Evaluation of Bacteriocins against Multi-drug Resistant Bacteria Implicated in Urinary Tract Infections

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Abstract: As the economic burden of treating urinary tract infections (UTIs) is gravely increasing due to multi-drug resistance (MDR) among uro-pathogens, the search for new sources of antimicrobial compounds including bacteriocins has been a priority recently. This study therefore, evaluated the antibacterial activity of bacteriocins against multi-drug resistant bacteria implicated in UTIs. Fermented food (ogi, tapioca, palm wine, kunu, raw milk, and unripe plantain) samples were collected and screened for bacteriocin-producing bacteria using standard microbiological methods. Cell-free bacteriocn was extracted from the screened isolates by centrifugation and tested against MDR *Escherichia coli (*MDREc) and *Staphylococcus aureus (*MDRSa) using agar-well diffusion method. Bacteriocin-producing bacterial isolates with high antibacterial activity were identified using PCR and sequencing methods. A total of eight isolates produced bacteriocins with antibacterial activity against *E. coli* MDREc (7.0 ± 0.40 mm to 19.0 ± 0.99 mm) and *S. aureus* MDRSa (4.0 ± 0.5 mm to 14.0 ± 0.49 mm). The MIC for the cell-free extracts ranged from 15.63 mg/ml to 31.25 mg/ml against *E. coli* MDREc and 31.25 mg/ml to 62.50 mg/ml against *S. aureus* MDRSa, while MBC was at 125 mg/ml to 250 mg/ml and 250 mg/ml against the organisms, respectively. Cell-free extracts of bacteriocin producing isolates obtained from kunu (KU-BPI(B)), raw milk (M-BPI(A)), and unripe plantain (PN-BPI(B)) had significantly ($P < 0.05$) higher antibacterial activity and the isolates were identified as *Morganella morganii* KU*,* *Proteus vulgaris* CM, and *Alcaligenes feacalis* PN, respectively. Hence, these isolates produced bacteriocins with broad spectrum bacteriostatic and bactericidal activity against multi-drug resistant *E. coli* MDREc and *S. aureus* MDRSa implicated in UTIs.

Key word: Bacteriocins, Antibacterial activity, Multi-drug resistant bacteria, Urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) are infections of the urinary system, mostly affecting the urethra, ureter, bladder, and the kidneys (Newstead *et al.*, 2020; Mancuso *et al.,* 2023). UTIs are hospital or community-acquired infections, predominantly caused by bacteria in the Enterobacteriaceae family particularly *Escherichia coli* and some Gram positive bacteria including *Staphylococcus aureus* (Bader *et al.,* 2020; Baldiris-Avila *et al.,* 2020; Santos *et al.,* 2022; Sujith *et al.,* 2024). Urinary tract infection (UTI) is one of the leading infections with high rate of morbidity and economic burden, affecting about one hundred and fifty (150) to two hundred and fifty (250) million people annually on a global scale (Flores-Mireles *et al.,* 2015; McCann *et al.,* 2020; Santos *et al.,* 2022; Sujith *et al.,* 2024). The economic burden due to the high cost of treatment of urinary tract infections is escalated by the development of multi-drug resistance to available effective antimicrobial agents against the bacterial uro-pathogens and this constitute a grave threat to public health (WHO, 2019; Benítez-Chao *et al.,* 2021; Larsson and Flach, 2022; Abiom *et al.,* 2024; Charkhian *et al.,* 2024). Multi-drug resistance in bacteria may be due to biofilm formation, mutation, and possible spread of drug resistant genes on mobile genetic elements such as plasmid and transposons among bacterial pathogens (Behrens *et al.,* 2020; Yakubu *et al.,* 2020; Bedenic *et al.,* 2021; Oyedum *et al.,* 2022). This selective pressure and prevalence of multi-drug resistant bacteria is attributed to over-use or indiscriminate use of antibiotics for treatment of human diseases or prolonged use in animal husbandry for disease control or growth enhancement and have reduced the effectiveness of many classes of available antimicrobial drugs (Gupta and Datta, 2019; Wenjing *et al.,* 2025). It has been estimated that by the year 2050 the few available antimicrobial drugs would be completely resistant to and inefficient for the treatment of human infections except new classes of drugs are manufactured (Rolain *et al.,* 2016; Gradisteanu *et al.,* 2021).

