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Cracking the Code: Molecular Insights into Antibiotic Resistance in Nigeria Dairy

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ABSTRACT

The extensive use and misuse of antibiotics in both human medicine and livestock production has accelerated the spread of antimicrobial resistance (AMR), creating a major challenge at the human–animal–environment interface. This study employed an in silico strategy to identify and characterize antimicrobial peptide candidates associated with antibiotic resistance pathways in livestock-related organisms, with particular emphasis on dairy cattle. Genomic sequences were screened using the CAMPR3 platform to predict antimicrobial peptides derived from biosynthetic genes. Two peptides originating from Terpene Synthase and Lycopene Cyclase genes demonstrated strong predicted antibacterial potential. Among them, the peptide designated PD101 showed favorable properties, including predicted antibacterial activity, cell-penetrating capacity, and intracellular targeting potential. Its physicochemical profile suggests preferential interaction with bacterial cells, indicating a potentially low risk of host cytotoxicity. These findings demonstrate the value of machine learning–driven computational tools for prioritizing novel antimicrobial peptides and identify PD101 as a promising candidate for reducing reliance on conventional antibiotics in livestock systems. Experimental studies are nevertheless required to validate its stability, delivery efficiency, and feasibility for large-scale production.

INTRODUCTION

Antimicrobial resistance (AMR) represents a growing constraint on dairy production systems, particularly in developing countries such as Nigeria, where antibiotics are frequently used for disease prevention and treatment in cattle (Adesanwo *et al.,* 2024). The persistence of resistant bacteria in milk and dairy products threatens food safety, public health, and the economic sustainability of the dairy sector (Ahsan *et al.,* 2024). Conventional antibiotics are increasingly losing effectiveness, creating an urgent need for alternative antimicrobial strategies that can be integrated into livestock management (Masato *et al.,* 2022). Antimicrobial peptides (AMPs) have emerged as attractive candidates due to their broad-spectrum activity, rapid mechanisms of action, and lower likelihood of inducing resistance (Adeluwoy *et al.,* 2024). Advances in bioinformatics and machine learning have made it possible to explore large genomic datasets for peptide sequences with antimicrobial potential before undertaking labor-intensive laboratory experiments (Marc *et al.,* 2012). In silico prediction approaches therefore offer a cost-effective pathway for identifying novel AMPs relevant to livestock-associated pathogens (Duan *et al.,* 2021).

The problem of Mastitis in Cattle is significant concern because not only does it affect the health and well being of cattle but has economic implications to dairy Farmers (Johabsson and Flach, 2021). Furthermore, the emergence of ESBL-producing Enterobacteria in mastitic Cattle has added a new later of complexity to this issue because study suggest that 70% of raw milk samples contains E.coli which is one among the member of Enterobacteriaceae (Lupo and Papp, 2018). The isolates were separated with gel electrophoresis and visualize with ethidium bromide and staining reveals Extended Spectrum Beta-lactamase lactamse genes (Kimera *et al.,* 2020). These bacteria are increasly prevalent and there is

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limited data on their present in mastitic cattle in Birnin kebbi. This lack of understanding makes it challenging to control strategies, which is why this research is crucial. Globally, antimicrobial resistance (AMR) has been identified as a rapidly expanding One Health issue (Azimun *et al.,* 2023). The improper use of antibiotics in people and animals is the main cause of AMR. Understanding AMR in animals is crucial to resolving this growing One Health issue since the animal business uses a significant amount of antimicrobials to treat Escherichia coli (E. coli) infections, which is difficult (Avanika *et al.,* 2020). Since milk is regarded as a complete food and a significant component of everyday human diets everywhere, including in Nigeria, the rise of antimicrobial-resistant bacteria like Escherichia coli in milk is a major public health problem (Kenneth *et al.,* 2022). The molecular characterization and antibiotic resistance profile of E. coli that produces extended-spectrum beta-lactamase (ESBL) from the milk of healthy Nigerian cows, however, have not been reported. Thus, the purpose of this study was to identify and describe ESBL-producing E. coli (ESBL-Ec) in milk samples from healthy cows (Sascha *et al.,* 2016). Dairy cattle and other animals raised for food are possible sources of antibiotic resistance. On the other hand, little is known about the selection of resistant bacteria and the usage of antimicrobials (Sascha *et al.,* 2016).

Antimicrobial resistance (AMR) is a quiet issue in the worldwide cattle business (Sasch et al ., 2016). Superbugs are a serious threat to human and animal health because of the uncontrolled use of antibiotics in animals (Garba *et al.,* 2025). Antibiotic residues in tissues can have negative impacts on human health, and eating tainted animal products, such beef, and can spread resistant bacteria (Nan-Lingri *et al.,* 2024). Beta-lactam antibiotic residues have been found in fresh and smoked beef samples in recent research, such as the one by, underscoring the possible hazards to human health. The extensive use of antibiotics in animals as well as inadequate regulation and oversight are blamed for the existence of these residues. (Garba *et al.,* 2025) Offers important information about the amount and presence of beta-lactam antibiotic residues in beef samples. Building on previous discoveries, the current in silico work looks into the molecular traits of antibiotic resistance genes (ARGs) linked to different livestock organisms, such as cattle bacteria (Eugeni B. *et al.,* 2019). This work will deepen our understanding of the genetic basis of antibiotic resistance in livestock by functionally annotating ARGs and predicting and generating antibiotic resistance peptides (Ke *et al.,* 2015). The objectives of this work are to identify and retrieve genomic sequences of antibiotic-resistant organisms from the NCBI database, use machine learning tools to predict and generate antibiotic resistance peptides, annotate ARGs functionally, and display the genetic context and relationships of identified ARGs. By accomplishing these goals, we will learn more about the molecular causes of antibiotic resistance in cattle, which will help develop solutions to reduce the spread of AMR, enhance animal health, and safeguard human health worldwide (Josman & Helena, 2020). In the worldwide cattle sector, antimicrobial resistance (AMR) has become a major but frequently disregarded problem (Kauthar M. O. *et al.,* 2024).

