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RESISTANCE-AWARE SPECTRUM PROFILING OF ANTIMICROBIAL PEPTIDES AGAINST PRIORITY BACTERIAL PATHOGENS

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ABSTRACT

Antimicrobial resistance increasingly compromises the effectiveness of conventional antibacterial therapy, creating a need for alternative agents capable of maintaining activity against resistant pathogens. This study evaluated antimicrobial peptides (AMPs) using a resistance-aware spectrum-profiling framework integrating physicochemical characteristics, minimum inhibitory concentration (MIC), hemolytic activity, and selectivity. A total of 225 AMP–pathogen observations covering six priority bacterial pathogens were analyzed. Resistance profiles were classified as susceptible, multidrug-resistant (MDR), or extensively drug-resistant (XDR). Statistical comparisons were performed in Python using the Kruskal–Wallis H test. Peptides exhibited substantial physicochemical and antimicrobial heterogeneity, with MIC values ranging from 0.43 to 104.30 µM. Pathogen-specific MIC distributions did not differ significantly ($p = 0.439$), despite numerical variation among bacterial species. In contrast, resistance phenotype was significantly associated with AMP potency ($p = 0.004$). Mean MIC increased from 10.04 µM in susceptible profiles to 14.69 µM in MDR and 26.04 µM in XDR profiles. Selectivity declined concurrently from 22.03 to 9.05, whereas hemolytic activity remained comparatively stable. These findings indicate that resistance phenotype provides an important dimension for interpreting AMP performance beyond pathogen identity alone. Integrating antimicrobial potency with resistance status and selectivity may support more informative prioritization of peptide candidates for subsequent experimental evaluation.

Keywords: Antimicrobial peptides, Antimicrobial resistance, Priority bacterial pathogens, Minimum inhibitory concentration, Peptide selectivity

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1. Introduction

The ever growing problem of antimicrobial resistance (AMR) has emerged as a major limitation in the successful treatment of bacterial diseases, especially when the pathogens develop resistance to known antimicrobial drugs. This therapeutic impact of resistance is not limited to the actual loss of a specific antibiotic as resistance can gradually erode the management of difficult to treat infections, and limit treatment options. Current strategies thus focus more on approaches that complement or are an alternative to the use of conventional antibacterial drugs (MacNair et al., 2024). These include bacteriophages, antimicrobial peptides, antibody-based therapies, microbiome targeting and other non-traditional therapies that target resistant bacterial populations (Wang et al., 2020). Meanwhile, there is still the need to continue advancing the antimicrobial pipeline as current antimicrobial therapeutic options have not caught up to the ongoing emergence and prevalence of antimicrobial resistance (Tarin-Pello et al., 2022). Variations in the recommendations for antibiotic use among healthcare systems also highlight the role of antimicrobial-use factors in AMR (Jamil et al., 2025).

Among the various candidates for developing alternative antimicrobial agents, antimicrobial peptides (AMPs) are receiving a great deal of attention, as they are structurally diverse molecules that can have antimicrobial effects, different from those of classical antibiotics. Their activity may range from interactions with microbial membranes to intracellular targets and some peptides are also immunomodulatory. Peptides derived from proteins that are not typically associated with host immunity can also exhibit antimicrobial and immune-regulatory properties and greatly broaden the molecular landscape in which potentially therapeutic peptides can be found (Torres et al., 2025). There is also interest in using AMP mimetic compounds as antibiotic substitutes in medical, pharmaceutical, food and animal-health use (Rahman, 2025). Furthermore, AMPs could be used in combination with conventional antibiotics, as there might be synergistic interactions that enhance the antibiotic's effect on the inhibition of the growth of the bacteria, providing alternative treatments for antibiotic-resistant infections (Zhu et al., 2022).

