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A novel bioengineered derivative of nisin displays enhanced antimicrobial activity against clinical *Streptococcus agalactiae* isolates

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ABSTRACT

Objectives: Streptococcus agalactiae is the leading cause of neonatal disease worldwide, and infections caused by this opportunistic pathogen are becoming increasingly more prevalent in adults. With the global incidence of antimicrobial resistance continuing to rise, there is a recognised need for new therapeutic agents. Nisin is a potent antimicrobial peptide with demonstrated broad-spectrum activity against a range of clinically significant pathogens. This study aimed to examine the efficacy of nisin against a clinical population of *S. agalactiae* isolates and further to investigate the bioactivity of a novel bioengineered derivative of the peptide, designated nisin PV.

Methods: A deferred antagonism assay was used to assess the bioactivity of wild-type nisin and nisin PV against 122 *S. agalactiae* isolates. Minimum inhibitory concentrations (MICs) were evaluated to determine the specific activity of both peptides. The genetic basis of nisin resistance among the isolate collection was investigated by PCR detection of the *nsr* gene.

Results: In total, 91.0% (111/122) of the collection showed some level of susceptibility to nisin, whilst 9.0% (11/122) displayed complete resistance. Interestingly, the nisin derivative exhibited enhanced antimicrobial activity for 64.8% of the isolates. The frequency of the *nsr* gene conferring nisin resistance was 98.4% (120/122), suggesting that resistance may be linked to levels of expression of the protein or other regulatory elements.

Conclusion: This study indicates that there is potential for the use of nisin and its derivatives as therapeutic agents against *S. agalactiae* infections.

1. Introduction

Streptococcus agalactiae is a frequent coloniser of the gastrointestinal and genitourinary tracts of healthy adults. It remains a leading cause of neonatal disease worldwide, and invasive diseases amongst adults are becoming more prevalent [1]. Penicillin is an effective antimicrobial agent for treating *S. agalactiae* infections, however there have been reports of reduced susceptibility to this antibiotic [2]. In addition, resistance to second-line antibiotics, used in cases of penicillin allergy, continues to rise [1]. Indeed, as the burden of antimicrobial resistance increases globally, there is an urgent need for novel therapeutic options.

Bacteriocins are small, ribosomally synthesised antimicrobial peptides produced by many Gram-positive bacteria [3]. Within the bacteriocin family, a large subgroup called lantibiotics have been identified that undergo extensive post-translational modification [4–6]. Lantibiotics are characterised by the formation of lanthionine rings that stabilise the peptide, protect it from proteolytic activity and, importantly, ensure high antimicrobial activity. Nisin, produced by *Lactococcus lactis*, is the most extensively characterised lantibiotic [7] and has demonstrated antimicrobial activity against a wide variety of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) [8],*Clostridioides* (*Clostridium*) *difficile* [9] and *Listeria monocytogenes* [10]. Nisin is a 34-amino acid peptide with five lanthionine-based rings in its structure [6]. The first three rings (A–C) are located at the N-terminus and are separated from the last two rings (D and E) by a hinge region (Fig. 1) [11,12]. Active nisin exerts its antimicrobial action by initial binding of the A–C rings to lipid II, a bacterial peptidoglycan and an important precursor in cell wall synthesis. Nisin uses lipid II as a docking molecule and then employs its hinge region to flip into the membrane bilayers, where rings D and E perforate the bacterial cell membrane [4,13–17].

