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Virulence Signatures, Integrons, and Antibiotic Resistance Genes in Bacterial Strains Recovered from Selected Commercial Dairy Products and Fresh Raw Meat

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Abstract

Bacterial species responsible for food infections and intoxication are sometimes carried through the food production and processing. Very few published literatures exist on integrons among antibiotic-resistant staphylococcal strains from foods of animal origin in Gauteng Province, South Africa, hence this study. A total of 720 samples (360 meat and 360 dairies) from a community abattoir of a research farm in South Africa, using conventional bacteriological and molecular methods. Nine (9) bacterial strains, including *Bacillus subtilis* AYO-123, *Acinetobacter baumannii* AYO-241, *Staphylococcus lentus* AYO-352, among others were identified and submitted to GenBank. More bacterial strains were recovered from raw meat (90.5%) than dairy products (9.5%). Resistance was shown (0–100%) to Imipenem, Meropenem, Norfloxacin, Clindamycin, and 22 other antibiotics, without any carbapenem-resistant *Acinetobacter baumannii* and methicillin/vancomycin-resistant *Staphylococcus* species (MRSS/VRSS). Virulence genes for fibronectin-binding protein A (*FnbA*) were predominant (56.24%) followed by the circulating nucleic acids (*cna*) gene (43.75%). Others were staphylococcal enterotoxin A (*sea*, 41%), staphylococcal enterotoxin B (*seb*, 23.5%). Co-presence of *sea* and *seb* genes occurred in 11.76% of the isolates, but no *coa* genes was amplified. Antibiotic resistance genes (ARGs), *tetK* (70.58%), *linA* (29.4%), and *ermA* (11.76%) were detected, but none of the *mecA* and *vat* genes was amplified. Class 2 integron (50%) was more predominantly detected than integron 1 (25%), but no Class 3 integron was detected. Bacteria with both the detected virulence and antibiotic resistance genes are of potential risks to human health.

Introduction

The success associated with antibiotics discovery and their subsequent management are repeatedly marred by microbial response with antibiotic resistance [1]. This has a direct correlation to therapeutic antibiotic failures, prolonged hospitalization, and unwarranted cost of treatments [2, 3]. Diseases that were previously controlled by antibiotics are now re-emerging as incurable [4]. The global phenomenon of multi-drug-resistant bacterial strains limits the efficacies of current antibiotics, and thus, treatment failures [2, 5]. On the list of the major pathogenic bacterial groups that are

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resistant to standard antibiotic therapies, *Staphylococcus* and *Bacillus* species are noticeable, and are implicated as leaders in contributing to the global infectious disease burden [6–9].

Staphylococcus and *Bacillus* spp. are predominant etiological agents of foodborne illnesses worldwide [10] as they are often implicated in a wide variety of acute and chronic diseases affecting both animals and humans [7, 11]. The two genera possess appreciable degree of pathogenicity (virulence), possessing several virulence factors [12]. The pathogenicity of foodborne disease (FBD) causing bacteria depends on their potentials for intra-intestinal toxigenicity or ingestion of preformed toxins in foodstuff leading to intoxication. For instance, poisoning resulting from *Staphylococci* might be subsequent to ingestion of preformed enterotoxins [13], which persist and remain stable in heat. *Bacillus* species can cause two distinct types of food poisoning, viz diarrhea and emesis, which are caused by two different types of toxins. Immediately after consumption of contaminated food with *Bacillus* species, the enterotoxins are released into the small intestinal cells [10].

Administration of antimicrobial agents in breeding the animals meant for human consumption may contribute to the elevation of uncontrollable zoonotic bacterial pathogens. These antimicrobial agents are often used for therapeutic, growth promotion, and prophylactic purposes [7]. *S aureus* was the first among staphylococci to exhibit penicillin resistance and such attributes have been linked with the β-lactamase enzyme produced by the bacteria [3]. Resistance to various types of narrow spectrum beta lactam antibiotics can be linked to methicillin-resistant *S. aureus* [14, 15]. Such resistance began just after the discovery of methicillin [16] and MRSA remains persistent threat ever.

Antimicrobial resistance limits treatment option, leading to higher death rate and economic loss [17–19]. Antibiotic resistance genes can spread to other microbes in food, leading to worrisome epidemiological outcomes [15, 17]. Antibiotic resistance genes/determinants in antibiotic-resistant bacteria (ARB) in contaminated food of animal origins can be transferred to other bacteria affecting humans [10, 20].

This transfer of genes or mobile genetic elements, particularly integrons, leads progressively to multiple antibiotic resistance, extended spectrum antibiotic resistance, pandrug resistance, extreme drug resistance, and possibly totally drug resistance [3, 11, 21]. Integrons make up a significant proportion of these elements that are often found either in plasmids and/or transposons that further amplify the spread of resistance genes [22]. The presence of integrons in bacterial strains is often missed because it cannot be detected by routine phenotypic microbiological methods [23]. Screening for integrons in bacterial strains and their relationship with the severity and extent of antibiotic resistance can be of empiric importance in the management and treatment of infection [24]. There are very few published literatures on integrons among antibiotic-resistant staphylococcal strains from foods of animal origin in Gauteng Province, South Africa. Evaluation of antibiotic resistance genes allows access to their potential reservoirs. Therefore, this study was conducted to document the antibiotic susceptibility profiles and classes of integrons in *Acinetobacter baumannii*, *Bacillus* species and *Staphylococcus* species as well as to detect some selected virulence and antibiotic resistance genes among the *Staphylococcus* species recovered from raw meat and dairy products collected from a local abattoir and a farm shop in Irene, South Africa.

