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Article

Antimicrobial Resistance Profiles of *Staphylococcus Aureus* Isolated from Meat Carcasses and Bovine Milk in Abattoirs and Dairy Farms of the Eastern Cape, South Africa

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Abstract:

1) Background: *Staphylococcus aureus* (*S. aureus*) occasionally threatens the life of the host as a persistent pathogen even though it is normal flora of humans and animals. We characterized drug resistance in *S. aureus* isolated from animal carcasses and milk samples from the abattoirs and dairy farms in the Eastern Cape Province.

Methods: A 1000 meat swab samples and 200 raw milk samples were collected from selected abattoirs and dairy farms in the Eastern Cape Province, South Africa. *S. aureus* was isolated and positively identified using biochemical tests and confirmed by molecular methods. Antibiotic susceptibility test against 14 different antibiotics was performed against all isolates. Antibiotic resistance genes were also detected.

Results: Of the 1200 samples collected, 134 (11.2%) samples were positive for *S. aureus*. Resistance ranged from 71.6% for penicillin G to 39.2% for tetracycline. Resistance gene (*bla*Z) was detected in 13 (14.9%), while *msr*A was found in 31 (52.5%) of *S. aureus* isolates.

Conclusions: The present result shows the potential dissemination of multidrug-resistant *S. aureus* strains in the dairy farms and abattoirs in the Eastern Cape. Therefore, this implies that the organism may rapidly spread through food and pose serious public health risk

Keywords: *Staphylococcus aureus*, meat, raw milk, antibiotics; antibiotic resistance genes

1. Introduction

Staphylococci asymptomatically colonizes the skin and mucous membranes in the nostrils of humans and animals [1-5]. An important outcome bearing in mind the fact that nasal carriage of *S. aureus* has been associated with subsequent infection [5]. Several studies have reported the identification of coagulase-positive and coagulase-negative species in warm-blooded animals [2-3, 6-9]. Carriers are therefore an important source of spread of infections in the communities. *S. aureus* causes diseases in humans and animals which including toxic syndrome and staphylococcal food poisoning (SFP) [10-13]. The work of Hatakka et al. [14]

has revealed that *S. aureus* in meat is as a result of improper hygienic practices during handling by the slaughter personnel during meat production.

South Africa studies have reported that a high percentage of the population largely depends on beef and pork meat as a protein [15-16]. Additionally, some research has demonstrated that antibiotic resistant strains are caused by foods contaminated with antibiotic resistant bacteria [17-19] making them an ideal vehicle for transmission of antibiotic resistance.

Studies have reported that prolonged use and misuse of antimicrobial agents in agriculture, stock farming and in treatment of human diseases have resulted in rapid resistance of many bacteria to several antibiotics of different classes [20-22]. The development of antibiotic resistance has been noticed to variety of antimicrobial agents which include aminoglycosides, macrolides, glycopeptides, fluoroquinolones and tetracyclines [23]. Many antibiotic resistance genes play a role in *S. aureus* resistance and these include macrolide resistance encoded by *erm* gene, *aphA3* and *sat* genes for kanamycin and streptomycin resistance and *accA-aphD* and *tet* genes for gentamicin, tobramycin and tetracycline resistance [24-25].

There is paucity of information on the molecular characterization of *S. aureus* in most developing countries [23, 26]. Better understanding of *S. aureus* antibiotic susceptibility profiles and molecular characterization of genes causing resistance are of paramount importance for initiating effective control measures and reducing staphylococcal infections [23, 26]. The aim of the study was to identify, and characterize antibiotic resistance susceptibility patterns including antibiotic resistance genes in *S. aureus* strains isolated from selected dairy farms and abattoirs in the Eastern Cape Province, South Africa.

2. Materials and Methods

A total of two hundred milk samples were collected from cows with subclinical and clinical mastitis cases at Dairy Farm A (100 samples) and Dairy Farm B(100 samples). Milk samples were collected using the method of Caine *et al.* [27]. Briefly, in each milking station there is a small collection bottle with a small hole that open and closed using a tap. The bottles were properly washed and used for sampling another cows' milk. All the milk samples were stored on ice and transported to the Biochemistry and Microbiology Laboratory for analysis.