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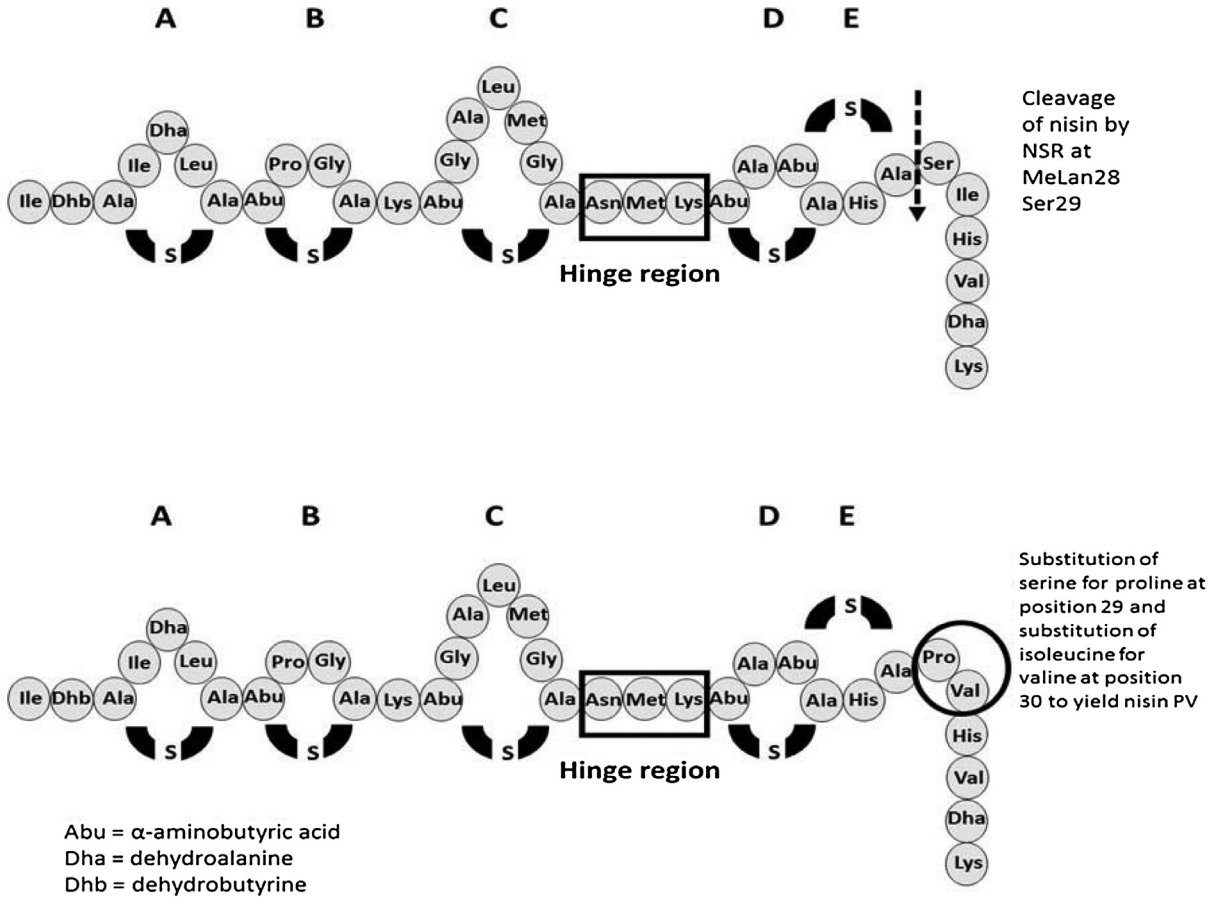


Fig. 1. Schematic structures of nisin A and nisin PV. The schematics highlight the amino acid substitutions in the nisin PV derivative and the site of nisin cleavage of nisin resistance protein (NSR) in nisin A. The nisin PV modification confers resistance to NSR cleavage.

Resistance to nisin is infrequently reported, however some clinically relevant bacteria exhibit innate resistance through cell wall modification or biofilm formation [18]. Resistance can also occur through the presence of two-component systems (TCSs) linked to ATP-binding cassette (ABC) transporters and through the presence of nisin resistance proteins (NSRs) [18]. Froseth and McKay identified a nisin resistance protein in *L. lactis* subsp. *lactis* biovar *diacetylactis* DRC3 strain and confirmed that the encoding *nsr* gene was harboured on a plasmid [19]. Further studies revealed that the mode of action of the NSR protein involves proteolytic cleavage of nisin at the C-terminus mediated by a conserved tail-specific protease domain [6]. NSR cleaves nisin at residues MeLan-28 and Ser-29, generating a truncated nisin (nisin$^{1-28}$) with reduced affinity for the cell membrane and subsequently reduced antimicrobial activity [20].

Recently, a gene encoding a nisin resistance protein (*nsr*) was discovered in the reference strain *S. agalactiae* ATCC 13813 and was shown to confer resistance to nisin when expressed in *L. lactis* [21]. The *nsr* gene in *S. agalactiae* was found to be part of a conserved operon that encodes six proteins including a lipoprotein, an NSR protein, an ABC transporter designated NsrFP, a response regulator denoted NsrR and a histidine kinase NsrK. The histidine kinase and response regulator are thought to be part of a TCS that regulates the expression of both the NSR and the ABC transporter, which cleave nisin and export nisin out of the cell, respectively [21,22]. Expression of NSR in *S. agalactiae* is thought to be under tight regulation by this TCS and is conditional upon the amount of external nisin [21].

