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Molecular characterization and detection of antibiotic resistant genes of bacteria isolated from yoghurt in Port Harcourt Metropolis, Nigeria

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ABSTRACT

Currently, molecular identification is replacing the conventional method because of its precision and reliability whereas the effectiveness of antibacterial treatments has continuously declined due to antimicrobial resistance (AMR). This study aimed to identify the bacterial isolates; phenotypically and molecularly as well as detect the resistant genes after susceptibility testing of the isolates obtained from yoghurt samples. Standard microbiological techniques and molecular analysis were applied on both samples (commercial and home-made yoghurt) for species validation. Forty-four (44) bacterial species were identified, phenotypically belonging to three (3) genera; *Bacillus, Staphylococcus* and *Lactobacillus* and an additional genus *Bifidobacterium* emerged from molecular analysis. The microbial load of the yoghurt samples was not statistically significant at ($p \geq 0.05$). A sensitivity test on the species was carried out using Kirby-Bauer disc diffusion method with some standard antibiotics. The results revealed that *Bacillus* and *Staphylococcus* species were resistant to ampicillin and augmentin (100%) but susceptible to ofloxacin and gentamicin respectively. *Lactobacillus spp*. were susceptible to ofloxacin and ceftazidime (100%), and resistant to ampicillin, augmentin, and ciprofloxacin (100%). The six most resistant species were molecularly identified as *S. aureus* CP019117, *S. epidermidis* AB68833, *B. megaterium* KC246043, *B. cereus* NC004722, *Lactobacillus casei* NC008526 and *Bifidobacterium lactis* CP003941. Resistant bacteria with *mecA* gene are *S. aureus* and *S. epidermidis* and those with *ampC* gene are *Bifidobacterium lactis* and *Lactobacillus casei*. However, neither gene was found in the genome of any *Bacillus species.* However, the data also revealed that the bacterial species in home-made yoghurt samples were negative for *mecA* and *ampC* resistant genes but positive in the commercial samples. These genes contributed to the bacterial isolates' high levels of multidrug resistance (MDR). The presence of resistant genes in bacterial species from commercial yoghurt samples remains a challenge for food safety. Therefore, good manufacturing practices, proper hygiene and sanitation are hereby advocated to avoid serious emerging foodborne illnesses.

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1. Introduction

One of the dairy products that is fermented is yogurt, commonly derived from whole or skimmed milk powder. Since homemade yoghurt processing, production, and retailing began, yogurt consumption has grown steadily in Nigeria. However, the hygienic condition and handling processes may be inadequate (1).

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Yoghurt is a very nutritious food for people of all ages and contains probiotics. Probiotics are living microorganisms which when taken into the body sufficiently has health benefits that are conveyed to the host. The most significant probiotic with favourable effects on the gastrointestinal tract (GIT) is lactic acid bacteria (LAB), which is a member of the *Lactobacillus* and/or *Bifidobacterium* genera (2-4). Among their many uses, milk, milk powder, and dairy products serve as a vital source of protein, calcium, vitamins, and micronutrients. They are also the main component of infant formula (5-7). Yoghurt, one of the functional and nutraceutical foods with multiplex nutrition and health advantages are popular in the dairy sector and have garnered a lot of scientific attention over the years (8-13).

Medicinal advantages of food products containing probiotic microorganisms include; stimulation of immune system, decreasing hypercholesterolemia, decrease in lactose intolerance, prevention of diarrhoea and allergies and treating constipation and urogenital tract infections furthermore a decreased risk of colon cancer (14-16). The lowest concentration of probiotic live microorganisms at the time of consumption has been advised to be 10^6–10^7 cfu/mL or g in order to elicit favourable therapeutic effects (16).

Several investigators have reported that prebiotics support probiotics' functionality and viability throughout the food preparation process (17, 18).

Lactobacillus casei and *Bifidobacterium bifidum* are probiotic strains known for their gastrointestinal, enhance digestibility and for biofunctional effects and therapeutic applications (19-22).

There have been numerous cases of *S. aureus* and other germs being found in dairy products despite the fact that a variety of procedures, including high temperature, high pressure, drying, and nonthermal processing, have been used to reduce microbial survival and growth (23-25). Food that hasn't been properly sterilized could pose a risk. The source of the raw milk is also crucial in ensuring the safety of the finished goods. Dairy product *S. aureus* residues may increase your chance of getting sick from food. The features of *S. aureus* in the raw milk from independent dairy retail establishments must thus be studied. Bacterial contamination can result in food poisoning occurrences and subpar products, which is a major global economic issue (26). Antimicrobial resistance (AMR), however, has been created as a result of the indiscriminate and excessive use of antibiotics, which has slowly reduced the efficacy of current antibacterial therapy (27, 28). Nearly all clinical bacterial isolates have resistance mechanisms, and persistent bacteria can cause recurring infections that make it difficult to treat infections effectively (29, 30). This circumstance emphasizes the criticality of finding fresh therapeutic options and less harmful treatment targets. Hence, there is need to identify the bacterial community structure as well as resistant genes in ready-to-eat (RTE) dairy food products. The research is focused to investigate the molecular characterization, antibacterial susceptibility and detection of resistant genes from yoghurt samples in Port Harcourt metropolis.

