for the combination of 3.56 μg/ml penicillin G and 1.8 μg/ml lacticin 3147 after both 1 and 3 h of treatment resulting in 1.7 and 3.5 log reductions compared with the untreated controls, respectively ($p < 0.001$ in both cases). In contrast, penicillin G alone resulted in 0.5 log reduction after 1 h of treatment ($p < 0.05$) and a 2.1 log reduction after 3 h of treatment ($p < 0.01$), while lacticin 3147 alone generated a 1.3 log reduction after 1 h ($p < 0.001$) and a 2.6 log reduction after 3 h ($p < 0.01$). Likewise, all treatments resulted in significant reductions in cell numbers for *E. faecium* UCC2 compared with the untreated control after 1 and 3 h of treatment (Figure 2C). After 1 h of treatment, 1.44 μg/ml lacticin 3147 alone generated a 1.9 log reduction in cell numbers compared with the untreated control ($p < 0.001$), 3.56 μg/ml penicillin generated a 0.6 log reduction ($p < 0.01$), while the combination resulted in a 3.4 log reduction ($p < 0.001$). After 3 h, no growth was recorded for the combination compared with 3.67×10^3 cfu/ml for lacticin 3147 and 2.33×10^5 cfu/ml for penicillin G. Compared with the untreated control (1.40×10^8 cfu/ml), all of these reductions were significantly different ($p < 0.001$). Lacticin 3147 (1.2 μg/ml) alone and in combination with 0.91 μg/ml vancomycin resulted in significant reductions in *C. xerosis* DPC 5629 compared with the untreated control after 1 h of treatment (Figure 2D). In this case, the combination generated a 2.4 log reduction in growth compared with the untreated control ($p < 0.01$); whereas lacticin 3147 alone generated a 0.8 log reduction ($p < 0.05$) and vancomycin alone generated a 0.3 log reduction that was not considered significant ($p < 0.4$). After 3 h of treatment, *C. xerosis* did not grow in the presence of 1.2 μg/ml lacticin 3147 combined with 0.91 μg/ml vancomycin but reached 2.67×10^5 cfu/ml in the control ($p < 0.01$). Lacticin 3147 resulted in a 2.1 log reduction compared with the control ($p < 0.01$) while vancomycin generated a 0.9 log reduction that was not considered significant ($p < 0.06$). Finally, 11.55 μg/ml lacticin 3147 alone or in combination with 0.91 μg/ml vancomycin resulted in significant reductions in MRSA ST291 growth compared with the untreated control ($p < 0.001$) after 1 h of treatment (Figure 2E). Indeed, the combination resulted in a 2.6 log reduction in cell numbers compared with no reduction for vancomycin alone and a 1.7 log reduction for lacticin 3147 alone. After 3 h, all treatments generated significant reductions in cell numbers when compared with the untreated control. In this case, lacticin 3147 generated a 4.6 log reduction ($p < 0.001$), vancomycin generated a 1.9 log reduction ($p < 0.01$), and the combination generated a 5.2 log reduction ($p < 0.05$).

FIC assays & time-kill assays using nisin Z, vancomycin & methicillin against MRSA ST291

In a further attempt to confirm that bacteriocins can enhance antibiotic efficacy, FIC assays were performed to determine whether a bacteriocin can work synergistically with an antibiotic against a bacterium that is known to be resistant to the same antibiotic. In this case, nisin Z was chosen because it has already been approved by the US FDA, making it a potentially more easily and rapidly implemented therapeutic. The MIC of more than 64 μg/ml for methicillin confirms that the *S. aureus* strain used is indeed a MRSA. The FIC assays confirmed that a combination of vancomycin and nisin Z had only an additive effect against MRSA ST291 (FIC: 1.01), while a combination of methicillin and nisin Z gave a more promising result, having a partially synergistic effect (FIC: 0.75) (Table 3).