However, the presence of antimicrobial activity does not necessarily imply the therapeutic potential of AMPs. Peptides are very diverse in amino-acid composition, length, molecular weight, charge, hydrophobicity, structural organization, antimicrobial potency and host-cell toxicity. Systematic profiling is therefore needed to differentiate between peptides possessing desirable antibacterial properties and those that have weak, pathogen specific or have undesirable toxicity properties. This is especially relevant to resistant bacteria as microorganisms can also develop mechanisms that decrease susceptibility to antimicrobial peptides. New findings on anti-AMP resistance highlight the need for an understanding of the mechanisms of resistance and environmental reservoirs for future peptide therapeutics developments (Lu et al., 2026). The research on alternative therapies generally supports the notion that a single alternative to traditional antibiotics is unlikely to solve the AMR crisis; rather, a combination of different therapies will be necessary (Berger & Loewy, 2024; Singha et al., 2024).

Resistance-aware spectrum profiling thus offers a convenient framework to assess the performance of AMP. This is not focusing on activity against a single bacterial species, but on how potency of peptides is against clinically relevant organisms and resistance phenotypes. For quantitative comparisons, minimum inhibitory concentration (MIC) can be used, and hemolytic activity and selectivity can be used to provide further information on the balance between antimicrobial effectiveness and potential host-cell effects. To further advance antimicrobial research, computational strategies can be leveraged to uncover intricate patterns in multidimensional datasets; recent studies have shown how artificial intelligence and predictive modeling are increasingly being employed in AMR-related analyses (Ahvar et al., 2025). Other antimicrobial settings also show the importance of pathogen characterisation and susceptibility testing in the evaluation of potential control measures (Yusuf Abubakar et al., 2025).

Resistance is not limited to clinical use of antibacterial drugs, but is a general biological phenomenon that occurs under continued selection pressure. Studies on insecticide resistance also highlight the need to grasp the mechanisms of resistance and employ a combination of management strategies to prevent its emergence. In similar fashion, antimicrobial problems are not limited to bacteria; but the

emergence of hard to control fungal infections, like oral candidiasis among the immunocompromised, underscores the ongoing need for better anti-infective strategies (Suresh et al., 2025). In the case of bacterial therapeutics, however, a specific need to combine AMP physicochemical properties, pathogen range, resistance phenotype, antimicrobial activity, and selectivity in a single analytical framework exists. Such integration can help to determine if the activity of the peptides is retained during target pathogens or not, and if the resistance is increased, what are the changes in the susceptibility of the AMPs?

Hence, antimicrobial peptide activity was investigated for priority bacterial pathogens in the present study in a spectrum-profiling framework with consideration of resistance. The study concentrated on peptide physico-chemical features, antimicrobial activity based on minimum inhibitory concentration (MIC), activity patterns about resistance, hemolytic effects, and selectivity to offer a comprehensive evaluation of AMP performance. Objectives of the study are as follows:

- To characterize physicochemical and antimicrobial profiles of peptides against priority bacterial pathogens.
- To compare AMP potency across bacterial species and antimicrobial resistance phenotypes.
- To evaluate antimicrobial potency, hemolytic activity, and peptide selectivity.

2. Materials and Methods

2.1 Study Design and Data Framework

The number of observations was 225 AMP–pathogen, and a quantitative analytical design was used to evaluate the same. The parameters included in each observation were peptide physicochemical properties, bacterial species, resistance phenotype, antimicrobial activity, haemolytic activity and selectivity. Characterization of AMP profiles and comparison of their activity against priority pathogens and resistance categories only was done.

2.2 Antimicrobial Peptide Characterization

The peptides were classified based on sequence length, molecular weight, net charge, hydrophobicity, and predicted structural characteristics. Minimum inhibitory concentration (MIC, μM) was used to evaluate the antimicrobial potency of the compounds; the lower the MIC, the more potent the antimicrobial activity. Peptides were also classified into three groups namely high, moderate and low antimicrobial activity. Hemolysis (%) and selectivity index were used as measures to describe the antimicrobial activity and selectivity of cells.

