Systematic Review

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Future Medicinal Chemistry

Efficacy of natural antimicrobial peptides versus peptidomimetic analogues: a systematic review

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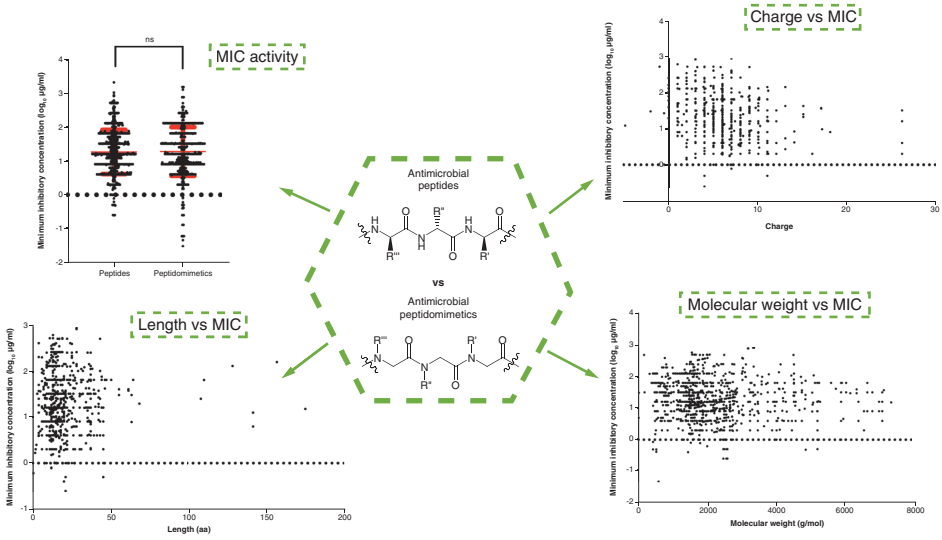
Aims: This systematic review was carried out to determine whether synthetic peptidomimetics exhibit significant advantages over antimicrobial peptides in terms of *in vitro* potency. Structural features – molecular weight, charge and length – were examined for correlations with activity. **Methods:** Original research articles reporting minimum inhibitory concentration values against *Escherichia coli*, indexed until 31 December 2020, were searched in PubMed/ScienceDirect/Google Scholar and evaluated using mixed-effects models. **Results:** *In vitro* antimicrobial activity of peptidomimetics resembled that of antimicrobial peptides. Net charge significantly affected minimum inhibitory concentration values ($p < 0.001$) with a trend of 4.6% decrease for increments in charge by +1. **Conclusion:** AMPs and antibacterial peptidomimetics exhibit similar potencies, providing an opportunity to exploit the advantageous stability and bioavailability typically associated with peptidomimetics.

Graphical abstract:



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Keywords: antibiotic • antimicrobial resistance • Gram-negative • peptides • peptidomimetics

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Antimicrobial peptides (AMPs), also commonly denoted as host-defense peptides, form a fundamental part of the innate immune response mounted by all higher life forms against pathogenic bacteria, fungi, parasites and enveloped viruses. In addition, some AMPs exert anticancer cytotoxic effects. Importantly, AMPs may provide a valuable source of lead compounds for novel antibiotics/potentiators based on their key roles in the innate immune system in most multicellular organisms [1,2].

The majority of AMPs identified to date can be divided into four main categories based on their secondary structure: β-sheet, α-helix and extended and loop conformations – with α-helical peptides appearing to be the most abundant/studied to date. Although these AMPs share several common features (albeit consisting of a limited number of overall structural motifs), their sequences, activities and targets differ considerably [3]. The mode of action (MoA) of AMPs is dependent on specific physico-chemical properties, including the sequence, content of certain amino acids, charge, amphipathicity and overall molecular shape (i.e., secondary structure) [4]. However, the most common MoA considered and analysed for Gram-negative bacteria is their interaction with the lipopolysaccharide layer that constitutes a main part of the outer membrane [5]. Measures of permeability of Gram-negative pathogens are often used to assess the MoA for cationic AMPs [6]. For example, AMPs have been shown to potentiate the effects of antimicrobial compounds that otherwise exhibit poor or slow uptake in Gram-negative bacteria. This was demonstrated when the effect of penicillin was monitored in the presence of nisin Z, where the minimum inhibitory concentration (MIC) was lowered by 155-fold as compared with that of penicillin alone [7].

Although AMPs have the potential to contribute to the fight against antimicrobial resistance, their clinical use has unfortunately been hampered by a variety of adverse pharmacological properties [8]. Importantly, natural AMPs are not proteolytically stable and hence are rapidly degraded by endogenous proteases *in vivo*, which reduces their bioavailability (due to short half-lives), which makes it difficult to reach and maintain a therapeutically relevant concentration *in vivo* [9]. Additionally, there are many more adverse properties that are associated with the clinical use of AMPs, including concomitant toxic side effects on mammalian cells (e.g., many AMPs give rise to unacceptable levels of hemolysis) [6].

Nevertheless, in the continuous search for novel antimicrobial drugs, AMPs serve as useful pharmacophores amenable to structural optimization [10]. Thus, their structures can be manipulated into stable analogues of AMPs that retain the activity and selectivity of AMPs while displaying improved bioavailability, metabolic stability and lowered immunogenicity. This field has gained considerable attention in recent years, and numerous research groups have reported the incorporation of various modified residues into peptide structures, resulting in analogues termed peptidomimetics [1,8,10–12].

Similar to AMPs, peptidomimetics can also be divided into four main classes, and in this classification peptidomimetics are categorized based on their degree of peptide character [12]. Class A compounds have high similarity to their parent peptide (limited number of local modifications). Class B compounds contain more pronounced modifications, which may include non-natural amino acids. Class C compounds have a nonpeptide unnatural structure, where the backbone of the parent peptide is completely replaced. Class D compounds have the least similarity to their parent peptide while mimicking the MoA.

The field of peptidomimetics has undergone significant development over the years, and thus the strategies in peptidomimetic design have matured alongside. Strategies have migrated from localized modifications of AMPs, such as peptide bond substitution and side chain isosteres, to completely synthetic peptidomimetics based on rational design [12]. Such design processes involve the development of new backbones and side chains, such as by incorporating unnatural amino acids, β-amino acids and peptoid residues and constructing hybrid peptidomimetic structures as well as lipidation (Supplementary Figure 1) [1,10,13].

AMPs and their synthetic analogues (including synthetic peptidomimetics) hold vast potential for the development of novel antimicrobial drugs due to their often rapid, membrane-disruptive killing mechanisms (thereby reducing the risk of resistance development), ease of structural modification and many options for improvement of stability conferring improved pharmacokinetic profiles [14–19]. However, it is important to ask whether the antimicrobial activity of peptidomimetics indeed provides significant advantages over AMPs composed of natural amino acids.