Furthermore, time-kill assays were performed to establish the effect of nisin Z and methicillin alone or in combination on MRSA ST291 cell numbers following 3, 6, 9, 12 and 24 h treatment periods (Figure 3A). After 3 h, all treatments resulted in significant reductions in cell growth of MRSA ST291 compared with the untreated control. However, the combination of 1 μg/ml nisin Z and 32 μg/ml methicillin proved the most effective resulting in a 3.1 log reduction ($p < 0.001$), methicillin resulted in a 2.0 log reduction ($p < 0.001$) and nisin Z generated a 0.5 log reduction ($p < 0.01$). After 6 h, the combination treatment and methicillin alone generated significant reductions in cell numbers when compared with the untreated control at the same time point. In this case, cell numbers reached 9.3×10^7 cfu/ml in the presence of methicillin, representing a 1.1 log reduction compared with the untreated control (1.13×10^9 cfu/ml; $p < 0.05$), while cell numbers reached 9.02×10^8 cfu/ml in the presence of nisin, representing a 0.1 log reduction (not significant). However, after 6 h, the combination resulted in a 4.8 log reduction in cell numbers compared with the untreated control ($p < 0.001$). After 9 h of treatment, significant reductions continued to be observed for methicillin ($p < 0.001$) and the combination ($p < 0.001$). However, cell numbers for the combination were recorded at 9×10^3 cfu/ml, compared with 6.5×10^8 cfu/ml for methicillin, 1.12×10^9 cfu/ml for nisin and 2.26×10^9 cfu/ml for the untreated sample. After 12 h of treatment, a significant reduction in cell numbers was observed for the combination when compared with the untreated control ($p < 0.01$), which resulted in a 4.6 log reduction in cell numbers. Nisin reduced cell numbers by 0.2 logs compared with the untreated control, which was considered significant at a p-value of 0.04. However, at the same time point methicillin failed to significantly inhibit cell growth. By 24 h, no significant reductions were observed between the untreated control and any of the other treatments. Indeed, by the 24 h time point, colony counts from the nisin and methicillin combination were equal to that of untreated cells, however, colonies were much smaller indicating that the cells were stressed (Figure 3B).

Ex vivo porcine skin model infected with MRSA ST291 & treated with nisin Z & methicillin

A porcine skin model contaminated with approximately 10^7 cfu/ml MRSA ST291 was used to determine if the benefits of the nisin Z and methicillin combination could be used to impede bacterial growth *ex vivo* (Figure 5). Following 1 h of incubation in the presence of the pathogen, the pig skin samples were treated with nisin Z (1 μg/ml), methicillin (32 μg/ml) or a combination of both for 3 h. Pathogen numbers reached 5.9×10^8 cfu/ml in the untreated sample after the 3-h period. Nisin Z alone had minimal impact on cell numbers after the same time period (recorded at 5.1×10^8 cfu/ml in the presence of nisin Z), while methicillin reduced numbers to 4.61×10^8 cfu/ml. These results were not significantly different from the untreated control. In contrast, the combination of nisin Z and methicillin resulted in a 0.7 log reduction in cell numbers that was considered significant when compared with the untreated control ($p < 0.01$).

Discussion

In this study, we examined the use of bacteriocins in combination with different antibiotics for the treatment of various clinically significant microorganisms. An initial screening for antimicrobial activity using a bank of bacteriocins against five different strains of bacteria highlighted the potential of nisin Z and lacticin 3147 for antibiotic combination studies. Nisin Z and lacticin 3147 are both broad-spectrum lantibiotics (class I bacteriocins) and are produced by *L. lactis*. Nisin has been used within the food industry as a food preservative since 1953 and has been approved by the EU, WHO and FDA, making it an ideal candidate for further research as a potential therapeutic. Lacticin 3147 is comprised of two peptides, α and β, and has demonstrated potent activity against