2. Material and Methods

2.1. Area of study

The yoghurt samples were prepared and purchased also in Port Harcourt metropolis from supermarket. Port Harcourt is the capital city of Rivers State in the Niger Delta Region of Nigeria. It is bounded by Longitude 6°56° to 7°07°E and Latitude 4°44° to 4°52°N of the equator, a home to people of different nationalities and bubbling with commercial, industrial and crude oil business activities.

2.2. Sample collection

Home-made yoghurt samples were produced under aseptic conditions (Fig.1) and stored in the refrigerator at 4°C whereas commercially processed samples were purchased from supermarket in Port Harcourt. The samples were labeled and put into an ice-chest and conveyed to the Department of Microbiology Laboratory, Rivets State University for microbiological analyses.

2.3. Sample preparation

The home-made yoghurt samples were prepared as described in the flow chart below.

2.4. Bacteriological analysis

2.4.1. Enumeration and preservation of isolates

One millilitre (1 mL) of the yoghurt samples was aseptically dispensed into a beaker containing 9 mL of normal saline and stirred to form a homogenate. A serial tenfold decimal dilutions (10^{-1} -10^{-6}). Dilutions of (10^{-1} and 10^{-2}) spread plated in duplicates onto Titan Biotech Limited's Nutrient agar (NA) and De Man Rogosa and Sharpe agar (MRS), Titan Biotech Ltd, Netaji Subhash Place, Delhi, India.

The plates were incubated for 24 h at 37°C. Colony forming units (CFU) of representative discrete colonies on/in the media were counted by sub-culturing on freshly made sterile NA plates and incubating at 37°C for 24 h to obtain pure culture. For additional analyses, the pure cultures were kept in McCartney bottles and chilled to -4°C.

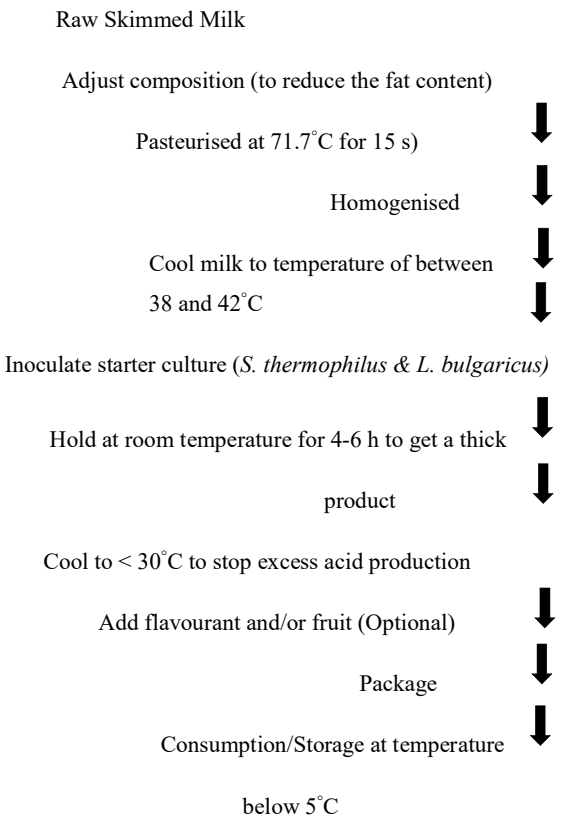

Figure 1. Flow chart for the processing of homemade yoghurt.

2.4.2. Isolation and phenotypic identification of the bacterial isolates

Bacterial colonies were isolated for identification based on their colonial and morphological properties, including size, margin, surface, color, elevation, texture, and transparency. Gram staining and biochemical tests to identify the bacteria, including oxidase, catalase, coagulase, citrate utilization, methyl red, indole, Voges Proskauer, and sugar fermentation tests (31, 32).

2.5. Antibacterial susceptibility test

On hardened, sterile Mueller-Hinton agar, the antibacterial susceptibility test was conducted using the Kirby-Bauer disc diffusion method (MHA). The overnight pure culture of x 10^8 cells of the bacterial isolate combined in a tube with 5 mL of sterile peptone water produced the 0.5 McFarland turbidity criteria. A sterile cotton swab was gently rotated against the surface of the tube after being dipped into the suspension to remove any surplus. The entire surface of MHA was equally covered with the inoculum-containing swab. The plates were dried for three to five min. Eight antibiotic discs were aseptically inserted with sterile forceps on the infected surface of MHA, including Chloramphenicol (300 g), Erythromycin (5 g), Gentamicin (10 g), Ofloxacin (5 g), Ceftazidime (30 g), Ampicillin (10 g), Ciprofloxacin (5 g), and Augmentin (30 g). Zone of inhibition, including the disc, was measured in millimeters (mm) after 24 h of incubation at 37°C and classified as resistant, intermediate, or susceptible using the meter rule (33).

2.6. Molecular Identification

2.6.1. DNA Extraction and Quantification

According to Bell *et al.*, the extraction process was carried out using the boiling method (34). The bacterial isolate's pure culture was placed in Luria-Bertani (LB) Broth and incubated at 37°C. The DNA at the base of the supernatant was decanted after being centrifuged at 14000 rpm for three min with zero milliliter (0.5 mL) of the broth culture of the Luria Bertani (LB) bacterial isolates. The Eppendorf tubes were appropriately labeled. Three times this technique was carried out. The cells were heated at 95°C for 20 min while being re-suspended in 500 ul of normal saline. The heated bacterial suspension was spun at 14000 rpm for three min after cooling on ice for around ten min. For use in additional downstream procedures, the supernatant containing the DNA was transferred to a 1.5 mL microcentrifuge tube and kept at -20°C (31) Bell *et al.*, 1998). According to Olsen and Marrow's instructions, the Nanodrop 1000 Spectrophotometer was used to measure the extracted DNA (35).