The global rise of Antimicrobial resistance demands urgent attention (Algammal *et al.,* 2020). Further research is needed to understanding the transmission mechanisms of antimicrobial in household which is critical Public health concern (Krisha *et al.,* 2025). By investigating the prevalence, predispossing factors, antimicrobial resistance pattern and molecular mechanisms underlaying the ESBL-production in Enterobacteria (Eugen *et al.,* 2019). We Sha be taking a step towards unveiling the hidden threats and reducing the burden of antimicrobial resistance. This research findings will guide clinicians in developing effective treatment strategies for infection caused by ESBL-producing Enterobacteriaceae, Support surveillance effort to track the spread of ESBL -producing genes and enhance our understanding of the molecular mechanisms underlaying ESBL-production (Josman and Helena, 2020). This study focuses on PD101, a computationally predicted antimicrobial peptide derived from biosynthetic genes associated with organisms linked to dairy cattle (Armen. D. *et al.,* 2015). Using machine learning–based prediction tools, the study aims to evaluate the predicted structure–function relationship of PD101, infer its mechanism of antibacterial action, and assess its potential effectiveness against antibiotic-resistant bacteria commonly associated with dairy products in Nigeria. By exploring the possible application of PD101 within dairy production and processing systems, this work contributes to ongoing efforts to reduce antibiotic dependence and mitigate the spread of AMR in livestock.

MATERIALS AND METHODS

Antimicrobial Peptides (AMPs) from biosynthetic genes were carefully collected using the CAMPR3 program, which is renowned for its specific machine-learning capabilities. This ensured the accuracy of peptide selection for further research. Two steps comprised the methodology: first, CAMPR3 had to predict a single AMP with a length of 99 amino acids, ranking the selection according to the highest likelihood among all models that were provided. Twenty AMPs, each containing 20 amino acids, were carefully selected based on the highest likelihood across the majority of models after the prediction was painstakingly broken down. In order to enable a thorough investigation of possible antimicrobial peptides and guarantee diversity and feasibility for experimental validation, this strategy sought to simplify and concentrate the analysis.

Table 1: Phytochemical Properties Prediction

Secondary Metabolites	S/N	Encoding Gene	Protein	Length	Predicted AMP within Peptides		AMP Probability				Predicted as AMP Across All Models
					SEQUENCE	POSITION	SVM	RF	ANN	DA	
Terpene	1	ctg2_19	phytoene synthase	292	99 AMP						
					GRCYIPATWLGKADIT PGEHMKPHYRKALAG IGAKLAEMAEGYEASA RIGARRLPFRSRWAVLSA AGIYGDIAREVARRGEH AWDSRVFTRKTDKLRWV	186-284	0.999	0.882	AMP	1.000	YES
					20 AMP						
					ARIGARRLPFRSRWAVLSAA	47-66	0.952	0.989	AMP	0.995	YES
	2	ctg2_22	Lycopene cyclase	386	99 AMP						
					NCDLAIVGGGLAGGLIA LALKHHRPELDVRVIEG DARLGGNHVWSFFDTD IAPEDWPIVEPLIGWRWQ DYDIIFPAHSRTLEAVYNS IDSEHFDSVVRA	6-104	0.987	0.885	AMP	0.885	YES
					20 AMP						
					LAIVGGGLAGGLIALALKHH	4 - 23	0.977	0.931	AMP	0.988	YES

lanthipeptideclass	3	GGR89_ _RS12515	Lanthionine synthetase C-like								

Identification and Retrieval of Genomic Sequences

The NCBI database was searched for genomic sequences of antibiotic-resistant organisms using keywords such as antimicrobial resistance, Escherichia coli, and dairy cattle study conducted by (Azimun *et al.,* 2023) indicate that used a similar strategy to extract genomic sequences of ESBL-producing E. coli from the raw milk of healthy cows in Bangladesh (Ahsan *et al.,* 2024). Following this methodology, we used a similar procedure in this work to identify interesting genomic sequences for future analysis (Kenneth *et al.,* 2022).

Prediction and Generation of Antibiotic Resistance Peptides

We predicted and synthesized antibiotic-resistant antimicrobial peptides (AMPs) from biosynthetic genes using the CAMPR3 program, which is known for its superior machine learning methods. Our methodology is similar to the in silico strategy used by Duan *et al.* (2021) to predict and examine AMPs from rapeseed protein. CAMPR3 was chosen because of its excellent AMP prediction accuracy and robust ability to analyze big datasets as implicated by study conducted by (Kauthar *et al.,* 2024).