Materials and Methods

Study Site and Sample Collection

The study was done at the Agricultural Research Council (ARC), Irene Campus, situated about 25 km south of Pretoria 25°52′S 28°13′E/25.867°S 28.217°E/− 25.867; 28.217 in Gauteng, South Africa. Fresh raw beef, pork, mutton, and dairy products including yoghurts, cheese (cottage and Italian), and "amasi" (naturally fermented milk) all produced within the ARC were randomly sampled monthly for 6 months. Seven hundred and twenty samples (360 meat and 360 dairies) were collected which were composited to 36 for ease of processing. The thirty-six (18 meat and 18 dairies) composite samples were processed which means 6 (3 meat and 3 dairy) samples at a time for six times.

Isolation and Primary Identification of Bacteria

Bacteria were isolated from samples following a method described by Harrigan [25]. The composited meat and milk samples were individually separately homogenized together. One gram of each sample was put into 9 mL of sterile bacteriological peptone (Oxoid, Hampshire, England), homogenized separately, and incubated at 37 °C for 1–3 h, as a pre-enriched culture to enhance the isolation of targeted bacteria. One mL of each sample was serially diluted (10^{-1} to 10^{-6}) and plated on mannitol salt agar and MacConkey agar. The plates were incubated at 37 °C for 24 h. Distinct colonies were picked from these primary plates, purified on trypticase soy agar, and subjected to Gram staining and biochemical tests.

Molecular Detection of Bacteria and Genes

Extraction of DNA

The genomic DNA was isolated as described by Styers [7]. Few colonies from fresh culture at exponential phase on mannitol salt agar were suspended in 100 µL distilled water.

The suspension was then homogenized by vortexing for 5 s, followed by boiling on the heating block at 94–95 °C for 15 min. After this period, the suspension was centrifuged at 15,000 rpm for 10 min at 4 °C, after which the supernatant containing the DNA was removed and stored at − 20 °C for further assays.

Polymerase Chain Reaction (PCR)

The method of Goja [26] was utilized. The PCR assay for the 16S rRNA region was done in 50 µL reaction volumes containing 25 µL of PCR master mix, 5 µL of template DNA, 18 µL of molecular grade water, and 1 µL containing 20 µM each of each of the primers 27F: 5′-AGAGTT TGATCMTGGCTCAG-3′ and 1 µL of 1525R: 5′-AAGGAG GTGWTCCARCC-3′. PCR was done using the following conditions: initial denaturation at 94 °C for 1 min, followed by 29 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1.5 min, and a final extension step at 72 °C for 5 min, and cooling to 4 °C. *S. aureus* ATCC 25923; *A. baumannii* DSM 30007 and *Bacillus subtilis* ATCC 6633 utilized as positive controls were processed along. Gel electrophoresis of the amplicons was done in 2% agarose gel and 100 bp ladder ran at 110 V for 45 min.

16S rRNA Sequencing and Analyses of Sequenced Data

The purified PCR products were sequenced with an Automated DNA sequencing Analyzer (ABI 3130×1 genetic analyzer, Applied Biosystems), and the resulting chromatograph was viewed using Finch TV (Version 14.0; www.geospiza.com/finchtv). The identities of generated DNA sequences were determined by subjecting them to the Basic Local Alignment Search Tool (BLAST) program alignment tool on GenBank of the National Center for Biotechnology Information (NCBI). After the base calling, comparison of the sequences was made to the reference sequences in the GenBank database by multiple alignments using Bioedit (Version 7.0.5.3). The phylogenetic tree showing ancestry and relatedness was constructed using the Maximum Likelihood method in MEGA 7 (Version 7.0.26).

Antimicrobial Susceptibility Testing

Standard disk diffusion method as recommended by the Clinical and Laboratory Standards Institute [19] was utilized. The plates were prepared from 0.5 McFarland turbidity of the isolates. The ATCC 25923 *S. aureus, A. baumannii* DSM 30007, and *Bacillus subtilis* ATCC 6633 strains were used as quality control microorganisms. All isolates were classified as resistant, intermediate, or susceptible as described earlier [19, 27]. The following antibiotics were used: Ampicillin (AP2), Methicillin (MH30), Norfloxacin (NOR10), Ciprofloxacin (CIP5), Levofloxacin (LEV5), Cephalothin (KF30), Penicillin G (PG10), Cefotaxime (CTX5), Ceftazidine (CAZ30), Chloramphenicol (C30), Imipenem (IMP10), Meropenem (MEM10), Oxytetracycline (OT30), Tetracycline (T30), Doxycycline (DO30); Amikacin (AK30), Clindamycin (DA10), Gentamicin (CN10), Lincomycin (L10), Tobramycin (TOB10), Erythromycin (E15), Nalidixic acid (NA30); Tylosin (TY30), Vancomycin (VA30), Co-trimoxazole (TS25), kanamycin (KA30), and Nitrofurantoin (NI300) were dispensed.