2.6.2. Amplification of *16S rRNA and mecA and ampC* Gene

According to Srinivasan et al., the 16S rRNA amplification was performed using an ABI 9700 Applied Biosystems, Thermal Cycler (36). Using the forward primer 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and the reverse primer 1492R: 5'- CGGTTACCTTGTTACGACTT-3' primers and for mecA primers, the 16s rRNA region of the rRNA gene of the bacterial isolates was amplified. Primer combinations for forward and reverse are as follows: 5'-AAAATCGATG- GTAAAGGTTGGC-3' and

mecA 5'TTCCTGATGATCGTTCTGCC-3' Int-B2F. On the ABI 9700 Applied Bio-systems thermal cycler, 35 cycles of 5'-AAAAGCGGAGAAAGGTCCG-3' were performed. The PCR mixture consists of water, Taq polymerase, DNTPs, and $MgCl_2$, as well as primers at a concentration of 0.5 M and extracted DNA as the template. The following were the PCR conditions: Initial denaturation took place at 95°C for 5 min, followed by subsequent denaturation at 95°C for 30 s, 52°C for 30 s, extension for 35 cycles, and 72°C for 5 min of final extension. The result was visualized using a blue light trans-illuminator for 1500 bp amplicons after being resolved on a 1% agarose gel at 130 V for 30 min (36). For the mecA and ampC genes, the product was resolved on a 1% agarose gel prepared with EZ vision dye at 120 V for 25 min and seen on a blue light trans-illuminator.

2.6.3. DNA Sequencing

The Big-Dye Terminator kit was used to sequence the amplified product on a 3510 ABI sequencer. Big Dye® terminator v1.1/v3.1, 2.25 ul of 5 x Big-Dye sequencing buffer, 10 uM Primer PCR primer, and 2–10 ng PCR template per 100 bp were the components used in the sequencing, which was done at a final volume of 10 ul. There were 32 cycles of 96°C for 10 s, 55°C for 5 s, and 60°C for 4 min in the sequencing conditions (36).

2.6.4. Phylogenetic Analysis

Before the acquired sequences were edited using the bioinformatics tool Trace edit, similar sequences were downloaded using BLASTN from the National Center for Biotechnology Information (NCBI) database. These sequences were aligned via MAFFT.

The Neighbor Joining method in MEGA 6.0 was used to infer the evolutionary history (37). The Jukes-Cantor technique was used to compute the evolutionary distances (38).

2.7. Data Analysis

The susceptibility pattern in percentages underwent statistical investigation utilizing descriptive analysis. A computer-based program called SPSS 25 was used for this. Tables were used to display the data.

3. Results

The microbial load in yoghurt samples are represented in Table 1. Unsweetened homemade yoghurt sample A had the highest total heterotrophic bacterial count (THBC) and least was sweetened commercial yoghurt sample E. There was no significant differences in THBCs between the samples at ($p \geq 0.05$). Sample A had the highest TFC and nil in sample F. The highest TLC was obtained in sample F and least in A.

Table 1. Microbial load of homemade and commercial yoghurt samples

Sample	THBC $\times 10^4$	TFC $\times 10^3$	TLC $\times 10^2$
A	4.65±2.19[a]	1.75±0.28 [a]	2.34±1.94[b]
B	3.21±1.08[a]	1.00±0.00 [a]	3.13±1.94[b]
C	3.67±1.72[a]	1.13±0.05 [a]	3.55±1.86[b]
D	3.32±1.93[a]	1.63±0.11 [a]	2.44±0.11[b]
E	2.20±0.63[a]	1.35±0.06 [a]	6.05±1.42[a]
F	2.27±0.69[a]	0.00±0.00 [a]	12.28±8.76[a]

Legend: THBC = Total heterotrophic bacteria count; TFC = Total fungal count; TLC = Total *Lactobacillus* count. A-unsweetened homemade; B-sweetened homemade; C-unsweetened commercial;

D-Sweetened commercial; E-sweetened commercial; F-unsweetened commercial.
Mean±SD with the same superscript along the columns is not significantly different (p≥0.05)

The results of the susceptibility pattern of *Bacillus* are shown in Table 2. *Bacillus* species were 100% resistant to ampicillin and augment and susceptible to ofloxacin (88.5%) and Gentamicin (80.8%).

Table 2. Susceptibility pattern of *Bacillus spp.* from the different yoghurt samples

Antibiotic	Conc. (µg)	*Bacillus spp.*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
CE	(10)	23(88.5)	0(0.00)	3(11.5)
CH	(10)	25(96.2)	0(0.00)	1(3.8)
E	(300)	6(23.1)	18(69.2)	2(7.7)
AM	(10)	26(100)	0(0.00)	0(0.00)
OFX	(5)	3(11.5)	0(0.00)	23(88.5)
AU	(30)	26(100)	0(0.00)	0(0.00)
CPX	(10)	20(76.9)	0(0.00)	6(23.1)
CN	(10)	4(15.4)	1(3.8)	21(80.8)

Legend: (CE) Ceftazidime, (CH) Chloramphenicol, (E) Erythromycin, (AM) Ampicillin, (OFX) Ofloxacin, (AU) Augmentin (CPX) Ciprofloxacin, (CN) Gentamicin; n = Number of isolate(s)