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A 1000 meat swab samples were collected from cow carcasses, pig carcasses and sheep carcasses in selected Abattoirs according to the method of Pearce and Bolton [18]. Permission to collect swab samples was obtained from abattoirs managers. Samples were collected from the available animal carcasses during the period of 10 months (August 2015 to May 2016). Samples were collected using sterile swab rinsing kit containing 10 ml isotonic buffer rinse solution, after the gastrointestinal tract was removed. A 100 cm^2 sterile disposable plastic template (Analytical Diagnostics, USA) was used to mark the area for swabbing. A total of 500 meat swab samples were collected from cow carcasses, 300 meat swab samples from sheep carcasses and 200 meat swab samples from pig carcasses. Each animal carcass was sampled in four areas which include rump, flank, brisket and neck and isolates from those four areas were counted consecutively. The meat swab samples were then stored on ice and transported to the Biochemistry and Microbiology Laboratory for analysis.

2.2 Isolation of *S. aureus* milk and meat samples

Ten microliter of each milk sample were inoculated onto Baird Parker Agar (Oxoid; country) and incubated at 37˚C for 24-48 hours. The meat swab samples were also inoculated onto the same culture media and incubated for the same period. Presumptive grey-black colonies surrounded by opaque halo of precipitation on Baird Parker agar were regarded as presumptive *S. aureus* isolates and were subjected to biochemical identification.

2.3 Biochemical Identification and DNA extraction

Gram-staining, catalase test and oxidase test were performed according to the method of Health Protection Agency [28-30] for biochemical identification of the organism. DNA extraction was performed based on the procedure of Maugeri *et al*. [31]. Briefly, a loop full 24-hour culture of *S. aureus* colonies grown onto Nutrient Agar plates were suspended into 200 µl of sterile nuclease free water and vortexed for 2 minutes using MS2 Minishaker (Digisystem Laboratory instruments Inc, New Taipei City, Taiwan) and the cells were lysed using a heat Dri-Block DB.2A (Techne, Johannesburg South Africa) for 15 min at 100ºC. The pellet was removed by centrifugation at 10 000 rpm for 5 min using a MiniSpin microcentrifuge (ThermoFisher Scientific; Waltham, MA USA) kept at 4ºC. The supernatant was transferred in new Eppendorf tubes and used for PCR reactions.

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2.4. Identification of *S. aureus*

2.4.1 The *nuc* gene amplification

The isolates were confirmed by PCR amplification of the *nuc* gene encoding the thermonuclease enzymes with the oligonucleotide primers shown in Table 1.1. The total PCR reaction volume of 25 µL containing 12 µL of master mix (Kapa Biosystems, South Africa) (containing, DNA Taq polymerase, dNTPs, $MgCl_2$ and PCR buffer), 5 µL DNA template , 1µL of the forward and reverse primers (10 ng), and 6 µL of nuclease free water was used. Polymerase Chain reaction (PCR) was performed using MyCycler™ (Biorad, Cape Town, South Africa). Amplicons were resolved on 1.5% agarose gel containing 5µL Ethidium bromide in 1X TAE buffer pH 8.0 for an hour at 100 volts before being visualized and captured under Alliance 4.7 transilluminator (UVITEC Limited, Cambridge, UK).

2.5. Antibiotic susceptibility testing

A disk diffusion antibiotic susceptibility test was conducted according to the Clinical and Laboratory Standards Institute [32]. Bacterial suspensions were prepared in 2.5 ml of Mueller-Hinton broth and the turbidity was adjusted to meet 0.5 McFarland turbidity standards (~1.5 x 10^8 cfu/ml). The isolates were inoculated onto a Mueller-Hinton Agar plate and tested against a panel of fourteen (14) antibiotics (Table 1.2) and results were interpreted according to the Clinical and Laboratory Standards Institute [32]. *Staphylococcus aureus* ATCC 25923 was used as a positive control.

Table 1.1. Oligonucleotide sequences used for Polymerase Chain Reaction for the identification of *Staphylococcus aureus* and detection of antibiotic resistance genes.

Target gene	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Reference
nuc	GCG ATT GAT GGT GAT ACG GTT CCA AGC CTT GAC GAA CTA AAG C	255	[33]
*mec*A	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	533	[6]
*bla*Z	ACT TCA ACA CCT GCT GCT TTC TGA CCA CTT TTA TCA GCA ACC	173	[34]
acc(6')-*aph*(2")	TTG GGA AGA TGA AGT TTT TAG A CCT TTA CTC CAA TAA TTT GGC T	174	[35]
aph(3')-1-IIIa	AAA TAC CGC TGC GTA CAT ACT CTT CCG AGC AA	242	[36]
Ant(4')-Ia	AAT CGG TAG AAG CCC AA GCA CCT GCC ATT GCT A	135	[36]
*erm*A	TAT CTT ATC GTT GAG AAG GGA TT CTA CAC TTG GCT TAG GAT GAA A	139	[35]
*erm*B	CTA TCT GAT TGT TGA AGA AGG ATT GTT TAC TCT TGG TTT AGG ATG AAA	142	[37]
*erm*C	CTT GTT GAT CAC GAT AAT TTC C ATC TTT TAG CAA ACC CGT ATT C	190	[35]
*msr*A	TCC AAT CAT TGC ACA AAA TC AAT TCC CTC TAT TTG GTG GT	163	[35]
*tet*K	GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA	360	[37]
*tet*M	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT GTC TA	158	[37]