Functional Annotation of ARGs

We functionally annotated antibiotic resistance genes (ARGs) using standard bioinformatics tools, following the methods described by (Kauthar *et al.,* 2024). in their metagenomic analysis of antimicrobial resistance genes in cattle from Kenya, Uganda, and Tanzania. This technique was adopted to acquire a better knowledge of the genomic architecture and interactions between the ARGs discovered throughout our analysis (Roschankis and Fischer, 2014; Kauthar *et al.,* 2024)

Analysis of Secondary Metabolite (PD101)

A comprehensive physicochemical investigation of PD101, a predicted antimicrobial peptide, was carried out to determine critical parameters such as hydrophobicity index, net charge, and molecular mass. This characterization method is similar with the analytical framework used by (Duan *et al.,* 2021) to profile multidrug-resistant Escherichia coli collected from dairy calves with mastitis in Algeria. Property evaluation was critical for deducing PD101's antibacterial mechanism and determining its therapeutic potential (Kenneth *et al.,* 2022). These strategies were chosen to fully leverage computational prediction capabilities for the identification of novel AMPs (Ejikeugwu and Esinone, 2018). Potential candidates such as PD101 were discovered by a swift, broad examination made possible by the combination of CAMPR3 and bioinformatics techniques. The goal was to develop a prioritized list of AMPs for therapeutic viability assessment and experimental validation as suggested by (Timoffe *et al.,* 2014; Ke *et al.,* 2015).

RESULTS AND DISCUSSION

Result

Promising insights into the prediction of antimicrobial peptides (AMPs) were obtained from the investigation of genes encoding proteins involved in secondary metabolite biosynthesis. In particular, computer models found a peptide sequence between locations 186 and 284 in the Terpene Synthase (ctg2_19) gene that was consistently categorized as an AMP by all models. The SVM, RF, and ANN models indicated probabilities of 0.999, 0.882, and 1.000, respectively, indicating high probabilities of antibacterial action (Dantas *et al.,* 2020). Furthermore, its classification as an AMP was validated by Discriminant Analysis, highlighting its strong antibacterial potential. Similarly, all models identified a peptide sequence from positions 6 to 104 in the Lycopene Cyclase (ctg2_22) gene as an AMP; the SVM, RF, and ANN models predicted probability of antimicrobial activity at 0.987, 0.885, and 0.885, respectively. Additionally, the DA supported its categorization as an AMP, suggesting that peptides produced from these proteins involved in secondary metabolite formation have a high likelihood of having antimicrobial properties (Duan *et al.,* 2021; Marc T. *et al.,* 2012).

Table 2: Phytochemical Properties Prediction

Peptide ID	Metabolite (peptide)	Hydrophobic amino acid								The number of G and P		Negatively charged amino acid		Positively charged amino acid			Other amino acids				total hydrophobic ratio (%)		The total net charge	Molecular Weight (MW)	WIMELY HYDROPHOBICITY	Boman index (kcal/mol)
		I	V	L	F	C	M	A	W	G	P	E	D	K	R	H	T	S	Y	Q	N					
PD101	GRCYSPATWLGKADTTPGEHMKPHYR KALAGIGAKLAEMAEGYEASARIGARR LPFRSRWAVLSAAGIYGDIAREVARRGE HAWDSRVFTRKTDKLRWV	5	4	6	2	1	2	16	4	10	4	6	4	6	14	3	4	5	4	0	0	40	9.74	11098.761	21.1	2.17

Size and Class Comparison

Pd101 Is not a classical short Antimicrobial Peptide, But rather a Large, Structured, Cationic Peptide, Closer To Bacteriocins, Ribosomal Proteins, Dna-Binding Peptides, Or Enzyme-Associated Antimicrobial Proteins.

Pd101 Is Exceptionally Cationic, Placing It Closer To:

Table 3: Comparison of PD101 with Other Peptides

Peptide Type	Typical Length	Typical Mw	How Pd101 Compares
Short Amps (Defensins, Magainins)	20–50 Aa	2–6 Kda	Much Larger
Helical Amps (Ll-37-Like)	30–40 Aa	~4 Kda	Much Larger & More Complex
Bacteriocins (Class Ii)	50–80 Aa	5–9 Kda	Slightly Larger
Enzymatic/Functional Peptides	80–120 Aa	9–14 Kda	Very Similar
Pd101	~100 Aa	~11 Kda	Falls Into Large Cationic Peptide / Mini-Protein Range

Table 4: Charge Comparison

Peptide	Net Charge
Typical Amps	+2 To +9
Strongly Cationic Amps	+10 To +15
Dna-Binding Proteins	+10 To +25
Pd101	**+21.1**

DNA/RNA-Binding Proteins

Cell-Penetrating Peptides

Highly Potent Membrane-Active Antimicrobials

Pd101 Is Not Strongly Lytic. Instead, It Likely:

Table 5: Hydrophobicity Comparison

Feature	Typical Membrane-Lytic Amps	Pd101
Hydrophobic Residue %	30–50%	~10%
Amphipathic Helices	Strong	Moderate
Aggregation Tendency	Moderate–High	Low