Table 3. *Staphylococcus spp.* were resistant to ampicillin, augmentin (100%) and ofloxacin (84.6%) respectively. However, it was observed to be susceptible to gentamicin (84.6%**).**

Table 3. Susceptibility Pattern of *Staphylococcus spp.* from the Different Yoghurt samples

Antibiotic	Conc. (µg)	*Staphylococcus spp.*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
CE	(10)	9(69.2)	1(7.7)	3(23.1)
CH	(10)	5(38.5)	7(53.8)	1(7.7)
E	(300)	9(69.2)	3(23.1)	1(7.7)
AM	(10)	13(100)	0(0.00)	0(0.00)
OFX	(5)	11(84.6)	1(7.7)	1(7.7)
AU	(30)	13(100)	0(0.00)	0(0.00)
CPX	(10)	9(69.2)	1(7.7)	3(23.1)
CN	(10)	2(15.4)	0(0.00)	11(84.6)

Legend: (CE) Ceftazidime, (CH) Chloramphenicol, (E) Erythromycin, (AM) Ampicillin, (OFX) Ofloxacin, (AU) Augmentin (CPX) Ciprofloxacin, (CN) Gentamicin

The susceptibility pattern of *Lactobacillus spp*. as shown in Table 4, indicates that *Lactobacillus spp*. were susceptible to ceftazidime and ofloxacin (100%), and showed a decreasing trend of resistance in the order: ampicillin, augmentin, and ciprofloxacin (100%)> chloramphenicol (80%)> gentamicin (60%).

Table 4. Susceptibility pattern of *Lactobacillus spp*. from different yoghurt samples

Antibiotic	Conc.	*Lactobacillus spp.*		
	(µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
CE	(10)	0(0.00)	0(0.00)	5(100)
CH	(10)	4(80)	1(20)	0(0.00)
E	(300)	1(20)	4(80)	0(0.00)
AM	(10)	5(100)	0(0.00)	0(0.00)
OFX	(5)	0(0.00)	0(0.00)	5(100)
AU	(30)	5(100)	0(0.00)	0(0.00)
CPX	(10)	5(100)	0(0.00)	0(0.00)
CN	(10)	3(60)	2(40)	0(0.00)

Legend: (CE) Ceftazidime, (CH) Chloramphenicol, (E) Erythromycin, (AM) Ampicillin, (OFX) Ofloxacin, (AU) Augmentin (CPX) Ciprofloxacin, (CN) Gentamicin

Table 5. Antibacterial susceptibility of the study isolates to the 8 antibiotics

Antibiotic class	Antibiotic	*Bacillus*	*Staphylococcus*	*Lactobacillus*
		Resistant n (%)		
β –lactams	AM	26(100)	13(100)	5(100)
Augmentin	AU	26(100)	13(100)	5(100)
Aminoglycosides	CN	0(0.00)	0(0.00)	3(60.00)
Cephalosporins	CE	0(0.00)	9(69.20)	0(0.00)
Chloramphenicol	CH	25(96.2)	5(38.50)	4(80.00)
Macrolides	E	6(23.1)	9(69.20)	1(20.00)
Fluoroquinolones	OFX	3(11.5)	11(84.60)	0(0.00)
	CPX	20(76.9)	9(69.20)	5(100)
Phenotype showing MDR and number of drugs		*4(76.9-100)	*6(69-100)	*5(60-100)

•= Number of drugs; Number in parenthesis is in (%)

Table 6. Accession Number and Representative Genes of Isolates

Isolate	Genetic ID	Accession Number	Representative Genes	
			mecA	***ampC***
1	*S. aureus*	CP019117	+	-
2	*S. epidermidis*	ABP68833	+	-
3	*B. cereus*	NC004722	-	-
4	*B. megaterium*	KC246043.1	-	-
5	*Bifidobacterium lactis*	CP003941	-	+
6	*L. casei*	NC008526	-	+

Antibacterial susceptibility profiles of the eight antibiotics to the bacterial isolates displayed different levels of activity (Table 5). Some of these bacteria were resistant to as much as four to six drugs, thus exhibiting multidrug resistance (MDR) the highest being Staphylococcus. However, substantial number of the isolates were 100% resistant to ampicillin (AM), augmentin (AU) and ciprofloxacin (CPX).

Before sequencing, the Agarose gel electrophoresis of several chosen bacterial isolates' amplified 16S rRNA gene reveals that Lanes 1-6 represent the 16S rRNA gene bands (1500 bp), while Lane L represents the 100 bp molecular ladder (Fig. 1). The six bacterial isolates with the highest antibiotic resistance are represented by the amplified mecA gene in the agarose gel electrophoresis image. Lanes 1 and 2 show the mecA gene bands at 500 bp, Lanes 4 and 5 show the ampC gene bands at 500 bp, and Lane L represents the 100 bp molecular ladder. Table 6 and Fig. 2 show the evolutionary distance between the bacterial isolates from this investigation, their accession numbers, and their closest relatives on the phylogenetic tree. This demonstrates that the genes were present in the genetic makeup of two out of the six bacterial isolates tested for the mecA gene and two out of the six tested for the ampC gene, as shown on Fig. 3-4.