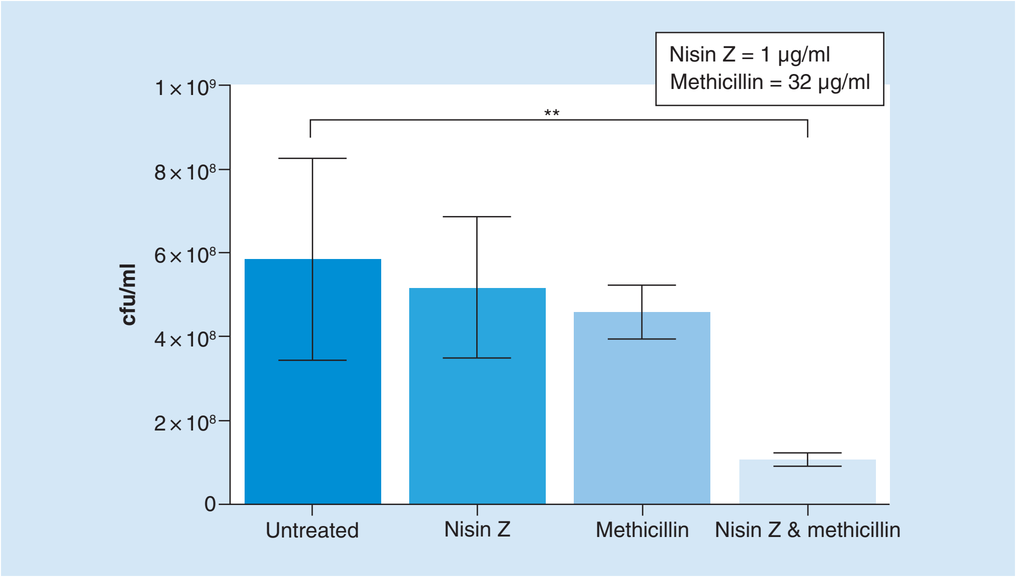

Figure 5. Effect of nisin and methicillin in a pig skin model. *Ex vivo* porcine skin model plate counts of methicillin-resistant *Staphylococcus aureus* ST291 after 3-h treatment with nisin Z (1 µg/ml), methicillin (32 µg/ml) and a combination of nisin Z (1 µg/ml) and methicillin (32 µg/ml).
**$p < 0.01$.

a wide range of Gram-positive pathogens [32]. Nisin and lacticin 3147 have also previously been shown to work synergistically with various other antibiotics against Gram-negative bacteria, such as polymyxin, amikacin and colistin [30,33,34]. Nisin Z demonstrated the highest level of activity against *C. acnes* LMG 16711 and has activity comparable to the two other bacteriocins with the highest level of activity against *E. faecium* UCC2 (nisin F and lacticin 3147). Lacticin 3147 demonstrated the highest level of activity against *E. faecium* UCC1 and *C. xerosis* DPC 5629 and exhibited comparable activity to the two other bacteriocins with the highest level of activity against *E. faecium* UCC2 (nisin F and nisin Z). For these reasons, nisin Z and lacticin 3147 were chosen for further combination studies with vancomycin, penicillin G and methicillin. It is noteworthy that in this initial screen no bacteriocin demonstrated solo activity against MRSA ST291.

MICs confirmed that the strains used in this study were sensitive to vancomycin; however, MRSA ST291 and *C. xerosis* DPC 5629 exhibited resistance to penicillin G. In the case of *C. acnes* LMG 16711, a higher concentration of bacteriocin was required to produce an antimicrobial effect than antibiotic. For the other strains tested the bacteriocins proved to be more effective in certain cases than the antibiotics tested, as was the case for nisin Z and lacticin 3147 against *E. faecium* UCC1 and *E. faecium* UCC2 and lacticin 3147 against *C. xerosis* DPC 5629.