As therapeutic agents, an attractive attribute of lantibiotics is that their proteinaceous structure is easily modified, and changing even a single residue can significantly alter their biological function [23]. Bioengineered derivatives of nisin have been shown to display enhanced antimicrobial activity against a variety of Gram-positive and Gram-negative pathogens [24–27]. This logic was applied to design a novel nisin peptide that could combat nisin resistance. Site-specific and site-saturation mutagenesis techniques were employed to identify derivatives of nisin that could resist proteolytic cleavage by NSRs [28]. The amino acid serine-29 was altered alone and in combination with isoleucine-30 to identify amino acid substitutions that would result in enhanced activity. A serine to proline substitution (Ser-29 → Pro) combined with an isoleucine to valine (Ile-30 → Val) substitution resulted in a novel nisin derivative, designated nisin PV, that was engineered to prevent cleavage by NSR proteins [28]. Resistance to cleavage was assessed using peptide release assays whereby both wild-type nisin and nisin PV were incubated with *L. lactis* DRC3 cells expressing NSR. Following incubation, reversed-phase high-performance liquid chromatography (RP-HPLC) detected truncated nisin$^{1-28}$, whereas nisin PV remained intact indicating that it had not been cleaved [28]. Furthermore, simulation studies demonstrated that NSR can still bind to nisin PV but cannot cleave it [28].

This study aimed to investigate the level of susceptibility to nisin of a clinical collection of *S. agalactiae* isolates. The efficacy of nisin A and the bioengineered derivative nisin PV were then assessed to identify whether the derivative displayed enhanced activity against *S. agalactiae*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

S. agalactiae isolates ($n = 122$) were collected over a 6-year period (2010–2016) from Cork University Hospital (Cork, Ireland) and University Hospital Limerick (Limerick, Ireland). Invasive isolates ($n = 47$) were obtained from blood culture samples, whilst colonising isolates ($n = 75$) were obtained from high vaginal swab (HVS) samples. Details of the bacterial isolates are listed in Table 1.

S. agalactiae isolates were grown in brain–heart infusion (BHI) broth (Cruinn Diagnostics, Dublin, Ireland) or Todd–Hewitt broth (THB) (Sigma-Aldrich Ireland Ltd., Arklow, Ireland) and were incubated aerobically at 37 °C. *Lactococcus lactis* strains were cultured in M17 broth (Sigma-Aldrich Ireland Ltd.) supplemented with 0.5% glucose (GM17) or on GM17 agar plates and were incubated at 30 °C. Nisin-producing *L. lactis* strains NZ9700 and NZ9800pCI-S29PV were grown in GM17 broth or on GM17 agar plates containing 10 μg/mL chloramphenicol by incubation at 30 °C.

2.2. Nisin purification

The wild-type peptide nisin A and the derivative nisin PV were purified according to published methods [25]. Briefly, 2 L of modified tryptic yeast broth (Oxoid) was inoculated with 1% fresh culture of *L. lactis* NZ9700 (producing nisin A) and was incubated overnight at 30 °C. The following day, the culture was centrifuged at 7000 rpm for 15 min and the supernatant was passed through a 60 g column of pre-equilibrated Amberlite XAD16 beads (Sigma-Aldrich Ireland Ltd.) to collect the peptide. The beads were then washed in 30% ethanol and the peptide was eluted with 70% isopropyl alcohol (IPA) containing 0.1% trifluoroacetic acid (TFA). The original cell pellet was re-suspended in 300 mL of 70% IPA:0.1% TFA solution and was stirred at room temperature for 3 h before centrifuging at 7000 rpm for 15 min. The supernatant was combined with the eluted supernatant and IPA was evaporated using a rotary evaporator (Buchi AG, Flawil, Switzerland). The sample was then adjusted to pH 4 and was applied to a 60-mL Varian C18 Bond Elut Column (Varian Inc., Harbor City, CA, USA) that had been pre-equilibrated with methanol and water. Subsequently, 120 mL of 30% ethanol was used to wash the column and the peptide was eluted in 60 mL of 70% IPA:0.1% TFA. Then, 10 mL aliquots were concentrated to volumes of 2 mL by rotary evaporation and were purified by HPLC using a Phenomenex C12 RP-HPLC column (Jupiter® 4 μm Proteo 90 Å, 250 × 10.0 mm, 4 μM; Phenomenex Ltd., Macclesfield, UK). The column was developed in a gradient of 30–50% acetonitrile:0.1% TFA. The relevant fractions were pooled and acetonitrile was removed by rotary evaporation before the sample was freeze-dried (Labconco). The nisin derivative (nisin PV) was purified by the same protocol using *L. lactis* NZ9800pCI-S29PV strain (nisin PV producer).

2.3. Deferred antagonism assay

Deferred antagonism assays were conducted according to the method described by Field et al. [25]. Fresh overnight cultures of *L. lactis* strains producing nisin A and nisin PV were spotted (5 μL) onto GM17 agar plates and were incubated overnight at 30 °C before being exposed to ultraviolet radiation for 30 min. The plates were then overlayed with 0.75% w/v agar (GM17 for *L. lactis* and BHI for *S. agalactiae*) that had been seeded with the indicator strains at 0.2%. *L. lactis* subsp. *diacetylactis* DRC3 and *L. lactis* MG1614/pNP40 were used as control strains for enhanced activity of nisin PV. Plates were incubated overnight in appropriate conditions (30 °C for *L. lactis* and 37 °C for *S. agalactiae*). The resulting zones of inhibition were measured to identify how sensitive the isolates were to both compounds. As there are no standardised cut-off points for lantibiotic resistance, the isolate collection was evaluated as having enhanced, equal or decreased susceptibility to nisin PV compared with nisin A. When there was no zone of inhibition, isolates were identified as resistant to the peptide.