2.3 Pathogen and Resistance Classification

AMP activity was tested with six priority pathogens, such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus and Enterococcus faecium. Microorganisms were then separated according to their Gram staining. A total of 81 observations were susceptible, 117 were multidrug-resistant (MDR) and 27 were extensively drug-resistant (XDR).

2.4 Statistical Analysis

Data was analyzed statistically in python using pandas, NumPy and SciPy. The quantitative variables were presented as mean ± standard deviation, median, interquartile range and range; the qualitative variables were presented in their frequencies and percentages. As the MIC values were found to be heterogeneously distributed, Kruskal–Wallis H test was performed to evaluate the antimicrobial potency of each pathogen and resistance group. All tests were two sided and $P < 0.05$ was deemed to be statistically significant. The pathogen specific MIC, activity-class and resistance-stratified potency and selectivity profiles were then displayed.

3. Results

3.1 Antimicrobial Peptide Characteristics and Activity Profile

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What are characteristics and activity profile of antimicrobial peptides? 225 AMP–pathogen observations were analysed. The peptides showed significant physicochemical variability, including a range of size from 10 to 45 amino acids with an average molecular weight of 3014.20 ± 1134.62 Da. Mean net charge was 2.86 ± 2.61, while hydrophobicity averaged 56.98 ± 9.37%. The antimicrobial response was variable and the range of MIC values was between 0.43 and 104.30 µM with a median value of 7.38 µM. The overall physicochemical, antimicrobial, hemolytic and selectivity characteristics are indicated in Table 1. Eighty (36.4%) of the observations were categorized as high, 76 (33.8%) as moderate and 67 (29.8%) as low activity. The mean selectivity index was 16.26 ± 17.37, which shows that there was a wide range of antimicrobial activity/cellular selectivity.

Table 1. Overall Physicochemical and Antimicrobial Characteristics of the Peptide Profiles (n = 225)

Characteristic	Mean ± SD	Median	Range
Peptide length (aa)	27.32 ± 10.29	27.00	10.00–45.00
Molecular weight (Da)	3014.20 ± 1134.62	2980.70	920.10–5091.00
Net charge	2.86 ± 2.61	3.00	−3.00–12.00
Hydrophobicity (%)	56.98 ± 9.37	56.80	21.40–85.70
MIC (µM)	14.38 ± 17.39	7.38	0.43–104.30
Hemolysis (%)	26.51 ± 12.81	25.30	0.00–68.70
Selectivity index	16.26 ± 17.37	8.55	0.64–79.90

Figure 1 shows the distribution of antimicrobial activity classes across the complete peptide profile. High- and moderate-activity observations together accounted for 70.2% of the dataset, although the presence of 67 low-activity profiles demonstrated that antimicrobial potency was not uniform across AMP–pathogen combinations.

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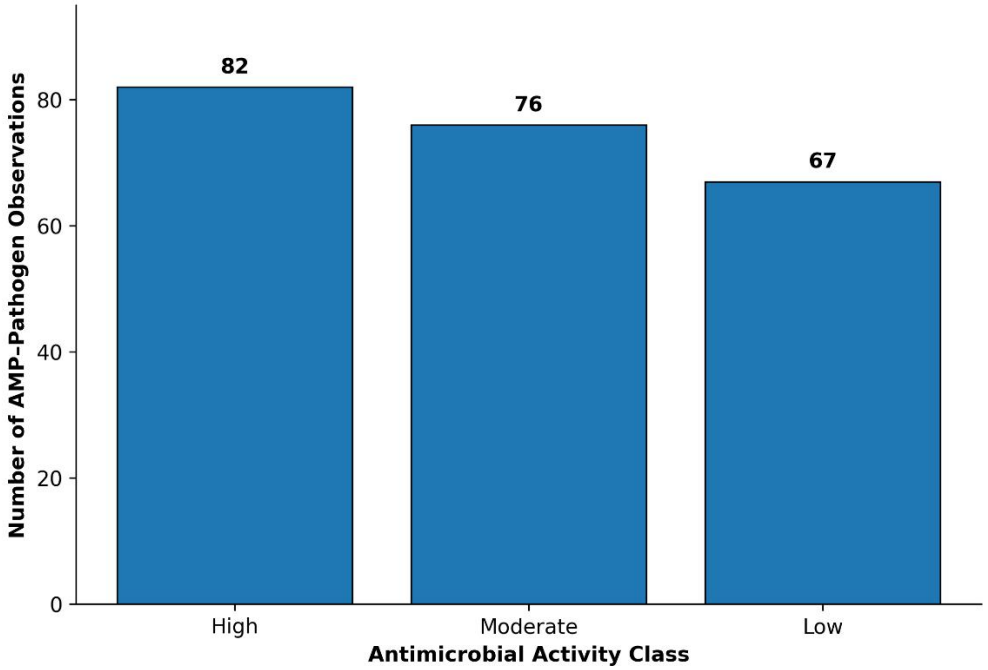