Responding to this challenge of drug resistance, alternative sources of antimicrobial compounds including bacteriocins production by bacteria, which may be sustainable and cost-effective have been suggested in recent times (Benítez-Chao *et al.,* 2021; Gradisteanu *et al.,* 2021; Kuznetsova *et al.,* 2022). Bacteriocins are bio-active antibacterial peptides synthesize in the ribosomes by Gram-positive/negative bacteria and exported extracellularly against closely related bacteria as antagonistic means to surviving in their environment (Nandan and Nagar, 2016; Costa *et al.,* 2019; Benítez-Chao *et al.,* 2021; Gradisteanu *et al.,* 2021; Ajiboye *et al.,* 2022). Bacteriocins are secondary metabolites produced at certain growth phase of bacteria and have been reported to demonstrate antibacterial activity against clinically susceptible bacteria Charkhian *et al.,* 2024). The appealing qualities which qualifies bacteriocins as suitable alternative antibacterial compounds to conventional antimicrobial agents include broad spectrum activity against diverse groups of bacteria including uro-pathogens, quick bactericidal/bacteriostatic activity against both metabolically inactive and active pathogens, low minimum inhibitory concentrations, and stability of activity over expanded range of environmental influences (Riley *et al.,* 2013; Nandan and Nagar, 2016; Gradisteanu *et al.,* 2021). Therefore, this study evaluated the antibacterial activity of bacteriocins against multi-drug resistant bacteria implicated in urinary tract infections.

MATERIALS AND METHODS

Collection and confirmation of test multi-drug resistant bacteria: Test multi-drug resistant *Escherichia coli* and *Staphylococcus aureus* isolated from high vaginal swab (HVS) samples were collected from the Department of Microbiology, Akwa Ibom State University in sterile agar slants. These organisms were further identified and confirmed using the standard cultural, morphological, and biochemical protocols in the Department of Microbiology of the Akwa Ibom State University, as well as the molecular method by polymerase chain reaction (PCR) and sequencing (Jacob et al, 2024a).

Isolation and screening for bacteriocin-producing bacteria: Fermented food samples such as ogi, tapioca, palm wine, kunu, row milk, and unripe plantain, were freshly purchased from vendors in the local markets and streets in Mkpat Enin and Eket, Akwa Ibom State, and aseptically transported within 1 h on ice-packed cooler to the Microbiology Laboratory for analyses. The samples were serially diluted in normal saline, inoculated on deMan Rogosa and Sharpe (MRS) agar by pour-plating technique and incubated both aerobically and anaerobically at 37°C for 24 h (Khodaei and Soltani, 2018; Antia *et al.,* 2024). Different colonies, particularly those forming halo, were picked and sub-cultured on fresh MRS agar to purify. The pure colonies were preserved on agar slants at 4±3°C in the refrigerator (Jacob *et al.,* 2024b). The pure colonies were screening for bacteriocin production using the deferred sandwich antagonism method (Muriana *et al*., 2015). Exactly 0.1 ml aliquot of 24 h broth culture (1%) of each isolate was inoculated on the surface of MRS agar plates by spread plate technique. The plates were covered with about 5.0 ml molten MRS agar to form a sandwich layer, allowed to set and incubated both aerobically and anaerobically at 37 °C for 24 h. Then, the colonies which developed on the sandwich layer of the plates were overlaid with about 10 ml of 1.5% molten MRS agar inoculated with 1 OD_{600nm} of 24 h broth culture of indicator bacteria (*Escherichia coli*) and re-incubated at 37°C for 24 h. The plates were examined and the isolates with colonies surrounded with halo and zones of clearance were selected and preserved on slants at 4±3°C in the refrigerator as bacteriocin producers (Prasanth *et al.,* 2019).