2.4. Minimum inhibitory concentration (MIC) assays

MICs of nisin A and nisin PV were determined as described by Field et al. [25]. Initially, 96-well microtitre plates (Sarstedt) were pre-treated with 200 μL of phosphate-buffered saline (PBS) (Sigma-Aldrich Ireland Ltd.) containing 1% (w/v) bovine serum albumin and were incubated at 37 °C for 30 min before washing with 200 μL of PBS. When dry, 100 μL of fresh THB was added to each test well. Purified nisin A or nisin PV was added to the first well (100 μL) and 2-fold serial dilutions of each peptide were made, resulting in 12 dilutions ranging from 7.5 μM to 0.0036 μM. Overnight cultures of test *S. agalactiae* isolates were subcultured into fresh THB (10 mL) and were grown to an optical density at 600 nm (OD_{600}) of 0.5 before adjusting to a final concentration of 5×10^5 CFU/mL when 100 μl was transferred into each well. Plates were incubated at 37 °C for 16 h. The MIC was defined as the lowest peptide concentration causing visible inhibition of growth. Experiments were performed in triplicate.

2.5. Growth curves

S. agalactiae isolates were grown overnight in THB at 37 °C and were diluted to an OD_{600} of 0.05 in fresh THB broth supplemented with nisin A or nisin PV (using equimolar concentrations of peptides at approximately one-third of the MIC of nisin A) [25]. Following this, 200 μL of each culture was added to a 96-well plate and cell growth was monitored spectrophotometrically at 600 nm (SpectraMax® spectrophotometer) over a 24-h period at 37 °C. The assay was performed in triplicate for all test isolates.

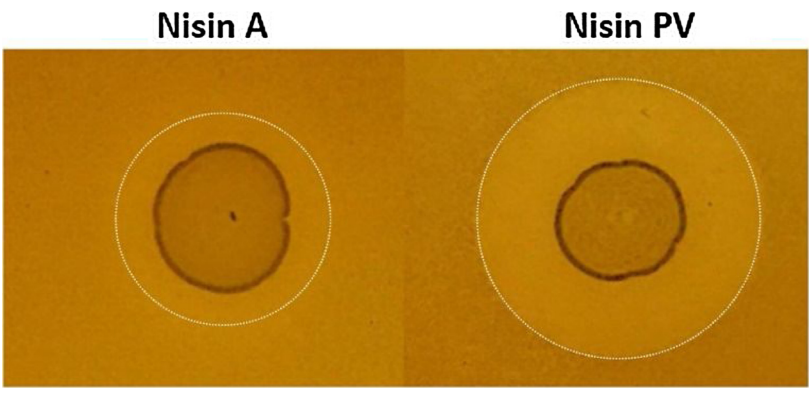
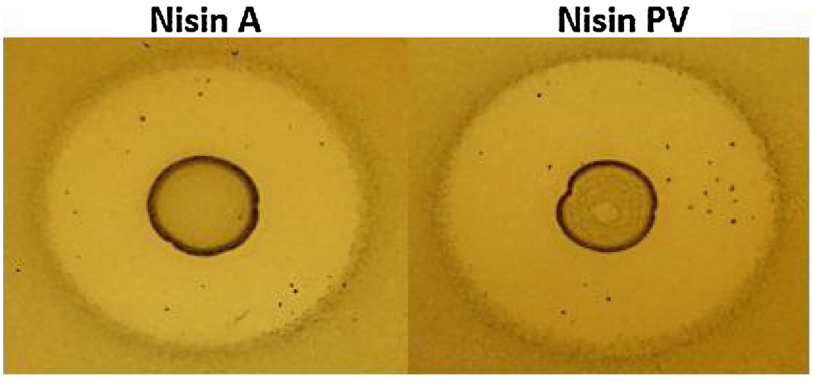
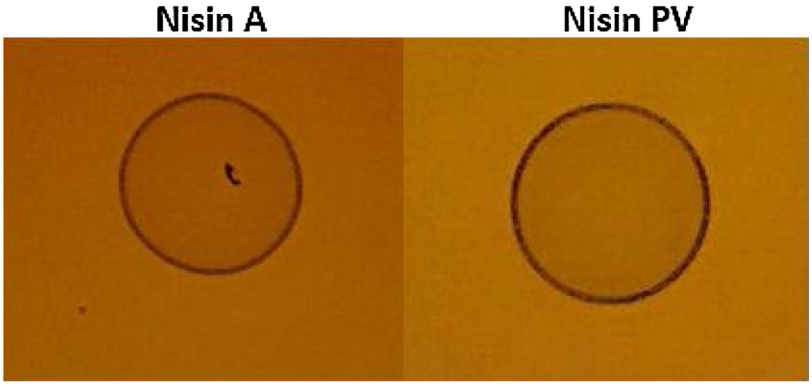
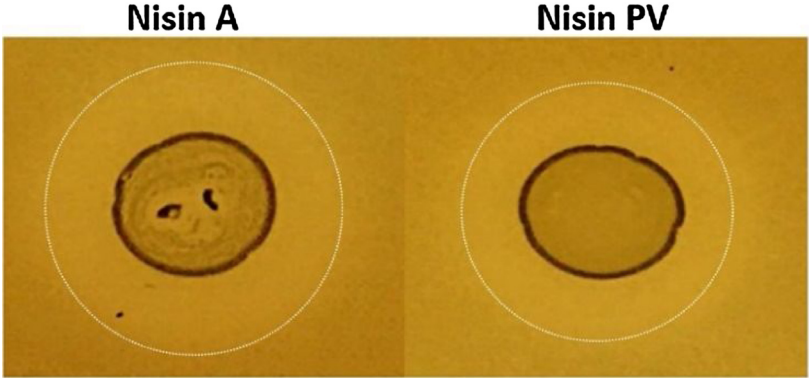
2.6. PCR investigation of the nisin resistance gene