Characterization of Virulence and Antibiotic-Resistant Genes

All the isolates were studied for distribution of virulence genes encoding coagulase (*coa*), enterotoxin A (*sea*), enterotoxins B (*seb*), circulating nucleic acid (*cna*), and fibronectic binding protein A (*FnbA)*. Resistant genes encoding resistance to methicillin (*mecA*), streptogramin (*vatA*), macrolide-lincosamides-streptogramin B (*ermA*), lincosamides (*linA*), and tetracyclines (*TetK*) were also assessed. All oligonucleotides were synthesized by Whitefield Scientific (Pretoria, South Africa). The amplification conditions and oligonucleotides used for these genes are shown in Table 1. Denaturation and extension temperatures were kept at 94 °C and 72 °C for each gene amplification. The PCR assay was done in 50 µL reaction volumes containing 25 µL of PCR master mix, 5 µL of template DNA, 18 µL of molecular grade water, and 1 µL each forward and reverse primers. The amplification was carried out in a thermal cycler (Bio-Rad, USA). Gel electrophoresis of the amplicons was done in 2% agarose gel and 100 bp ladder ran at 110 V for 45 min.

Characterization of Integron Genes

Integron genes were detected following a method described by Goja [26] with modifications. The amplification conditions and oligonucleotides used for these genes are shown in Table 1. Denaturation and extension temperatures were kept at 94 °C and 72 °C, respectively, for all genes' amplification. The PCR assay was done in 50 µL reaction volumes containing 25 µL of PCR master mix, 5 µL of template DNA, 18 µL of molecular grade water, and 1 µL each of forward and reverse primers. The amplification was carried out in a DNA thermal cycler (Bio-Rad, USA) as follows: initial denaturation at 94 °C for 10 min, followed by 30 to 40 repeated cycles of denaturation at 94 °C for 40 s, 50 s for annealing at 57 °C for int-1, int-2, and int-3 and 55 s for an extension at 72 °C, followed by 10 min at 72 °C for the final extension. The amplified products were analyzed by electrophoresis on 1% agarose gel and staining by the ethidium bromide.

Table 1 Oligonucleotides primers and amplification conditions for characterization of integrons, virulence and antibiotic resistant genes

Genes	Primer sequence	Amplification conditions	Amplicon size(bp)	References
mecA F	GCTTTGGTCTTTCTGCATTCCT	64 °C 30 cycles	91	Kumar et al. [39]
mecA R	ACGTTCAATTTAATTTTGTTAAAGAAGATG			
ErmA F	TAT CTT ATC GTT GAG AAG GGA TT	64 °C 30 cycles	139	Seydi et al. [51]
ErmA R	CTACAC TTG GCT TAG GAT GAA A			
tetK F	GTAGCGACAATAGGTAATAGT	55 °C 10 cycles	136	Strommenger et al. [29]
tetK R	GTAGTGACAATAAACCTCCTA			
VatA F	TGGTCCCGGAACAACATTTAT	55 °C 8 cycles	136	Strommenger et al. [29]
VatA R	TCCACCGACAATAGAATAGGG			
LinA F	GGTGGCTGGGGGGTAGATGTATTAACTGG	57 °C 30 cycles	323	Liu et al. [13]
LinA R	GCTTCTTTTGAAATACATGGTATTTTTCGA			
coa F	AACAAAGCGGCCCATCATTAAG	50 °C 15 cycles	850	Strommenger et al. [29]
coa R	TAAGAAATATGCTCCGATTGTCG			
sea F	5′-AAAATACAGTACCTTTGGAAACGGTT	50 °C 15 cycles	92	Kumar et al. [39]
sea R	5′-TTTCCTGTAAATAACGTCTTGCTTGA			
seb F	ACACCCAACGTTTTAGCAGAGAG	50 °C 15 cycles	81	Kumar et al. [39]
seb R	CCATCAAACCAGTGAATTTACTCG			
cna F	5′AGTGGTTACTAATACTG3	50 °C 15 cycles	744	Kumar et al. [39]
cna R	CAGGATAGATTGGTTTA			
FnbA F	GCGGAGATCAAAGACAA	50 °C 15 cycles	1280	Kumar et al. [39]
FnbA R	CCATCTATAGCTGTGTGG			
Int-1	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	50 °C 15 cycles	280	Xu et al. [47]
	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC			
Int- 2	ATGTCTAACAGTCCATTTTTAAATTCTA	50 °C 15 cycles	1471	Xu et al. [47]
	AAATCTTTAACCCGCAAACGC			
Int- 3	GCCTCCGGCAGCGACTTTCAG	50 °C 15 cycles	650	Xu et al. [47]
	ACGGATCTGCCAAACCTGACT			

Accession Numbers

Nine potential de novo bacterial strains were isolated and identified from the meat and dairy products as reported in Table 2. *Bacillus subtilis* AYO-123 (MT559272), *Bacillus subtilis* AYO-1474 (MT559279), and *Bacillus subtilis* AYO-2773 (MT559280) were the identified potential de novo *Bacillus* bacterial strains from meat.

