International Journal of Antimicrobial Agents 49 (2017) 668–676

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Review

A network perspective on antimicrobial peptide combination therapies: the potential of colistin, polymyxin B and nisin

Paula Jorge [a], Martín Pérez-Pérez [b], Gael Pérez Rodríguez [c], Maria Olívia Pereira [a], Anália Lourenço [b,c,*]

[a] Centre of Biological Engineering (CEB), Laboratory of Research in Biofilms Rosário Oliveira (LIBRO), University of Minho, Campus de Gualtar, Braga 4710-057, Portugal
[b] Escuela Superior de Ingeniería Informática (ESEI), Universidad de Vigo, Edificio Politecnico, s/n Campus As Lagoas, Ourense 32004, Spain
[c] Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, Braga 4710-057, Portugal

ARTICLE INFO

Article history:
Received 23 August 2016
Accepted 10 February 2017

Keywords:
Antimicrobial peptide combination
Bioinformatics
Network
Colistin
Polymyxin B
Nisin

ABSTRACT

Antimicrobial combinations involving antimicrobial peptides (AMPs) attract considerable attention within current antimicrobial and anti-resistance research. The objective of this study was to review the available scientific literature on the effects of antimicrobial combinations involving colistin (polymyxin E), polymyxin B and nisin, which are US Food and Drug Administration (FDA)-approved AMPs broadly tested against prominent multidrug-resistant pathogens. A bioinformatics approach based on literature mining and manual expert curation supported the reconstruction of experimental evidence on the potential of these AMP combinations, as described in the literature. Network analysis enabled further characterisation of the retrieved antimicrobial agents, targets and combinatory effects. This systematic analysis was able to output valuable information on the studies conducted on colistin, polymyxin B and nisin combinations. The reconstructed networks enable the traversal and browsing of a large number of agent combinations, providing comprehensive details on the organisms, modes of growth and methodologies used in the studies. Therefore, network analysis enables a bird's-eye view of current research trends as well as in-depth analysis of specific drugs, organisms and combinatory effects, according to particular user interests. The reconstructed knowledge networks are publicly accessible at http://sing-group.org/antimicrobialCombination/. Hopefully, this resource will help researchers to look into antimicrobial combinations more easily and systematically. User-customised queries may help identify missing and less studied links and to generate new research hypotheses.

1. Introduction

Antimicrobial agents have significantly improved the well-being and life expectancy of humans and animals, but their overuse has accelerated the emergence of multidrug-resistant (MDR) microorganisms and has raised an urgent need for novel antimicrobials [1]. Repurposing of natural compounds, such as antimicrobial peptides (AMPs), and the creation of synergistic antimicrobial combinations are two attractive and increasingly explored research approaches [2].

AMPs are widespread in nature as part of the immune system of plants and animals and can be also found in fungi and bacteria. In fact, AMPs played a fundamental role in the evolution of complex multicellular organisms and are currently still effective host defence agents [3]. In their majority, these peptides are short-length (between 15 and 30 amino acids), cationic, amphipathic, gene-encoded and directed to the cell membrane [4,5]. As single agents, the multiple mechanisms of action and the low specificity in terms of molecular targets reduce the propensity of AMP therapeutics to the development of antimicrobial resistance [4]; also, AMPs aid cellular processes such as cytokine release, chemotaxis, antigen presentation, angiogenesis and wound healing [5,6]. Synergistic combinations of AMPs with other antimicrobials often decrease individual effective concentrations and broaden the antimicrobial spectrum, whilst reducing antimicrobial resistance, toxicity and other side effects [2,7].

Most of these research outcomes are scattered across the ever-growing scientific bibliome, which impedes their systematic comparison. However, the development of computational workflows to integrate and analyse such textual information has the potential to automate compilation and to enable comprehensive data analysis. In previous work, we implemented the reconstruction of antimicrobial-centric knowledge networks based on literature mining and manual expert curation methodologies [8,9].

Here, our knowledge integration approach is applied to the study of polymyxins and bacteriocins, two families of AMPs widely used in healthcare and food-related studies. In particular, this paper discusses experimental findings retrieved from the scientific literature on antimicrobial combinations involving colistin (polymyxin E),

* Corresponding author. Escuela Superior de Ingeniería Informática (ESEI), Universidad de Vigo, Edificio Politecnico, s/n Campus As Lagoas, Ourense 32004, Spain. Fax: + 34 988 387001.
E-mail address: analia@uvigo.es (A. Lourenço).

http://dx.doi.org/10.1016/j.ijantimicag.2017.02.012
0924-8579/

polymyxin B and nisin, which are US Food and Drug Administration (FDA)-approved AMPs broadly tested against prominent MDR pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Candida albicans* [10–12].

Colistin, also known as polymyxin E, and polymyxin B belong to the polymyxin group of cationic polypeptides, i.e. cyclic, positively charged decapeptides bound to a fatty acid and derived from various species of *Paenibacillus* (*Bacillus*) *polymyxa* [11]; they differ in structure by only one amino acid, i.e. Leu in colistin versus Phe in polymyxin B [13]. The basic mechanism of action consists of disruption of the cell membrane by binding to the anionic part of the lipopolysaccharide (LPS). This causes a detergent effect with permeability changes in the cell envelope, leakage of cell contents and cell death [13,14]. The polymyxins are mainly active against Gram-negative pathogens, including major nosocomial pathogens such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa* and *Acinetobacter* spp. [11]. Colistin and polymyxin B are used as a last-resource treatment for infections caused by MDR Gram-negative bacteria, such as *P. aeruginosa* infections of the respiratory tract of cystic fibrosis patients [15,16].

Nisin is the main representative of the AMP class of lantibiotics (lanthionine-containing antibiotics) or class I bacteriocins. These small peptides (<5 kDa) are characterised by their unusual post-translationally modified residues (e.g. lanthionine or 3-methyllanthionine), which result in the formation of rings by covalent bonding with other amino acids [17]. Nisin was first isolated from *Lactococcus lactis* [18] and remains the only FDA-approved and commercially available bacteriocin, being normally used as a food additive [19]. In recent years, nisin has been increasingly studied in biomedical scenarios, exploring its ability to form poration complexes in cell membranes, mainly against Gram-positive bacteria [10,20]. Nisin has reported antimicrobial activity against major Gram-positive pathogens such as *L. monocytogenes* and *S. aureus* [21].

The three AMP-centric knowledge network reconstructions describe experimental results in an intuitive and user-customised way, enabling various analysis perspectives. AMP–drug combinations are described in terms of reported effects and experimental settings, e.g. strains, mode of growth and methodologies of analysis found in the literature. By focusing on various network features, we address different questions about the role of these AMPs in antimicrobial combinational therapy.