The presence of the nisin resistance gene *nsr* was investigated in each *S. agalactiae* study isolate using a novel PCR assay with the

Table 1
Bacterial strains used in this study.

Strain	Characteristic	References
Lactococcus lactis NZ9700	Wild-type nisin producer (nisin A)	[29]
L. lactis NZ9800pCI-S29PV	Nisin derivative producer (nisin PV)	[28]
L. lactis subsp. *diacetylactis* DRC3	Carries *nsr* gene on plasmid pNP40	[19]
L. lactis MG1614/pNP40	Carries *nsr* gene on plasmid pNP40	[30]
Streptococcus agalactiae ATCC 13813	Reference strain, carries *nsr* gene	[21]
S. agalactiae ($n = 47$)	Invasive isolates from blood culture samples	[31]
S. agalactiae ($n = 75$)	Colonising isolates from high vaginal swabs	[31]

Table 2
Characterisation of *Streptococcus agalactiae* isolates ($n = 122$) by deferred antagonism overlay assay.

Susceptibility characteristics (no. of isolates)	Representative overlay assay result
Enhanced susceptibility to nisin PV compared with nisin A ($n = 79$; 64.8%)	
Equal susceptibility to nisin A and nisin PV ($n = 29$; 23.8%)	
Complete resistance to nisin A and nisin PV ($n = 11$; 9.0%)	
Decreased susceptibility to nisin PV ($n = 3$; 2.5%)	

following primer pair: nsrFor, 5′-CTGGCGGCAATATGATCCCT-3′; and nsrRev, 5′- AGCACCGTCGTAAAGCATGA-3′. Briefly, primers were designed using NCBI Blastn software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with the *S. agalactiae* ATCC 13813 *nsr* gene as the query sequence to retrieve homologous sequences from other *S. agalactiae* genomes. All homologous sequences were aligned using MUSCLE software (https://www.ebi.ac.uk/Tools/msa/muscle) and a consensus sequence was generated. Primers were designed from this sequence using Primer3 software with default parameters. Bacterial DNA was extracted from all *S. agalactiae* isolates used in this study as previously described [31]. PCR amplification was carried out in a final volume of 25 μL using 12.5 μL of RED*Taq*® ReadyMix™ (Sigma-Aldrich Ireland Ltd.) with 0.4 μM of each primer and 100 ng of template DNA using the following thermocycling conditions: initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The 317-bp PCR products were analysed by electrophoresis using a 1% (w/v) agarose gel and were visualised using a Gel Doc viewing system (DNR Bio Imaging Systems, Neve Yamin, Israel). Selected PCR products were confirmed as the *nsr* gene by sequencing and were included as positive controls for screening of isolates.

2.7. Statistical analysis

A related samples *t*-test was used to analyse differences between groups using IBM SPSS Statistics v.24.0 (IBM Corp., Armonk, NY, USA). A *P*-value of $\leq$0.05 was considered statistically significant.