Statistical Analysis

Descriptive statistics and correlation analysis were employed to analyze the putative antibiotic resistance genes using IBM SPSS version 23. Frequencies of some phenotypic properties and virulence genes were compared using Chi-square (χ^2). Correlations were established using Pearson's correlation coefficient (r^2) in bivariate linear correlations ($P < 0.05$). P values of ≤ 0.05 were considered significant. Antibiotic resistance was estimated as percentage resistance. Maximum Likelihood method was used to estimate and plot the phylogenetic tree.

Results

Bacterial Isolation Rate

Among all the isolates identified using 16S rRNA sequencing in this study (Table 2a, b), *Bacillus* genus was the most predominant species (61.9%), among which *B. subtilis was* the most predominant species (52.4%). Staphylococcus was the most predominant genus in the list of identified potential de novo bacterial strains (55.5%). The phylogenetic tree (Fig. 1) showed close relationships between the de novo bacterial strains to known bacteria in the same speciation in the NCBI GenBank. The de novo bacterial strains, hereby referred to as phylotypes, were identified preliminarily to their speciation especially during the phenotypic characterization but exhibited slight genetic variations, justifying their classification into unique strains.

Table 2 (a) and (b): Bacterial Identities, Sources and Accession number

(a) Unique Identity of bacterial strains and sources as deposited and approved by NCBI, USA

Identity	Accession number	Source
Bacillus subtilis AYO-123	MT559272	Meat
Acinetobacter baumannii AYO-241	MT559273	Meat
*Staphylococcus lentus*AYO-352	MT559274	Meat
Staphylococcus simulans AYO-544	MT559275	Meat
Staphylococcus pasteuri AYO-637	MT559276	Dairy
Staphylococcus simulans AYO-1113	MT559277	Meat
Staphylococcus epidermidis AYO-1355	MT559278	Meat
Bacillus subtilis AYO-1474	MT559279	Meat
Bacillus subtilis AYO-2773	MT559280	Meat

(b) Identities of other bacteria and sources where they were isolated

Identity	% Identity	Accession number	Source
Bacillus subtilis	95.0	MG705985.1	Meat
Bacillus subtilis	96.5	KM894175.1	Meat
Staphylococcus simulans	96.61	CP015642.1	Meat
Bacillus subtilis	99.68	MT509757.1	Meat
Bacillus tequilensis	95.47	MK796031.1	Dairy
Bacillus subtilis	96.27	MT111062.1	Meat
Bacillus circulans	84.80	HQ678668.1	Meat
Bacillus subtilis	96.93	CP044498.1	Meat
Bacillus subtilis	99.67	MT525235.1	Meat
Staphylococcus pasteuri	88.72	EU379261.1	Meat
Bacillus subtilis	96.57	JQ308573.1	Meat
Bacillus subtilis	96.86	CP044498.1	Meat
Bacillus sp.	93.72	MN555648.1	Meat

Phylogeny of the Bacterial Isolates

These phylotypes showed reasonably close relationships and sometimes close ancestry to the other identified intraspecific or intrageneric bacteria, as shown in the phylogenetic tree, despite identification percentage below 98%. *Bacillus* spp. showed large diversities of strains, second to *Staphylococcus* spp. in species diversities, and they have close distance relationships as expected (Fig. 1). *Acinetobacter baumannii* AYO-241 aligned in Acinetobacter/Bacterium strain BS1910 subcluster.

Antibiogram

The resistance profile of the Staphylococci and *Bacillus* strains varied among the 26 antibiotics tested in our study (Table 3). Wide range of resistance (11.11–100%) to conventional antibiotics (Nalidixic Acid, Meropenem, Norfloxacin, Ciprofloxacin, Levofloxacin, Cephalothin, Cefotaxime, Erythromycin, Ceftazidine, Tylosin, Amikacin, Clindamycin) by the *Bacillus* species was observed. For *Staphylococcus* species, an observation of resistance range of 20–60% Erythromycin, Clindamycin, Lincomycin, and Cephalothin was made in our study. Resistance range of 60–100% was observed against the tetracycline group (Oxytetracycline, Doxycycline, and Tetracycline) with tetracycline having 100% resistance. Higher resistance of 80% against cefotaxime was observed but notably no vancomycin-resistant *Staphylococcus* species (VRSS). Multidrug-resistant (MDR) pattern of the Staphylococcus species is reported in Table 4. As high as 30.8% showed MDR pattern to 8 antibiotics at the same time. The only *Acinetobacter baumannii* identified did not show resistance to any of the two carbapenem antibiotics (imipenem and meropenem).