The reconstructed knowledge networks are publicly available at http://sing-group.org/antimicrobialCombination/.

2. Methods

2.1. Information retrieval

Information extracted from the literature using text mining methods was integrated with data from curated databases to reconstruct experimental evidence on the antimicrobial combinations of colistin, polymyxin B and nisin in a comprehensive way. The curation pipeline is depicted in Supplementary Fig. S1.

Emphasis was put on experimentally validated combinations involving any of the three AMPs and drugs, other AMPs or molecules with added antimicrobial potential. To this end, the scope of the PubMed queries was narrowed to experiments mentioning at least one of the three AMPs (common name or name variants), any term variants denoting common agent combination effects (e.g. synergy, antagonism) and experimental methods used in testing antimicrobial combination susceptibility (e.g. chequerboard method). PubMed document retrieval, relevance assessment and initial annotation of entities of interest (namely AMPs, drugs and molecules with antimicrobial potential) were conducted automatically. Then, the prioritised documents were manually curated. Notably, experts validated the relevance of the documents and annotated additional information (e.g. organisms, strains, mode of growth and experimental tests) on truly relevant documents. Curators also revised automatic normalisation of textual references to antimicrobial agents to existing ontology terms, namely DrugBank [22], PubChem [23], ChEBI [24], ChEMBL [25], LAMP [26], $CAMP_{R3}$ [27] and UniProt [28] database entries.

2.2. Data organisation and presentation

The annotated combinations are categorised as follows: 'synergy', i.e. the combined action is superior to the sum of the isolated actions; 'additiveness', i.e. the combined action is equal to the sum of the isolated actions; 'indifference', i.e. the combined action is equal to the action of the most active single agent; and 'antagonism', i.e. the combined action is inferior to the action of the most active single agent. Other categories, named 'synergy/additiveness', 'additiveness/indifference' and 'antagonism/indifference', are used to denote non-conclusive results.

Categorisation of the combinations was dependent on the type of methodology described in the paper. The two main standardised methods in use are the chequerboard assay, with fractional inhibitory concentration index (FICI) and fractional bactericidal concentration index (FBCI) assessments, and time–kill curves. In the case of the chequerboard assays, the common interpretation of the breakpoint values is as follows: 'synergy (S)', FICI or FBCI ≤ 0.5; 'additiveness (Ad)', 0.5 < FICI or FBCI ≤ 1; 'indifference (I)', 1 < FICI or FBCI ≤ 4; and 'antagonism (A)', FICI or FBCI > 4.0 [29]. In the case of time–kill curves, the action of the combinations is compared with the action of the most active individual agent and interpretation is as follows: 'synergy (S)', ≥2 log decrease; 'additiveness (Ad)', 1 ≤ log <2 decrease; 'indifference (I)', <1 log decrease; and 'antagonism (A)', ≥2 log increase [30].

Annotation of the combination category was primarily based on the textual descriptions presented in the paper. However, this was not always possible due to discrepancies between studies, including different types of analysis and different interpretations of the results, which did not always result in the above classification. In these cases, and in order to maintain a systematic and harmonised annotation, the experts used the above definitions in order to curate those results.

The reconstructed knowledge networks are publicly accessible at http://sing-group.org/antimicrobialCombination/. Network web visualisation is supported by Cytoscape Web v.2.6.1 [31] and advanced analyses are conducted in Cytoscape v.3.4.0 [32].

2.3. Data analysis

Data are analysed in relation to different types of totals, namely the number of total combinations (TC), the number of combinations across documents and species (CDS) and the number of combinations across documents (CD). TC represents all the combinations that are presently annotated in our knowledgebase, encompassing all documents, species and respective strains tested. This number is used, for example, to calculate the % synergy or other type of outcome for a given organism or drug. CDS counts antimicrobial combinations by unique species targets, i.e. it ignores tests of the same combination on multiple strains of the same species. This figure is important to calculate statistics such as the most tested organisms. Finally, CD computes unique antimicrobial combinations in order to assess the most used drugs. Other totals are used to present relative data and are described in the corresponding table caption/footnote.

3. Results and discussion

3.1. Overview

The number of documents retrieved from the literature was highest for colistin, followed by polymyxin B and nisin, with an

Table 1
General statistics on retrieved and annotated documents.

Antimicrobial peptide	No. of documents in query	No. (%) of relevant documents[a]	Total combinations (TC)	Combinations across documents and species (CDS)	Combinations across documents (CD)
Colistin	374	187 (50)	2829	352	231
Polymyxin B	278	100 (36)	993	367	220
Nisin	184	130 (71)	900	464	263

[a] The % is relative to the total number of documents in query.

approximate difference of 100 documents from one another (Table 1). The number of relevant documents was also greater for colistin, followed by nisin and polymyxin B. Interestingly, the TC was much higher for colistin than for the other two AMPs. However, when analysing the CDS, colistin has the lowest figure, which indicates that studies using colistin tend to test susceptibility over more strains.

Historically, the number of combination studies involving colistin, polymyxin B or nisin has grown exponentially, reflecting the interest that these studies have been receiving from researchers. The majority of documents retrieved from the literature are dated after 2000. It is also clear that the present tendency is still of growth, since the number of documents in the last 5 years is close (polymyxin B) or already higher (colistin and nisin) than the total achieved in the previous decade. More details are available in Supplementary Fig. S2.

Given the fact that colistin is already used as an antibiotic in clinical settings, it was expected that the great majority of studies included a varied array of clinically isolated strains. In fact, 90% of the strains used in the annotated colistin combinations are clinical isolates (data not shown). Similarly, 67% of the strains used for testing polymyxin B combinations are also clinical isolates (data not shown). In turn, only 40% of strains used for testing nisin combinations are clinical isolates, a fact that may be justified by the recent application of this food additive to biomedical scenarios [10].

In general, AMPs are mainly combined with antibiotics and antifungals (77%, 66% and 32% for each of the three AMPs, respectively), and AMP–AMP combinations represent just a small fraction of all the combinations tested. Current interest on the repurposing of antibiotics by combination with other antimicrobials or antimicrobial adjuvants can explain these percentages [7]. AMPs are known to disrupt the bacterial membrane [5], which makes them excellent partners for antibiotics whose antimicrobial action affects intracellular targets, by facilitating their entrance into the cell. In contrast to the other two AMPs, combinations of nisin with biomedical-associated drugs, such as antibiotics and antifungals, do not yet represent the main focus of the tests (<50%). This may relate with the fact that nisin is mainly used as a food additive and its biomedical application has only been explored in recent years [10]. More details can be found in Supplementary Table S1.