3. Results

3.1. Phenotypic assessment of the bioactivity of nisin A and nisin PV

Initially, deferred antagonism agar diffusion assays were employed using the producer strains *L. lactis* NZ9700 and *L. lactis* NZ9800pCI-S29PV to screen the bioactivity of nisin A and nisin PV against a bank of 122 clinical *S. agalactiae* isolates and the reference strain *S. agalactiae* ATCC 13813. Results indicated that 91.0% (111/122) of the clinical population displayed some level of susceptibility to nisin A, with 9.0% (11/122) of isolates identified as resistant to nisin A. Interestingly, 64.8% (79/122) of isolates displayed enhanced susceptibility to nisin PV compared with nisin A (Table 2), with a statistically significant increase in zone size ($P < 0.0005$). Of these 79 isolates, 41.8% ($n = 33$) were isolated from blood culture samples and 58.2% ($n = 46$) were HVS isolates. Equal levels of susceptibility to both peptides were detected in 23.8% (29/122) of isolates, with 34.5% of these strains ($n = 10$) being invasive isolates and 65.5% ($n = 19$) being colonising isolates. Only 9.0% (11/122) of this collection of clinical isolates displayed complete resistance (no zone of inhibition) to both peptides, with 2 of these isolated from blood culture samples and 9 isolated from HVS samples. Three isolates (2.5%) showed decreased susceptibility to nisin PV compared with nisin A (Table 2), with two of these being isolated from blood culture samples and one from a HVS sample.

3.2. Minimum inhibitory concentration-based investigation of the activity of nisin A and nisin PV

The specific activities of both nisin A and nisin PV were investigated using broth-based microdilution MIC assays on 10% of the total population reflecting the different phenotypes observed by the deferred antagonism assay (Table 3). Of the 14 isolates selected, 64.3% ($n = 9$) had lower MICs for nisin PV thereby demonstrating an overall increase in specific activity for this derivative of nisin.

Isolates exhibiting enhanced susceptibility to nisin PV by deferred antagonism assay had MICs ranging from 0.9375 to 2.5 μM, whilst the MICs for nisin A ranged from 0.029 to 7.5 μM (Table 3). Interestingly, nisin PV demonstrated an 8-fold increase in specific activity for one isolate (CIT 67). Another isolate (CIT 263) exhibited enhanced susceptibility to nisin PV using the overlay assay but appeared to be more susceptible to nisin A when evaluated by the MIC method. However, the MICs for both peptides established for this isolate were much lower than other isolates within this group. Overall, the MICs established for nisin PV were significantly lower ($P < 0.0005$) compared with nisin A for isolates with this phenotype.

MICs were established for three isolates that had equal susceptibility to both peptides by the overlay assay (CIT 276, 106 and 274) and the results indicated a lower MIC for the nisin A peptide for all three strains (Table 3), although this was not statistically significant ($P = 0.189$). Two isolates (CIT 221 and 395) displaying complete resistance to both peptides by the overlay assay had lower MICs with nisin PV (Table 3).

3.3. Growth curve analysis of S. agalactiae in the presence of nisin A and nisin PV

Growth curves provide a more detailed assessment of the bactericidal impact of purified peptides. Representative strains were cultured in the presence of equimolar concentrations of the peptides and growth was monitored over a 24-h period (Fig. 2). In the presence of 2.5 μM nisin A, an extended lag phase was observed for isolates CIT 67 and CIT 239 compared with the untreated control. The enhanced potency of nisin PV against these isolates was evident, with complete inhibition of the isolate at the concentration tested (2.5 μM) (Fig. 2A,B). For isolate CIT 395, an extended lag phase and short log phase was observed in the presence of 2.5 μM nisin PV compared with nisin A and the untreated control (Fig. 2C). Growth analysis of CIT 106 in equal concentrations of both peptides (0.4 μM) resulted in similar growth rates compared with the untreated control (Fig. 2D).

3.4. Detection of the nisin resistance gene

All isolates in the study ($n = 122$) were screened for the presence of the *nsr* gene using a novel PCR assay. In this clinical *S. agalactiae* collection, 98.4% of isolates ($n = 120$) were positive for the *nsr* gene, with only 2 isolates testing negative for the gene. Interestingly, for one of these isolates (CIT 263) nisin PV displayed a decrease in specific activity, whilst for the other isolate (CIT 351) nisin PV displayed an increase in specific activity (Table 3).

4. Discussion

S. agalactiae remains the leading cause of invasive neonatal disease worldwide [32] and has become increasingly more prevalent amongst non-pregnant adults [1]. Resistance to second-line antibiotics continues to rise [1] and a recent study conducted by us highlighted the emergence of new antimicrobial resistance patterns among Irish *S. agalactiae* strains [31]. Thus, there is a continuing need to monitor resistance and to identify alternative and novel treatment options for this opportunistic pathogen. In the current study, nisin and a novel derivative of nisin were examined for activity against clinical *S. agalactiae* isolates.