Antibiotic Resistance Genes

Detection of antibiotic resistance genes revealed that proportions of the tetracycline-resistant gene (*tetK*) recorded in this present study were (70.58%). There was correlation in the phenotypic expression of tetracycline resistance and the detection of tetracycline resistance genes. The amplification of *linA* gene revealed that 29.4% of the isolates harbored the gene while rest were negative for it. Although in

Fig. 1 Molecular Phylogenetic analysis of bacterial strains from meat and milk by Maximum Likelihood method. The potential de novo bacterial strains were compared with typed strains (tagged with T) from American Typed Culture Collection (ATCC) and German Collection of Microorganisms and Cell Cultures

low prevalence rate, *ermA* genes were detected, *mec* and *vat* genes were not detected. Also based on the PCR results, 4(26.66%) and 8 (53.33%) isolates contained Class 1 and 2 integrons, respectively (Table 4). Class 3 was not detected in any of the isolates. The results in Table 4 show that Class 2 integrons were predominant, accounting for 50% in all isolates. Exactly 25% of the integron 1 was found in *Staphylococcus* spp.

Virulence Genes

Our study showed that enterotoxin gene, *sea* was the most frequently detected in 41% of our samples (Fig. 2). Co-existence of the *sea* and *seb* genes was observed in 11.76% of our isolates. The *coa* gene was not detected in any of the isolates. Non-detection of *coa* gene in our isolates aligns with no coagulase production phenotypically. *cna* and *FnbA* genes to be 56.25% and 43.75%, respectively (Fig. 2). Detection of *cna* and *FnbA* genes was observed in 47.7% and 36.7% of the isolates.

Discussion

Raw meat samples and dairy products from an abattoir and a farm shop in Centurion (South Africa) were investigated for bacterial contamination. The abattoir environments and slaughter processes have long been documented to play leading roles in the spread of microbial contaminations [27, 28]. This informed the need for intermittent quality checks. In South Africa, meat and dairy products processed in the formal sector are inspected through various checkpoints for microbial quality. However, when these products are supplied from the informal sector, they are often not thoroughly checked. This makes the operators relax and compromise the quality of their supplies, exacerbating the possibility of FBD outbreaks [13].

This study showed the presence of *Acinetobacter baumannii, Bacillus subtilis,* and *Staphylococcus* species in both the raw meat and dairy samples. The presence of *Staphylococcus* species and *Bacillus* species in both the raw meat and milk samples corroborated an earlier report [17]. *Bacillus* genus were the most predominant (61.9%) with *B. subtilis*

Table 3 Antibiotic susceptibility profile of selected bacterial strains isolated from beef and dairy samples

Antibiotics	*Bacillus* group ($n=9$)			*Staphylococcus* group ($n=5$)			*Acinetobacter* group ($n=1$)		
	Sensitive intermediate resistant			Sensitive intermediate resistant			Sensitive intermediate resistant		
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Imipenem	8 (88.88)	1 (11.11)	0 (0)	2 (40)	0	3 (60)	1 (100)	0	0
Nalidixic Acid	0	0	9 (100)	2 (40)	0	3(60)	0	0	1 (100)
Meropenem	4 (44.44)	1 (11.12)	4 (44.44)	1 (20)	1 (20)	3 (60)	1(100)	0	0 (100)
Norfloxacin	5 (55.56)	0	4 (44.44)	1 (20)	2 (40)	2 (40)	0	0	1 (100)
Ciprofloxacin	7 (77.78)	0	2 (22.22)	0	1 (20)	4 (80)	0	0	1 (100)
Levofloxacin	2 (22.22)	1 (11.11)	6 (66.67)	2 (40)	2 (40)	1 (20)	0	0	1 (100)
Cephalothin	5 (55.56)	1 (11.11)	3 (33.33)	1 (20)	1 (20)	3 (60)	0	0	1 (100)
Cefotaxime	6 (66.67)	1 (11.11)	2 (22.22)	1 (20)	0	4 (80)	0	0	1 (100)
Erythromycin	5 (55.56)	1 (11.11)	3 (33.33)	3 (60)	1 (20)	1 (20)	0	0	1 (100)
Ceftazidine	5 (55.56)	1 (11.11)	3 (33.33)	1 (20)	1 (20)	3 (60)	0	0	1 (100)
Tylosin	5 (55.56)	0	4 (44.44)	0	1 (20)	4 (80)	0	0	1 (100)
Amikacin	4 (44.44)	0	5 (55.56)	3 (60)	1 (20)	1 (20)	1 (100)	0	0
Clindamycin	8 (88.89)	0	1(11.11)	3 (60)	1 (20)	1 (20)	1 (100)	0	0
Gentamicin	1 (11.11)	0	8(88.89)	1 (20)	0	4 (80)	0	0	1 (100)
Lincomycin	7 (77.78)	0	2(22.22)	3 (60)	0	2 (40)	0	0	1 (100)
Tobramycin	3 (33.33)	1 (11.11)	7 (77.77)	0	1 (20)	4 (80)	0	0	1 (100)
Vancomycin	8 (88.89)	1 (11.11)	0	5(100)	0	0	1 (100)	0	0
Ampicillin	1 (11.11)	0	8 (88.89)	0	0	5 (100)	0	0	1 (100)
Co-trimozole	2 (22.22)	0	7 (77.78)	2 (40)	1 (20)	2 (40)	0	0	1 (100)
Penicillin G	0	0	9 (100)	0	0	3 (100)	0	0	1 (100)
Nitrofurantoin	8 (88.88)	0	1 (11.11)	5 (100)	0	0	1 (100)	0	0
Oxytetracycline	1 (9.09)	1 (11.11)	7 (77.77)	2 (40)	0	3 (60)	0	0	1 (100)
Tetracycline	0	1 (11.11)	8 (88.88)	0	0	5 (100)	0	0	1 (100)
Kanamycin	8 (88.88)	0	2 (22.22)	3 (60)	2 (40)	0	0	0	1 (100)
Chloramphenicol	5 (55.55)	1 (11.11)	3 (33.33)	2 (40)	1 (20)	2 (40)	0	0	1 (50)
Doxycycline	2 (22.22)	0	7 (77.77)	1 (20)	0	4 (80)	0	0	1 (100)