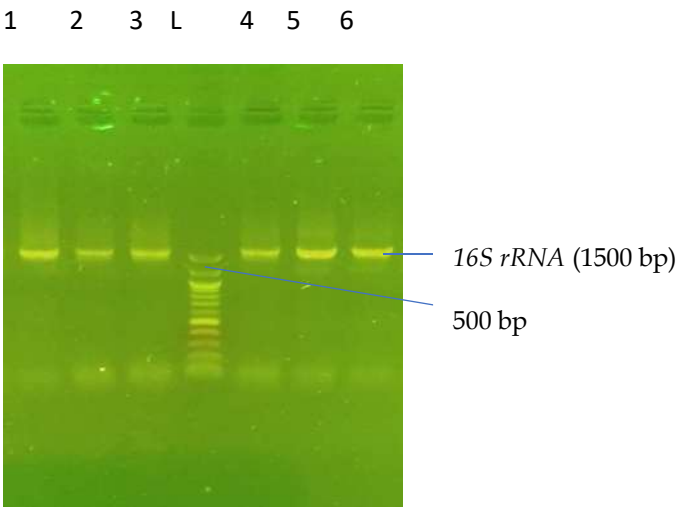

Figure 2. Agarose gel electrophoresis sowing the amplified 16S rRNA fragment. Lanes 1-6 represent the amplified *16SrRNA band*s at 1500 bp while L represents the 100 bp molecular.

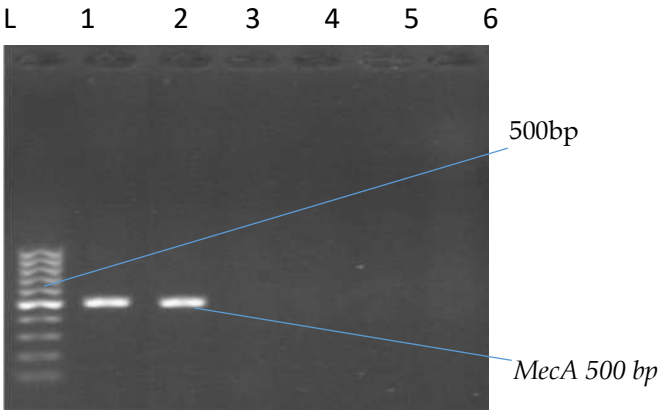

Figure 3. Agarose gel electrophoresis showing the amplified *AmpC* gene of the isolates

The accession number and resistant genes of bacterial isolates are presented in Table 4. The isolates positive for *mecA* are *S. aureus* CP019117 and *S. epidermidis* whereas *Bifidobacterium lactis* CP003941 and *L. casei* NC008526 were positive for *ampC* gene. The remaining isolates were negative for both genes.

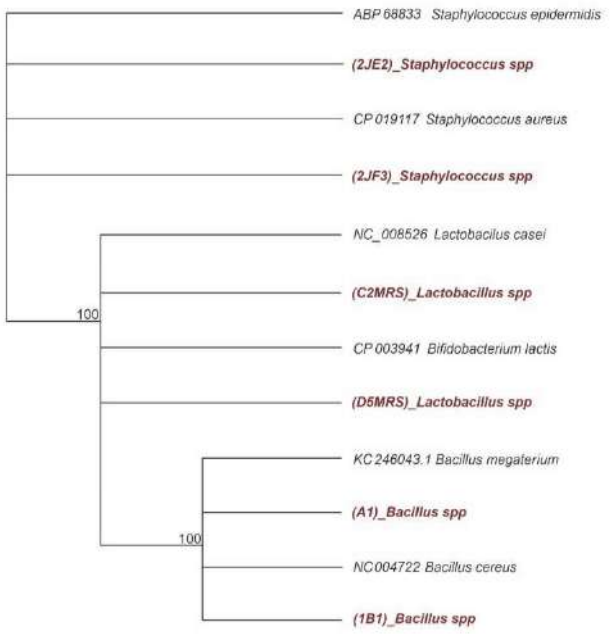

Figure 4. Phylogenetic Tree showing evolutionary distance between bacterial Isolates.

4. Discussion

The phenotypic results revealed that the 44 bacteria isolated from the yoghurt samples belonged to three genera; *Bacillus, Staphylococcus* and *Lactobacillus* whereas molecular analysis identified *Bifidobacterium* in addition. This bacterium is associated with yoghurt and gut microbiota. This phenomenon demonstrates the significance of complementing the conventional culture-dependent techniques with the molecular. The predominance of Gram-positive bacteria (GPB) and their load in this study is not uncommon with dairy products and are still below the lowest limits prescribed for probiotic products, 10^6–10^7 cfu/mL and corroborates previous (16, 39).

GPB in dairy products especially of the species of *S. epidermidis*, *S. aureus*, Bacillus and Lactobacillus had been reported in literature as due to contamination of skin, transmission through animal infection and soil and plant sources (2-4, 39, 40) which would have played out in this study. The non-detection of Gram-negative bacteria (GNB) may be attributed to inability to survive pasteurization and growth at low temperatures (41). However, persistence of GPB in dairy products and food system has been attributed to 'microbial protection' such as heat-shock proteins in staphylococcal species which enhances their survival after heat treatment at 80°C for 20 min, protective effects of food components (carbohydrate, fat, etc) for Lactobacillus and spore-forming ability of Bacillus species respectively (42-45).