Other interesting data are that AMP combinations are still primarily tested on planktonic cultures (82–97%). That is, experimental results are somewhat limited in terms of describing effects on real-world scenarios, namely over microbial biofilms (0.9–5.7%). Most bacteria are naturally present in a biofilm mode of growth and these consortia are related to persistent and chronic infections [33] and possess multiple resistance mechanisms that challenge eradication [34]. Therefore, testing AMP combinations over these microbial growth scenarios is an urgent necessity. In fact, AMPs have some characteristics that make them promising for treating biofilms, namely the fact that their main mechanism of action is independent of the cell's metabolic state (i.e. they are directed towards the membrane). This makes them effective against active and dormant cells, which are common types of cell populations in mature biofilms [35].

Moreover, the majority of the reported combinations have a synergistic effect, which may reflect both the predisposition of AMPs to be good adjuvants in antibiotic therapy [2] and the fact that scientific papers often tend to report only/majorly positive outcomes.

3.2. Web search and visualisation

The reconstructed networks are publicly accessible at http://sing-group.org/antimicrobialCombination/. The web interface supports user-customised network visualisation, search and navigation (Fig. 1). Network presentation was made simple and intuitive: the antimicrobial agents are displayed as nodes and the antimicrobial combinations are represented by the edges linking those nodes. The user may search combinations using different filter levels, namely find information by organism, antimicrobial agent or type of combination. Furthermore, different filters may be combined in more advanced searches. For example, this approach can be used to identify direct and indirect relations between two antimicrobial agents, or to find all combinations that produced a given effect in a specific organism.

For visualisation simplicity, the shape of the nodes stands for the type/family of the antimicrobial agents, and edges are coloured according to the effect of the combinations. Moreover, the interface takes advantage of topological metrics to highlight the representativeness and interconnection of antimicrobial agents. Specifically, node size is dependent of the node degree, i.e. node size is scaled according to the number of combinations that support its presence in the network.

3.3. Network content analysis

Table 2 presents the most annotated agents for each of the AMPs. The following subsections make an in-depth analysis of these annotations for each of the AMP networks.

Table 2
Top three antimicrobials, organisms and methods co-annotated with the antimicrobial peptides (AMPs) colistin, polymyxin B and nisin.

AMP	Combined AMP/drug[a]	Organism[b]	Experimental method[a]
Colistin	Rifampicin (7.8%)	*Pseudomonas aeruginosa* (32%)	Chequerboard assay (53%)
	Tigecycline (4.8%)	*Acinetobacter baumannii* (15%)	Time–kill curve (31%)
	Meropenem (4.3%)	*Klebsiella pneumoniae* (8.2%)	Etest (14%)
Polymyxin B	Rifampicin (5.0%)	*P. aeruginosa* (29%)	Chequerboard assay (37%)
	Erythromycin (4.6%)	*Escherichia coli* (14%)	MIC determination (28%)
	Novobiocin (4.1%)	*K. pneumoniae* (12%)	Time–kill curve (20%)
Nisin	EDTA (3.8%)	*Listeria monocytogenes* (16%)	Cell viability (41%)
	Vancomycin (2.3%)	*Staphylococcus aureus* (14%)	Chequerboard assay (24%)
	NaCl (2.3%)	*E. coli* (9.9%)	Cell growth (11%)

MIC, minimum inhibitory concentration; EDTA, ethylene diamine tetra-acetic acid.
[a] % relative to CD (see Table 1).
[b] % relative to CDS (see Table 1).