Associates with Membranes

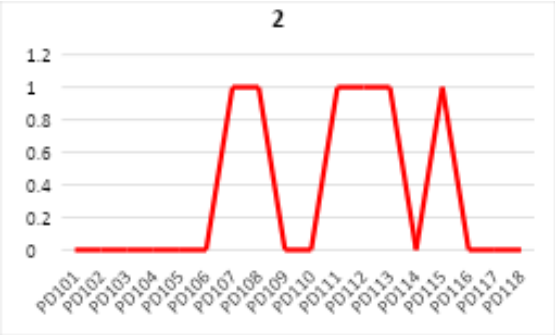
Penetrates Rather Than Destroys Them

Targets Intracellular Components

Peptide Identity: This is the unique identifier and sequence

Table 6: Possible Biological Functions

Activity	Likelihood	Reason
DNA/RNA Binding	Very High	Charge Density, Arg/Lys Richness
Cell-Penetrating Peptide (Cpp)	High	Size + Cationic Nature
Signaling Peptide	Moderate	Length Supports Structured Domains
Enzyme Inhibitor	Moderate	Boman Index + Size
Hemolytic/Toxic	Low–Moderate	Low Hydrophobic Ratio



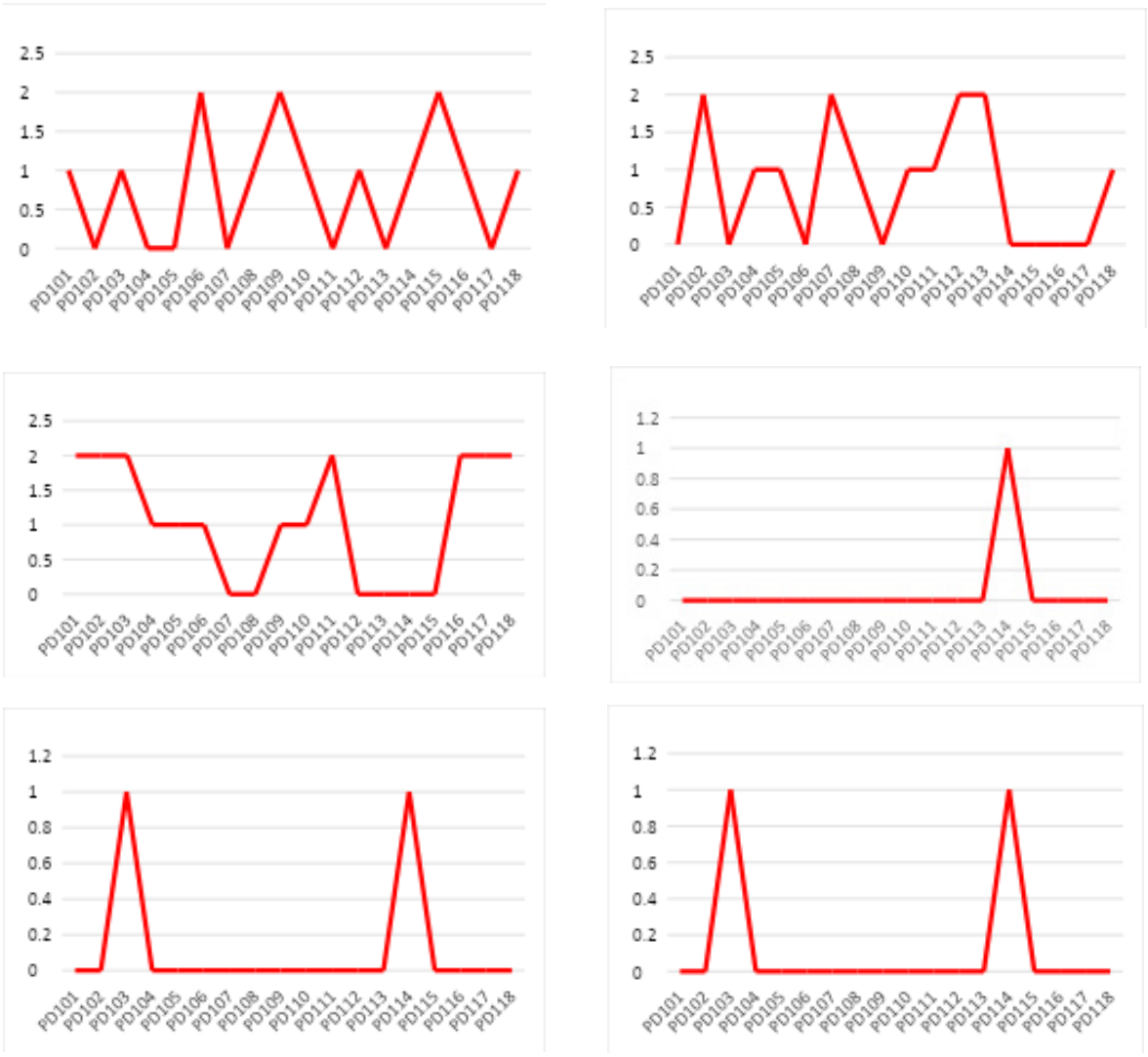


Figure 1: Hydrophobic amino acid I, V, L, F, C, N, A, W

of the peptide PD101, a 65-amino acid chain with specific functions already indicated by (Chien-Hao, Chia-Wei , & Po-Yu, 2021). PD101 is a large, structured, cationic peptide (~11 kDa, 65 aa), larger than typical AMPs but similar to bacteriocins or DNA-binding proteins. Its strong positive charge (+21.1) suggests high affinity for bacterial membranes and intracellular targets like DNA/RNA similar to the study conducted by (Arslan A., Herrison F., Nilton L., Elder S., & Mashkoor M., 2021).