To address this question and provide further insight into design parameters, a systematic review was undertaken, summarizing experimental studies that assessed the MICs of both AMPs and antimicrobial peptidomimetics in *Escherichia coli* provenances. Analysis of the existing data for AMPs and peptidomimetics demonstrated that there

Table 1. Advanced search strategy.

Science Direct	1	KEYWORDS (Antimicrobial AND peptides AND *Escherichia coli* AND Minimum Inhibitory Concentration), YEARS (2018–2020), ARTICLE TYPE (Research article) Total: 1353
	2	KEYWORDS (Antimicrobial AND peptidomimetics AND *Escherichia coli*), YEARS (2018–2020), ARTICLE TYPE (Research article) Total: 40
PubMed	1	Search: **antimicrobial peptides, *Escherichia coli*** Filters: **from 2016–2020** ("*anti infective agents*"[Pharmacological Action] OR "*anti infective agents*"[MeSH Terms] OR ("*anti infective*"[All Fields] AND "*agents*"[All Fields]) OR "*anti infective agents*"[All Fields] OR "*antimicrobial*"[All Fields] OR "*antimicrobials*"[All Fields] OR "*antimicrobially*"[All Fields]) AND ("*peptid*"[All Fields] OR "*peptidal*"[All Fields] OR "*peptide s*"[All Fields] OR "*peptides*"[MeSH Terms] OR "*peptides*"[All Fields] OR "*peptide*"[All Fields] OR "*peptidic*"[All Fields]) AND ("*Escherichia coli*"[MeSH Terms] OR ("*Escherichia*"[All Fields] AND "*coli*"[All Fields]) OR "*escherichia coli*"[All Fields]) Total: 3261
	2	Search: **antimicrobial peptidomimetics, *Escherichia coli*** Filters: **from 2016–2020** ("*anti infective agents*"[Pharmacological Action] OR "*anti infective agents*"[MeSH Terms] OR ("*anti infective*"[All Fields] AND "*agents*"[All Fields]) OR "*anti infective agents*"[All Fields] OR "*antimicrobial*"[All Fields] OR "*antimicrobials*"[All Fields] OR "*antimicrobially*"[All Fields]) AND ("*peptidomimetic*"[All Fields] OR "*peptidomimetics*"[Pharmacological Action] OR "*peptidomimetics*"[MeSH Terms] OR "*peptidomimetics*"[All Fields]) AND ("*escherichia coli*"[MeSH Terms] OR ("*Escherichia*"[All Fields] AND "*coli*"[All Fields]) OR '*escherichia coli*'[All Fields]) Total: 28
Google Scholar	1	Find articles: (Antimicrobial peptides) AND (*Escherichia coli*) WITH (Minimum inhibitory concentration) FROM 2016–2020, Exact phrase "*Minimum inhibitory concentration*" Total: 13,900
	2	Find articles: (Antimicrobial peptidomimetics) AND (*Escherichia coli*) FROM (2016–2020), Exact phrase "*Minimum inhibitory concentration*" Total: 712

is little variance in antimicrobial activity between the two types of compounds, but significant differences in MIC values could be correlated to changes in overall net charge and charge density. Knowledge about such differences, or lack of differences, provides useful information in the continued efforts to develop clinically relevant AMP-based and peptidomimetic lead compounds for antimicrobial chemotherapy.

Methods

Systematic literature searches

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 checklist was used as a guide for this systematic review [20]. A literature search within PubMed, Google Scholar and Science Direct databases was performed by using a defined set of keywords, selecting original research articles in any language (provided they could be translated into English) reporting on susceptibility test results (here MICs) for *E. coli* isolates *in vitro* (for details, see Table 1). Studies published from January 2016 to December 2020 were included in this review to compensate for fluctuations in research output within the peptide and peptidomimetic field over time, enabling sufficient data to be analysed – review articles were not included. The final search was conducted on 16 March 2021.

Inclusion & exclusion criteria

Research articles reporting the antibacterial activity of AMPs and/or peptidomimetics against pathogenic and laboratory strains of *E. coli* were included. Articles were not considered eligible for inclusion if they failed to mention any of the selected keywords (Table 1) describing the MIC values of AMPs/peptidomimetics against *E. coli*.

Eligible articles were screened by a two-step process, as described in Figure 1. Articles were first screened by examining titles and abstracts alone. If keywords and/or minimum inhibitory analysis in *E. coli* was not mentioned, articles were excluded. Duplicates were then removed before the full text was analysed; this included the title, abstract and main body of the article. Only experimental studies from original research articles were included. The selected studies were required to report MIC value(s) for one or more AMPs/peptidomimetics against an *E. coli* provenance (laboratory strain or clinical isolate). In addition, studies including the MIC stated in μM or μg/ml were included. However, if the necessary information was not available for the conversion of μM into μg/ml, these values were excluded. Review articles and studies that could not be assessed (as described above) were excluded. Articles in languages other than English were included if a transcript was available.