The potential for nisin to be used as an antimicrobial agent has been examined previously [23,33]. Nisin is approved by the US Food and Drug Administration (FDA) for use in cheese production and has long been used as a preservative in the food industry [34]. Use of nisin as an antibiotic has also been investigated and it has been shown to exert antimicrobial activity against a variety of

Table 3
Susceptibility testing with nisin A and nisin PV, and PCR screening of representative *Streptococcus agalactiae* isolates.

Isolate	Zone size (mm)[a]		MIC (μM)[b]		*nsr* gene[c]
	Nisin A	Nisin PV	Nisin A	Nisin PV	
Isolates with enhanced susceptibility to nisin PV					
CIT 10	0	12.33	6.25	1.875	+
CIT 239	0	15.33	7.5	1.875	+
CIT 351	0	11.33	1.875	1.4	–
CIT 223	7.33	15.67	7.5	1.875	+
CIT 67	10.33	15.33	7.5	0.9375	+
CIT 113	10.67	12.67	7.5	1.875	+
CIT 114	15.33	18.33	5	2.5	+
CIT 263	22.33	24.33	0.029	0.156	–
Isolates with equal susceptibility to both peptides					
CIT 106	15.33	15.33	1.25	1.875	+
CIT 276	16	16	0.546	1.875	+
CIT 274	23	23	0.058	0.234	+
Isolates with complete resistance to both peptides					
CIT 221	0	0	6.25	1.56	+
CIT 395	0	0	>7.5	3.75	+
Isolates with decreased susceptibility to nisin PV					
CIT 211	12.33	8.33	1.875	3.75	+

MIC, minimum inhibitory concentration.
[a] Deferred antagonism agar diffusion-based assessment of the bioactivity of nisin A- and nisin PV-producers against *S. agalactiae* isolates. Values represent the zone of inhibition and are the mean of triplicate assays.
[b] Broth-based MIC assays of nisin A and nisin PV for selected *S. agalactiae* isolates. Values are the mean of triplicate assays.
[c] PCR screening assay for detection of the *nsr* gene.

clinically relevant pathogens such as MRSA [8] and vancomycin-resistant enterococci [35]. *In vitro* studies have also demonstrated the antibiofilm properties of nisin [36] as well as its ability to work synergistically with other antibiotics to improve therapy [37]. However, to the best of our knowledge, the efficacy of nisin has never been examined with respect to a clinical collection of *S. agalactiae*.

The current study investigated rates of susceptibility to nisin amongst a clinical population of *S. agalactiae* isolates and additionally assessed whether a novel derivative (nisin PV) was