Penetrating peptides, or potent membrane-active antimicrobials.

Amino-acid composition: This breaks down the peptide's

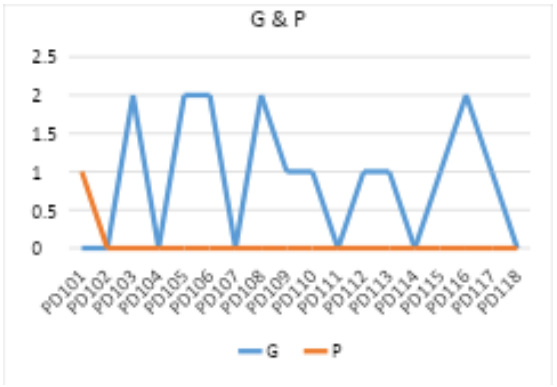


Figure 2: The Number of G and P

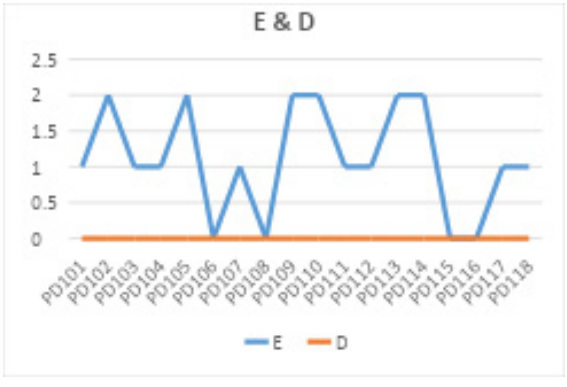


Figure 3: Negatively charged amino acids

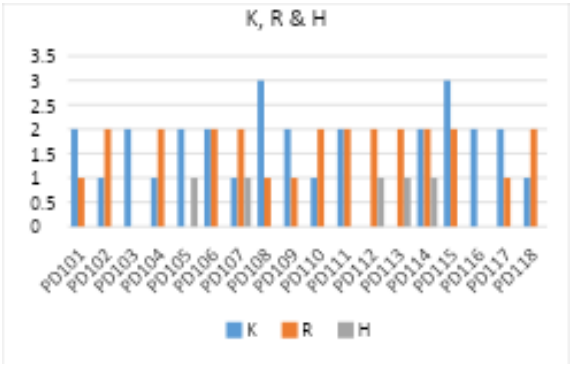


Figure 4: Positively charged amino acids

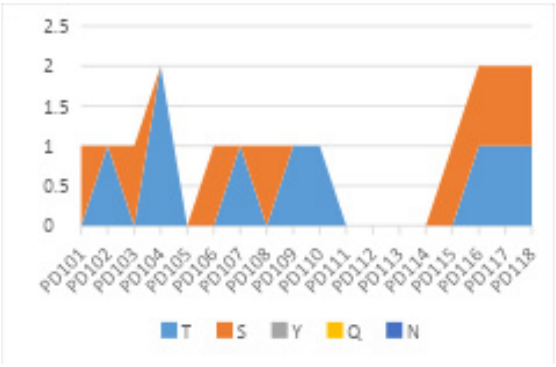


Figure 5: Other amino acids

amino acids into categories (hydrophobic, charged, etc.), influencing its structure, function, and interactions. Positive residues are distributed throughout the sequence, supporting global electrostatic interaction and structural stability.

Hydrophobic ratio with 9.74% hydrophobic residues, PD101 has moderate hydrophobicity, allowing interactions without strong aggregation.

Molecular weight: The peptide's mass is approximately 11 kDa (11,098.761 Da), indicating it's a relatively large peptide.