To guarantee food safety, adequate, sustainable control and protective measures must be put in place to ensure minimization of contamination from farm-to-fork chain. However, it is obvious that commonly used antibiotics are becoming less efficacious due to AMR, mutated pathogens, wide spread use, abuse and overuse of antibiotics in food animal production and selective pressure has led to the appearance of new drugs (46, 47).

The use of these antibiotics in this study was also justified by their potency in stifling bacterial development and broad-spectrum properties.

Data indicates that the isolates displayed varying degrees of high resistance (100%) to some commonly used antibiotics such as ampicillin, augmentin and ciprofloxacin. On the hand, *Lactobacillus spp.* were susceptible to ofloxacin and ceftazidime (100%) whereas the susceptibility of Bacillus and *Staphylococcus spp.* to ofloxacin and/or gentamicin respectively were (<100). In Nigeria, β-lactam antibiotics is one of those drugs regularly used, and resistance to these antibiotics has been reported globally especially in bacteria from humans, wastewater, food products and the wider environment (48, 49). The high level of resistance of *S. aureus* to β-lactams and fluoroquinolones antibiotics in this study confirms previous report (50). According to Pontes et al. (51) co-existence of MDR bacteria with susceptible ones accentuates the chances of transfer of antibiotic resistance to the sensitive ones and this could also be responsible for the high resistance observed. Nonetheless, substantial number of the isolates showed resistance from 4 to 6 antibiotics, indicative of high MDR which may be multifactorial. These factors include drug abuse, poor handling and hygienic standards, improper food safety rules and regulatory systems (39, 52) as well as presence of resistant genes as demonstrated in this study. Consequently, the emergence of MDR *S. aureus*, *S. epidermidis*, Bifidobacterium lactis and *L. casei* is worrisome for a ready-to-eat (RTE) beverage like yoghurt and may represent a potential hazard to consumers.

Several investigators have earlier reported that the continued presence of cephalosporins tend to induce the over production of β-lactamase enzyme coded by the ampC gene and this gene is the probable precursor for MDR in the bacterial isolates (53, 54). The detection of the ampC gene in the genome of Bifidobacterium lactis and Lactobacillus casei was largely responsible for imparting resistance to the cephalosporins and can further increase the capability of isolates to resist these antibiotics completely (55). Additionally, many intrinsic resistance mechanisms, such as target alteration, decreased permeability, and efflux, can take place in the same cell at the same time, resulting in fluoroquinolone and other antibiotic resistance at a high degree (56, 57). mecA genes has been known to cause resistance in most GPB apart from the methicillin-resistant *Staphylococcus aureus* on the transposonsin mecA complex (58). This research on the evolution of resistant bacterial strains and genes provides an understanding of bacterial genomics (59). However, the occurrence of such genes in GPB may cause harm to public health security. To mitigate this potential health risk and contamination, retail food products should be subjected to extensive processing and handling, at all levels, the "One Health" philosophy and hygienic packaging should be used (60, 61).

5. Conclusions

This study revealed that Bacillus, Staphylococcus and Lactobacillus were the phenotypes detected in homemade and commercially processed yoghurt

samples but genotypic analysis resulted in the addition of Bifidobacterium. Four (4) of the bacterial species had resistant genes; mecA for *Staphylococcus aureus* and *S. epidermidis*, and ampC for *Bifidobacterium lactis* and *L. casei* resident in their genome. The genus with highest MDR was Staphylococcus but still susceptible to Gentamicin. These genes are known to confer resistance to bacteria, especially on GPB which resulted in MDR to some antibiotics (β-lactam (ampicillin), augmentin, chloramphenicol, fluoroquinolone (ciprofloxacin and ofloxacin) aminoglycoside (gentamicin) and macrolide (erythromycin) used this research. Such high-level of resistance depicted in the study is worrisome and calls for adequate monitoring and tracking of emerging and resistant foodborne bacteria.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

1. Bellio A, Chiesa F, Gallina S, et al. Insight into the distribution of Staphylococci and their enterotoxins in cheeses under natural conditions. Front Microbiol 2019; 9: 3233.
2. Lone KD, Dhole JA. Formulation and evaluation of probiotic tablets containing anti-inflammatory drug. Int J Pharma Sci Res 2013; 4: 341-46.
3. Moayednia N. Quality evaluation of new developed symbiotic yoghurt over the storage at refrigerator. J Food Biosci Technol 2014; 4:57-64.
4. Shafiei Y, Razavilar V, Javadi A, et al. Survivability of free and microencapsulated *Lactobacillus plantarum* with alginate and resistant starch in simulated gastrointestinal conditions. J Food Agri Environ 2012; 10: 207-12.
5. Yang M, Cong M, Peng X, et al. Quantitative proteomic analysis of milk fat globule membrane (MFGM) proteins in human and bovine colostrum and mature milk samples through iTRAQ labelling. Food Function 2016; 7: 2438-50.
6. Qiao Q, Guo X, Wen F, et al. Aptamer-based fluorescence quenching approach for detection of Aflatoxin M1 in Mmilk. Front Chem 2021; 9: 653869.
7. Zhang L, Van Dijk ADJ, Hettinga K. An interatomic overview of the human and bovine milk proteome over lactation. Proteome Sci 2016; 15: 1.
8. Zhou K, Li C, Chen D, et al. A review on nanosystems as an effective approach against infections of *Staphylococcus aureus.* Int J Nanomed 2018; 13: 7333-47.
9. Atwaa ESH, Shahein, MR, El-Sattar ESA, et al. Bioactivity, physicochemical and sensory properties of probiotic yoghurt made from whole milk powder reconstituted in aqueous fennel extract. Ferment 2022; 8: 52.
10. Shahein MR, Atwaa ESH, E-Zahalr KM, et al. Remedial action of yoghurt enriched with watermelon seed milk on renal injured hyperuricemic rats. Ferment 2022; 8: 41.