The amount of penicillin or vancomycin required to kill the microorganisms under investigation was reduced by the addition of lacticin 3147. This is significant given that antibiotic-related toxicity can result from the use of high doses [35]. Both nisin and lacticin 3147 produce antimicrobial activity by interacting with lipid II, forming pores in the cell membrane and inhibiting cell wall synthesis. These pores may facilitate intracellular access to antibiotics allowing them to act more effectively and thus producing an improved antimicrobial effect. Furthermore, the antimicrobial activity of penicillin G and vancomycin against each bacterial strain tested was significantly strengthened in the presence of lacticin 3147. Growth curves revealed that over 48 h (or 100 h in the case of *C. acnes* LMG 16711), the level of bacterial growth was substantial when treated with either lacticin 3147 or antibiotic alone compared with the lack of growth when treated with a combination. In the case of *C. acnes* LMG 16711, the concentration of penicillin G could be reduced from 0.2 µg/ml to 0.015 µg/ml when used in combination with 1.5 µg/ml lacticin 3147. For both *E. faecium* strains, penicillin G could be reduced from 3.56 to 0.22 µg/ml when used in combination with 1.8 µg/ml lacticin 3147 (*E. faecium* UCC1) or 2.4 µg/ml lacticin 3147 (*E. faecium* UCC2). The concentration of vancomycin could be reduced from 0.91 to 0.45 µg/ml when used in combination with 1.2 µg/ml lacticin 3147 for the treatment of *C. xerosis* DPC 5629, and from 1.81 to 0.91 µg/ml when used in combination with 38.5 µg/ml lacticin 3147 for the treatment of MRSA ST291. Penicillin acts by binding to a penicillin-binding protein, DD-transpeptidase, preventing cell wall synthesis, whereas vancomycin acts by binding to D-alanyl-D-alanine containing peptides and preventing their incorporation into the peptidoglycan matrix of the cell wall, thus preventing cell wall biosynthesis [36,37]. After encountering multiple approaches of attack on its cell wall from both lacticin 3147 and antibiotic, the bacterium cannot recover as easily as with one antimicrobial approach and thus a higher rate of death occurs. This was observed in both the growth curves and time-kill assays where the same concentration of lacticin 3147 or antibiotic (penicillin G or vancomycin) displayed greater antimicrobial activity, to varying degrees, against each indicator strain when used in combination than when used alone. Combining antimicrobials with different antimicrobial mechanisms rather than antimicrobials with the same or similar modes of action in general yield better antimicrobial results along with reducing the prospect of resistance development [38–40]. Previous studies have shown that combining vancomycin, which by inhibiting cell wall synthesis decreases access to lipid II, with a bacteriocin that interacts with lipid II, such as lacticin 3147 or nisin, can decrease the possibility of synergistic antimicrobial activity [38]. Therefore, combining lacticin 3147 or nisin Z with an antibiotic such as penicillin, which attacks by a different pathway, may be a better approach to achieve synergy.

Together with reducing the amount of antibiotic required, this study focused on the possibility of using antibiotics to which bacteria have already established resistance in combination with bacteriocins in an effort to reclaim the use of such antibiotics. Due to MRSA's clinical significance, methicillin was used as the subject for investigation in combination with the bacteriocin nisin Z for the treatment of MRSA. Nisin and methicillin attack the cell wall synthesis pathway at different points, thus potentially allowing them to generate greater disruption within the cell wall when used together. FIC results confirmed partial synergy between nisin Z and methicillin against MRSA ST291. FIC also revealed a lack of synergy between nisin Z and vancomycin, possibly due to vancomycin decreasing the accessibility of lipid II to nisin Z. Time-kill assays were used to further analyze the activity of nisin Z and methicillin against MRSA ST291 over a 24-h period. Interestingly, for cells treated with nisin Z or methicillin alone the cfu/ml generally increased over the 24-h period; however, the cfu/ml of the combination treated cells declined until the 9th h after which these cell numbers began to increase, indicating that the combination of nisin Z and methicillin worked optimally up to hour 9. Despite the fact that the cfu/ml of the combination-treated cells was equivalent to that of cells treated with either antimicrobial alone by hour 24 the colonies were much smaller, indicating that they were stressed.