Boman index: With 2.17 kcal/mol, PD101 shows

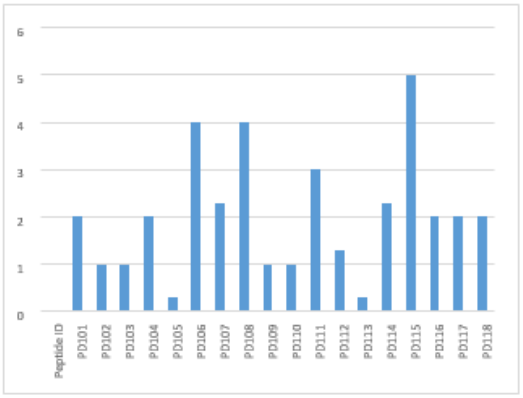


Figure 6: Total hydrophobic ratio

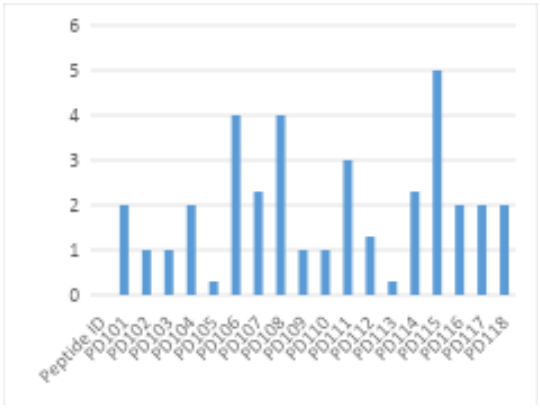


Figure 7: The total net charge

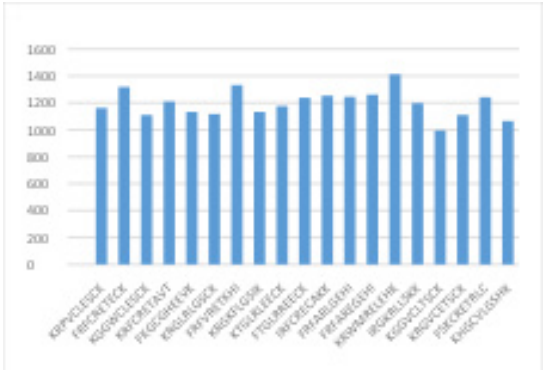


Figure 8: Molecular Weight

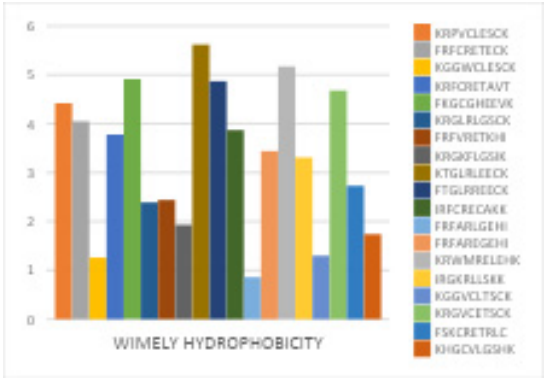


Figure 9: Wimely hydrophobicity

moderate affinity for protein-protein interactions.

PD101 is a large, strongly cationic, moderately hydrophobic peptide likely involved in antimicrobial activity (non-lytic), cell penetration, and intracellular targeting, comparable to bacteriocins or engineered therapeutic peptides.

Discussion

Antimicrobial resistance is of significant public health concern affect food security and domestication of livestock (WHO, 2020). In Nigeria, dairy cattle are crucial for livelihoods and nutrition (Adesokan *et al.,* 2015). However, the misuse and overuse of antibiotics

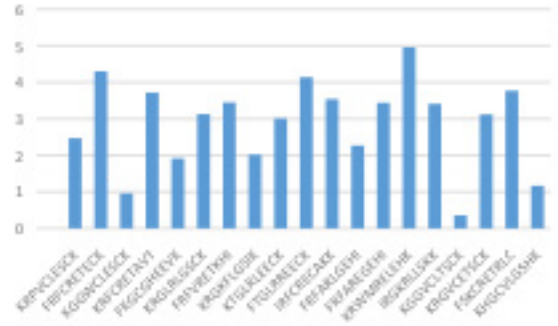


Figure 10: Boman index (kcal/mol)

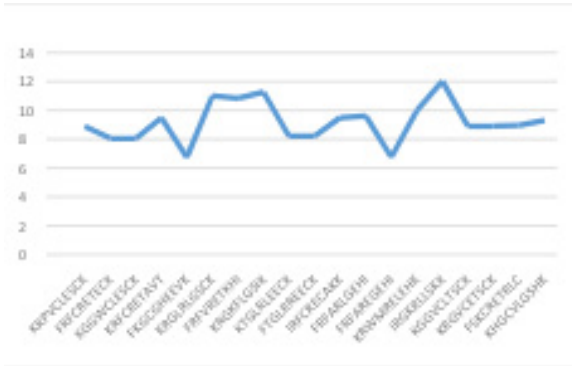


Figure 11: Theoretical pI

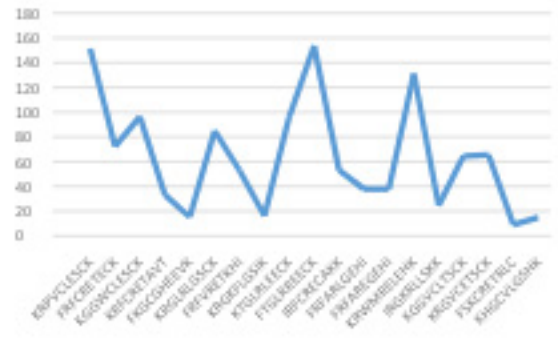


Figure 12: Instability index (II)