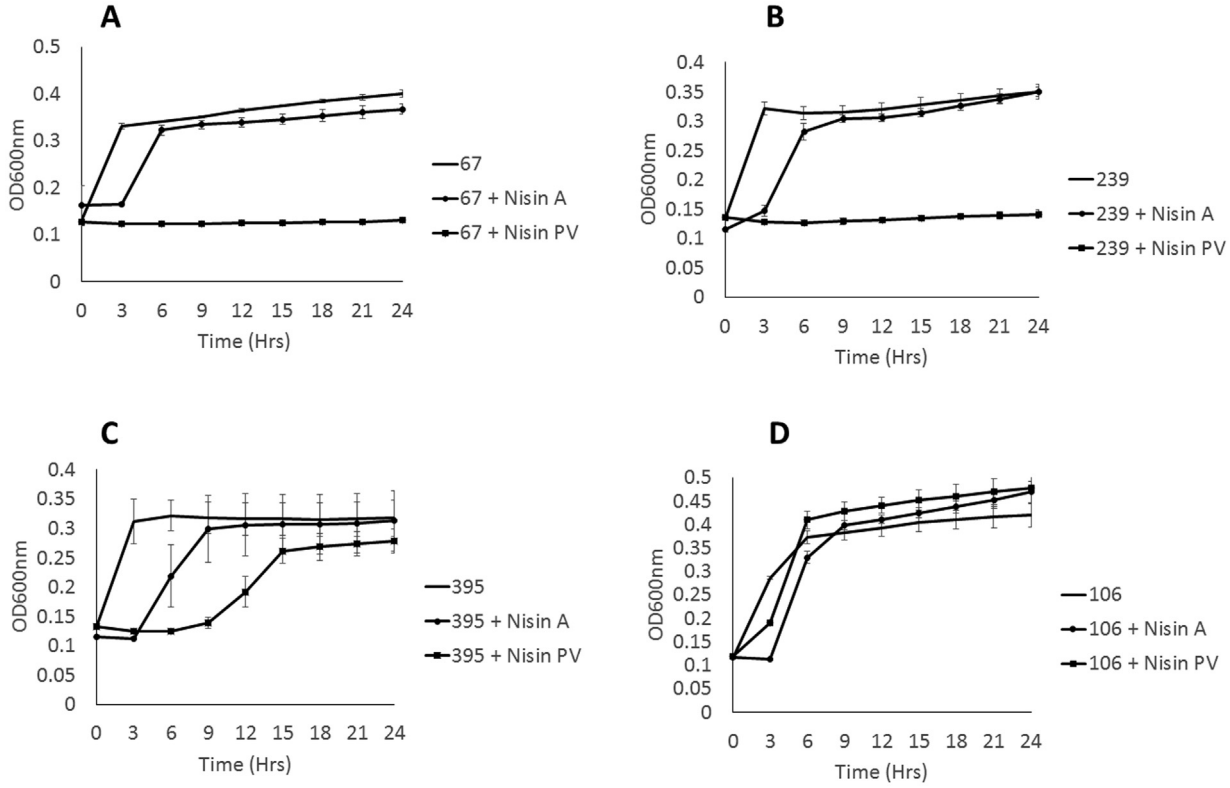


Fig. 2. Growth curve analysis of *Streptococcus agalactiae* isolates in the presence of nisin A and nisin PV. Comparison of the effect of equimolar concentrations (established from MIC data) of nisin A and nisin PV on the growth of representative *S. agalactiae* isolates. (A–C) Isolates CIT 67, CIT 239 and CIT 395, respectively, in the presence of 2.5 μM nisin A and nisin PV. (D) Isolate CIT 106 in the presence of 0.4 μM nisin A and nisin PV. Data represent the mean of triplicate experiments. MIC, minimum inhibitory concentration. OD_{600nm}, optical density at 600 nm.

a more potent peptide. In this collection, the nisin PV derivative was determined to be significantly more effective than nisin A ($P < 0.0005$). Furthermore, MICs were significantly lower for nisin PV compared with nisin A ($P < 0.0005$). The MIC results corresponded with the overlay assay results for isolates with enhanced susceptibility to nisin PV ($n = 8$), with the exception of 2 isolates (CIT 263 and 351). Interestingly, these were the only isolates to test negative for the *nsr* gene within the entire collection and this could explain the lack of concordance between the assay results. A number of factors can influence the zone of inhibition produced by a peptide in the deferred antagonism assay, including solubility, ability to diffuse through the agar, as well as the mechanisms of resistance to nisin that *S. agalactiae* strains possess. These factors may explain the discrepancies observed between the two assays.

Despite the considerable potential of nisin to be used in the clinical setting, there have been reports of resistance amongst certain bacteria through production of nisin resistance proteins (NSRs), including *S. agalactiae* strains [19,21]. Even more worrying is the fact that *nsr* genes are frequently located on naturally transmissible elements [19,38]. Bioengineered derivatives of nisin are an attractive counteract, as nisin is easily manipulated and mutant derivatives have been shown to be more stable and more effective against bacteria [26], including *S. agalactiae* ATCC 13813 [39]. Nisin PV was designed to resist proteolytic cleavage by NSR proteins owing to specific amino acid substitutions at the C-terminal cleavage site. We therefore postulated that strains producing an NSR would be potentially more susceptible to nisin PV. However, in the study some strains (26.2%) showed equal or reduced susceptibility to nisin PV compared with nisin A despite harbouring the *nsr* gene. This is possibly due to strains harbouring the *nsr* gene but not expressing it, and therefore the strains remain susceptible to proteolytic cleavage by nisin. Alternatively, other mechanisms of nisin resistance, including expression of the ABC transporter NsrFP [22], regulatory systems (NsrR and NsrK) [21] and cell wall modifications such as D-alanylation of bacterial membranes [18], may also explain the varied responses to nisin and nisin PV observed in this study. Another possibility is that nisin PV may not be recognised by the regulatory TCS and therefore its presence does not induce production of NSR proteins. As little is known about the NsrR/K TCS of the *S. agalactiae nsr* operon, further analysis of the operon is required to confirm this hypothesis.

The global threat of antimicrobial resistance remains a significant issue for the treatment of infections, particularly those caused by multidrug-resistant pathogens [40]. The rate of antimicrobial resistance has surpassed the rate of discovery and development of new and effective antibiotics, resulting in a public-health emergency [41]. More than ever there is a need for alternatives to antibiotics, or indeed novel derivatives of existing antibiotics. However, the future of such antimicrobials necessitates continued studies to investigate the bioavailability of nisin and its derivatives in vivo [42].

To the best of our knowledge, this is the first investigation demonstrating nisin susceptibility within a clinical population of clinical *S. agalactiae* isolates. Furthermore, the study reports a derivative of nisin that exhibited enhanced activity against *S. agalactiae* isolates. Resistance to certain antibiotics for the treatment of *S. agalactiae* infections continues to rise, and published guidelines no longer recommend the use of clindamycin owing to high levels of resistance, indicating that there is a need for alternative therapies. Nisin and its derivatives could prove a viable option in these cases.

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Competing interests

None declared.

Ethical approval

Not required.

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