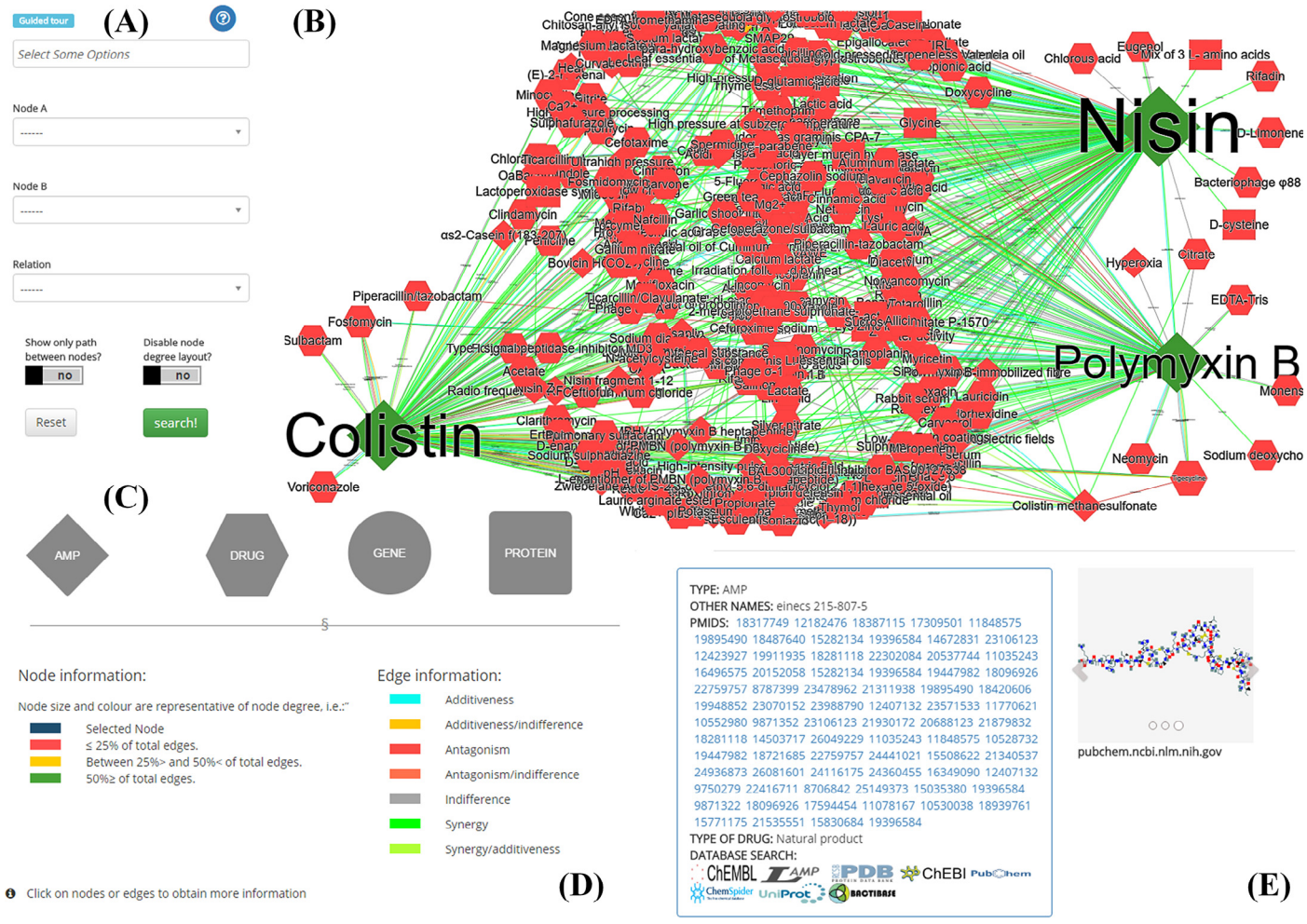

Fig. 1. Web visualisation of the antimicrobial combination networks of colistin, polymyxin B and nisin (B). Node shape corresponds to antimicrobial agent type (C). Edges are coloured according to the effect of the reported combination (D). Networks may be searched by organism, antimicrobial agent or combination effect (A). Both nodes and edges are clickable and enable the display of additional information, e.g. alternative agent names, chemical activity, cross-links to chemical and other external data sources (E).

3.3.1. *Colistin combinations*

Colistin is predominantly combined with the antibiotics rifampicin, tigecycline and meropenem (Table 2), which represent three distinct classes of antibiotics (rifamycins, glycylcyclines and carbapenems, respectively). All of these antibiotics possess intracellular targets, such as the bacterial DNA-dependent RNA polymerase, causing inhibition of RNA synthesis [36], the 30S ribosomal subunit, causing inhibition of protein synthesis [37], and the penicillin-binding proteins, causing inhibition of cell wall synthesis [38], respectively. The combination of colistin with intracellular-acting antibiotics was somewhat expected since the action of this AMP, i.e. membrane disruption, can help other antibiotics to enter the cell more easily. Chequerboard assays and time–kill curves are the most used test methods in these studies (Table 2), which is in accordance with the most used standardised methodologies for synergy testing [39].

Currently, the network reconstructed for colistin (Fig. 2) includes a total of 2829 antimicrobial combinations, which were extracted from 187 documents. Most of the combinations involving colistin are tested against Gram-negative bacteria (Table 3). This was somewhat expected given that the mechanism of action of colistin is more specific for this type of bacteria by targeting LPS, which is a major constituent of the outer membrane of these bacteria [11,13]. Infections by fungi and Gram-positive bacteria are addressed by a small fraction of the combinations (17%). *P. aeruginosa* is the most represented Gram-negative bacteria with 743 colistin combinations across 59 documents, 33% of which have synergistic effects. At the top of colistin combinations against *P. aeruginosa* is the combination with rifampicin (7.2% of the number of combinations across documents for that species).

S. aureus is the most Gram-positive bacteria used in these tests. Specifically, four documents describe the testing of seven combinations, with 57% of them resulting in synergy. These combinations involved six different drugs, three of which were antibiotics (namely ciprofloxacin, sodium sulfadiazine and erythromycin). In turn, *C. albicans* is the most tested fungi, with 12 colistin combinations annotated across two documents, with 75% of them resulting in synergy. Colistin was mainly combined with the antifungal caspofungin (38% of the number of combinations across documents for that species).

Apparently, there is no obvious correlation between the type of organism and the per cent of synergy or antagonism results obtained. Some of the less tested organisms showed more positive outcomes; however, the number of documents was substantially lower, which impairs the establishment of a statistically significant correlation. It is known that AMPs are able to enlarge the antimicrobial spectrum of certain antibiotics [2], which could explain the high per cent synergy seen with colistin combinations both for Gram-positive bacteria and fungi.

Analysis of the targets of the more and less successful combinations with colistin is presented in Supplementary Table S2. Apparently, the specific targets of the combined agents do not