Table 4 Frequency of integrons found in 15 selected bacterial strains from beef and dairy samples

Integron class	*Bacillus* group (9)	*Staphylococcus* group *(5)*	*Acinetobacter* group *(1)*
Class 1	2 (22.22%)	1 (20%)	1 (100%)
Class 2	5 (55.55%)	2 (40%)	1 (100%)
Class 3	0	0	0

being the most predominant *Bacillus* species (52.4%). *B. subtilis* have been implicated in food poisoning due to intoxication by one of two ways, viz (i) consumption of food containing preformed toxin, or (ii) toxins created by these bacteria in the human gut [13, 29].

The predominance of *Staphylococci* as milk contaminants has long been reported by Rall et al. [30]. The novel *Staphylococcus lentus* (strain AYO-352 and other Staphylococci were known floras of animal skin which contaminate milk and meat products easily [31, 32]. *Staphylococcus pasteuri* AYO-637 was detected in our study. The spread of *S. pasteuri* has previously been reported as contaminants in foodstuffs sold in South Korea [33, 34], animal farms in the Netherlands [35], and a source of infection among animals in Brazil [36].

Contamination of raw meat by Staphylococcal and *Bacillus* bacteria may also be accredited to workers' skin lesions, sneezing, or coughing or to an already contaminated working surface, reclaimed water [37] or equipment used for slaughtering. The muscular tissues and body fluids of healthy animals are customarily pathogenic bacteria free; therefore, contamination is likely to occur during the slaughtering, processing, and transportation. Bacterial recovery in contamination cases is not uniform due to dissimilar way of caring for the critical quality control points in the slaughtering and milking processes [13, 38, 39]. Contamination in some cases might have been a result of direct excretion of staphylococci and bacilli into milk from infected udders

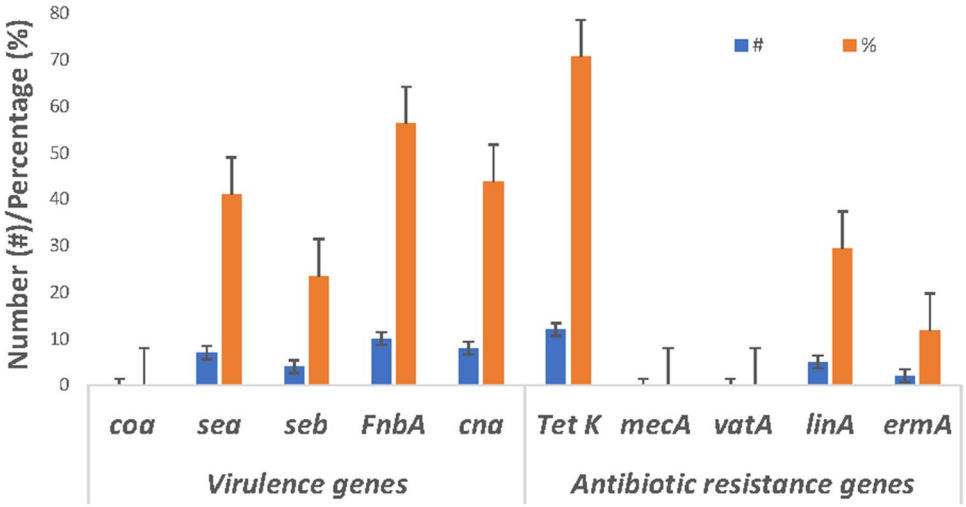

Fig. 2 Detection of virulence genes and antibiotic resistance genes among the *Staphylococcus* species. The numbers (#) for each of the genes detected were represented in blue-colored bar, while the percentages (%) detected were represented with orange-colored bar

or by contamination from fodder, equipment, air, or milk handlers [25, 39].