Table 1.2. A list of antimicrobial agents used for antibiotic susceptibility testing for *Staphylococcus aureus* isolates and their limits as shown on CLSI (2016)

Antimicrobial Class	Antibiotics	Concentration (µg)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
B-lactams	penicillin G	10 units	≤28	-	≥29
	oxacillin	1	≤17	-	≥18
Tetracyclines	tetracycline	30	≤14	15-18	≥19
	doxycycline	30	≤21	13-15	≥16
	minocycline	30	≤14	15-18	≥19
Macrolides	erythromycin	15	≤13	14-22	≥23
Aminogycosides	amikacin	30	≤14	15-16	≥17
	gentamicin	10	≥12	13-14	≥15
Fluoroquinolones	ciprofloxacin	5	≥21	16-20	≥15
Lincosamides	clindamycin	30	≤14	15-20	≥21
Phenicols	chloramphenicol	30	≤12	13-17	≥18
Sulfonamides	Trimethoprim/sulfamethoxazole	1.25/23.75	≤10	11-15	≥16
Cephems	Ceftaroline	5	≤20	21-23	≥24
Oxazolidinones	Linezolid	30	≤20	-	≥21

2.5.1. Detection of antibiotic resistance genes of *Staphylococcus aureus*

The confirmed *S. aureus* isolates were screened for antibiotic resistance genes using the oligonucleotide primers listed in Table 3.1. The total reaction volume of 25 µL containing 12

µL of master mix (Kapa Biosystems, South Africa) (containing, DNA Taq polymerase, dNTPs, $MgCl_2$ and PCR buffer), 5 µL DNA template , 1µL of the forward and reverse primers (10 ng), and 6 µL of nuclease free water was used for amplication. Polymerase Chain reaction (PCR) was performed using MyCycler™ (Biorad, Cape Town, South Africa).The amplified products were separated on 1.5% agarose gel containing 5µL Ethidium bromide in 1X TAE buffer pH 8.0 for an hour at 100 volts before being visualized and photographed under Alliance 4.7 transilluminator (UVITEC Limited, Cambridge, UK.

3. **Results**

3.1. Study population

From 1200 meat and milk samples a total of 134 samples were positive for *S. aureus* by culture, biochemical tests and molecular confirmed by Polymerase chain targeting the *nuc* gene. There were 102/500 (3.3%) isolates from beef samples, 10/300 (3.3%) from sheep samples, 14/100 (7%) from pork samples were and 8/200 (4%) from milk samples. All the 134 *S. aureus* revealed a 255 base pair size in an agarose gel electrophoresis. Figure 3.1 shows the representatives of 134 positive *Staphylococcus aureus*.

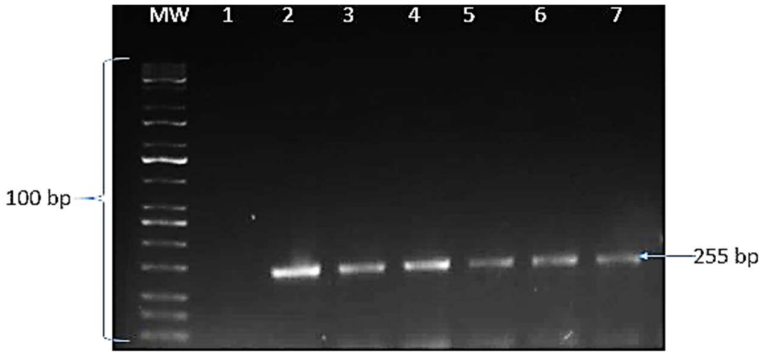

Figure 1.1. Confirmation of *S. aureus* isolates using the thermonuclease gene (*nuc* gene). Lane MW: 100 bp universal Kappa ladder (Biosystems), Lane 1: Negative control, Lane 2-7: *S. aureus* isolates.

3.2. Antimicrobial resistance screening of *S. aureus* isolates.

The resistance profiles of isolated *S. aureus* is shown in Table 1.3. Sheep and pork isolates were resistant to one to five antimicrobial agents. Multidrug resistance (MDR) among all *S.*

aureus isolates was noted in penicillin G, oxacillin, clindamycin, erythromycin and tetracycline (information not shown).