Determination of antibacterial activity of bacteriocin extracts using agar-well diffusion assay: Cell-free bacteriocin extracts were obtained by centrifuging (21010009, 6x20 ml, 800D Centrifuge) one milliliter (1 ml) of 24 h broth culture of each bacteriocin-producing bacterial isolate at 4000 rpm for 10 minutes at 30°C (Prasanth *et al.,* 2019; Sukmawati *et al.,* 2022; Al-Qudah *et al.,* 2023). Sterile-prepared Mueller-Hinton (MH) agar plates were uniformly seeded with 0.1 ml aliquot of 1 OD_{600nm} (measured with LabMed spectrophotometer Spectro UV-Vis Rs UV-2500 at 600 nm) of each multi-drug resistant test bacteria (*E. coli* and *S. aureus)* in triplicates and allowed to dry **(**Pato *et al.,* 2022; Wei *et al.,* 2022). Three evenly spaced wells of about 8 mm in diameter were cut through each plate using a sterile cork-borer. One well per each triplicate plate was aseptically filled with 100 µl of the cell-free bacteriocin extracts of each bacteriocin-producing isolate. The remaining two wells were filled with 100 µl of gentamycin (10 µg) and sterile MRS broth without the extract to serve as standard positive and negative controls, respectively. The plates were allowed to stay on the bench for 1 h in order to achieve complete diffusion before inverted and incubated at 37°C for 24 h. The plates were examined and the inhibitory zones measured in millimeters using a transparent ruler. The bacteriocin extracts with wider zones of inhibition against the test were selected for further assay.

***Determination of MIC and MBC of the Cell-free bacteriocin extracts*:** The minimum inhibitory concentration of the cell-free bacteriocin extracts with wider zones inhibition against test organisms was carried out using broth dilution technique, a modification of the method described by Al-Qudah *et al.* (2023). A two-fold serial dilution of each bacteriocin extracts at 1000 mg/ml was carried out in sequence of test tubes containing 2.0 ml of nutrient broth to obtain varying concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml, 7.81 mg/ml, 3.91 mg/ml, 1.95 mg/ml and 0.98 mg/ml. Exactly 200 µl aliquot of 24 h broth culture (1 OD_{600nm}) of each test bacteria was inoculated into each test tube and incubated at 37°C for 24 h. The MIC was read after incubation as the least concentration of the cell-free extracts that inhibited the growth of the test bacteria, using turbidity as an index against a control (cell-free extract without test bacteria). Minimum bactericidal concentration (MBC) was determined by first selecting the tubes that showed no growth (turbidity) during the MIC test, and then a loopful of the aliquot from each tube was cultured on bacteriocin extract free agar plates at 37°C for 24 h. The least concentration in the MIC test at which no growth occurred in the sub-cultured plates was noted as the minimum bactericidal concentrations (MBC) of the cell-free bacteriocin extract of the isolates (Charkhian *et al.,* 2024).

Molecular characterization and identification of isolates: Bacteriocin-producing isolates with high antibacterial activity were identified molecularly using PCR and Sanger sequencing method. Genomic DNA of the bacterial isolates was extracted using Zymo Research (ZR) fungal/bacterial DNA mini prep extraction kit and procedure (supplied by Inqaba South Africa). The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer, the 16S rRNA region amplified using PCR master mix with 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ as a forward primer) and 1492R:5'-CGGTTACCTTGTTACGACTT-3’ as a reverse primer on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microliters for 35 cycles, and the product was visualized on 1% agarose gel using UV trans-illuminator at 120 V for 15 minutes. Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa under sequencing conditions of 32 cycles at 96°C for 10 seconds, 55°C for 5 seconds, and 60°C for 4 minutes. Obtained sequences were edited using the bioinformatics algorithm trace edit and search for similarity in the National Center for Biotechnology Information (NCBI) database using BLASTN. Phylogenetic analysis and evolutionary history was inferred using the Neighbor-Joining method in MEGA 11.0 (Jacob *et al.,* 2024a).

RESULTS

Table 1 shows the morphological and biochemical characteristics of the multi-drug resistant test bacteria coded MDREc and MDRSa isolated from high vaginal swab (HVS) samples obtained from patients with urinary tract infections. Isolate MDREc formed colonies with pink pigmentation on McConkey agar medium incubated aerobically at 37°C for 24 h. Microscopic examination of the pure colony of the organism showed Gram negative short rods. The organism was indole/methyl red positive, Voges-Proskaur/citrate negative, produced metallic-sheen colour on eiosin methylene blue (EMB) agar after 24 h of growth at 37°C and was confirmed *Escherichia coli*. Similarly, the test bacterium MDRSa formed golden-yellow pigmentation when grown on nutrient agar medium at 37°C for 24 h. Microscopic examination of the organisms showed Gram positive cocci-shape in clusters. It was coagulase positive and formed β-haemolysis on blood agar medium incubated at 37°C for 24 h. These characteristics confirmed the organism as *Staphylococcus aureus.*