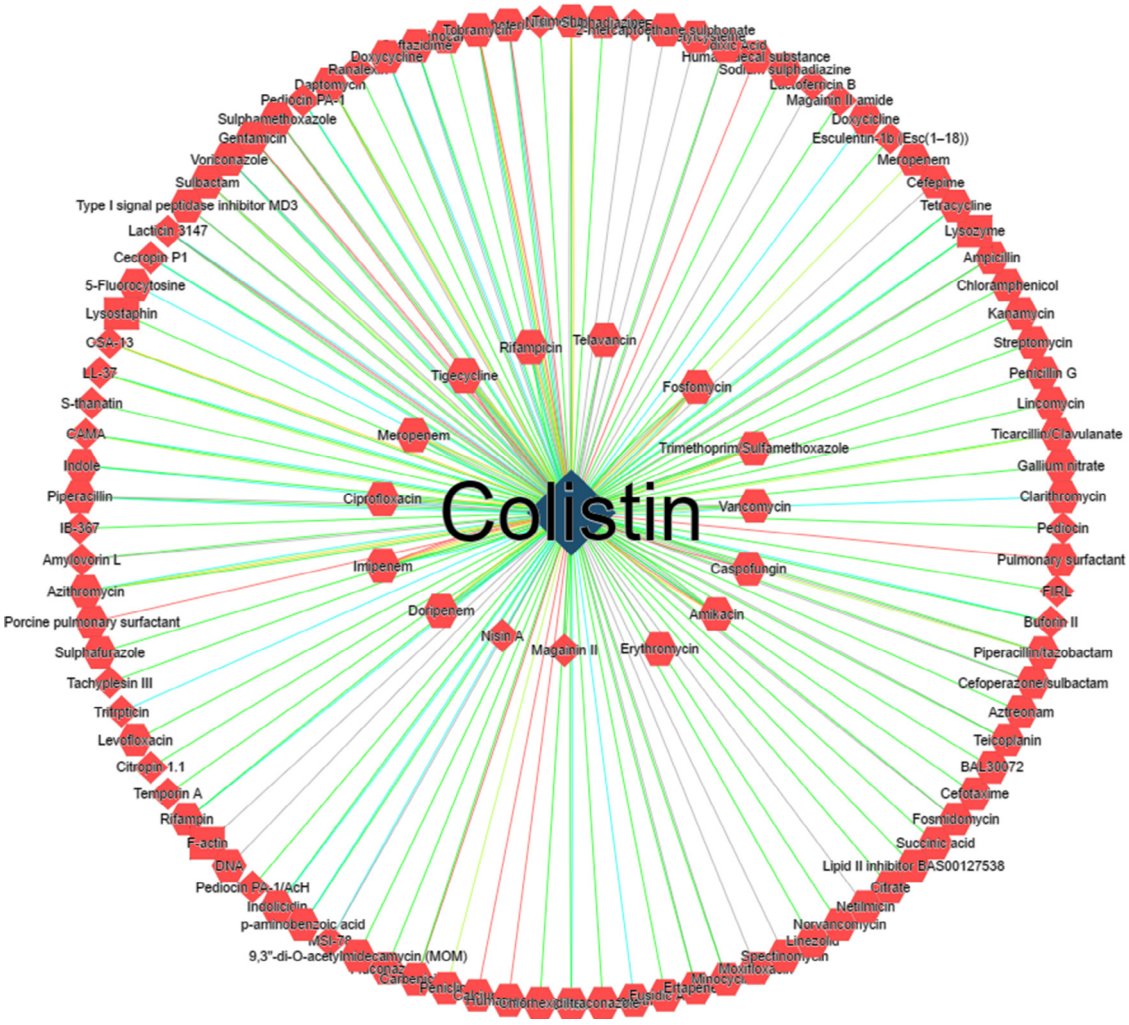

Fig. 2. Antimicrobial combination network reconstructed for colistin.

Table 3
Distribution of organisms and synergistic/antagonistic (Syn/Ant) combinations in the antimicrobial combination network of colistin.

Type of organism (%)[a]	Top 3 (%)[a]	% Syn/% Ant[b]	No. of documents (%)[c]
Gram-negative bacteria (83%)	*Pseudomonas aeruginosa* (32%)	33/1.1	59 (50%)
	Acinetobacter baumannii (15%)	45/1.0	29 (24%)
	Klebsiella pneumoniae (8.2%)	34/11	18 (15%)
Gram-positive bacteria (7.5%)	*Staphylococcus aureus* (1.7%)	57/0	4 (3.4%)
	Listeria monocytogenes (0.6%)	88/0	1 (0.8%)
	Actinomyces spp., *Bacillus cereus*, *Bifidobacterium adolescentis*, *Clostridium* spp., *Enterococcus faecium*, *Eubacterium limosum*, *Lactobacillus* spp., *Peptococcus* spp., *Peptostreptococcus* spp., *Streptococcus* spp. (0.28%)	5/0	2 (1.7%)
Fungi (9.5%)	*Candida albicans* (2.3%)	75/0	2 (1.7%)
	Pseudallescheria boydii, *Pseudallescheria apiospermum*, *Scedosporium prolificans*, *Geosmithia argillacea*, *Exophiala dermatitidis* (0.9%)	9.0/14	1 (0.8%)
	Batrachochytrium salamandrivorans (0.6%)	100/0	1 (0.8%)

[a] % relative to CDS (see Table 1).
[b] % relative to TC (see Table 1) for that species.
[c] % relative to the number of relevant documents.

correlate with the four different combination categories since the top three targets are equal and/or similar amongst them. In the case of synergistic combinations, all of the targets are also reported for the other combination categories. This might suggest that the synergy amongst colistin and other agents is not dependent on the target of the latter but rather on their mechanism of entering the cell and the efficacy of colistin to facilitate that entrance depending on the selected organism/conditions.

3.3.2. *Polymyxin B combinations*

Polymyxin B is mainly combined with antibiotics belonging to three different classes, i.e. rifamycins (rifampicin), macrolides

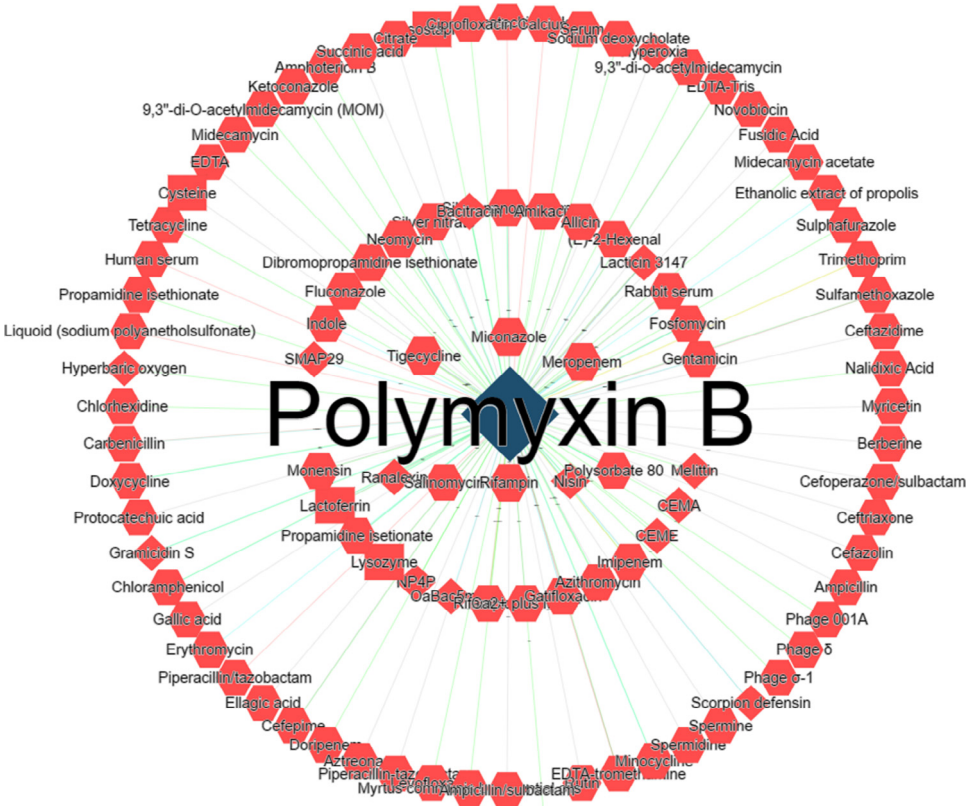

Fig. 3. Antimicrobial combination network reconstructed for polymyxin B.

(erythromycin) and aminocoumarins (novobiocin) (Table 2). Apparently, combinations that use polymyxins tend to use rifampicin, as seen in the case of colistin. These antibiotics act intracellularly, namely on the bacterial DNA-dependent RNA polymerase, causing inhibition of RNA synthesis [36], the 23S rRNA molecule in the 50S ribosomal subunit, causing inhibition of protein synthesis [40], and the bacterial DNA gyrase, causing inhibition of DNA supercoiling [41], respectively. The use of intracellular-acting antibiotics in these combinations follows the same line of reasoning as in the case of colistin, as previously explained. Polymyxin B combinations are tested mainly through chequerboard assay and minimum inhibitory concentration (MIC) determination (Table 2).