The enhanced antimicrobial activity of the nisin Z-methicillin combination was further analyzed for its potential clinical use by means of an *ex vivo* porcine skin model. Pig skin samples are commonly used to test the antimicrobial efficacy of substances [41–44]. When applied to porcine skin contaminated with MRSA ST291, the combination of antimicrobials (nisin Z and methicillin) had a greater effect (0.7 log reduction) than the sum of antimicrobial activity of either alone (~0.16 log reduction), following 3 h of exposure. However, the antimicrobial effect of the nisin Z-methicillin combination appears to be reduced when applied to the skin model, with the reduction in cell numbers being much less than that achieved *in vitro* (3 log reduction *in vitro*). Nevertheless, a greater antimicrobial effect was still observed for the combination *ex vivo* and was considered significant ($p < 0.01$) when compared with the untreated control. Furthermore, treatment duration is an important consideration for antimicrobial treatment regimens with repeated doses usually administered for a specified duration. Indeed, Paterson *et al.* [45] reported that high initial doses followed by extended tapering of doses improved antibiotic efficacy. Thus, the model presented here could be further optimized.

The exact mechanism by which nisin Z and methicillin produce a synergistic effect against MRSA ST291 is still unclear, as resistance in MRSA to methicillin is reliant upon its ability to produce the enzyme β-lactamase, which results in the digestion of methicillin rendering it inactive against MRSA. Further studies need to be conducted to determine if, in the presence of both methicillin and nisin Z, the production of β-lactamase is downregulated, possibly as a result of cellular stress, thus allowing for methicillin to again have antimicrobial activity against MRSA. Similar studies should be performed on lacticin 3147 in combination with penicillin G against β-lactamase producing pathogens.

Conclusion

With antibiotic resistance becoming of greater concern the need for alternative therapies is becoming more prevalent. In this study, the combined results of the deferred antagonism, MIC, growth curve, time kill and FIC assays show the synergy/partial synergy between the bacteriocins lacticin 3147 or nisin Z and antibiotics vancomycin, penicillin G or methicillin. Furthermore, the *ex vivo* assay demonstrates that the combined antimicrobial effect of nisin Z and methicillin on a porcine skin model is of greater statistical significance than when either is used alone. These *in vivo* and *ex vivo* assays suggest that both nisin Z and lacticin 3147 may: aid in prolonging the use of certain antibiotics that are under threat of becoming ineffective due to antibiotic resistance; and be used in combination with currently ineffective antibiotics, like methicillin, to revive the use of such antibiotics.

Summary points

Lacticin 3147 improves antimicrobial activity of penicillin G & vancomycin

- Combining lacticin 3147 with penicillin G or vancomycin improves antimicrobial efficacy of both antibiotics against *Cutibacterium acnes*, *Corynebacteroim xerosis*, *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* (MRSA).
- The amount of penicillin or vancomycin required to kill these microorganisms is reduced by the addition of lacticin 3147.

Nisin Z revives the antimicrobial activity of methicillin against MRSA

- The nisin Z-methicillin combination exerts partial synergistic antimicrobial activity against MRSA.
- The combination works optimally against MRSA for up to 9 h but continues to stress the cells at 24 h.
- The nisin Z-methicillin combination proved more effective than either antimicrobial alone for reducing MRSA numbers in an *ex vivo* porcine skin model.

Author contributions

C Hill and RP Ross conceived and designed the study; J-C Ellis performed the experiments. C Hill, RP Ross and J-C Ellis analyzed the data and wrote the paper.

Financial & competing interests disclosure

This publication has emanated from research conducted with the financial support of Science Foundation Ireland under grant number SFI/12/RC/2273. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. The White House. National action plan for combating antibiotic resistant bacteria (2015). www.cdc.gov/drugresistance/pdf/national_action_plan_for_combating_antibotic-resistant_bacteria.pdf

   The highlighted articles emphasize the importance of synergism with azoles, the aim of this work.