A total of twenty seven (27) bacterial strains were isolated from all the food samples cultured, and of these, only eight (8) (29.6%) produced bacteriocin and were coded KU-BPI(A), KU-BPI(B), PN-BPI(A), PN-BPI(B), PN-BPI(D), TA-BPI(B), CM-BPI(A), and CM-BPI(B) (Table 2). Most (75%) of the bacteriocin-producing isolates were Gram positive rods, while the least (25%) were Gram negative rod-shaped bacteria. Plantain had the highest number, 3(37.5 %) of bacteriocin producers, while tapioca had the least number 1(12.0 %) of bacteriocin producers among all the food samples (Figure 1).

Among all the bacteriocins-producing isolates, the cell-free extract obtained from isolate PN-BPI(B) had the highest zone of inhibition of 19.0 ± 0.99 mm and 14.0 ± 0.49 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively (Table 3). Similarly, the cell free extract obtained from isolate PN-BPI(A) had the lowest zone of inhibition (6.0 ± 0.52 mm and 4.0 ± 0.52 mm) against the test bacteria, respectively. When compared to the antibacterial activity of the positive control (14.0 ± 0.45 mm against *E. coli* and 12.0 ± 0.45 mm against *S. aureus*), the cell free extract PN-BPI(A) had significantly ($P < 0.05$) higher zone of inhibition against the test bacteria. The cell free extracts obtained from isolates TA-BPI(B), CM-BPI(B), PN-BPI(D), and KU-BPI(A) had zone of inhibition which are not statistically different from PN-BPI(A) and are all lower than the positive control. The zone of inhibition exhibited by the cell-free bacteriocin extract obtained from isolate KU-BPI(B) against the test bacteria were not significantly ($P = 0.075$) different from the zone of inhibition exhibited by the cell-free extract PN-BPI(B), CM-BPI(A) and the positive control. The negative control (sterile MRS broth) had no zone of inhibition against the test bacteria. Considering the higher zone of inhibition exhibited by the cell-free extracts KU-BPI(B), CM-BPI(A), and PN-BPI(B) against the test MDR bacteria, the isolates were selected for further studies.

Table 4 shows the MIC and MBC of the cell-free bacteriocin extracts obtained from the bacteriocin-producing isolates KU-BPI(B), CM-BPI(A), and PN-BPI(B) which had higher antibacterial activity against the test MDR bacteria (*Escherichia coli* and *Staphylococcus aureus*). The MIC of the cell-free extracts against *E. coli* ranged from 15.63 mg/ml (isolate PN-BPI(B)) to 31.25 mg/ml (both isolates KU-BPI(B) and CM-BPI(A)), and 31.25 mg/ml to 62.5 mg/ml against *S. aureus.* Similarly, the MBC of the cell free extracts ranged from 125 mg/ml to

250 mg/ml against both *E. coli* and 250 mg/ml against *S. aureus.* The MIC and MBC of the cell-free bacteriocin extracts from all the isolates were comparatively lower for the MDR *E. coli* than *S. aureus.*

Plate 1 shows the PCR amplicons of the 16S rRNA of the selected bacteriocin-producing isolates KU-BPI(B), CM-BPI(A), and PN-BPI(B), and the test MDR bacteria MDREc and MDRSa. The 16S rRNA sequences of the organisms showed 100% similarity with *Morganella morganii* strain DZ1 (CP148043), *Proteus vulgaris* 2023JQ-00005 (CP137920), *Alcaligenes feacalis* strain SN6A (KX281149), *Escherichia coli* strain EGE 415981-41 (CP099138) and *Staphylococcus aureus* strain 12 (OQ626022), respectively (Figure 2).