Currently, the network reconstructed for polymyxin B (Fig. 3) has 993 antimicrobial combinations, which were extracted from 100 documents. Polymyxin B combinations are mainly tested on Gram-negative bacteria (Table 4), probably due to its mechanism of action, which is similar to colistin and affects the outer membrane of Gram-negative bacteria [11,13]. Testing in Gram-positive bacteria and fungi represents only 20% of the targeted organisms. Similar to colistin, the most tested Gram-negative and Gram-positive bacteria and fungi were *P. aeruginosa*, *S. aureus* and *C. albicans*, respectively. In the case of *P. aeruginosa*, 317 polymyxin B combinations were annotated across 43 documents, with 54% of them showing synergy. Here, polymyxin B was mostly combined with novobiocin (3.7% of the number of combinations across documents for that species).

S. aureus was used to test 24 polymyxin B combinations across 14 documents, and 50% of them showed synergy. The most combined drugs were the antibiotic neomycin, the AMP bacitracin, the protein lysostaphin and the antifungal miconazole, each of which representing 9.5% of the number of combinations across documents for that species.

A total of eight polymyxin B combinations, belonging to five different documents, were tested against *C. albicans*, and all demonstrated synergistic outcomes. These combinations involved eight different drugs, four of which are antifungals, i.e. amphotericin B, ketoconazole, miconazole and fluconazole. The majority of the outcomes for all organisms were positive, which further illustrates the potential of the AMP combinations.

Analysis of the targets of the more and less successful combinations with polymyxin B is presented in Supplementary Table S3. Similar to colistin, all top three targets for synergistic combinations are also reported for the other combination categories, namely indifference. Since the mechanisms of action of colistin and polymyxin B are similar, this was somewhat expected. Notably, synergy between polymyxin B and other agents appears independent of the target but might be correlated with other agent features, such as the mechanism of cell entrance and the efficacy of polymyxin B to facilitate that entrance.

Table 4
Distribution of organisms and synergistic/antagonistic (Syn/Ant) combinations in the antimicrobial combination network of polymyxin B.

Type of organism (%)[a]	Top 3 (%)[a]	% Syn/% Ant[b]	No. of documents (%)[c]
Gram-negative bacteria (80%)	*Pseudomonas aeruginosa* (30%)	54/3.2	43 (45%)
	Escherichia coli (14%)	70/2.4	30 (32%)
	Klebsiella pneumoniae (12%)	60/0	13 (14%)
Gram-positive bacteria (12%)	*Staphylococcus aureus* (5.7%)	50/0	14 (15%)
	Bacillus subtilis (1.6%)	33/0	4 (4.2%)
	Staphylococcus epidermidis (0.8%)	67/0	2 (2.1%)
Fungi (8.0%)	*Candida albicans* (2.2%)	100/0	5 (5.3%)
	Saccharomyces cerevisiae (1.6%)	100/0	5 (5.3%)
	Aspergillus niger (0.8%)	67/0	2 (2.1%)

[a] % relative to CDS (see Table 1).
[b] % relative to TC (see Table 1) for that species.
[c] % relative to the number of relevant documents.

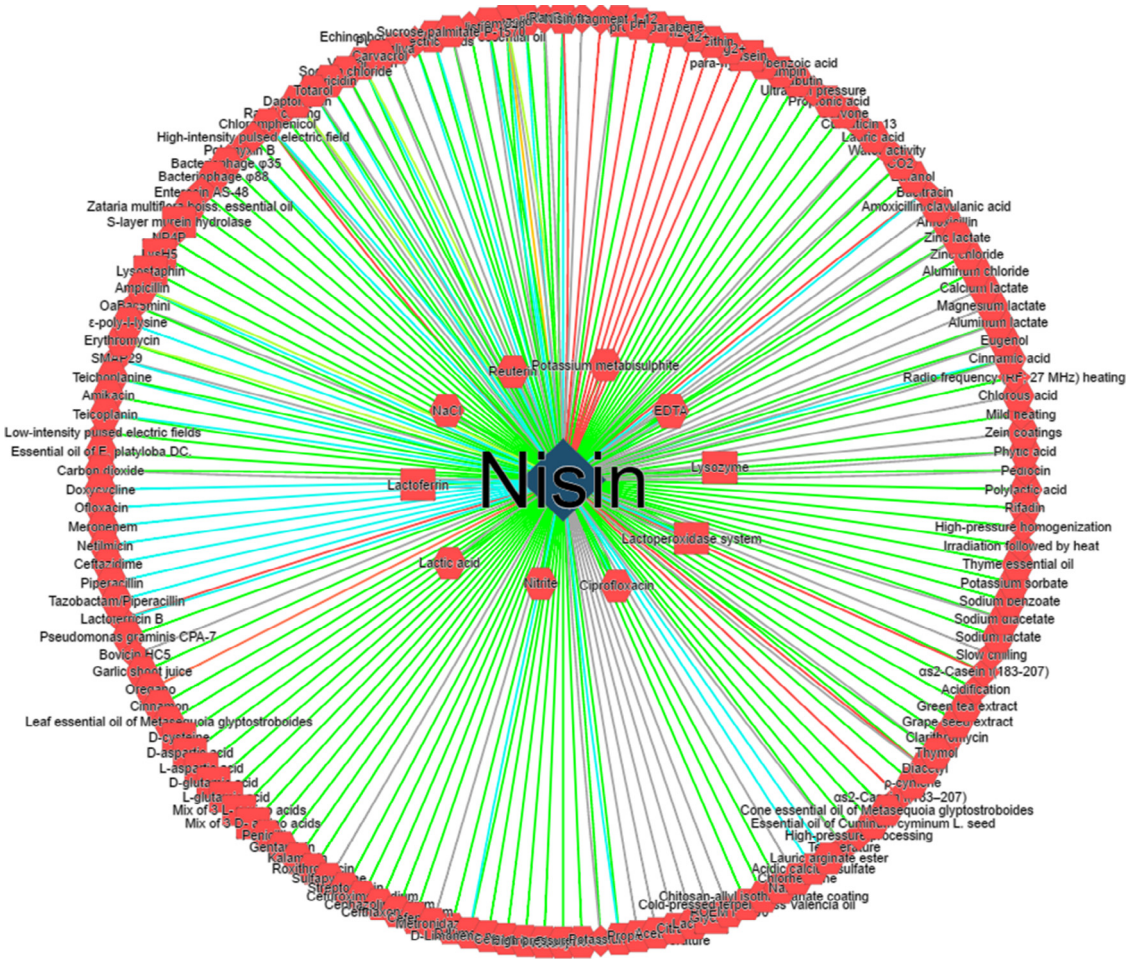

Fig. 4. Antimicrobial combination network reconstructed for nisin.