2. World Health Organization. Global action plan on antimicrobial resistance (2015). http://apps.who.int/iris/bitstream/handle/10665/193736/9789241509763_eng.pdf?sequence=1
3. European Commission. AMR summary action plan (2017). https://ec.europa.eu/health/amr/sites/amr/files/amr_summary_action_plan_2017_en.pdf

• **Provides information on the new EU one health action plan against antimicrobial resistance.**

4. Blaser M. Antibiotic overuse: stop the killing of beneficial bacteria. *Nature* 476(7361), 393–394 (2011).
5. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host–microbiota mutualism. *Nat. Rev. Micro.* 9(4), 233–243 (2011).
6. Cotter PD, Stanton C, Ross RP, Hill C. The impact of antibiotics on the gut microbiota as revealed by high throughput DNA sequencing. *Discov. Med.* 13(70), 193–199 (2012).
7. Bierbaum G, Sahl H-G. Lantibiotics. Mode of action, biosynthesis and bioengineering. *Curr. Pharm. Biotechnol.* 10(1), 2–18 (2009).
8. Cotter PD, Ross RP, Hill C. Bacteriocins – a viable alternative to antibiotics? *Nat. Rev. Micro.* 11(2), 95–105 (2013).

•• **In depth discussion on the use of bacteriocins as alternatives to antibiotics including the risk of bacteriocin resistance and what is required to make bacteriocins a viable antimicrobial therapy.**

9. Okuda K, Zendo T, Sugimoto S *et al.* Effects of bacteriocins on methicillin-resistant *Staphylococcus aureus* biofilm. *Antimicrob. Agents Chemother.* 57(11), 5572–5579 (2013).

10. Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *Proc. Natl Acad. Sci. USA* 104(18), 7617–7621 (2007).

11. Gong HS, Meng XC, Wang H. Mode of action of plantaricin MG, a bacteriocin active against *Salmonella typhimurium*. *J. Basic Microbiol.* 50(S1), S37–S45 (2010).

12. Rea MC, Clayton E, O'Connor PM *et al.* Antimicrobial activity of lacticin 3,147 against clinical *Clostridium difficile* strains. *J. Med. Microbiol.* 56(7), 940–946 (2007).

13. Behrens HM, Six A, Walker D, Kleanthous C. The therapeutic potential of bacteriocins as protein antibiotics. *Emerg. Top. Life Sci.* 1(1), 65–74 (2017).

•• **Discusses state of the art in relation to therapeutic uses of two types of Gram-negative bacteriocins. Along with *in vitro* data, results involving animal models of infections are also discussed.**

14. Kirschbaum JO, Kligman AM. The pathogenic role of *Corynebacterium acnes* in acne vulgaris. *Arch. Dermatol.* 88, 832–833 (1963).

15. Nakase K, Sakuma Y, Nakaminami H, Noguchi N. Emergence of fluoroquinolone-resistant *Propionibacterium acnes* caused by amino acid substitutions of DNA gyrase but not DNA topoisomerase IV. *Anaerobe* 42, 166–171 (2016).

16. Nakase K, Nakaminami H, Noguchi N, Nishijima S, Sasatsu M. First report of high levels of clindamycin-resistant *Propionibacterium acnes* carrying erm(X) in Japanese patients with acne vulgaris. *J. Dermatol.* 39(9), 794–796 (2012).

17. Nakase K, Nakaminami H, Takenaka Y, Hayashi N, Kawashami M, Noguchi N. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. *J. Med. Microbiol.* 63(5), 721–728 (2014).

18. Vela AI, Gracía E, Fernández A, Domínguez L, Fernández-Garayzábal JF. Isolation of *Corynebacterium xerosis* from animal clinical specimens. *J. Clin. Microbiol.* 44(6), 2242–2243 (2006).