11. Swelam S, Zommara MA, Abd El-Aziz AE, et al. Insights into chufa milk frozen yoghurt as cheap functional frozen yoghurt with high nutritional value. Ferment 2021; 7: 255.
12. Elkot WF, Ateteallah AH, Al-Moalem MH, et al. Functional, physicochemical, rheological, microbiological, and organoleptic properties of symbiotic ice cream produced from camel milk using black rice powder and *Lactobacillus acidophilus* LA-5. Ferment 2022; 8: 187.
13. Hui W, Jiawei S, Chengfeng Z, Kai, et al. Antibiotics resistance and virulence of *Staphylococcus aureus* isolates isolated from raw milk from handmade dairy retail stores in Hefei city, China. Food 2022; 11: 2185.
14. Karlton-Senaye BD, Tahergorabi R, Giddings VL, et al. Effect of gums on viability and β-galactosidase activity of *Lactobacillus* spp. in milk drink during refrigerated storage. Int J Food Sci Technol 2015; 50: 32–40.
15. Ray RC, Aly FEl, Sheikha AF, et al. Oriental fermented functional (probiotic) foods chapter 9: In; Microorganisms and Fermentation of Traditional Foods. 2014; 283-311.
16. Shahein MR, Elkot WF, Albezrah NKA, et al. Insights into the microbiological and physicochemical properties of bio-frozen yoghurt made with probiotic strains in combination with Jerusalem Artichoke tubers powder. Ferment 2022; 8: 390.
17. Akın M, Akın M, Kırmacı Z. Effects of inulin and sugar levels on the viability of yogurt and Probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem 2007; 104: 93–99.
18. Cruz AG, Antunes AE, Sousa ALO, et al. Ice-cream as a probiotic food carrier. Food Res Int 2009; 42: 1233-39.
19. Ren Q, Liao G, Wu Z, et al. Prevalence and characterization of *Staphylococcus aureus* isolates from subclinical bovine mastitis in southern Xinjiang, China. J Dairy Sci 2020; 103: 3368-80.
20. Yateem A, Balba MT, Al-Surrayai T, et al. Isolation of lactic acid bacteria with probiotic potential from camel milk. Int J Dairy Sci 2008; 34: 194-199.
21. Dai Y, Liu J, Guo W, et al. Decreasing methicillin-resistant *Staphylococcus aureus* (MRSA) infections is attributable to the disappearance of predominant MRSA ST239 clones, Shanghai, 2008–2017. Emerg Microbes Infect 2019; 8: 471-78.
22. Ku S, Park MS, Ji GE, et al. Review on *Bifidobacterium bifidum* BGN4: functionality and nutraceutical applications as a probiotic microorganism. Int J Mol Sci 2016; 17: 1544.
23. Aragão BB, Trajano SC, Silva JG, et al. Short communication: High frequency of β-lactam-resistant *Staphylococcus aureus* in artisanal coalho cheese made from goat milk produced in northeastern Brazil. J Dairy Sci 2019; 102: 6923-27.
24. Song Q, Zhu Z, Chang Y, et al. Prevalence and characteristics of enterotoxin b-producing *Staphylococcus aureus* isolated from food sources: a particular cluster of st188 strains was identified. J Food Sci 2016; 81: M715–M718.
25. Verras C, Vlaemynck G, Van Weyenberg S, et al. A review of the microbiological hazards of dairy products made from raw milk. Int Dairy J 2015; 50: 32-44.
26. Hernández-Cortez C, Palma-Martínez I, Gonzalez-Avila LU, et al. Food poisoning caused by bacteria. Food Toxin 2016; 565-21.
27. Aydemir DH, Cifci G, Aviyente V, et al. Quorum-sensing inhibitor potential of trans-anethole against *Pseudomonas aeruginosa*. J Appl Microbiol 2018; 125: 731-39.