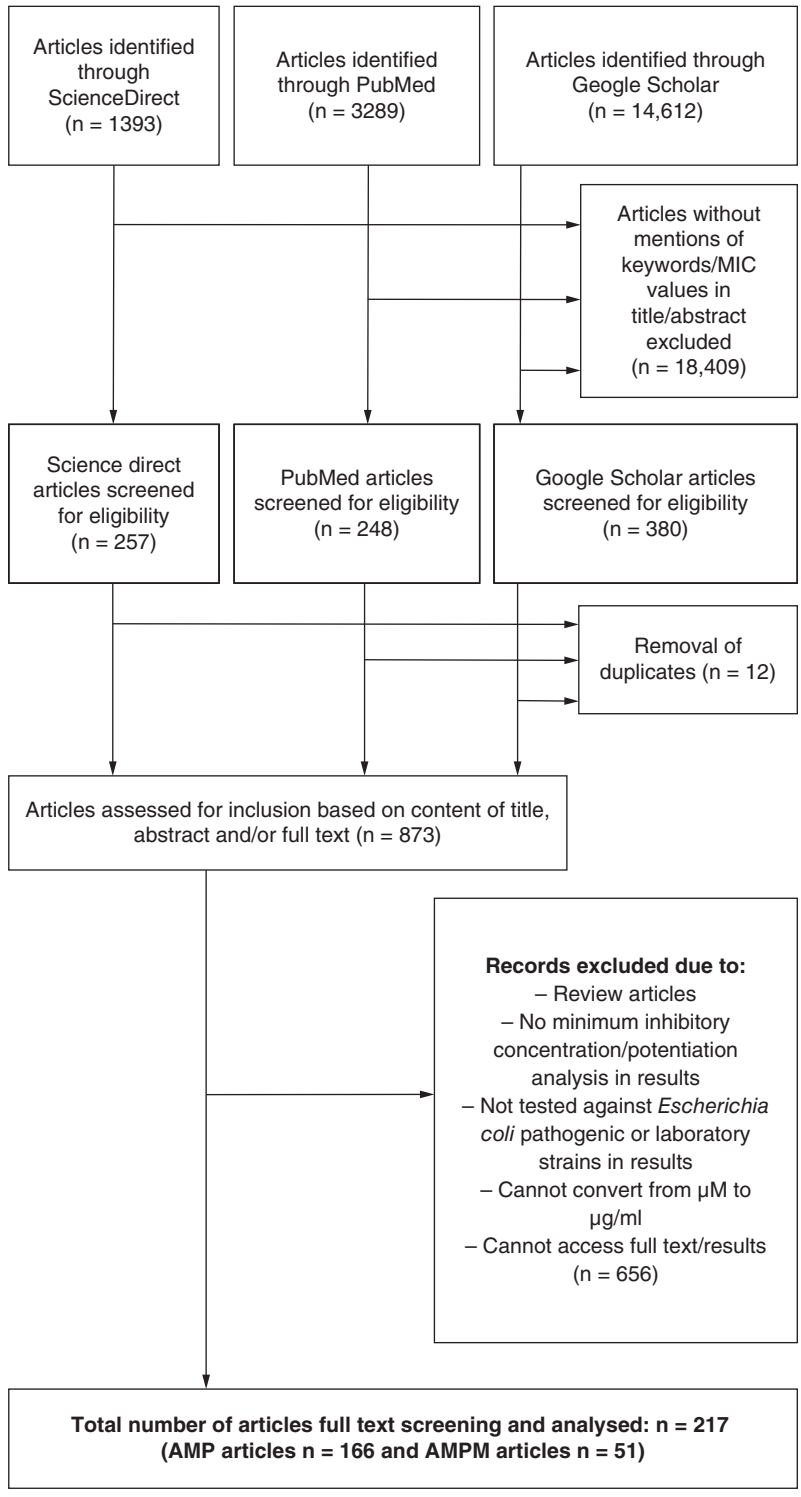

Figure 1. Study selection. Flow chart adapted from the PRISMA guidelines, showing the process of including articles starting from a systematic search of Science Direct, PubMed and Google Scholar.
AMP: Antimicrobial peptide; AMPM: Antimicrobial peptidomimetics; MIC: Minimum inhibitory concentration.

Titles and abstracts were screened, and the inclusion criteria were applied by two authors (LH, NMG). Full-text articles of potentially relevant publications were obtained and reviewed independently by two authors (LH, NMG), who made the final decision on whether the data were to be subjected to inclusion. In cases where inclusion was uncertain, other authors were consulted, and final unanimous decisions were made after an in-depth discussion (see Figure 1). For a full list of studies used, see Supplementary Tables 10 (for AMPs) & 11 (for antibacterial peptidomimetics).

Definitions

The conventional definition of MIC was used – that is, the lowest drug concentration that completely inhibits growth of a bacterial culture after overnight incubation as determined by either optical density measurements or visible analysis.

AMPs were defined as naturally occurring peptides, including truncated analogues and analogues based on *de novo* design to mimic natural AMPs, while semisynthetic or synthetic analogues of naturally occurring peptides were grouped together and defined as antibacterial peptidomimetics.

Data extraction

The following data were extracted from each article: year of publication, molecular weight of the peptide/peptidomimetic, length (i.e., number of residues) of the peptide/peptidomimetic, net charge of the peptide/peptidomimetic, MIC, study design (methodology), type of *E. coli* provenance (laboratory strain or clinical isolate), controls used, media used and research group. Quality assessment of different research groups and risk of bias included comparisons between each laboratory method by looking into the following: whether a standardized method was employed, controls and medium used and *E. coli* provenance used. Comparisons were then made for each of these sections across research groups. In cases of missing data, no attempts were made to contact the authors of such studies.

Data analysis

For data synthesis and analysis, various documentational and statistical packages were used: Microsoft Excel version 16.40, Prism 8 for macOS version 8.4.2 and IBM SPSS Statistics 26. The linear mixed-effects model was used to evaluate the effects of compound type (i.e., peptide vs peptidomimetic), length, molecular weight, net charge, standardized MIC determination method and type of *E. coli* provenance(s) on the log-transformed MIC values; lab ID was included in the model as a random effect to account for multiple observations from the same lab. Statistical significance was assessed in IBM SPSS Statistics 26 for all analyses. Statistically significant differences were set as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****). All error bars are standard deviations from the mean unless specified otherwise. Prism 8 was used to construct graphical representations of the data.

Results

Systematic search

Initially, a list of criteria for selection of publications was developed as well as an advanced search strategy (Table 1). This gave a starting number of possible publications to include. Figure 1 shows the study selection process according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses. The initial search yielded 19,294 studies in total. After screening titles and abstracts and exclusion of duplicates, 873 articles were selected for full-text screening, after which 51 and 166 articles on antibacterial peptidomimetics and AMPs, respectively, remained for data extraction and analysis. Included studies are compiled in Supplementary Tables 10 & 11 [8,21–231].

Publication bias analysis

To avoid undermining the validity of the review and to demonstrate that all publications met the criteria set (no matter their outcome), publication bias was evaluated by comparing overall MIC values using a linear mixed-effects model to evaluate the variation caused by the separate lab groups themselves (see Figure 2). For further data, see Supplementary Tables 2–8. A linear mixed-effects model was also used to evaluate possible bias, focusing on which *E. coli* provenances were used and whether a standard method of analysis was used. This enabled identification of any significantly different laboratory results that could affect comparisons among peptides and peptidomimetics. For each comparison, no significant differences between laboratories were found (p-values are included in the footnote of Figure 2).