19. Dumas ER, Michaud AE, Bergeron C, Lafrance JL, Mortillo S, Gafner S. Deodorant effects of a supercritical hops extract: antibacterial activity against *Corynebacterium xerosis* and *Staphylococcus epidermidis* and efficacy testing of a hops/zinc ricinoleate stick in humans through the sensory evaluation of axillary deodorancy. *J. Cosmet. Dermatol.* 8(3), 197–204 (2009).

20. Coyle MB, Lipsky BA. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. *Clin. Microbiol. Rev.* 3(3), 227–246 (1990).

21. Willems RJ, van Schaik W. Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. *Future Microbiol.* 4(9), 1125–1135 (2009).

•• **Discusses the transition of *Enterococcus faecium* from commensal microorganism to nosocomial pathogen with a focus on population-based studies. The review also discusses the accessory genome of *E. faecium* and how it has contributed to its infectious phenotype.**

22. Eymard D, Dascal A, Hiscott J *et al.* Non-beta-lactamase-producing penicillin-resistant *Enterococcus faecium* in a clinical setting. *Can. J. Infect. Dis.* 1(3), 73–76 (1990).

23. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant *Staphylococcus aureus*: report of a clinical failure. *Antimicrob. Agents Chemother.* 49(3), 1222–1224 (2005).

24. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J. Antimicrob. Chemother.* 48(2), 315–316 (2001).

25. CDC. Laboratory Testing for MRSA. www.cdc.gov/mrsa/lab/index.html

26. Wilson SE, O'Riordan W, Hopkins A *et al.* Telavancin versus vancomycin for the treatment of complicated skin and skin-structure infections associated with surgical procedures. *Amer. J. Surg.* 197(6), 791–796 (2009).

27. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin. Microbiol. Rev.* 23(3), 616–687 (2010).

28. Lowy FD. *Staphylococcus aureus* infections. *N. Engl. J. Med.* 339(8), 520–532 (1998).

29. Ruppen C, Sendi P. Time kill assays for *Streptococcus agalactiae* and synergy testing. *Protocol Exchange* doi: 10.1038/protex.2015.126 (2015).

30. Draper LA, Cotter PD, Hill C, Ross RP. The two peptide lantibiotic lacticin 3147 acts synergistically with polymyxin to inhibit Gram negative bacteria. *BMC Microbiol.* 13, 212 (2013).

•• **Reports that combining lacticin 3147 with polymixin results in synergy and enables a reduction in levels of polymixin. This mixture was capable of targeting *Escherichia coli* and *Cronobacter*.**

31. Neu HC. Mecillinam – an amidino penicillin which acts synergistically with other beta-lactam compounds. *J. Antimicrob. Chemother.* 3(Suppl. B), 43–52 (1977).

32. Galvin M, Hill C, Ross RP. Lacticin 3147 displays activity in buffer against gram-positive bacterial pathogens which appear insensitive in standard plate assays. *Lett. Appl. Microbiol.* 28(5), 355–358 (1999).

33. Landersdorfer CB, Ly NS, Xu H, Tsuji BT, Bulitta JB. Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design. *Antimicrob. Agents Chemother.* 57(5), 2343–2351 (2013).

34. Field D, Seisling N, Cotter PD, Ross RP, Hill C. Synergistic nisin-polymyxin combinations for the control of *Pseudomonas* biofilm formation. *Front. Microbiol.* 7, 1713 (2016).

35. Yilmaz C, Özcengiz G. Antibiotics: pharmacokinetics, toxicity, resistance and multidrug efflux pumps. *Biochem. Pharmacol.* 133, 43–62 (2017).

36. Yocum RR, Rasmussen JR, Strominger JL. The mechanism of action of penicillin. *Penicillin acylates the active site of Bacillus stearothermophilus* D-alanine carboxypeptidase. *J. Biol. Chem.* 255(9), 3977–3986 (1980).

37. Watanakunakorn C. Mode of action and *in-vitro* activity of vancomycin. *J. Antimicrob. Chemother.* 14(Suppl. D), 7–18 (1984).