Table 1: Morphological and biochemical characteristics of the MDR test bacteria implicated in UTI

Isolate code	Pigmentation		Gram' s reaction	Growth on EMB	Growth on blood agar	Coagulase	Catalase	Oxidase	Citrate	Indole	MR	VP	Organism
MDREc	Creamy	-	Short rods	Metallic-sheen color	X	x	x	-	-	+	+	-	*Escherichia coli*
MDRSa	Golden-yellow	+	Cocci in clusters	x	β-haemolysis	+	+	-	+	x	x	x	*Staphylococcus aureus*

Key x = Not applicable

Table 2: Morphological and biochemical characteristics of bacterial strains isolated from different food samples on MRS agar medium

Isolate code	Size	Shape	Colour	Elevation	Margin/edge	Surface		Gram' s reaction	Catalase	Oxidase	Citrate	Aerobic growth	Anaerobic growth	Bacteriocin production
KU-BPI(A)	Large	irregular	Creamy	Flat	Lobate	Dry	+	Shot rods	+	+	-	+	++	+
KU-BPI(B)	Large	Circular	Opaque	Raised	Convex	Dry	+	Short rods	+	-	-	+	++	+
KU-BPI(C)	Large	Irregular	White	Flat	Entire	Moist	+	Short rods	+	-	+	+	+	-
KU-BPI(D)	Small	Round	Opaque	Raised	Entire	Dry	+	Cocci	+	-	+	+	+	-
KU-BPI(E)	Small	Round	White	Raised	Entire	Moist	+	Cocci	+	-	+	+	+	-
PW-BPI(A)	Small	Round	White	Raised	Entire	Moist	-	Short rods	+	-	-	+	-	-
PW-BPI(B)	Small	Circular	Creamy	Raised	Convex	Moist	+	Cocci (chain)	+	+	-	+	-	-
PN-BPI(A)	Small	Circular	White	Raised	Convex	Dry	+	Short rods	+	+	-	+	+	+
PN-BPI(B)	Small	Round	Opaque	Raised	Entire	Dry	-	Coccobacilli	+	+	+	+	+	+
PN-BPI(C)	Large	irregular	Creamy	Flat	Lobate	Dry	+	Shot rods	+	+	-	+	++	-
PN-BPI(D)	Small	Round	Creamy	Flat	Lobate	Dry	+	Long rods	+	+	-	+	-	+
PN-BPI(E)	Medium	Circular	Creamy	Raised	Convex	Slimy	-	Short rod	+	-	-	+	+	-
PN-BPI(F)	Small	Circular	Creamy	Raised	Convex	Slimy	-	Short rod	+	-	-	+	+	-
TA-BPI(A)	Large	Round	White	Flat	Convex	Slimy	+	Shot rods	+	+	-	+	++	-
TA-BPI(B)	Large	Irregular	White	Flat	Lobate	Dry	+	Short rods	+	-	+	+	-	+
TA-BPI(C)	Medium	Circular	Creamy	Raised	Convex	Slimy	+	Short rod	+	-	-	+	+	-
TA-BPI(D)	Small	Circular	Creamy	Raised	Convex	Slimy	+	Short rod	+	-	-	+	+	-
TA-BPI(E)	Large	irregular	Creamy	Flat	Lobate	Dry	+	Shot rods	+	+	-	+	++	-
OG-BPI(F)	Small	Round	Creamy	Flat	Lobate	Dry	+	Long rods	+	+	-	+	-	-
OG-BPI(G)	Medium	Circular	Creamy	Raised	Convex	Moist	-	Short rod	+	-	-	+	+	-
OG-BPI(H)	Small	Circular	Creamy	Raised	Convex	Moist	+	Short rod	+	-	-	+	+	-
OG-BPI(I)	Small	Circular	Creamy	Raised	Convex	Slimy	+	Short rod	+	-	-	+	+	-
CM-BPI(A)	Small	Round	White	Raised	Entire	Slimy	-	Short rods	+	-	-	+	+	+
CM-BPI(B)	Large	Irregular	White	Flat	Entire	Moist	+	Long rods	+	-	+	+	+	+
CM-BPI(C)	large	irregular	White	Flate	Lobate	Dry	+	Rods	+	-	+	+	+	-
CM-BPI(D)	Small	Circular	White	Raised	Entire	Moist	+	Short rod	+	-	-	+	+	-
CM-BPI(E)	Small	Circular	Creamy	Raised	Convex	Slimy	+	Short rod	+	-	-	+	+	-