3.2.1. Antimicrobial resistant of the four areas of the animal carcasses

Table 1.3 shows that most of the *Staphylococcus aureus* isolates were resistant to penicillin G, rump (40-62.9%), flank (60-100%), brisket (50-100%) and the neck (20-100%) from pork, sheep and beef isolates. A similar antibiotic resistant pattern was also observed for oxacillin, where isolates from rump (40-75%), flank (50-100%), brisket (50-100%), and neck (20-100%) showed resistance while resistance to tetracycline was observed on isolates from rump (40-75%), isolates from flank (45-50%), brisket (25-50%) and neck (20-50%).

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Table 1.3. Antibiotic resistance of *Staphylococcus aureus* isolates in beef, sheep and pork samples against 14 different antibiotics

	Antimicrobial resistance				
Antibiotics	**Rump np/n** (%)	**Flank np/n** (%)	**Brisket np/n** (%)	**Neck np/n** (%)	**Total np/n** (%)
Penicillin G					
Beef	22/35 (62.9)	12/20 (60)	23/28 (82.1)	16/19 (84.2)	73/102 (71.6)
Sheep	2/4 (50)	2/2 (100)	1/2 (50)	2/2 (100)	7/10 (70)
Pork	2/5 (40)	0/0 (0)	4/4 (100)	1/5 (20)	7/14 (50)
Oxacillin/Methicillin					
Beef	23/35 (65.7)	10/20 (50)	16/28 (57.1)	19/19 (100)	68/102 (66.7)
Sheep	3/4 (75)	2/2 (100)	1/2 (50)	1/2 (50)	7/10 (70)
Pork	2/5 (40)	0/0 (0)	4/4 (100)	1/5 (20)	7/14 (50)
Tetracycline					
Beef	14/35 (40)	9/20 (45)	11/28 (39.3)	6/19 (31.6)	40/102 (39.2)
Sheep	3/4 (75)	1/2 (50)	1/2 (50)	1/2 (50)	6/10 (60)
Pork	0/5 (0)	0/0 (0)	1/4 (25)	1/5 (20)	2/14 (14.3)
Doxycycline					
Beef	9/35 (25.7)	5/20 (25)	6/28 (21.4)	6/19 (31.6)	26/102 (25.5)
Sheep	2/4 (50)	0/2 (0)	0/2 (0)	0/2 (0)	2/10 (20)
Pork	1/5 (20)	0/0 (0)	0/4 (0)	1/5 (20)	2/14 (14.3)
Minocycline					
Beef	4/35 (11.4)	3/20 (15)	2/28 (7.1)	4/19 (21.1)	13/102 (12.7)
Sheep	1/4 (25)	0/2 (0)	0/2 (0)	0/2 (0)	1/10 (10)
Pork	1/5 (20)	0/0 (0)	0/4 (0)	0/5 (0)	1/14 (7.1)
Erythromycin					
Beef	15/35 (42.9)	6/20 (30)	15/28 (53.6)	13/19 (68.4)	49/102 (48)
Sheep	2/4 (50)	2/2 (100)	1/2 (50)	1/2 (50)	6/10 (60)
Pork	1/5 (20)	0/0 (0)	2/4 (50)	1/5 (20)	4/14 (28.6)
Amikacin					
Beef	9/35 (25.7)	2/20 (10)	5/28 (17.9)	6/19 (31.6)	22/102 (21.6)
Sheep	1/4 (25)	0/2 (0)	0/2 (0)	1/2 (50)	2/10 (20)
Pork	0/5 (0)	0/0 (0)	0/4 (0)	0/5 (0)	0/14 (0)
Gentamicin					
Beef	1/35 (2.9)	2/20 (10)	2/28 (7.1)	5/19 (26.3)	10/102 (9.8)
Sheep	2/4 (50)	1/2 (50)	0/2 (0)	2/2 (100)	5/10 (50)
Pork	0/5 (0)	0/0 (0)	0/4 (0)	0/5 (0)	0/14 (0)