Figure 1. Distribution of Antimicrobial Peptides According to Antimicrobial Activity Class

3.2 Pathogen-Specific Antimicrobial Spectrum

The antimicrobial potency of the six priority bacterial pathogens was numerically different and the difference between the pathogens was not statistically significant (Kruskal – Wallis H = 4.82, p = 0.439). The lowest mean and Median MIC value of 10.67 ± 14.09 µM and 3.79 µM respectively was observed for Pseudomonas aeruginosa in the Table 2. In comparison, Acinetobacter baumannii had the highest median MIC of 10.87 µM and its mean MIC was 16.30 ± 15.92 µM. The mean MIC of Klebsiella pneumoniae was also relatively high at 16.06 ± 20.44 µM. The median MICs for the Gram-positive pathogens were 7.69 µM for Enterococcus faecium and 6.88 µM for Staphylococcus aureus. In general, the median MICs for Gram-negative and Gram-positive organisms were similar (8.16 and 7.13 µM, respectively).

Table 2. Pathogen-Specific Antimicrobial Potency and Selectivity Profiles

Priority pathogen	n	MIC, mean ± SD (µM)	Median MIC (µM)	IQR (µM)	Mean selectivity index
Acinetobacter baumannii	44	16.30 ± 15.92	10.87	3.73–23.55	14.26
Enterococcus faecium	37	14.48 ± 17.64	7.69	2.04–19.10	15.66
Escherichia coli	30	12.69 ± 13.46	8.66	3.43–15.37	16.39
Klebsiella pneumoniae	36	16.06 ± 20.44	8.09	2.64–19.81	15.79
Pseudomonas aeruginosa	39	10.67 ± 14.09	3.79	2.30–10.64	18.95

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Staphylococcus aureus	39	15.55 ± 21.34	6.88	2.57–19.78	16.71

The median MIC profile, pathogen-specific, is represented in figure 2. The minimum median MIC was found to be for P. aeruginosa while the maximum median MIC was found to be for A. baumannii. Given that these numerical differences did not result in significant differences in overall pathogen effect, this suggests that there was considerable heterogeneity of AMP activity within pathogens.