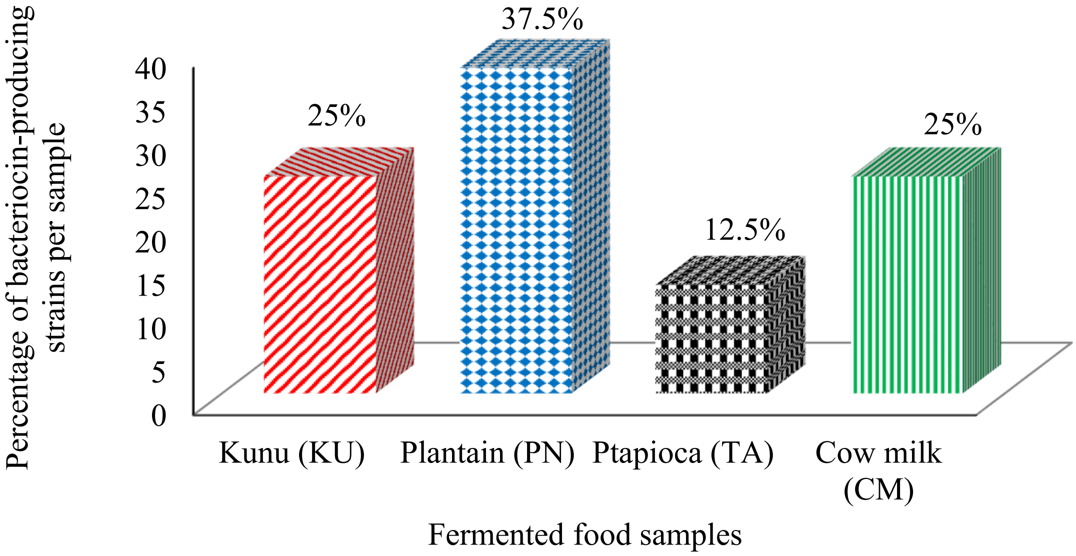

Figure 1: Percentage (%) occurrence of bacteriocin-producing bacterial strains per food sample

Table 3: Antibacterial activity of the cell-free bacteriocin extracts of the bacteriocin-producing isolates against test MDR bacteria implicated in UTI using agar-well-diffusion method

MDR bacteria	Zones of inhibition (mm) of cell-free bacteriocin and controls against MDR bacteria									
	KU-BPI(B)	CM-BPI(B)	KU-BPI(A)	CM-BPI(A)	TA-BPI(B)	PN-BPI(A)	PN-BPI(B)	PN-BPI(D)	*Positive control	**Negative control
E. coli	17.0±0.86^{ab}	8.0±0.32^{c}	7.0±0.40^{c}	12.0±0.21^{b}	9.0±0.54^{c}	6.0±0.52^{c}	19.0±0.99^{a}	8.0±0.40^{c}	14.0±0.45^{b}	0.0±0.00^{d}
S. aureus	13.0±0.43^{ab}	7.0±0.32^{c}	5.0±0.22^{c}	13.0±0.43^{b}	7.0±0.27^{c}	4.0±0.52^{c}	14.0±0.49^{a}	6.0±0.42^{c}	12.0±0.45^{b}	0.0±0.00^{d}

Keys: Data are expressed as mean ±S.E of duplicate trial. Values with different superscript across the rows are statistically different at 5% level of significance *Gentamycin (10µg), **MRS broth KU,= Kunu, CM = Raw cow milk, TA = Tapioca, PN = Unripe Plantain, BPI = Bacteriocin-producing isolate

Table 4: MIC and MBC of the bacteriocin extracts produced by the isolates against test MDR bacteria implicated in UTI

MDR indicator bacteria	Cell-free bacteriocin extract	Concentration of cell-free bacteriocin extract (mg/ml)											MIC (mg)	MBC (mg)
		1000	500	250	125	62.5	31.25	15.63	7.81	3.91	1.95	0.98		
E. coli	KU-BPI(B)	-	-	-	-	-	-	+	+	+	+	+	31.25	250
	CM-BPI(A)	-	-	-	-	-	-	+	+	+	+	+	31.25	250
	PN-BPI(B)	-	-	-	-	-	-	-	+	+	+	+	15.63	125
S. aureus	KU-BPI(B)	-	-	-	-	-	+	+	+	+	+	+	31.25	250
	CM-BPI(A)	-	-	-	-	-	+	+	+	+	+	+	62.50	250
	PN-BPI(B)	-	-	-	-	-	+	+	+	+	+	+	31.25	250