The phylogenetic tree (Fig. 1) showed close relationships between the bacterial strains to bacteria. The bacterial strains, hereby referred to as phylotypes, were identified preliminarily to their speciation especially during the phenotypic characterization but exhibited slight genetic variations, justifying their classification into unique strains. *Bacillus* spp. showed large diversities of strains, second to *Staphylococcus* spp in species diversities, and they have close distance relationships as expected (Fig. 1). *Acinetobacter baumannii* AYO-241 aligned in Acinetobacter/Bacterium strain BS1910 sub cluster showing relatedness to *Acinetobacter baumannii* strain HSTU-ABk29 and others of previously known species.

Meanwhile, *Acinetobacter baumannii* has been identified as ubiquitous commensal bacteria and neglected pathogens in veterinary health [40, 41]. Tavakol et al. [42] reported the potential human health threat associated with the presence of *A. baumannii* in meat. The impact of this is usually high among individuals with preferred undercooked meat as highlighted by Askari et al. [43].

As noted, the resistance profile to 26 antibiotics of both strains of Staphylococci and *Bacillus* varied (Table 3). Wide resistance range (11.11–100%) to the conventional antibiotics by the *Bacillus* species was observed. An observation of 20–60% resistance range by *Staphylococcus* spp. to Erythromycin, Clindamycin, Lincomycin, and Cephalothin was made. Resistance range from 60 to 100% was observed against the tetracycline group with tetracycline having 100% resistance, which is in tandem with the earlier report of Adegoke and Okoh [15], where very high (83.3%) resistance to tetracycline by bacterial isolates from animal source was detected. Higher resistance of 80% against cefotaxime, but notably no vancomycin-resistant *Staphylococcus* species (VRSS) was observed. The rate of 0% VRSS in our study is in contrast to 50% ratio in human samples reported by Dewan [21], and 24% observed in a study by Fluit et al. [6] in Europe. The difference could not have been sample types as vancomycin is administered in both human and animals [44]. Rather, the difference in vancomycin resistance might be other factors like geographical variations [45]. Contamination from fomites depending on the extent of hygiene is also possible [27] Thus, the frequency of antibiotic resistance varies greatly across countries and continents. Our data, thus, confirm the efficacy of this vancomycin antibiotics in the treatment of staphylococcal infections. VRSS rattles clinicians and was classified with high priority for research and development by WHO [46]. Many strains of *Staphylococcus* species have been reported with multidrug resistant [15] as we have observed (Table 4). The human health risks of exposure to such strains can be very high.

The only *Acinetobacter baumannii* identified did not show resistance to any of the two carbapenem antibiotics (imipenem and meropenem) in this study. This makes the bacteria of no critical concern in the interim as WHO [46] classified carbapenem-resistant *Acinetobacter baumannii* with critical criteria for urgent research and development. No external factors were immediately identified that bring about the development of resistance especially in amikacin and kanamycin because of reduced intra-resistance among aminoglycosides [13, 38]. Some level of pronounced resistance noted might be due to availability and affordability of aminoglycosides and gentamicin as well as being the pertinent antibiotic administrable for severe staphylococcal infections. The high resistance further points to the potential health risk associated with the source of the isolates [15]. It is quite in order to presume potential common source infection by the resistant strains in such places [25, 38].

Characterization of antibiotic resistance genes revealed that proportions of the tetracycline-resistant gene (*tetK*) recorded in this present study were 70.58% (Fig. 2). In

contrast, a study by Malachowa and DeLeo [11] revealed *tetK* to be 24% among their isolates. The amplification of *linA* gene revealed that 29.4% of the isolates harbored the gene. This was lower than the report of Malachowa and DeLeo [11]. Where *linA* was discovered to be present in 51.6% of their isolates. Both our study and the study of Goja et al. [26] reported the occurrence of the *ermA* gene in staphylococci isolates, though in different prevalent rates, 11.76% in the present study is compared to 63.2% in the former. The low recovery rate of the gene in the present study may be because the gene is commonly widespread in methicillin-resistant strains [29], and none of our isolates were positive for the *mecA* gene. The presence of the *ermA* gene, though low prevalence rate is a significant discovery because antibiotics belonging to Macrolides, Lincosamides, and Streptogramines (MLS) are not often used in animal treatment, frequent administration of macrolide-class tylosin to animals resulted in the development of cross-resistance to the MLS group. The *mec* and *vat* genes were not discovered in all the isolates. Elsewhere Cuevas et al. [14] and Fluit et al. [6] reported a 50% and 56% prevalence of the *mec* gene from their meat isolates in contrast to our non-detection of the gene.

Our study showed that fibronectin-binding protein A (*fnbA*) gene was the most frequently detected in our samples (56.24%) (Fig. 2), which was similar to an earlier report [25]. Harrigan [25] earlier reported that the fibronectin-binding protein A *(fnbA)* is the most commonly recovered enterotoxin gene in staphylococci bacteria from food. The *sea* (41.5%) gene has also been reported in different prevalence rates elsewhere: Xu et al. [47] reported 16.2%, Syed and Sarangi [48] reported 58%, and Cuevas et al. [14], reported 38.7% compared to *seb* (23.5%). The *seb* was recovered in 23.5% of our isolates (Fig. 2). In contrast to our results, *seb* was a more prevalent gene in a study by Malachowa and DeLeo [11].