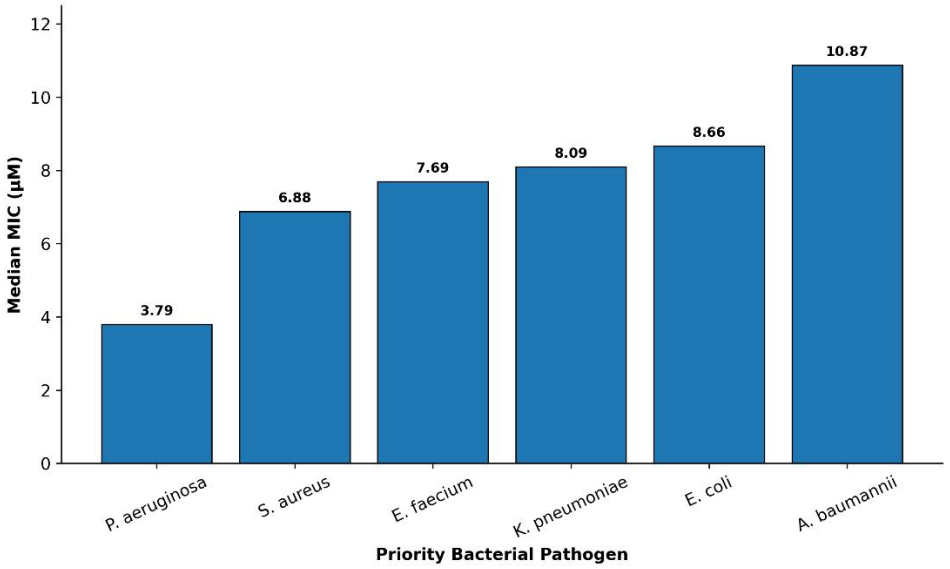

Figure 2. Pathogen-Specific Minimum Inhibitory Concentration Profile of Antimicrobial Peptides

3.3 Resistance-Associated Changes in Antimicrobial Potency and Selectivity

Resistance phenotype caused a greater differentiation in AMP activity than pathogen identity. There were 81 susceptible, 117 multidrug-resistant (MDR) and 27 extensively drug-resistant (XDR) profiles in the 225 observations. Table 3 demonstrates a progressive increase in the mean MIC value associated with susceptible organism, MDR organism and XDR organism which are 10.04 ± 10.56, 14.69 ± 16.11 and 26.04 ± 30.09 µM respectively. High differences were observed in the corresponding median MIC values of 14.59 µg/mL for XDR, 6.86 µg/mL for susceptible and 6.88 µg/mL for MDR. The difference between all the resistance groups was statistically significant (Kruskal–Wallis H = 10.91, p = 0.004).

Table 3. Antimicrobial Potency and Selectivity According to Bacterial Resistance Profile

Resistance profile	n (%)	MIC, mean ± SD (µM)	Median MIC (µM)	IQR (µM)	Mean selectivity index
Susceptible	81 (36.0)	10.04 ± 10.56	6.86	1.50–14.62	22.03
MDR	117 (52.0)	14.69 ± 16.11	6.88	3.02–20.40	13.92
XDR	27	26.04 ± 30.09	14.59	4.45–29.79	9.05

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MDR, multidrug resistant; XDR, extensively drug resistant; IQR, interquartile range.

The increase in the MIC associated with the resistance was accompanied with reduction in selectivity. Mean selectivity index of susceptible profiles was 22.03, of MDR profiles was 13.92 and of XDR profiles was 9.05. However, there was no significant difference in the mean hemolysis between susceptible (26.57%), MDR (26.58%) and XDR group (26.02%) which suggests that the observed reduction in selectivity was mainly due to decrease in antimicrobial activity and not due to significant change in the hemolytic activity.

The gradient in antimicrobial potency is shown for each resistance in figure 3. The mean MIC for the XDR group was about 2.6-fold higher than the susceptible group (26.04 versus 10.04 µM) and the lowest selectivity index was observed in the XDR group. These data illustrate that a change in the activity landscape of these AMPs was observed with the XDR bacterial profiles showing the lowest apparent susceptibility.