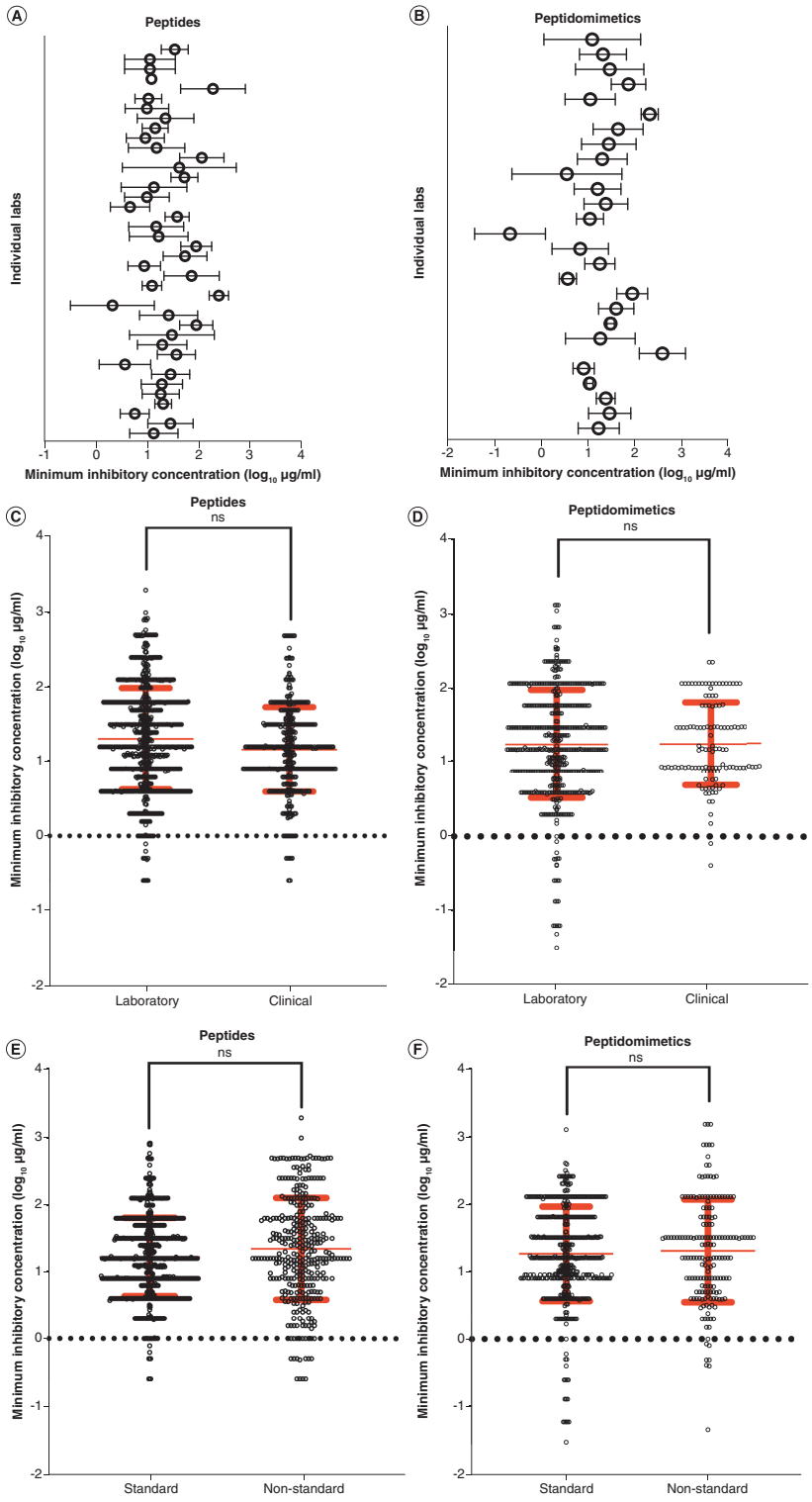

Figure 2. Bias analysis. (A–B) Laboratory comparison. Similar covariance parameters indicate (Supplementary Table 1) variation in minimum inhibitory concentration results and within labs is comparable for both peptide and peptidomimetic data. Zero corresponds to an minimum inhibitory concentration of 1ug/ml. **(C–D)** *Escherichia coli* provenance comparisons. Minimum inhibitory concentration values generated using laboratory vs clinical strains are not significantly different for both peptide and peptidomimetic data ($p = 0.957$). **(E–F)** Method comparisons. Minimum inhibitory concentration values generated for method comparisons are not significantly different for both the peptide and peptidomimetic data ($p = 0.477$).
ns: Not significant.

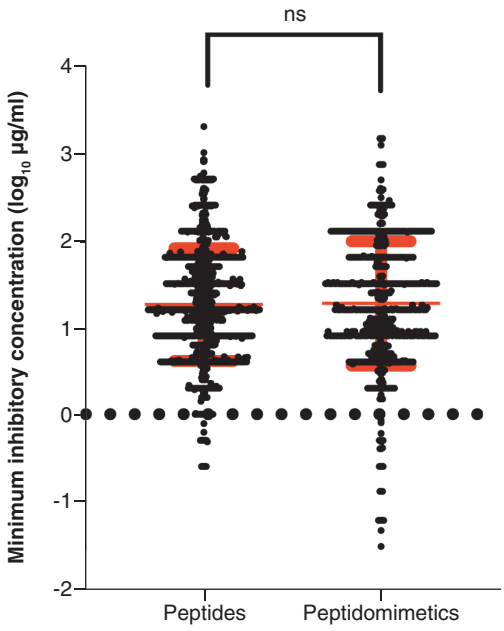

Figure 3. Minimum inhibitory concentration comparison between peptide and peptidomimetic compounds. Minimum inhibitory concentration values generated for peptides vs peptidomimetics are not significantly different (p = 0.381). ns: Not significant.

Comparison of MICs between AMPs & peptidomimetics

MIC values reported for AMPs were not significantly different from those reported for peptidomimetics, with mean Log_{10} values of 1.24 µg/ml and 1.30 µg/ml, respectively (see Figure 3).

Factors affecting MIC values

Structural features, length, molecular weight and charge, of AMPs and antibacterial peptidomimetics, factors that could influence the MIC value of each compound were assessed. It was found that neither length or molecular weight had a significant influence on MICs (see Figure 4).

In an investigation of the effect of the net charge of the AMPs and antimicrobial peptidomimetics, MIC values appeared to be clearly correlated to this property. Thus, a decrease in MIC by 4.6% ($p < 0.001$) was observed for each +1 increment in net charge, as depicted in Figure 4 & Supplementary Table 8. However, this effect was found to be most pronounced in the range between 0 and +6, where a significant lowering of the MIC values was observed. On the other hand, the incremental improvement in antibacterial activity (as measured by MIC) declined as the net charge was increased beyond +6, as demonstrated in Figure 5. While these two net charge groupings (i.e., 0–+6 and +6–+10) had varying effects on the MIC values, a substantial overlap was found (as depicted in Figure 6).

To continue the investigation of the relationship identified between MIC values and overall net charge, charge density was also considered to be of importance (see Supplementary Table 12). Charge density is defined as the charge per residue in each peptide, and it was found to be correlated with high significance (p = 0.000013) to the MIC values (see Figure 7), as demonstrated by a decrease in MIC by 44% for each +1 increment in charge density (see Supplementary Table 16). The significance of charge density versus length (amino acid) was also analysed (see Supplementary Tables 13–15). As the peptide length increased, the significance of the charge density was found to be more pronounced. For peptides/peptidomimetics ranging from 0 to 15, 16 to 30 and 31 to 45 residues in length, a decrease in MIC by 26%, 45% and 465%, respectively, was observed for each +1 increment in charge density (see Supplementary Tables 17–19).