Co-existence of *sea, seb*, and *sec, sed* with *seg, seh,* and *sei* has been reported by other researchers [7, 14]. In our study, the co-existence of the *sea* and *seb* genes was observed in 25.76% of the isolates. The *coa* gene was not detected in any of the isolates. The zero prevalence rates of the *coa* gene observed were similar to a study by Bashir et al. [49], where none was detected but contrary to a study by Styers et al. [7] where the prevalence rate was 5%. Non-discovery of *coa* gene in our isolates aligns with phenotypes as no coagulase as the isolates could not coagulate plasma. *FnbA* and *cna* genes to be 56.25% and 43.75% respectively (Fig. 2). Detection of *FnbA* and *cna* genes in 47.7% and 36.7% of the isolates, respectively, have also been reported by Rall et al. [30]. The detected virulence genes so far in these isolates posited them as a potential threat for human health as these genes play significant roles in causing infection via colonization and invasion of the host [50].

Due recognition has been adduced to integron gene sequences as an origin of resistance genes, prenoting them to be the repository of antimicrobial resistance genes in a microbiota [4, 29]. Fifteen beef and dairy associated bacterial strains were assayed by PCR amplification for the three classes of integron genes. According to the PCR results, 8 (53.33%) and 4 (26.66%) isolates contained Class 2 and class 1 integron, respectively (Table 4). Class 3 was not detected in any of the isolates. The results shown in Table 4 revealed that Class 2 integrons were predominant in all isolates. This corroborates the reported detection of Class 2 integrons in similarly large percentage of isolates by Cuevas et al. [14]. A 100% detection of Class 2 intergrons was reported by Xu et al. [47], which was far higher than our observations from this present study.

Class 1 integrons are known to play a key role in the acquiring, expressing, and disseminating antibiotic resistance genes and may accommodate genes like those coding for β-lactamases, aminoglycosides, chloramphenicol, sulfonamides, macrolides, trimethoprim, quinolone, and quaternary ammonium compound to propagate multidrug resistance in bacterial isolates. None of the isolates contained class 3 integrons. This is in line with a study by Otalu et al. [17], and Goja [26] where none of their isolates were positive for Class 3 integron. The differences in the prevalence of integron genes can be accredited to the different geographic regions, the bacteria strains, or the indiscriminate use of antibiotics in a particular region. The low prevalence of integrons in our study could imply that the antibiotic resistance genes may be carried by other genetic elements [47].

Characterization of antibiotic resistance genes revealed that the proportions of the tetracycline-resistant gene (*tetK*) recorded in this present study was 70.58%. The amplification of *linA* gene revealed that 29.4% of the isolates harbored the gene. The presence of the *ermA* gene, though in low prevalence rate is a significant discovery because antibiotics belonging to MLS are not often used in animal treatment, but frequent administration of macrolide-class tylosin to animals resulted in the development of cross-resistance to the MLS group. The *m*ecA and *vat* genes were not discovered in all the isolates. Novel virulent and antibiotic-resistant bacterial strains were recovered from the samples in the study area. Some of the bacterial strains were repositories of virulence genes including circulating nucleic acids *(cna)*, fibronectin-binding protein A (*Fnb*A), coagulase (*coa*), staphylococcal enterotoxin A, and staphylococcal enterotoxin B. These strains also exhibited co-occurrence of *sea* and *seb* genes. ARGs including integron class 1 and 2 were also reserved in these strains. In the entire process, the role of fomites like currency in dissemination of pathogens harboring resistance determinants cannot be ruled out in contaminations of meat or milk [27]. Although methicillin-resistant *Staphylococcus* species (MRSS) or vancomycin-resistant *Staphylococcus*

species (VRSS) and carbapenem-resistant *Acinetobacter baumannii* classified with high and critical priorities respectively by WHO were not present, resistance to other antibiotics as well as the presence of critical genes make these bacterial strains and their sources (meat and dairy products) to be of public health concern [50].

Conclusion

The selected commercial dairy products and fresh raw meat studied contained several bacterial species out of which nine potential de novo bacterial species were identified and the sequences deposited in GenBank of the NCBI. The raw meat products were more contaminated than the dairy products. Large proportions of the samples were contaminated with multidrug-resistant bacteria and some of the bacterial strains were repositories of virulence genes including circulating nucleic acids (*cna*), fibronectin-binding protein A (*FnbA*), coagulase (*coa*), staphylococcal enterotoxin A, and staphylococcal enterotoxin B. This showcases their high degree of pathogenicity. The bacteria also housed antibiotic resistance genes (ARGs), including the class 1 and 2 integrons. These critically important genes portrayed these bacterial isolates with associated potential risks to human health, especially the consumers of minimally processed meat and unpasteurized dairy products, who are still large population in the study area. The identified limitation of this study is that it did not encompass expression study of the virulence and antibiotic genes. This would be considered in our future study.

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Data Availability All the data are available at the GenBank of the National Center for Biotechnology Information (NCBI).

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent to Participate Not applicable as no human was sampled.

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