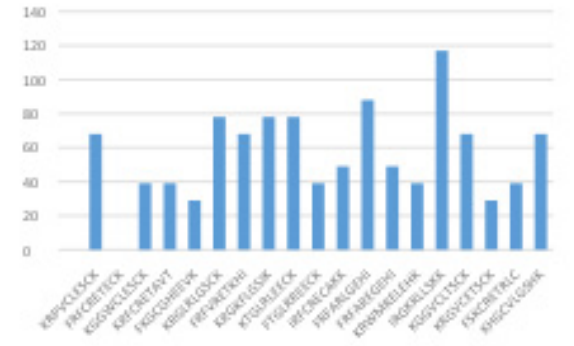


Figure 13: Aliphatic index

in dairy farming significantly contribute to antimicrobial resistance (AMR), particularly the emergence of Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae (Eze *et al.,* 2021). Several studies have reported worrying levels of antibiotic resistance among dairy cattle in Nigeria. For example, Adesokan *et al.* (2015) found that 71% of E. coli isolates from dairy cattle in Oyo State were resistant to tetracycline. Similarly, Eze *et al.* (2021) detected ESBL-producing E. coli in 43% of mastitic milk samples collected in Lagos. In another study, Ojo *et al.* (2019) identified multidrug-resistant Enterobacteriaceae in 60% of dairy cattle sampled in Ibadan. Akindolire *et al.* (2020) also reported that 80% of ESBL-producing isolates obtained from dairy farms in Kwara State carried the blaCTX-M gene. More recent research has further documented the presence of ESBL-producing *E. coli not only in cattle but also in humans, beef cattle, and abattoir environments in Nigeria, suggesting possible transmission across different settings (Ojo *et al.,* 2022). ESBL-producing Enterobacteriaceae commonly carry bla genes, such as blaCTX-M and blaTEM, on plasmids, which allows these resistance traits to spread easily between bacteria (Carattoli, 2013; Okeke *et al.,* 2018). Okeke *et al.* (2018) reported that blaCTX-M was the most prevalent gene among ESBL-producing E. coli isolates from Nigerian cattle, while Igunma *et al.* (2022) found a high prevalence of blaTEM genes in ESBL isolates from dairy farms. Additionally, genetic studies of antibiotic-resistant Enterobacteriaceae from bovine animals and surrounding environments in Nigeria have revealed a wide range of resistance mechanisms (Adekanmbi *et al.,* 2023).

The growing problem of AMR in dairy cattle poses serious risks to public health and food safety (WHO, 2020). As a result, adopting responsible antibiotic use and improving hygiene and farm management practices are essential measures to control the spread of resistance (Adesokan *et al.,* 2015). Recent research has also highlighted the importance of identifying priority areas for intervention to address AMR in both the human and animal health sectors in Nigeria (Abubakar *et al.,* 2022).

The present in silico investigation identified two peptide sequences derived from Terpene Synthase (ctg2_19) and Lycopene Cyclase (ctg2_22) genes with a high probability of antibacterial activity, as supported by discriminant analysis. The physicochemical properties of these peptides, particularly PD101, provide insight into their predicted antimicrobial behavior. The strong positive net charge (+21.1) combined with moderate hydrophobic content (~10%) (Tables 3 and 4) suggests a mechanism that involves electrostatic interaction with bacterial membranes followed by intracellular penetration, a characteristic commonly associated with cationic antimicrobial and cell-penetrating peptides. These findings support earlier reports proposing AMPs as viable alternatives to conventional antibiotics in livestock systems, especially in the context of rising resistance among dairy-associated pathogens. Machine learning–based prediction tools, such as CAMPR3, have proven effective in identifying peptide candidates with

desirable antimicrobial features, reinforcing the relevance of computational screening in antimicrobial discovery efforts. The need for such alternatives is particularly evident in studies documenting the widespread occurrence of multidrug-resistant Escherichia coli in dairy farms (Falgenhaver *et al.,* 2019).

These observations support earlier studies that highlight the growing relevance of AMPs as viable alternatives to conventional antibiotics, especially in the context of antimicrobial resistance (AMR) in livestock production systems (Kauthar M. O. *et al.,* 2024; Yuvaneswary *et al.,* 2025). For instance, Yuvaneswary *et al.* (2025) documented widespread antimicrobial resistance among Escherichia coli isolates from dairy farms, emphasizing the urgency of developing non-traditional antimicrobial strategies. Similarly, Kauthar M. O. *et al.* (2024) demonstrated the robustness of machine-learning approaches for AMP discovery, reinforcing the predictive strength of computational screening platforms such as CAMPR3.

The predicted peptide PD101 exhibits properties comparable to those of bacteriocins and DNA-binding proteins, indicating a potential role in targeting bacterial genetic material rather than relying solely on membrane lysis (Marc T. *et al.,* 2012). Its hydrophobic amino acid distribution (Figure 1), coupled with a strong net positive charge (Figure 6), further supports a CPP-like mode of action that facilitates cellular entry and intracellular interference. This mechanism is particularly relevant given the documented public health burden posed by extended-spectrum β-lactamase (ESBL)-producing E. coli in dairy cattle (Azimun *et al.,* 2023; Adesanwo *et al.,* 2024), underscoring the need for novel antimicrobial solutions such as PD101.

A notable contribution of this study is the application of machine-learning-based prediction tools (CAMPR3) to identify and characterize novel AMPs from biosynthetic gene clusters. This approach illustrates the efficiency and scalability of in silico methodologies for antimicrobial discovery, especially in resource-limited settings. The distinctive physicochemical profile of PD101 (Tables 1 and 2) suggests that it may exhibit reduced cytotoxicity while maintaining antibacterial selectivity, positioning it as a promising candidate for therapeutic development.