Keys: + = turbidity, - = absent of turbidity, MIC = Minimum inhibitory concentration, and MBC = Minimum bactericidal concentration

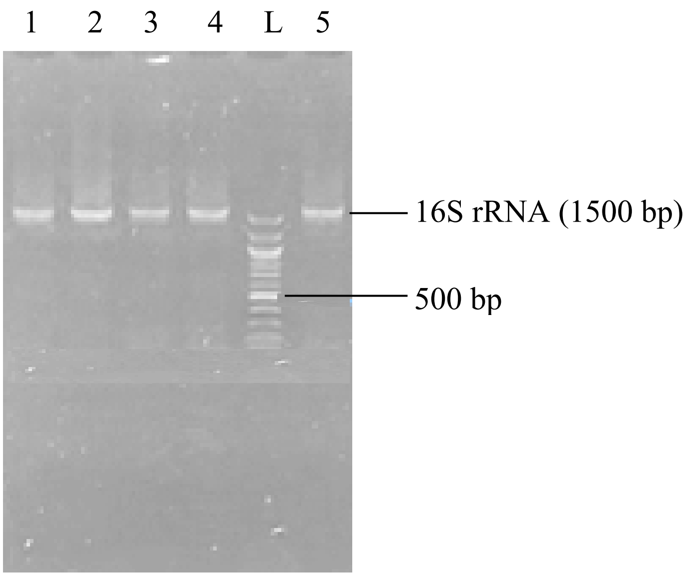

Plate 1: Agarose gel-image of the 16S rRNA gene amplicons (1500 bp) of the selected bacteriocin-producing isolates and the test MDR bacteria Lanes 1 = K-BPI(B), 2 = M-BPI(A), 3 = P-BPI(B), 4 = MDREc, 5 = MDRSa, L = 100bp DNA ladder.

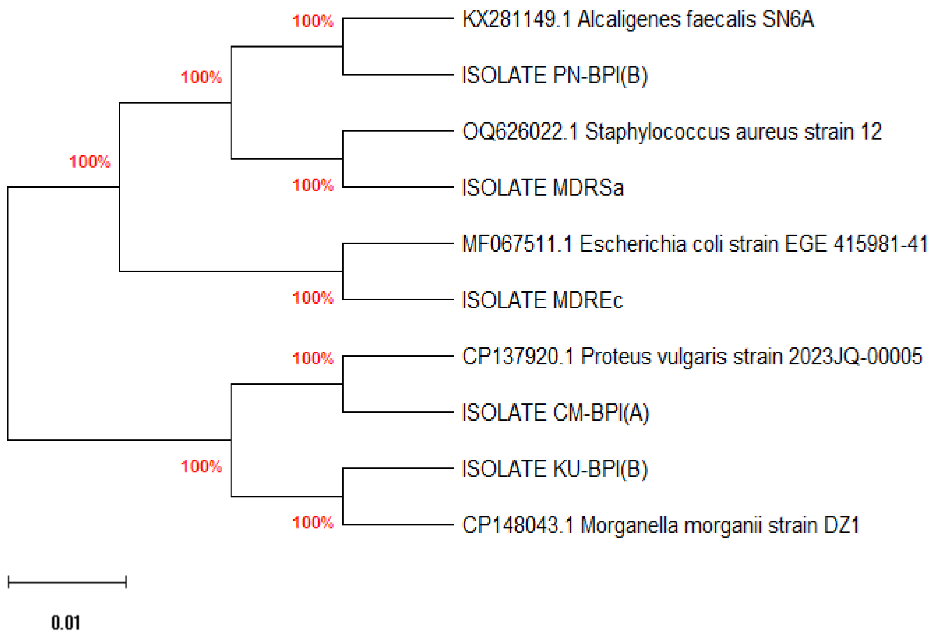

Figure 2: Phylogenetic tree of the evolutionary relationship between the bacterial isolates. The numbers at the nodes indicate the percentage of replicate trees based on 1000 bootstrap. The scale bar corresponds to a 0.01 nucleotide base substitution per site.