Discussion

This review provides a comprehensive and systematic comparative analysis of 217 studies reporting the antibacterial activity against *E. coli* of AMPs and antibacterial peptidomimetics, spanning the years 2016–2020. Its focus was to assess whether significant differences between AMPs and peptidomimetics could be identified with respect to the influence of simple structural features on their MIC values for this Gram-negative pathogen. Throughout the literature, it is stated that the development of novel AMPs and antibacterial peptidomimetics via appropriate chemical alterations is an efficient means of improving on the functionality of natural AMPs while tuning their

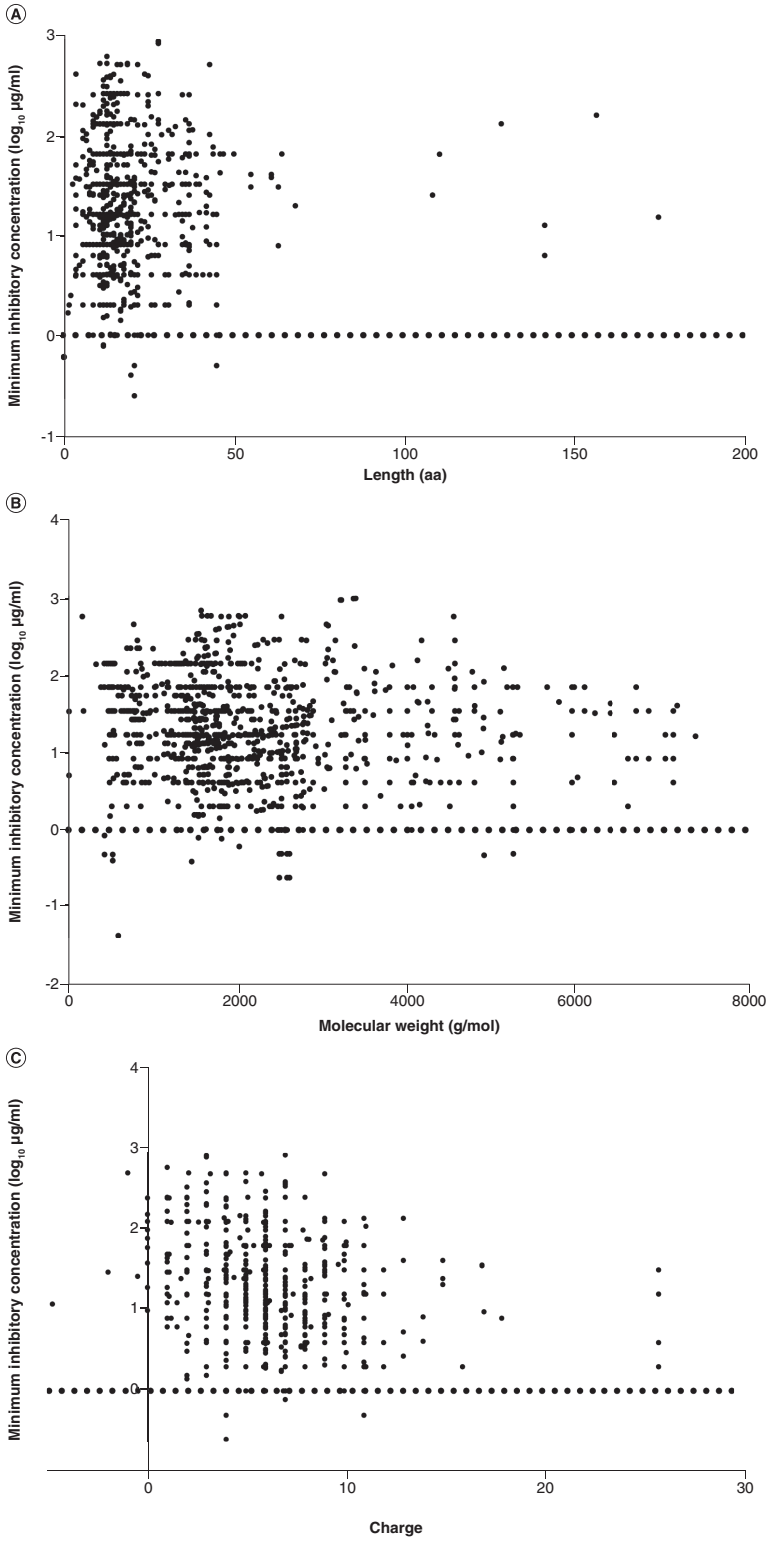

Figure 4. Effect of length, molecular weight and charge on minimum inhibitory concentrations in peptide and peptidomimetic compounds. (A) Length does not have a significant effect on minimum inhibitory concentration values ($p = 0.250$). **(B)** Molecular weight does not have a significant effect on minimum inhibitory concentration values ($p = 0.138$). **(C)** Charge does have a significant effect on minimum inhibitory concentration values ($p < 0.001$). Estimates of fixed effects demonstrates that an increase in charge causes a decrease in Log_{10} minimum inhibitory concentration by 4.6% ($p < 0.001$).