np= number positive isolates, n= number of collected isolate

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Table 1.3 continued

	Antimicrobial resistance				
Antibiotics					
	Rump np/n (%)	**Flank np/n (%)**	**Brisket np/n (%)**	**Neck np/n (%)**	**Total np/n (%)**
Ciprofloxacin					
Beef	2/35 (5.7)	0/20 (0)	2/28 (7.1)	2/19 (10.5)	6/102 (5.9)
Sheep	0/4 (0)	0/2 (0)	0/2 (0)	1/2 (50)	1/10 (10)
Pork	0/5 (0)	0/0 (0)	0/4 (0)	0/5 (0)	0/14 (0)
Clindamycin					
Beef	20/35 (57.1)	8/20 (40)	13/28 (46.4)	13/19 (68.4)	54/102 (52.9)
Sheep	1/4 (25)	1/2 (50)	2/2 (100)	2/2 (100)	6/10 (60)
Pork	1/5 (20)	0/0 (0)	2/4 (50)	0/5 (0)	8/14 (57.1)
Chloramphenicol					
Beef	0/35 (0)	3/20 (15)	0/28 (0)	0/19 (0)	3/110 (2.7)
Sheep	0/4 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/10 (0)
Pork	0/5 (0)	0/0 (0)	0/4 (0)	1/5 (20)	1/14 (7.1)
Trimethoprim/sulfomethoxazole					
Beef	9/35 (25.7)	5/20 (25)	8/28 (28.6)	3/19 (15.8)	25/102 (24.5)
Sheep	1/4 (25)	0/2 (0)	0/2 (0)	0/2 (0)	1/10 (10)
Pork	1/5 (20)	0/0 (0)	1/4 (25)	0/5 (0)	2/14 (14.3)
Ceftaroline					
Beef	5/35 (14.3)	5/20 (25)	9/28 (32.1)	5/19 (26.3)	24/102 (23.5)
Sheep	1/4 (25)	0/2 (0)	0/2 (0)	0/2 (0)	1/10 (10)
Pork	2/5 (40)	0/0 (0)	2/4 (50)	0/5 (0)	4/14 (28.6)
Linezolid					
Beef	1/35 (2.9)	3/20 (15)	1/28 (3.6)	4/19 (21.1)	9/102 (8.8)
Sheep	0/4 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/10 (0)
Pork	1/5 (20)	0/0 (0)	1/4 (25)	0/5 (00	2/14 (14.3)

np= number positive isolates, n= number of collected isolate

3.3.5 Detection of penicillin (*blaZ* gene) antibiotic resistance gene.

Penicillin antibiotic resistant gene, *blaZ* was detected from 13 isolates, of which 9/13 (69.2%) were from beef isolates, 3/13 (23.1%) were detected in pork isolates and only 1/13 (7.7%) was detected from a milk isolate. Figure 3.2 shows *blaZ* gene of 173 base pairs for 13/87(14.9%) *S. aureus* isolates alongside the 100 bp universal kappa ladder.

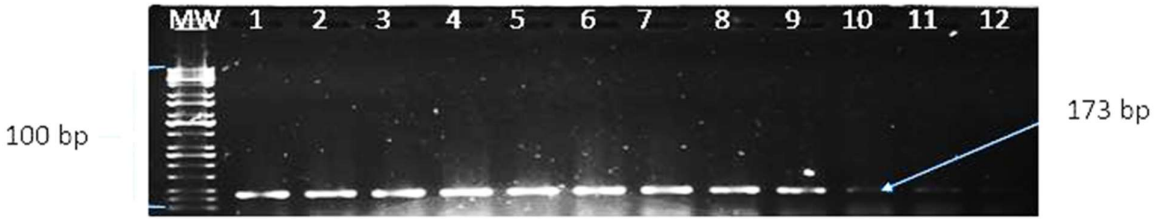

Figure 1.2. Penicillin antibiotic resistance gene (*blaZ* gene) of *S. aureus*. Lane MW: 100 bp universal Kappa ladder, Lane 1-12: *S. aureus* isolates.

Amplification of tetracycline (*tet*K) antibiotic resistance gene.

Tetracycline resistant gene, *tet*K was detected in 26 *S. aureus* isolates of which, 20/26 (76.9%) were from beef isolates, 2/26 (7.7%) from pork isolates, 1/26 (3.8%) was identified in sheep isolates and 3/26 (11.5%) from milk isolates. Figure 1.3 shows the 360 bp amplification of 26/48 (54.2%) tetracycline antibiotic resistance gene representatives in *S. aureus* with a 100 bp molecular weight marker.

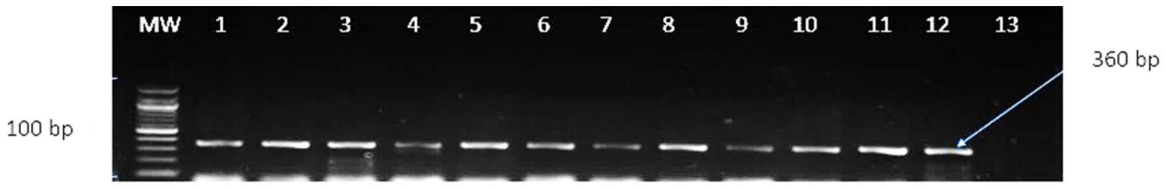

Figure 1.3. Tetracycline antibiotic resistance gene (*tet*K) of *S. aureus* isolates. Lane MW: 100 bp Molecular weight marker (biolabs), Lane 13: Negative control, Lane 2-12 *S. aureus* isolates.

