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Molecular Detection of spa, mecA, and mecC genes in *Staphylococcus aureus* Isolated from Soils and Wooden Table Surfaces from Abattoirs in Ilorin, Kwara, Nigeria

Ismaila O. Sule[1], Mutiat B. Odebisi-Omokanye[2], Olutoyin C. Adekunle[3], Zainab B. Abdulsalam, Favour C. Elemi[1]

[1]Department of Microbiology, University of Ilorin; Department of Microbiology, University of Ilorin; [2]Department of Medical Microbiology and Parasitology, Osun State University, Nigeria; [3]Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria;

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***Correspondence to:**
suleism@unilorin.edu.ng

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ABSTRACT

The resistance of bacteria to some antibiotics is rampant nowadays. One of the genera of bacteria that has shown resistance to notable antibiotics is *Staphylococcus aureus*. Therefore, this study aimed at isolation of *S. aureus* from the soils and wooden table surfaces in some abattoirs within Ilorin, Nigeria. The objectives of this study were to determine the counts of heterotrophic bacteria in the soils and wooden table surfaces at some abattoirs in Ilorin, Nigeria; characterize and identify the *S. aureus* isolates; determine the antibiotic resistance patterns of the isolates; and detect the presence of spa, mecA, and mecC in the isolates. The counts of viable bacteria and *S. aureus*, antibiotic susceptibility as well as the physicochemical characteristics were determined using standard methods. The heterotrophic bacterial counts of the soils and wooden table surfaces ranged from $1.0 \times 10^5 – 3.4 \times 10^7$ and $2.1 \times 10^5 – 1.0 \times 10^8$ cfu/g respectively. In addition, the counts of *S. aureus* in the soils and wooden table surfaces ranged from $1.0 \times 10^3 – 3.0 \times 10^7$ and $1.0 \times 10^3 – 3.1 \times 10^6$ cfu/g respectively. The *S. aureus* isolates were positive for Gram staining, catalase, oxidase, mannitol fermentation and coagulase test. All the *S. aureus* were resistant to ampicillin, and ceftazidime, followed by augmentin (82%), vancomycin, cefuroxime, and nitrofurantoin (55% each); gentamicin and ciprofloxacin (36%) and ofloxacin (18%). None of the isolates possess mecA gene while mecC and spa genes were detected in 45.5 and 54.5% of the isolates respectively. This is the first study in which mecC gene was detected in any *S. aureus* in Nigeria. It is concluded from this study that the soils and wooden table surfaces from the abattoirs harboured *S. aureus* some of which possessed mecC for antibiotic resistance and the virulent factor, spa genes.

1. INTRODUCTION

Animals such as cattle, sheep and goats are slaughtered daily in the abattoirs in most cities in order to provide beef for the teeming population. The meat is highly susceptible to contamination by microorganisms such as viruses, fungi and bacteria. The bacterium, *Staphylococcus aureus* is one of the contaminants of meats, abattoir soils and surfaces. Although *S. aureus* is one of the commensals of the human body, it can become an opportunistic pathogen causing infections and food poisoning (Kluytmans *et al.*, 1997). *S. aureus* is also known to cause disease such as mastitis in ruminants and this could be one of the sources of zoonotic transfer of the agent to humans (Ali *et al*., 2022).

Many strains of *S. aureus* have become resistant to antibiotics notably methicillin and they are regarded as methicillin-resistant *S. aureus* (MRSA) (Fitzgerald, 2014). The resistance

to methicillin and other β-lactam antibiotics is due to the possession of mecA gene, which is found on a mobile genetic element, the staphylococcal cassette chromosome (SCCmec). Besides mecA, a homologue of mecA, called mecC is also known to be responsible for methicillin resistance in some *S. aureus* (Foster, 2017). Glycopeptide antibiotics such as vancomycin are effective against most MRSA strains but some isolates of MRSA have reduced susceptibility to glycopeptides (Abhijit and Ian, 2005).