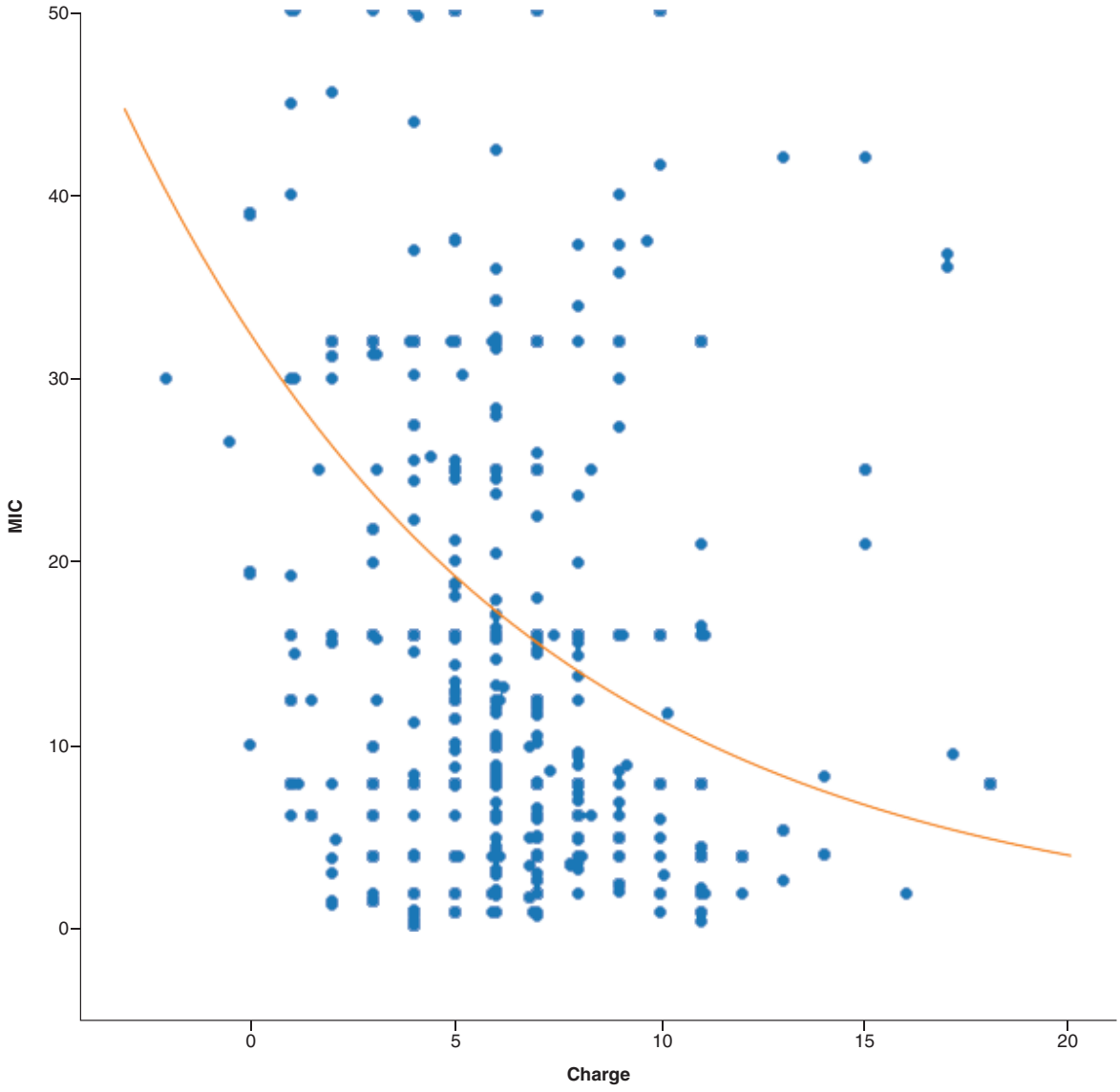

Figure 5. Relationship between charge and minimum inhibitory concentration (μg/ml). The trend of MIC vs charge shows an exponential decay. Between charges 0 and 6 there is a significant improvement in MIC with increased charge. As charge increases over 6, the incremental improvement in MIC decreases.
MIC: Minimum inhibitory concentration.

pharmacological properties [232]. Therefore, peptidomimetics, whose essential elements (pharmacophore) mimic those of a natural peptide in 3D space, are expected to retain the ability to interact with the biological target and produce equal or higher biological effects [233,234].

There are multiple publications discussing natural AMPs, their current use as clinical agents and their key limitations as compared with synthetic antibacterial peptidomimetics as well as research papers on the antimicrobial activity of AMPs and antibacterial peptidomimetics on various pathogens [21–26,235,236], detailing how effective these compounds are and highlighting their potential for clinical applications [233]. However, a direct comparison of general biological efficacy *in vitro* between antimicrobial peptidomimetics and AMPs has not been investigated systematically within a large dataset until now. Within this review, it has been identified that antimicrobial peptidomimetics, albeit retaining potency, do not provide an advantage over AMPs in terms of *in vitro* biological potency. Additionally, it was found that the overall charge and charge density of AMPs and antimicrobial peptidomimetics significantly affected antibacterial potency.

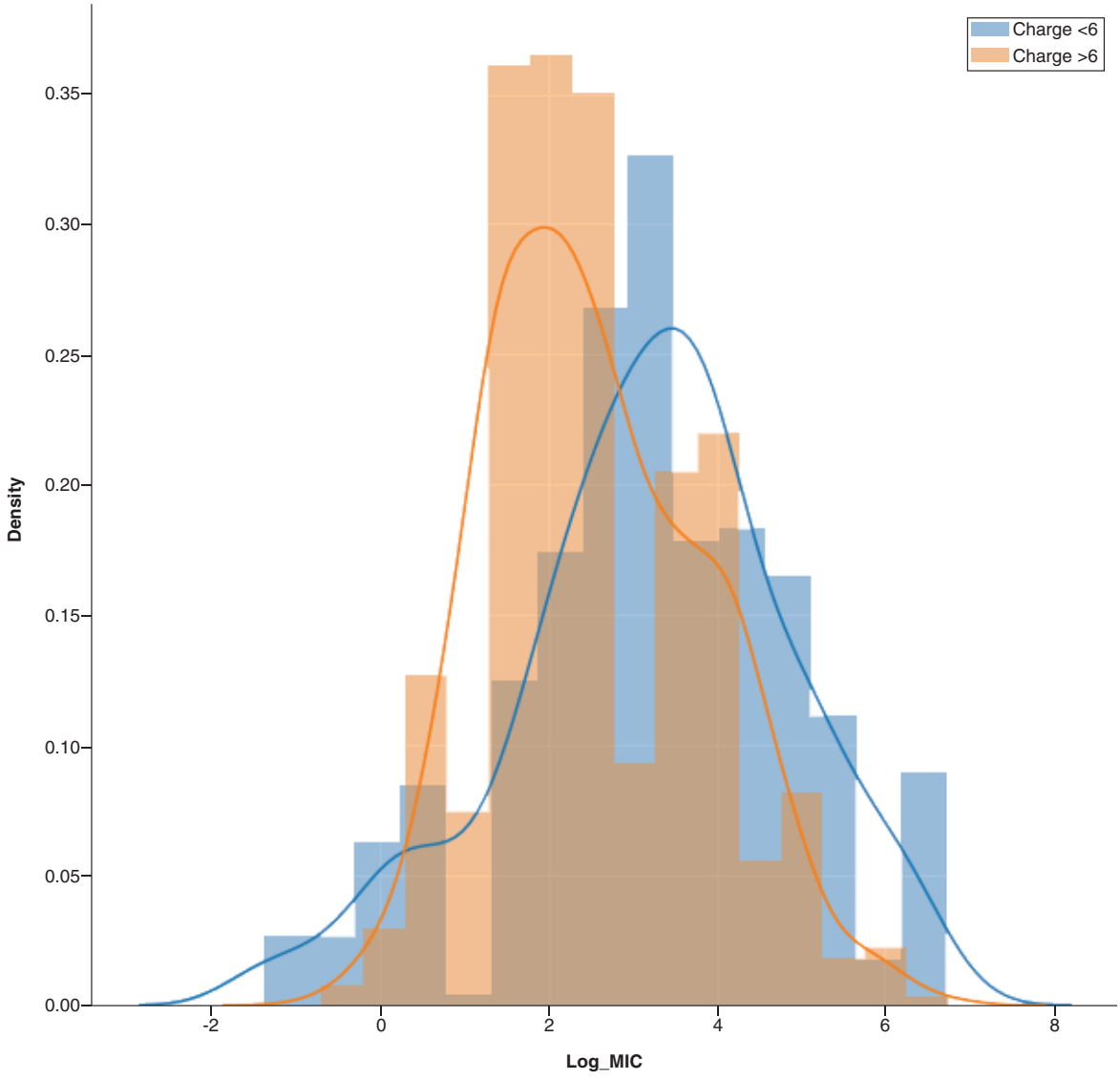

Figure 6. Comparison of minimum inhibitory concentrations above and below charge 6. The mean Log_{10} MIC in peptides and peptidomimetics with charge >6 is higher than those with charge <6. However, there is extensive overlap of Log_{10} MICs between peptides and peptidomimetics in both charge ranges.
MIC: Minimum inhibitory concentration.