Beyond its biomedical implications, the findings carry important economic and agricultural significance as suggested from study conducted by (Ke *et al.,* 2015). Effective AMP-based interventions could reduce antibiotic dependence in cattle production, thereby mitigating AMR-associated costs to animal health and public healthcare systems (Nan-Ling *et al.,* 2024). Additionally, limiting antibiotic usage may enhance food safety, support sustainable livestock practices, and improve international trade competitiveness. Nevertheless, despite its promising in silico profile, PD101 requires experimental validation to assess its antimicrobial potency, stability, delivery efficiency, and production feasibility (Marc *et al.,* 2012). As emphasized by Mandal *et al.* (2012), further studies addressing peptide stability, bio distribution, and manufacturing costs are essential prior to clinical or agricultural application (Partridge and Fischer, 2014).

Phytochemical Properties of the Predicted Metabolite

The phytochemical analysis presented in Table 1 reveals that PD101 possesses a distinctive combination of physicochemical attributes. The presence of hydrophobic amino acids including I, V, L, F, C, M, A, and W accounts for a hydrophobic content of 9.74% (Figure 6), which may facilitate interactions with bacterial membranes while maintaining intracellular accessibility. PD101 has a molecular mass of 11,098.761 Da and a Boman index of 2.17 kcal/mol, indicating a stable peptide with a strong propensity for protein–protein interactions which was not in consistency with the study conducted by (Timofte, 2014). The pronounced net positive charge (+21.1) (Table 3) arises from a high abundance of basic residues (K, R, and H), which likely enhances electrostatic attraction to negatively charged bacterial membranes and intracellular components. Additionally, the Wimley–White hydrophobicity value of 21.1 further supports the peptide's ability to associate with membrane interfaces (Armen. D. *et al.,* 2015). Collectively, these properties suggest that PD101 as secondary metabolites is well suited for intracellular targeting and antibacterial activity implicated from study conducted by (Krishna J. *et al.,* 2025).

Comparison of Secondary Metabolite (PD101) with Other Peptides

Table 2 compares PD101 with established antimicrobial peptides, highlighting its unique structural and functional attributes as suggested by (Waseem *et al.,* 2023). Unlike shorter AMPs such as defensins or magainins, PD101 is relatively large (~65 amino acids; ~11 kDa) and highly cationic. These features align more closely with bacteriocins or nucleic-acid-binding proteins, suggesting enhanced intracellular activity (Okechukwu *et al.,* 2020). Its moderate hydrophobicity may contribute to improved selectivity toward bacterial cells while minimizing toxicity to host tissues. This comparison underscores PD101's potential as a novel antimicrobial agent with a mechanism of action distinct from classical AMPs, making it particularly attractive for targeting antibiotic-resistant pathogens (Martina *et al.,* 2024).

Amino Acid Composition and Structural Features of PD101

Figures 1–5 collectively illustrate the amino acid composition and physicochemical characteristics of PD101, providing insight into its functional potential. Figure 1 shows a balanced distribution of hydrophobic residues, supporting moderate membrane interaction without excessive cytotoxicity similar to the study conducted by (Lara *et al.,* 2018). Figure 2 highlights the presence of proline and glycine residues, which may enhance peptide flexibility and structural stability,

contributing to resistance against proteolytic degradation implicated from study conducted by (Olusolabomi *et al.,* 2020). Figure 3 demonstrates the low abundance of negatively charged residues (E and D), reinforcing the peptide's strong overall cationicity and affinity for negatively charged bacterial surfaces. Figure 4 illustrates the widespread distribution of positively charged residues (K, R, and H) throughout the sequence, facilitating global electrostatic interactions with bacterial membranes and intracellular targets. Finally, Figure 5 depicts the remaining polar and non-polar residues that collectively influence PD101's three-dimensional structure and functional versatility (Shereen *et al.,* 2022).

CONCLUSION

The Terpene Synthase and Lycopene Cyclase genes produced promising antimicrobial peptides (AMPs) in this in silico investigation, with PD101 exhibiting promise as a treatment option. It may be effective against bacteria that are resistant to antibiotics because of its strong cationicity, mild hydrophobicity, and intracellular targeting abilities. This study shows the effectiveness of computational methods in finding new AMPs by utilizing machine learning (CAMPR3). PD101 may provide a practical substitute for conventional antibiotics, reducing cattle antimicrobial resistance. To fully achieve its medicinal potential, more study on stability, delivery, and production costs is required. Together, the data presented in Figures 1–5 support the classification of PD101 as a promising antimicrobial peptide with intracellular targeting capabilities, warranting further experimental investigation. Overall, this study demonstrates the value of computational strategies in identifying novel antimicrobial peptides and reinforces the importance of continued exploration of alternative approaches to combat AMR. The results provide a foundation for future experimental studies aimed at developing new antimicrobial agents capable of reducing AMR in livestock systems, with broader benefits for public health and economic development in Nigeria and globally. The discovered peptides present a viable substitute for traditional antibiotics and can be used to combat antimicrobial resistance in cattle production. To fully exploit the promise of these peptides, more investigation is required to examine their stability, delivery, and production costs. PD101 is a promising therapeutic candidate having antibacterial action (non-lytic), cell penetration, and intracellular targeting potential. Its selectivity for bacterial cells over mammalian cells suggests that it has the potential to cure infections with low cytotoxicity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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