3.3.7 Detection of tetracycline (*tet*M) antibiotic resistance gene.

The *tet*M gene was detected in 23/29 (79.3%) of beef *S. aureus* isolates, 4/29 (13.8%) from pork isolates and 2/29 (6.9%) were detected in sheep isolates. Figure 1.4 shows the representatives of the amplified 158 bp tetracycline (*tet*M) antibiotic resistance gene in 29/48 (60.4%) *S. aureus* isolates with a 100bp molecular DNA ladder.

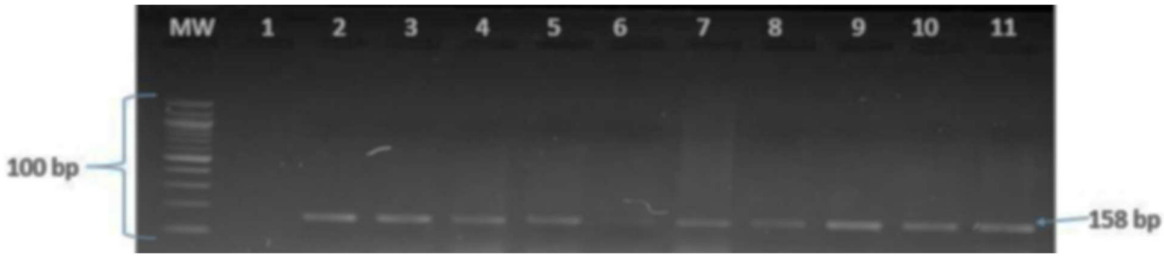

Figure 1.4. Tetracycline antibiotic resistance gene (*tet*M) of *S. aureus*. Lane MW: 100 bp Molecular weight marker (biolabs), Lane 1: Negative control, Lane 2-11 *S. aureus* isolates.

Detection of erythromycin antibiotic resistance (msr*A*) gene.

A total of 59 (46.8%) *S. aureus* isolates showed phenotypic resistant to erythromycin antibiotic, however, 31 (52.5%) contained erythromycin antibiotic resistance gene. Twenty-five (80.6%) were from beef isolates, 4(12.9%) were identified in pork isolates and 3(9.7%) were detected in milk isolates. Figure 1.5 demonstrates the agarose gel for the amplified erythromycin antibiotic resistance *S. aureus* isolates.

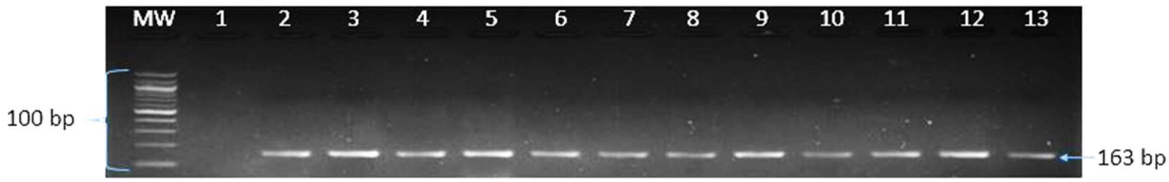

Figure 1.5. Erythromycin antibiotic resistance gene (msr*A*) of *S. aureus* isolates. Lane MW: 100 bp Molecular weight marker (biolabs), Lane 1: Negative control, Lane 2-13 *S. aureus* isolates.

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Detection of Gentamicin antibiotic resistance genes (*ant (4')-Ia*) and *aph* (3')-1-IIIa

Fifteen (11.9%) isolates were phenotypically resistant to gentamicin, however, only 5 had resistant genes to gentamicin. Gentamicin antibiotic resistance (*ant (4')-Ia*) gene was detected only 3(60%) isolates and *aph* (3')-1-IIIa in 2(40%) isolates. Figure 1.6. shows the amplified gentamicin antibiotic resistance genes from beef *S. aureus* isolates.