As already mentioned, the aim of the present systematic review was to evaluate whether antimicrobial peptidomimetics provide an increased advantage over AMPs by giving a consolidated and quantitative overview of recently reported MIC data for these compound classes. Systematic reviews are seen as increasingly useful tools to reveal patterns that might not be obvious from analysis of a few studies, thereby offering new opportunities to critically evaluate and statistically assess results from comparable studies [237]. With an increasing number of observations, the statistical power is obviously improved. In addition, quantifying sources of variability between studies is possible only by evaluating large, comprehensive datasets. While systematic reviews are not without limitations (including the effects of publication bias and the complexity of the statistical analysis), they provide an increased degree of generalization of the results obtained in individual studies, which may enable the resolution of apparent conflicts between studies, and thus yield conclusive results, when individual studies are inconclusive [238].

Initially, possible biases were eliminated by using several methods. First, individual MIC results from each laboratory were compared. Significant overlap of MIC results for both AMPs and peptidomimetics was observed (Figure 2A & B), which highlights no significant differences between the laboratories that could skew the compar-

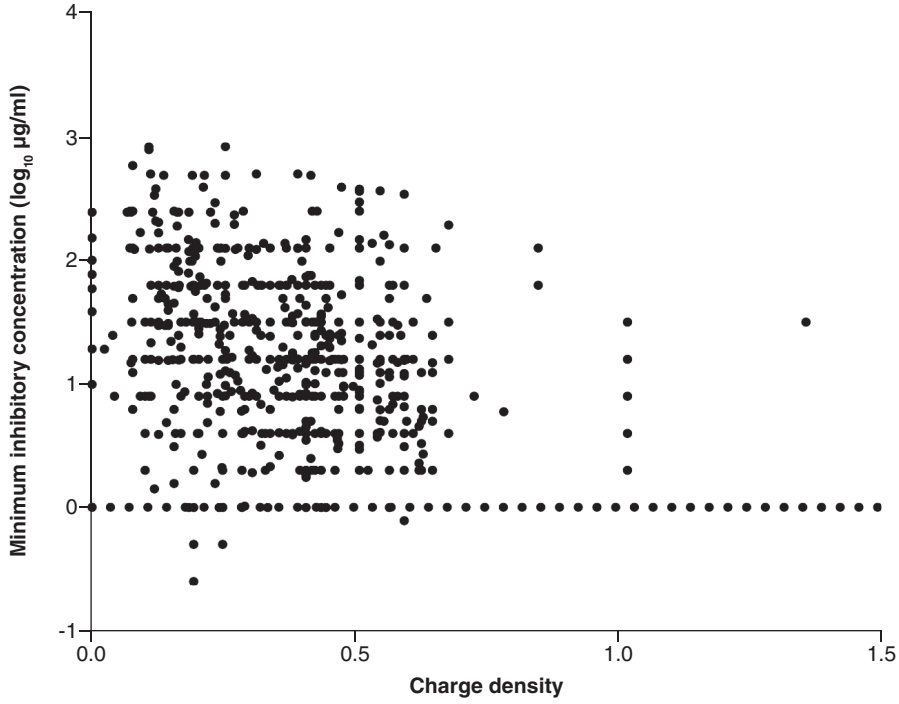

Figure 7. Effect of charge density on minimum inhibitory concentrations in peptide compounds. Charge density does have a significant effect on minimum inhibitory concentration values ($p < 0.0001$) with significance increasing with peptide length (Supplementary Tables 13–15).

ative analysis. This is further highlighted through similar covariance parameter estimates (Supplementary Table 1), indicating low variation between and within the individual laboratory groups. Therefore, while a variety of AMPs and antibacterial peptidomimetics were analysed, their MIC values were still comparable, with the variation arising from differences in structure. The same method was repeated for analysis of *E. coli* provenance and laboratory methods. Many MIC results were obtained by using various *E. coli* laboratory strains and clinical isolates as well as various methods for MIC determination (i.e., Clinical & Laboratory Standards Institute standard vs nonstandard protocols). For the MIC results to be comparable, these biases were analysed, but these were not found to be significantly different (Figure 2).

As stated above, it was found that antibacterial peptidomimetics do not have a significant advantage over AMPs in terms of their *in vitro* antibacterial potency. The variation in MIC values found for peptides versus those seen for peptidomimetics was not significantly different. Thus, AMPs displayed Log_{10} MIC values with a mean of 1.24 µg/ml, while peptidomimetics had Log_{10} MIC values with a mean of 1.30 µg/ml ($p = 0.381$), as depicted in Figure 3. It had been assumed that antibacterial peptidomimetics have the potential to provide a more potent alternative to AMPs [239]. However, direct comparison of *in vitro* efficacy has yet to be established. Therefore, these findings suggest that antibacterial potency will not be reduced upon backbone modifications or the introduction of synthetic amino acids, although it may instead allow for improved pharmacological features (e.g., hemolysis and general cell toxicity).

In addition, the structural features' length (i.e., number of residues in the sequence), molecular weight and charge were evaluated in relation to *in vitro* efficacy of AMPs and antibacterial peptidomimetics. Here a relationship between overall compound charge/charge density and *in vitro* efficacy of AMPs and antimicrobial peptidomimetics was discovered. The net charge was assessed against individual MIC values, and it proved to have a significant effect. A fixed-effects model demonstrated a decrease in MIC by 4.6% for each charge increment of +1.

This relationship was to be expected for membrane-disruptive peptides/peptidomimetics, ultimately corroborating the assumption that highly positively charged compounds will be strongly attracted to the negatively charged outer membrane of Gram-negative pathogens, causing such compounds to exhibit an increased *in vitro* efficacy.