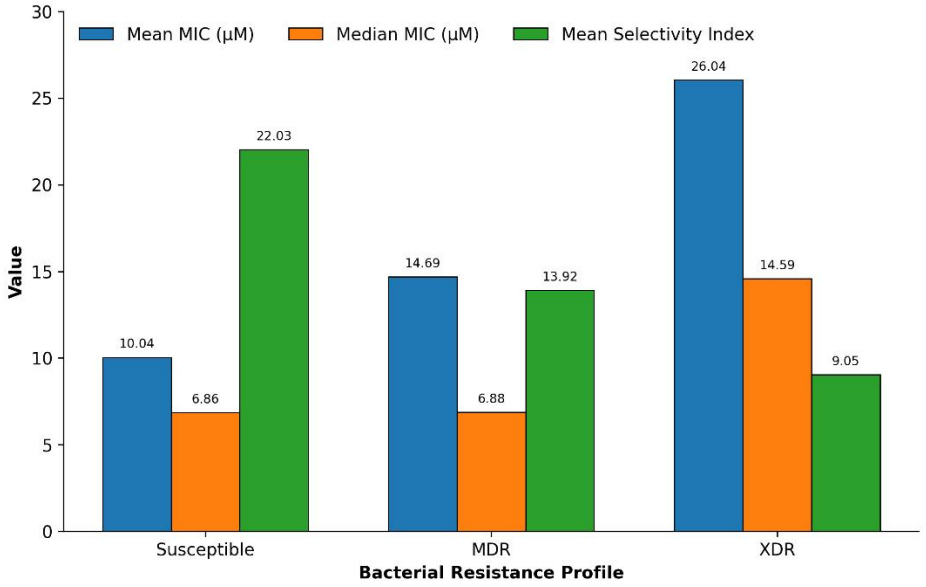

Figure 3. Resistance-Associated Gradient in Antimicrobial Peptide Potency

The results in general indicated that resistance profile was a more significant source of variation in AMP potency as opposed to species identity of the bacteria in this dataset. There was extensive overlap between pathogen-specific MIC distributions, however, XDR profiles had higher MICs and lower selectivity. Thus, evaluation based on pathogen spectrum, resistance phenotype, antimicrobial potency and selectivity gave a more differentiated picture on the performance of AMPs than activity classification alone.

4. Discussion

The current analysis revealed that there was significant variability between the different AMP–pathogen combinations. The antimicrobial activity of the six bacterial species was not statistically different with respect to the distribution of the MICs ($p = 0.439$), although there were numerical differences. Resistance phenotype, on the other hand, was significantly correlated with peptide susceptibility ($p = 0.004$) with the median MIC going up from 6.86 µM in susceptible profiles to 14.59 µM in XDR profiles. This distinction indicates that the evaluation of antimicrobial activity of AMPs cannot be solely achieved based on the species of bacteria used and that resistance status can serve as an extra parameter for the assessment of antimicrobial activity. Biological proof of pathogen-

dependent host defence is also provided, with different Drosocin-derived peptides having different protective effects against different bacterial infections (Hanson et al., 2022). The variation in susceptibility among host–pathogen combinations is a further indication of the context-dependency of antimicrobial defence (Sun et al., 2025).

The lowest Median MIC value was obtained against Pseudomonas aeroginosa (3.79 µM), while the highest median MIC value was 10.87 µM against Acinetobacter baumannii. However, much variation within species, prevented these numerical differences from being interpretable as a pathogen effect overall. This heterogeneity is in line with the wider notion that antimicrobial susceptibility is a function of interplay between microbial traits, defence mechanisms and biology. Similarly, studies on social immunity to pathogens have shown that the protective response can vary depending on the pathogen involved (Masson et al., 2024). Similarly, evidence throughout the Drosophilidae suggests that susceptibility patterns vary between pathogens and may not necessarily have the same phylogenetic structure. These observations support the use of a spectrum-based approach to assessment, instead of a homogenous effectiveness of AMPs for bacterial targets.

A greater separation of antimicrobial potencies was seen with resistance phenotype. The mean MIC of the susceptible profile was 10.04µM, which was significantly lower than the mean MIC of the MDR profile (14.69µM) and XDR profile (26.04µM). It is important to note that this trend does not necessarily indicate that conventional antibiotic resistance is automatically correlated with AMP resistance. The results, however, show an association between resistance category and decreased AMP susceptibility within profiles. The ability of bacteria to reduce peptide activity can be achieved through a number of mechanisms, such as changing the electrostatic interactions of the peptides with components of the cell envelope. For instance, it was shown that lipopolysaccharide-mediated resistance is involved in the resistance of Providencia alcalifaciens to host antimicrobial peptides (Shaka et al., 2022). Microbial resistance phenotypes can also be influenced by environmental antimicrobial pressures, which is why the prolonged exposure of microorganisms to disinfectants is also an area of interest in relation to antimicrobial resistance in clinically relevant microorganisms (Fernandes et al., 2024).