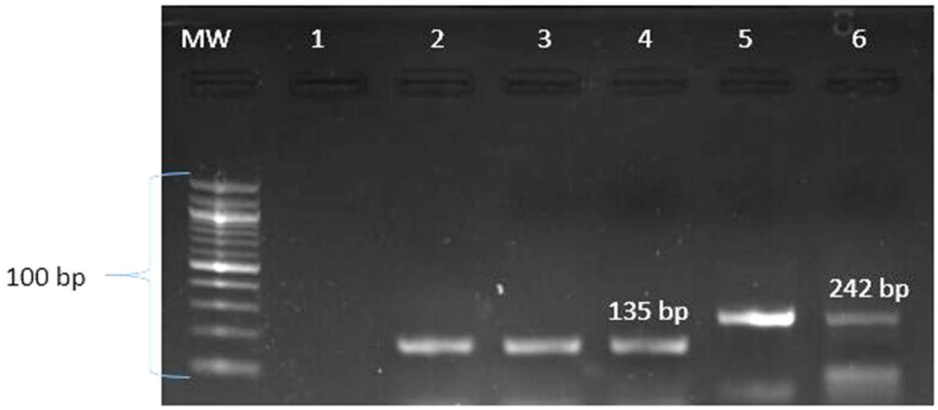

Figure 1.6. Gentamicin antibiotic resistance genes (ant (4')-Ia); 135 bp and aph (3')-1-IIIa; 242 bp) of S. aureus isolates. Lane MW: 100 bp Molecular weight marker (biolabs), Lane 1: Negative control, Lane 2-6 S. aureus isolates.The text continues here.

4. Discussion

Antibiotic use always selects for antibiotic resistance. Our task is to preserve the effectiveness of existing antibiotics by minimizing the emergence and spread of multidrug resistant microorganisms to maximize the time until existing antibiotics become ineffective. Over the past years, the dissemination of antimicrobial resistance (AR) in bacteria, including staphylococci has increased and poses public health risks. This is best narrated by the multidrug resistant *S. aureus* strains that causes infections that are difficult to treat (Simeoni *et al.*, 2008). In this study the most prevalent *S. aureus* strains were observed in beef samples and beef isolates showed resistance to several antibiotics including penicillin G (71.6%), oxacillin (66.7%), clindamycin (52.9%), erythromycin (48%) and tetracycline (39.2%) respectively. Sheep and pork samples were relatively resistant to one to five antimicrobial agents. The work conducted by Yang *et al.* [38] has reported similar levels of resistance of *S.*

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aureus isolates to penicillin, erythromycin and tetracycline [39-40]. Andreotti and Nicodeno [41] have revealed that the resistance of *S. aureus* to penicillin ranges from 20% to 100%, whilst the percentage of resistance to other antibiotics is relatively lower. Most of the isolates in our study showed high sensitivity to several antibiotics including ciprofloxacin, chloramphenicol, clindamycin, linezolid and trimethoprim-sulfamethoxazole. This implies that such antibiotics can be used to treat infections caused by *S. aureus*.

In this study 82 (67%) isolates showed phenotypic resistance to methicillin, however only one methicillin-resistant *Staphylococcus aureus* (MRSA) gene was detected using molecular method. All the other isolates that showed resistance to oxacillin could have a *mec*C gene, which was not investigated in our study. A study performed by Diederen and colleagues [42] in the Netherlands has demonstrated that 2.5% of pork and beef samples harbored MRSA isolates. The research performed by Fessler and co-workers [43] has shown that MRSA were resistant to oxacillin, and 62.5% demonstrated multidrug resistant. Similarly, the study of Hanson *et al.* [44] advocated that MRSA isolated from pork, beef, chicken and turkey were resistant to oxacillin and several other antibiotics.

Four main mechanisms used by bacteria to achieve resistance include: (i) reduction of membrane permeability to antibiotics; (ii) drug inactivation; (iii) rapid efflux of the antibiotic; and (iv) mutation of cellular target(s) [45], strategies foretold by chromosomal or mobile genetic elements (plasmids). Penicillin including, penicillin derivatives, cephalosporins, cephamycins, carbapenems, monobactams, monocarbams and ampicillin belong to the group of β-lactam antibiotics. Inhibition of the membrane-bound enzymes responsible for cell wall biosynthesis, makes this class of antibiotics to be bactericidal. Such inhibition is as a result of antibiotic binding to penicillin-sensitive enzymes, known as penicillin-binding proteins (PBPs). Penicillin resistant bacteria produce an extracellular β-lactamase which

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inactivates antibiotics through hydrolysis of the β-lactam ring [46]. The β-lactamase structural gene (*blaZ*) is coded for in the Tn*552*-like transposons [47], located on β-lactamase plasmids which display resistance to other antimicrobial agents. Four subgroups of these lactamase are known, including plasmids types that encodes resistance to inorganic ions and organomercurials in addition to β-lactamase production, plasmid that carries the erythromycin resistance transposon [47], and genes that encodes metal ion resistance too found in plasmids and in chromosomal genes. There were 87 (69%) *S. aureus* isolates that were phenotypically resistant to penicillin G and only 13 (14.9%) isolates were found to express *bla*Z gene in this study.