However, this relationship reached a plateau at charges of +6 and above. Hence, it was found that increments of +1 in net charge within the range 0–+6 conferred significantly improved MIC values, whereas the relative

incremental improvement of MIC values declined when the net charge was increased beyond +6. Thus, with +6 as the threshold value, it appears unnecessary to exceed this when trying to improve antibacterial potency by structure optimization.

In contrast, a comparison of MIC values between these two net charge groupings (i.e., 0–+6 and +6–+10) displayed substantial overlap, demonstrating that other structural factors must be influencing MIC values as well. This has also been highlighted throughout the literature, where it has been shown that antibacterial peptidomimetics with the same net charge can have substantially different MIC values due to varying backbone or side chain modifications (i.e., some side chains confer a higher potency than others) [27]. Therefore, while charge does significantly affect the *in vitro* efficacy of AMPs and antibacterial peptidomimetics, it cannot be ruled out that further modifications of the structure might increase the potency once it has reached the charge threshold value of +6.

In addition to the influence of net charge, charge density of the peptides was assessed against MIC values, and this property was found to be significant, highlighting a relationship with both charge and length. As peptide length increased, the significance of charge density also increased. This finding has also been previously discussed in several papers, whereby careful tuning of the charge density resulted in varying activity [240–243]. However, as the biological properties of synthetic and natural peptides result from the interplay of multiple parameters, it is not possible only to use the observed relationships between MIC and net charge as well as charge density to predict the exact properties for future designed peptidomimetics [240].

One crucial limitation that was discovered during the data search stage performed as the basis for this systematic review was the lack of information presented in each publication, which included pharmacokinetics/dynamics, toxicity and stability of the AMPs or antibacterial peptidomimetics. Therefore, establishing an overview on the safety profiles of these compounds was not possible. Although many peptidomimetics exhibit excellent antibacterial potency *in vitro* (as demonstrated in Figure 3), they may exert unexpected side effects when tested via *in vivo* animal models. Some publications have recognized that peptides and peptidomimetics designed for optimal antibacterial activity often possess undesired safety profiles *in vivo*, since the properties promoting efficient bactericidal effects typically also confer increased toxicity toward mammalian cells [26,244–246]. Importantly, careful adjustment of hydrophobicity can lower undesired cell toxicity without substantial loss of antibacterial potency [27,240]. With a sufficient amount of data on other chemical characteristics, such as hydrophobicity, further relationships between other structural features and *in vitro* antibacterial efficacy could have been drawn. The analysis of these factors may allow for a much more detailed conclusion regarding the potential for clinical application of antibacterial peptidomimetics as compared with AMPs.

Conclusions were further hampered by a lack of availability of peptide or peptidomimetic sequences in many reported studies. In such cases, in-depth structural comparisons could not be carried out and features that might have an additional effect on the *in vitro* efficacy, independent of net charge or charge density, could not be identified. These limitations include the lack of identification of various side chains or specific backbone modifications that could positively or negatively affect the MIC values. Additionally, computational software that recognizes unnatural amino acids present in antibacterial peptidomimetics is not available, which further obstructed the comparison of the limited AMP and antibacterial peptidomimetic sequences/structures available. While there has been significant progress in relation to AMP structural analysis [247,248], further understanding of differences in peptidomimetic structures, new programs and quantitative analyses should be developed [249,250]. Overall, to comment further on the comparison of AMPs and peptidomimetics, additional sequence analysis would be needed, including a comparison of side chains. This might allow for identification of other structural features that may contribute to the improvement of the pharmacological profiles (i.e., activity, toxicity and/or stability) of these compounds.

Conclusion

This systematic review of the literature investigated the activities of AMPs and antibacterial peptidomimetics against Gram-negative bacteria (represented by *E. coli*). While the findings provide an insight into the antibacterial potency of AMPs versus that of peptidomimetics, they also highlight the importance of sequence comparisons in future efforts to design peptidomimetics.

Synthetic peptidomimetics were found to possess antibacterial potency in terms of *in vitro* efficacy similar to that of naturally occurring AMPs. When designing peptidomimetics (or close analogues of AMPs), the net charge should have priority over length and molecular weight. However, the charge density of peptidomimetics may be kept almost constant (relative to the length) to retain or increase potency for shorter/longer analogues. To make

further correlations between *in vitro* efficacy and structural features, more detailed structural information must be made more accessible. This would allow additional structure–activity relationships to be revealed, which would assist in the design of future peptidomimetics.

The potential applications of modified AMPs (e.g., into peptidomimetics) with improved stability and pharmacokinetic properties is stimulating an extensive research effort within this field, and the present work infers that synthetic antibacterial peptidomimetics typically match the *in vitro* potency of naturally occurring AMPs. Therefore, future design should focus on structural modifications that provide more favorable pharmacological activity profiles, and a prerequisite to achieving this is that the net charge or charge density of the compound is retained or increased toward the optimum.

Future perspective

Resistance to antibiotics constitutes a continuous threat, causing major public health concerns globally, in part, due to insufficient discovery and development of new antimicrobials [251]. AMPs are potential alternatives to conventional antimicrobials, and in animal models they have proved suitable for the treatment of multidrug-resistant infections while also having a lower risk of inducing rapid antimicrobial resistance as compared with traditional antibiotics [3]. However, the therapeutic application of AMPs is often limited due to their pharmacokinetic/pharmacodynamic properties, such as toxicity and stability [252]. Therefore, antibacterial peptidomimetics, retaining the potency of AMPs, constitute favorable replacements for these, which may be fine-tuned to achieve more desirable pharmacokinetic/pharmacodynamic profiles, thereby effectively bypassing the limitations of AMPs [1]. The potencies and structural features of both AMPs and antibacterial peptidomimetics described here are the starting points for understanding the significant differences observed within both compound classes. However, further research, including methods enabling evaluation of the structural features of antibacterial peptidomimetics, is required. At present, general methods allowing the identification of unnatural amino acids, side chains and backbones that either diminish or enhance the potency (as well as how these affects pharmacokinetic/pharmacodynamic properties) of antibacterial peptidomimetics do not exist. Thus, accurate evaluation of AMP structures in comparison with analogous synthetic peptidomimetics can aid the design process to create effective therapeutic candidates that might alleviate the continuous emergence of antimicrobial resistance in pathogenic bacteria.

Summary points

Comparison of minimum inhibitory concentrations (MICs) between antimicrobial peptides & peptidomimetics

- Antimicrobial peptides (AMPs) and their synthetic counterparts (antibacterial peptidomimetics) display similar potency against various *Escherichia coli* laboratory strains and clinical isolates.

Factors affecting MIC values

- This study briefly investigated some structural features expected to influence the MIC of either AMPs or antibacterial peptidomimetics.
- Net charge and charge density are important determinants for the potency of both AMPs and antibacterial peptidomimetics.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.4155/fmc-2022-0160

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