3.3.3. *Nisin combinations*

Nisin was primarily combined with three different types of compounds: a surfactant [ethylene diamine tetra-acetic acid (EDTA)]; an antibiotic (vancomycin); and a salt (NaCl) (Table 2). This is probably due to the role of nisin as a food addictive [21], which changes the types of agents that this AMP can be combined with in comparison with the polymyxins. Nevertheless, combination with antibiotics for application in biomedical scenarios is attracting increasing attention [10]. The salts are used to test the stability of nisin in different ionic concentrations, which obviously varies amongst foods and can dictate the effectiveness of the AMP [42]. Finally, EDTA is a chelating agent and an FDA-approved food additive used to prevent oxidation and other deteriorations. It also has antimicrobial activity and it is known to potentiate the activity of nisin and other antimicrobials against Gram-negative micro-organisms [43]. For nisin, the chequerboard assay was also one of the most used methods, alongside cell viability analysis.

Currently, the network reconstructed for nisin (Fig. 4) encompasses 900 antimicrobial combinations, which were extracted from 130 documents. In contrast to the polymyxins, the combinations involving nisin were tested in their majority against Gram-positive bacteria (Table 5), which is the main target of this peptide [20]. Still, the testing of combinations against Gram-negative bacteria represented a sizable proportion (32%), with fungi being the least tested organisms. *E. coli*, *L. monocytogenes* and *Saccharomyces cerevisiae* were the most used Gram-negative bacteria, Gram-positive bacteria and fungi, respectively, and all of them are considered food pathogens [44]. A total of 50 nisin combinations were tested against *E. coli* across 25 documents, and 44% of them demonstrated synergy. Nisin is mainly combined with three different compounds, namely EDTA, lactoferrin and colistin, each of which represents 6.5% of the number of combinations across documents for that species. *L. monocytogenes* is used in 107 combination tests across 41 documents, and 62% of

Table 5
Distribution of organisms and synergistic/antagonistic (Syn/Ant) combinations in the antimicrobial combination network of nisin.

Type of organism (%)[a]	Top 3 (%)[a]	% Syn/% Ant[b]	No. of documents (%)[c]
Gram-negative bacteria (32%)	*Escherichia coli* (9.9%)	44/8.0	25 (22%)
	Salmonella enterica (6.7%)	24/8.8	15 (14%)
	Pseudomonas aeruginosa (3.7%)	31/0	4 (3.6%)
Gram-positive bacteria (66%)	*Listeria monocytogenes* (16%)	62/0	41 (37%)
	Staphylococcus aureus (14%)	32/9.1	31 (28%)
	Enterococcus faecalis (5.8%)	42/0	3 (2.7%)
Fungi (1.7%)	*Saccharomyces cerevisiae* (0.6%)	67/0	2 (1.8%)
	Mesophiles, *Aspergillus parasiticus*, *Zygosaccharomyces bailii*, *Fusarium moniliforme*, *Aspergillus ochraceus* (0.2%)	80/20	3 (2.7%)
	–[d]	–	–

[a] % relative to CDS (number of combinations across documents and species).
[b] % relative to TC (number of total combinations) for that species.
[c] % relative to the number of relevant documents.
[d] Indicates there were no more combinations.

the outcomes were of synergy. Here, nisin was combined more frequently with the lactoperoxidase system and EDTA (5.5% of the number of combinations across documents for that species).

Finally, for *S. cerevisiae*, there are three combinations across two documents, and 67% of them were synergistic. Nisin was combined with two formulations of a drug, namely D-limonene and D-limonene nanoemulsion, and was also combined with a method, namely ultrahigh pressure. In contrast to the polymyxins, nisin was frequently tested alongside physical methods such as high pressure or pulsed electric fields in order to improve food preservation [42].

Analysis of the targets of the more and less successful combinations with nisin is presented in Supplementary Table S4. Unlike the case for colistin and polymyxin B combinations, some of top targets are unique of synergistic combinations, i.e. they are not repeated in the other combination category's targets. These unique targets, namely the 50S ribosomal protein L10, the DNA-directed RNA polymerase subunit β and the DNA-directed RNA polymerase subunit β′, could be used as 'guidance' to test other agents with equal or similar targets and to validate the possibility of additional synergistic combinations with nisin. This type of directed approach has more probability of success and reduces costs in terms of time and resources, which are often significant in combination studies.

4. Conclusions

This work presented an integrative knowledge methodology for the reconstruction of relevant experimental results on antimicrobial combination tests, based on text mining and network mining methods and techniques. This methodology enabled the reconstruction of antimicrobial combinations involving colistin, polymyxin B and nisin and supports its periodical update, i.e. the curation of new publications on these topics.

This methodology holds great potential in mining combination networks for other AMPs. The aim would be to retrieve documents with similar text 'profiles', i.e. with similar core contents and semantics. So, the only adjustment would be changing the name of the AMP in question, alongside any possible synonyms or name variants, in the PubMed query and thus retrieve new pools of potentially relevant articles (i.e. describing experimental testing of combinations involving that AMP). Therefore, in the near future, the database will cover combination networks for a broader scope of AMPs.

Both the continuous update and broader scope of the database will be invaluable for exploring the possibility of building mathematical models capable of inferring other novel combinations that could be further experimentally validated. Such mathematical modelling will likely incorporate various pharmacological/biochemical features on both the agents and the reported combinations and will emerge from the testing of multiple data mining algorithms.

Funding: This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 [POCI-01-0145-FEDER-006684] and BioTecNorte operation [NORTE-01-0145-FEDER-000004], funded by the European Regional Development Fund under the scope of Norte2020—Programa Operacional Regional do Norte. The authors also acknowledge the support received from FCT and the European Community fund FEDER, through Program COMPETE, under the scope of the Project RECI/BBB-EBI/0179/2012 [FCOMP-01-0124-FEDER-027462], the [14VI05] Contract-Programme from the University of Vigo (Vigo, Spain), the INOU-16-05 project from the Provincial Council of Ourense, and the Agrupamento INBIOMED from DXPCTSUG-FEDER unha maneira de facer Europa [2012/273]. SING group thanks CITI (Centro de Investigación, Transferencia e Innovación) from University of Vigo for hosting its IT infrastructure. Finally, the authors acknowledge the PhD grant of Paula Jorge [grant no. SFRH/BD/88192/2012], funded by FCT, the PhD grants of Martín Pérez-Pérez and Gael Pérez-Rodríguez, funded by the Xunta de Galicia and the University of Vigo, and the Research grant 2014 of Anália Lourenço by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).