28. Chang Y, Wang PC, Ma HM, et al. Design, synthesis and evaluation of halogenated furanone derivatives as quorum sensing inhibitors in *Pseudomonas aeruginosa*. Eur J Pharm Sci 2019; 140: 105058.
29. Pacios O, Blasco L, Bleriot I, et al. Strategies to combat multidrug-resistant and persistent infectious diseases. Antibio 2020; 9: 65.
30. Klauss ECD, Edelberto SG, Bárbara DMM, et al. Natural and enantiopure alkylglycerols as antibiofilms against clinical bacterial isolates and quorum sensing inhibitors of *Chromobacterium violaceum* ATCC 12472. Antibio 2021; 10: 430.
31. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge University Press, Cambridge. 2005; 159-162.
32. Forbes BA, Sahm DE, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. International ed. 12th ed. Mosby, Inc., an affiliate of Elsevier, Inc., USA. 2007.
33. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, twenty-first informational supplement. CLSI document M100-S21 (ISBN 1-56238-742-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA. 2017; 30: 68-70.
34. Bell JM, Paton JC, Turnidge J. Emergence of vancomycin resistant Enterococci in Australia: phenotypic and genotypic characteristics of isolates. J Adv Biol 1998; 36: 2187-90.
35. Olsen ND, Morrow JB. DNA extract characterization process for microbial detection Methods, development and validation. BMC Res Notes 2012; 5: 668.
36. Srinivasan R, Karaoz U, Volegova M, et al. Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. Plos One 2015; 10: 1-22.
37. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biol Evolution 1987; 4: 406-25.
38. Jukes TH, Cantor CR. Evolution of protein molecules. In Munro HN, Editor, Mammalian Protein Metabolism, Academic Press, New York. 1969; 21– 132.
39. Ejaz H, Junaid K, Yasmeen H, et al. Multiple antimicrobial resistance and heavy metal tolerance of biofilm-producing bacteria isolated from dairy and non-dairy food products. Foods 2022; 11: 2728.
40. Fischer-Tenhagen C, Theby V, Krömker, V, et al. Detecting *Staphylococcus aureus* in milk from dairy cows using sniffer dogs. J Dairy Sci 2018; 101: 4317-24.
41. Özer B, Yaman H. Milk and milk. In Encyclop Food Microbiol 2nd ed.; Elsevier: Amsterdam, The Netherlands, 2014; 721–27.
42. Singh VK, Utaida S, Jackson LS, et al. Role for dnaK locus in tolerance of multiple stresses in *Staphylococcus aureus*. Microbiol 2007; 153, 3162-73.
43. Montanari C, Serrazanetti DI, Felis G, et al. New insights in thermal resistance of staphylococcal strains belonging to the species *Staphylococcus epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus aureus*. Food Control 2015; 50, 605–12.
44. Gould GW. Mechanisms of action of food preservation procedures. Elsevier Applied Science, London, UK. 1989.
45. Efiuvwevwere BJO, Amadi LO. Microbiological characteristics and deteriorative changes of 'Kwoka' (a Nigerian non-fermented Maize dish) produced using potassium sorbate and various steaming treatments. J Sci Food Agric 1992; 60: 443-50.

46. Hamzeh Pour S, Vaziri S, Molaee Aghaee E. Survey on the contamination rate and determination of antibiotic resistance of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* strains isolated from traditional cheeses distributed in Mahabad, Iran. Iran J Health Env 2019; 11: 465-76.
47. Roshanzamir M, Jafari M, Molaee Aghaee E, et al. The survival of probiotic bacteria and sensory properties of yogurt affected by microencapsulation with resistant starch. J Food Safe Hyg 2018; 3: 59-64.
48. Ogbolu DO, Daini OA, Ogunledun A, et al. Dissemination of IncF plasmids carrying beta lactamase genes in gram negative bacteria from Nigerian hospitals. J Infect Develop Count 2013; **7**: 382–90.
49. Lupo A, Coyne S, Berendonk TU. Origin and evolution of antibiotic resistance: The common mechanisms of emergence and spread in water bodies. Front Microbiol 2012; 3: 18.
50. Akanbi OE, Njom HA, Fri J, et al. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach sand in eastern cape province of south Africa. Int J Environ Res Pub Health 2017; 14: E1001.
51. Pontes DS, Pinheiro FA, Lima-Bittencourt CI, et al. Multiple antimicrobial resistance of gram negative bacteria from natural oligotrophic lakes under distinct anthropogenic influence in a tropical region. Microbial Ecolog 2009; 58: 762-72.
52. Davis R, Brown PD. Multiple antibiotic resistance index, fitness and virulence potential in respiratory *Pseudomonas aeruginosa* from Jamaica. J Med Microbiol 2016; 65: 261-71.
53. Dreier J, Roggerone P. Interaction of antibacterial compounds with RND efflux Pumps in *Pseudomonas aeruginosa.* Front Microbiol 2015; 6: 1-21.
54. Bush K. Past and present perspectives on β-Lactamases. Antimicrob Agent Chemo 2018; 16: 1076-18.
55. Jain P, Bepari AK, Sen PK, et al. High prevalence of multiple antibiotic resistance in clinical *E. coli* isolates from Bangladesh and prediction of molecular resistance Determinants using WGS of an XDR isolate. Sci Rep 2021; 11: 228-59.
56. Patrick F, McDermott PF, Walker RD, et al. Antimicrobials: Modes of action and mechanisms of resistance. Intl J Toxicol 2003; 22:135-43.
57. Goldberg E, Mical P, Talker O, et al. Co-trimoxazole versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia: A retrospective cohort study. J Antimicrob Chemo 2010; 65: 1779-83.
58. Rossolini GM, Thaller MC. Coping with antibiotic resistance: contributions from genomics. Genomic Med 2010; 2: 15-20.
59. Bakhtiari R, Javadi A, Aminzadeh M, et al. Association between presence of RmpA, MrkA and MrkD genes and antibiotic resistance in clinical *Klebsiella pneumoniae* isolates from hospitals in Tehran, Iran. Iran J Public Health 2021; 50: 1009-16.
60. Thung TY, Mahyudin NA, Basri DF, et al. Prevalence and antibiotic resistance of *Salmonella enteritidis* and *Salmonella typhimurium* in raw chicken meat at retail markets in Malaysia. Poult Sci J 2016; 95: 1888-93.
61. Kong-Ngoen T, Santajit S, Tunyong W, et al. Antimicrobial resistance and virulence of non-typhoidal *Salmonella* from retail foods marketed in Bangkok, Thailand. Food 2022; 11: 661.