The observed variation in activity is further in perspective of the physicochemical diversity of the peptides. The peptides analyzed here were highly variable in length, molecular weight, net charge and hydrophobicity. These properties are biologically relevant since they influence physicochemical properties of attraction to bacterial surface, association to membrane, insertion and disruption. Pirtskhalava et al. (2021) highlighted the importance of peptide charge, hydrophobicity, structure and membrane properties in the interplay of the interactions of AMPs with microbial membranes. So, it is improbable that any one molecular descriptor can be used to determine antimicrobial activity. The broad literature on antimicrobials also shows that the physicochemical properties of antimicrobial compounds play crucial roles in their interactions with the biological target, such as in structure- and property-based studies on antimicrobial molecules targeting bacterial enzymes (Azzam et al., 2022).

One important discovery was that there was a correlation between resistance phenotype and selectivity. The mean selectivity index decreased from susceptible to MDR and XDR to 22.03 and 13.92 and 9.05 respectively. In contrast, the mean hemolysis was rather constant at about 26% in all three resistance classes. The decrease in selectivity thus was largely associated with a decrease in antimicrobial activity, and not a significant rise in hemolytic activity. This is of practical significance, as the MIC alone should NOT be the criterion for selecting promising AMPs. A good therapeutically attractive peptide will have good inhibitory activity against bacteria, with a not too bad effect on host cells. The same applies when designing functional biomaterials that possess an antimicrobial function, as physical and physicochemical antimicrobial mechanisms need to be taken into account in addition to the biological compatibility (Nowotnick et al., 2024).

The observed resistance-associated gradient also reminds us that the environment of the host and microbes is an important factor to consider when interpreted in terms of antimicrobial defence. Microbial communities in the environment can impact immune function and evolutionary relationships between organisms and infectious agents, which can in turn change the conditions of

when antimicrobial mechanisms take effect (Hanson, 2024). Therefore, one should consider that the activity of AMPs against a single bacterial profile is only one aspect of a much more complicated biological play that includes the properties of the pathogen, the host defence, microecology and resistance mechanisms.

These findings all suggest that a resistance-aware approach to AMP spectrum profiling should be taken where antimicrobial potency, bacterial target, resistance phenotype and selectivity are all considered together. The fact that the MIC of the XDR profiles is significantly higher suggests that the resistance background needs to be taken into consideration when prioritizing peptide candidates. The results presented here should be expanded on through experimental susceptibility testing in more extensive strain panels, mechanistic evaluation of peptide resistance, membrane-interaction studies, and evaluation of combinations of peptides with existing antimicrobials. This validation would enable to assess the potential of peptides with low MIC and high selectivity across the spectrum of resistant pathogens as strong candidates for further antimicrobial drug development.

5. Conclusion

This study illustrates that more than pathogen identity alone is the determinant of antimicrobial peptide activity; bacterial resistance phenotype is a contributing factor as well. The MIC values for the six priority pathogens showed variation however there were no significant differences between species ($p = 0.439$) suggesting significant overlap in the susceptibility of peptides across the species. Another significant association was observed between resistance profile and antimicrobial activity ($p = 0.004$), where the mean MIC for the susceptible profiles was 10.04 µM, and the mean MIC for XDR profiles was 26.04 µM. This loss of potency was also paralleled by a decrease in mean SI (22.03 to 9.05) with hemolytic activity being relatively similar within resistance groups. These results suggest the need to assess AMPs within a multi-faceted perspective that takes into account the resistance phenotype, pathogen spectrum, antimicrobial activity and selectivity in addition to MIC. Thus, resistance-aware profiling could be a useful approach for prioritizing peptide candidates that have activity and a good selectivity in challenging bacterial profiles. More experiments with larger strain collections are needed to determine therapeutic relevance.

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