Of the 82 (67%) *S. aureus* isolates that were phenotypically resistant to oxacillin, only one isolate (1.2%) was found to possess *mec*A gene. This small proportion of isolates that showed amplification of *mec*A gene compared to phenotypic resistance to oxacillin was not surprising. Oxacillin has been proposed as an alternative antibiotic for testing susceptibility/resistance to methicillin and to all β-lactams [48]. This could explain why all oxacillin-resistant isolates were not carrying the *mec*A gene, because they were showing resistance to β-lactams. Phenotypic resistance witnessed to oxacillin in this study could have been attained through other mechanisms, including the reduction in membrane permeability to β-lactam antibiotics.

Binding of tetracycline antibiotics to the 30S ribosomal subunit prevents association of aminoacyl-tRNA with its acceptor site, thereby inhibiting protein synthesis [49]. *S. aureus* uses two mechanisms of tetracycline resistance; active efflux via *tet*A (*K*) and *tet*A(*L*) and ribosomal protection via *tet*A(*M*) [49].Tetracycline efflux in *S. aureus* strains is mediated by *tet*A(*K*), which is commonly carried by plasmid pT181. Integration of this plasmid into Type III *SCCmec* makes this kind of resistance to be named chromosomally encoded resistance. Resistance to tetracycline can also be mediated by mutations that cause increased expression of various

chromosomally encoded efflux pumps, such as Tet38 ([49-50]. In this study, a high detection rate was observed in *tetM* 29 (46.7%) while *tetK* detection rate was 26 (41.9%) for tetracycline resistant genes. Seven (11.3%) isolates contained both *tet*K and *tet*M gene.

The *erm*(C) determinant was found in 2 isolates (3.4%) while no *erm*A and *erm*B were detected. This results are different from the results of Cetin et al. [51] who showed that *ermA* was the most prevalent phenotype among *S. aureus*. Macrolide antibiotic (including azithromycin, clarithromycin, erythromycin), resistance in *Staphylococcus aureus* may be due to an active drug efflux mechanism encoded by *msr*A and *msr*B (conferring resistance to macrolides and type B streptogramins). In our study, *msr*A determinant was detected in 31 (52.5%) isolates. It is likely that other erythromycin resistance genes such as *msrA*, *Ere* A–B or *mef,* which we did not include in our study, might be present among these isolates and account for the remainder of the isolates showing phenotypic resistance to erythromycin.

Aminoglycosides antibiotics plays an important role in the treatment of staphylococcal infections [52]. Aminoglycosides inactivates antibiotics using amino-glycoside-modifying enzymes (AMEs) that are encoded by genetic elements (52-53]; Schmitz et al., 1999). The *aac (6′)-Ie + aph (2′′)*, ant (4′)-Ia, *aph (3′)-IIIa*, and *ant (6)-Ia* genes that encode aminoglycoside-6'-N-acetyltransferase/2"-O-phosphoryltransferase,aminoglycoside-4'-O-nucleotidyltransferase I, aminogly-coside-3'-O- phosphoryltransferase III, and streptomycin modifying enzyme, respectively, are the most important genes in this regard. Resistance to gentamicin, kanamycin, and tobramycin in staphylococci is mediated by a bi-functional enzyme displaying AAC (6') and APH (2") activity. The ANT (4')-IA enzyme inactivates neomycin, kanamycin, tobramycin, amikacin, and kanamycin, while the APH (3')-III, enzyme inactivates neomycin ([54]. In our study, we found 15 *S. aureus* isolates which were phenotypically resistant to gentamicin, however, only 5 (33.3%) isolates

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were found to express one of these gentamicin resistance genes. Antibiotic resistance can be classified into three main categories: intrinsic, adaptive, and acquired resistance [55]. We speculate that intrinsic antibiotic resistance, being the naturally low permeability of the bacterial cell wall, which limits uptake of many antibiotics including aminoglycosides is responsible for the other 10 isolates that did not show amplicons of the investigated target genes.

5. Conclusion

There is no doubt about the potential rapid spread of antibiotic resistant *S. aureus* strains as several studies have indicated this around the world. Resistance to the tested antibiotics indicates that they are no longer effective against *S. aureus.* Meat and milk form part of the human diet on a daily basis and it is for that reason that proper hygienic habits in the dairy farms and abattoirs should be prioritized. The managers should be on the fore-front to educate their employees about the importance of employing strict hygienic practices before, after and during the milk processing and meat production. Better supervision of new antibiotics use will be required. In retrospection, it was a mistake to permit usage of molecules in veterinary medicine and animal husbandry that are identical as or closely associated to antibiotics used in human medicine.

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