DISCUSSION

The growing development and spread of multi-drug resistant bacteria in the clinical system has become a threat of public health (Charkhian *et al.,* 2024). Exploring diverse natural sources including the screening of bacterial species from different fermented food substances for the discovery of novel antibacterial compounds, such as bacteriocins, has become necessary in our battle against multi-drug resistant pathogens. The findings of the agar-well diffusion assay showed significant ($P < 0.05$) difference in the antibacterial activity of the cell-free bacteriocin extracts obtained from the selected isolates against the multi-drug resistant *E. coli* MDREc and *S. aureus* MDRSa. All the extracts had antibacterial activity against the test bacteria, but the cell-free bacteriocin extracts obtained from isolates KU-BPI(B), CM-BPI(A), and PN-BPI(B) had significantly higher antibacterial activity than others. Though not significantly ($P < 0.05$) different, the extracts had wider zone of inhibition against *E. coli* MDREc than *S. aureus* MDRSa. The bacteriocin extracts obtained from all the isolates had lower MIC and MBC against the test bacteria isolates. This demonstrated a broad spectrum bacteriostatic and bactericidal activity against the test multi-drug resistant bacteria. The broad spectrum of activity is attributed to the ability of the bacteriocins to interact with the outer membrane receptor protein in the Gram negative bacteria, resulting to the outer membrane alteration and breakdown (Barraza *et al.,* 2017). This is in agreement with other studies which have established that bacteriocins are effective against Gram-positive and Gram-negative bacteria including *E. coli* and *S. aureus* (Khodaei and Soltani, 2018; Pandey and Gupta, 2018; Sukmawati *et al.,* 2022; Al-Qudah *et al.,* 2023). The finding on the minimum inhibitory and bactericidal concentrations (MIC and MBC) was higher than what has been reported in other work (Al-Qudah *et al.,* 2023). This is possibly due to the differences in the bacterial species producing the bacteriocin and the type of test bacteria which the bacteriocin was used against. Moreover, some other studies had reported very low or no antibacterial activity for their bacteriocin extracts against Gram negative bacteria. This is owed to the presence of lipopolysaccharide (LPS) in the outer membrane of this group of bacteria (Todorov *et al*., 2010; Ahmadova *et al*., 2013).

The selected bacteriocin-producing isolates KU-BPI(B), CM-BPI(A), and PN-BPI(B) were identified to the species levels as *Morganella morganii* KU, *Proteus vulgaris* CM, and *Alcaligenes feacalis* PN, respectively. These species of bacteria have been reported in previous studies as bacteriocin-producing bacteria, usually producing bacteriocin in antagonism for self-defense and surviving in different natural environments especially one with limited resources for growth and survival (Zahir *et al.,* 2013; Nandan and Nagar, 2016; Markovi´c *et al.,* 2022). *Morganella morganii* KU and *Proteus vulgaris* CM are members of the Enterobacteriaceae family. *Morganella morganii,* formally known as *Proteus morganii,* is a facultative anaerobic Gram-negative rod enteric bacterium which was first isolated by Morgan in 1906 in the faeces of an infant who suffered diarrhea and is known for the production of bacteriocin known as morganocin (Liu *et al.,* 2016; Liu *et al.,* 2021). *Alcaligenes faecalis* is a Gram's-negative rod aerobic bacterium belonging to the family of proteobacteria, having probiotic and antimicrobial characteristics (Ray and Pattnaik, 2024). The genus *Alcaligenes* is known among the bacteria having antagonistic activity. The presence of these bacterial species in the food samples analyzed in this study is a clear indication of faecal contamination (Ema *et al.,* 2022). Enterobacteriaceae produces high and low molecular weight bacteriocins known as colicin and microcin, respectively, usually under stress conditions (Baquero *et al.,* 2019; Ema *et al.,* 2022). Colicins and microcins can inhibit the growth of competing *E. coli* strains and other phylogenetically related bacteria (Cascales *et al.,* 2007).

CONCLUSION

Based on the findings of the investigation, it is concluded that the cell-free bacteriocin extracts obtained from the bacterial strains *Morganella morganii* KU*, Proteus vulgaris* CM*,* and *Alcaligenes feacalis* PN isolated from fermented ogi, tapioca, palm wine, kunu, raw milk, and unripe plantain had significantly (P < 0.05) higher antibacterial activity against the multi-drug resistant *Escherichia coli* MDREc and *Staphylococcus aureus* MDRSa implicated in UTIs.

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