38. Tong Z, Zhang Y, Ling J, Ma J, Huang L, Zhang L. An *in vitro* study on the effects of nisin on the antibacterial activities of 18 antibiotics against *Enterococcus faecalis*. *PLoS ONE* 9(2), e89209 (2014).

39. Jia J, Zhu F, Ma X *et al.* Mechanisms of drug combinations: interaction and network perspectives. *Nat. Rev. Drug Discov.* 8(2), 111–128 (2009).

40. Mathur H, Field D, Rea MC, Cotter PD, Hill C, Ross RP. Bacteriocin-antimicrobial synergy: a medical and food perspective. *Front. Microbiol.* 8, 1205 (2017).

•• **Discusses effective bacteriocin-antimicrobial combinations used to target clinical and foodborne pathogens. Also discusses issues hindering development of bacteriocins as viable therapeutics.**

41. Nakatsuji T, Chen TH, Narala *et al.* Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci. Transl. Med.* 9(378), eaah4680 (2017).

42. Wang Y, Leng V, Patel V, Philips KS. Injections through skin colonized with *Staphylococcus aureus* biofilm introduce contamination despite standard antimicrobial preparation procedures. *Sci. Rep.* 7, 45070 (2017).

43. Rubinchik E, Dugourd D, Algara T, Pasetka C, Friedland HD. Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models. *Int. J. Antimicrob. Agents* 34(5), 457–461 (2009).

44. Bush LW, Benson LM, White JH. Pig skin as test substrate for evaluating topical antimicrobial activity. *J. Clin. Microbiol.* 24(3), 343–348 (1986).

45. Paterson IK, Hoyle A, Ochoa G, Baker-Austin C, Taylor NGH. Optimising antibiotic usage to treat bacterial infections. *Sci. Rep.* 6, 37853 (2016).

46. McAuliffe O, Ryan MP, Ross RP, Hill C, Breeuwer P, Abee T. Lacticin 3147, a broad-spectrum bacteriocin which selectively dissipates the membrane potential. *Appl. Environ. Microbiol.* 64(2), 439–445 (1998).

47. O'Sullivan L, Morgan SM, Ross RP, Hill C. Elevated enzyme release from lactococcal starter cultures on exposure to the lantibiotic lacticin 481, produced by *Lactococcus lactis* DPC5552. *J. Dairy Sci.* 85(9), 2130–2140 (2002).

48. Kuipers OP, Beerthuyzen MM, de Ruyter PG, Luesink EJ, de Vos WM. Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. *J. Biol. Chem.* 270(45), 27299–27304 (1995).

49. Piper C, Hill C, Cotter PD, Ross RP. Bioengineering of a nisin A-producing *Lactococcus lactis* to create isogenic strains producing the natural variants Nisin F, Q and Z. *Microb. Biotechnol.* 4(3), 375–382 (2011).

50. Wirawan RE, Klesse NA, Jack RW, Tagg JR. Molecular and genetic characterization of a novel nisin variant produced by *Streptococcus uberis*. *Appl. Environ. Microbiol.* 72(2), 1148–1156 (2006).

51. Navaratna MADB, Sahl H-G, Tagg JR. Two-component anti-*Staphylococcus aureus* lantibiotic activity produced by *Staphylococcus aureus* C55. *Appl. Environ. Microbiol.* 64(12), 4803–4808 (1998).

52. Yamaguchi T, Hayashi T, Takami H *et al.* Complete nucleotide sequence of a *Staphylococcus aureus* exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. *Infect. Immun.* 69(12), 7760–7771 (2001).

53. O'Shea EF, Gardiner GE, O'Connor PM, Mills S, Ross RP, Hill C. Characterization of enterocin- and salivaricin-producing lactic acid bacteria from the mammalian gastrointestinal tract. *FEMS Microbiol. Lett.* 291(1), 24–34 (2009).

54. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, Version 9.0 (2019). www.eucast.org