Competing interests: None declared.

Ethical approval: Not required.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2017.02.012.

References

[1] O'Neill J. Review on antimicrobial resistance. Antimicrobial resistance: tackling a crisis for the future health and wealth of nations. London, UK: HM Government/Wellcome Trust; 2014.
[2] Pletzer D, Coleman SR, Hancock RE. Anti-biofilm peptides as a new weapon in antimicrobial warfare. Curr Opin Microbiol 2016;33:35–40.
[3] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415:389–95.
[4] Jorge P, Lourenço A, Pereira MO. New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 2012;28:1033–61.
[5] Pletzer D, Hancock REW. Anti-biofilm peptides: potential as broad-spectrum agents. J Bacteriol 2016;198:2572–8.
[6] Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 2009;30:131–41.
[7] Worthington RJ, Melander C. Combination approaches to combat multidrug-resistant bacteria. Trends Biotechnol 2013;31:177–84.
[8] Jorge P, Pérez-Pérez M, Rodríguez GP, Fdez-Riverola F, Pereira MO, Lourenço A. Reconstruction of the network of experimentally validated AMP–drug combinations against *Pseudomonas aeruginosa* infections. Curr Bioinform 2016;5:523–30.
[9] Jorge P, Pereira MO, Lourenço A. Networking the way towards antimicrobial combination therapies. In: 8th international conference on practical applications of computational biology & bioinformatics (PACBB 2014). Advances in Intelligent Systems and Computing; 2014. 294, p. 201–6.
[10] Shin JM, Gwak JW, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Biomedical applications of nisin. J Appl Microbiol 2016;120:1449–65.
[11] Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. Clin Microbiol Rev 2008;21:449–65.
[12] Velkov T, Roberts KD, Nation RL, Thompson PE, Li J. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. Future Microbiol 2013;8:711–24.
[13] Velkov T, Thompson PE, Nation RL, Li J. Structure–activity relationships of polymyxin antibiotics. J Med Chem 2010;53:1898–916.
[14] Falagas ME, Kasiakou SK. Colistin: the revival of polymyxins for the management of multidrug-resistant Gram-negative bacterial infections. Clin Infect Dis 2005;40:1333–41.
[15] Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589–601.
[16] Cassir N, Rolain J-M, Brouqui P. A new strategy to fight antimicrobial resistance: the revival of old antibiotics. Front Microbiol 2014;5:551.
[17] Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Fact 2014;13(Suppl. 1):S3.
[18] Mattick ATR, Hirsch A. Further observations on an inhibitory substance (nisin) from lactic streptococci. Lancet 1947;2:5–8.
[19] Juneja VK, Dwivedi HP, Yan X. Novel natural food antimicrobials. Annu Rev Food Sci Technol 2012;3:381–403.
[20] Islam MR, Nagao J-I, Zendo T, Sonomoto K. Antimicrobial mechanism of lantibiotics. Biochem Soc Trans 2012;40:1528–33.
[21] Tong Z, Ni L, Ling J. Antibacterial peptide nisin: a potential role in the inhibition of oral pathogenic bacteria. Peptides 2014;60:32–40.
[22] Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, et al. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res 2006;34(Database issue):D668–72.
[23] Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem substance and compound databases. Nucleic Acids Res 2016;44(Database issue):D1202–13.
[24] Hastings J, De Matos P, Dekker A, Ennis M, Harsha B, Kale N, et al. The ChEBI reference database and ontology for biologically relevant chemistry: enhancements for 2013. Nucleic Acids Res 2013;41(Database issue):D456–63.
[25] Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, et al. The ChEMBL bioactivity database: an update. Nucleic Acids Res 2014;42(Database issue):D1083–90.
[26] Zhao X, Wu H, Lu H, Li G, Huang Q. LAMP: a database linking antimicrobial peptides. PLoS ONE 2013;8:e66557.

[27] Waghu FH, Barai RS, Gurung P, Idicula-Thomas S. $CAMP_{R3}$: a database on sequences, structures and signatures of antimicrobial peptides. Nucleic Acids Res 2016;44(Database issue):D1094–7.
[28] UniProt Consortium. UniProt: a hub for protein information. Nucleic Acids Res 2015;43(Database issue):D204–12.
[29] Saiman L. Clinical utility of synergy testing for multidrug-resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis: 'the motion for. Paediatr Respir Rev 2007;8:249–55.
[30] White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time–kill, checkerboard, and E test. Antimicrob Agents Chemother 1996;40:1914–18.
[31] Lopes CT, Franz M, Kazi F, Donaldson SL, Morris Q, Bader GD. Cytoscape Web: an interactive web-based network browser. Bioinformatics 2010;26:2347–8.
[32] Saito R, Smoot ME, Ono K, Ruscheinski J, Wang P-L, Lotia S, et al. A travel guide to Cytoscape plugins. Nat Methods 2012;9:1069–76.
[33] Bjarnsholt T, Alhede M, Alhede M, Eickhardt-Sørensen SR, Moser C, Kühl M, et al. The in vivo biofilm. Trends Microbiol 2013;21:466–74.
[34] de la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock R. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol 2013;16:580–9.
[35] Strempel N, Strehmel J, Overhage J. Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. Curr Pharm Des 2015;21:67–84.
[36] Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 2001;104:901–12.
[37] Rose WE, Rybak MJ. Tigecycline: first of a new class of antimicrobial agents. Pharmacotherapy 2006;26:1099–110.
[38] Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. Antimicrob Agents Chemother 2011;55:4943–60.
[39] Doern CD. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. J Clin Microbiol 2014;52:4124–8.
[40] Mazzei T, Mini E, Novelli A, Periti P. Chemistry and mode of action of macrolides. J Antimicrob Chemother 1993;31(Suppl.C):1–9.
[41] Sugino A, Higgins NP, Brown PO, Peebles CL, Cozzarelli NR. Energy coupling in DNA gyrase and the mechanism of action of novobiocin. Proc Natl Acad Sci USA 1978;75:4838–42.
[42] Gálvez A, Abriouel H, López RRL, Ben Omar N. Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 2007;120:51–70.
[43] Branen J, Davidson P. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. Int J Food Microbiol 2004;90:63–74.
[44] Adam K, Brülisauer F. The application of food safety interventions in primary production of beef and lamb: a review. Int J Food Microbiol 2010;141(Suppl. 1):S43–52.