Protein A (spa) represents an important virulence factor of *S. aureus* (Frénay *et al.*, 1996). It has been shown to be involved in the pathogenesis of *S. aureus* pneumonia (Gómez *et al.*, 2004). Also, it plays a role in proteinaceous biofilm formation (Merino *et al.*, 2009).

This study is justified since it is the first study that attempted to detect mecA homologue, mecC in any sample whether medical or environmental in Nigeria. Most of the studies done were on humans and mecA.

The objectives of this study were to determine the counts of heterotrophic bacteria and *S. aureus* in the soils and wooden table surfaces from abattoirs within Ilorin, Nigeria; characterized and identified the isolates; determine the antibiotic resistance patterns of the isolates, detect the presence of spa, mecA, and mecC genes in the isolates; and determine the physicochemical properties of the abattoirs' soils.

2. MATERIALS AND METHODS

2.1 Collection of soils and wooden table surface samples

Soils and wooden table surface samples were collected from some abattoirs within Ilorin metropolis, Nigeria. These abattoirs were Ajanaku, Tipper Garage, Oja-Tuntun, Akerebiata I, Akerebiata II, Akerebiata III, Mandate and Oloje within Ilorin metropolis, Kwara, Nigeria. The soil samples were collected into sterile polythene bags according to standard method while wooden table surfaces samples were taken with sterile swab stick that was rolled over the entire table surface (Sule and Awe, 2019).

2.2 Counts of heterotrophic bacteria

One gramme of the soil was serially diluted up to 10^{-5}. The swab sample was dipped into 10ml of normal saline and shaken thoroughly to dislodge the microbial content; representing 10^{-1} dilution. The serial dilution of the swab sample was also done up to 10^{-5}. Then, 0.1ml of aliquot from dilutions 10^3, 10^4 and 10^5 were aseptically transferred into separate sterile Petri dishes and sterile molten nutrient agar was added to each plate. The plates were swirled and allowed to solidify; incubated upside down at 37^{o}C for 48 hours; and the colonies were counted and expressed in cfu/g (Sule and Awe, 2019).

2.3 Isolation and enumeration of *Staphylococcus aureus*

Sterile set plate of mannitol salt agar (MSA) was inoculated with 0.1ml aliquot from 10^{-1} to 10^{-3} dilutions. The inoculum was spread on the agar plates using sterile L- shaped glass spreader. The plates were incubated upside down at 37^{o}C for 24-48 hours. The number of yellow colonies were taken tentatively as *Staphylococcus aureus* (Aneja, 2014).

2.4 Purification and preservation of isolates

Distinct yellow colonies on the MSA plates were streaked on sterile nutrient agar plates and incubated at 37^{o}C for 24 hours. The colonies obtained were further streaked on nutrient agar slants and incubated at 37^{o}C for 24 hours. These slants were kept in a refrigerator until they were needed for further analysis (Aneja, 2014).

2.5 Characterization and identification of *S. aureus* isolates

Twenty-eight putative *S. aureus* isolates obtained from the soil and wooden table surface swab samples were characterized and identified based on their colonial, cellular and biochemical characteristics. Colonial characteristics such as colour, size, shape, and optical characteristics were observed while the cellular characteristics observed were Gram's reaction, shape and arrangement of the cells. The biochemical tests performed include catalase, oxidase, coagulase, and mannitol fermentation (Khademi *et al*., 2016).

2.6 Antimicrobial susceptibility testing

The 11 colonies that showed typical *S. aureus* characteristics were used for antimicrobial susceptibility test and molecular characterization. Mc Farland's standard (0.5) was prepared as described by Cheesbrough (2006). Twenty-four hours old culture of *S. aureus* on nutrient agar plate was picked with sterile inoculating loop and inoculated into 9ml of sterile normal saline solution (0.85% NaCl) and shaken to dissolve completely. The turbidity of the broth and the 0.5 McFarland was compared and adjusted by adding more culture or normal saline until they matched. Sterile set plate of Mueller Hinton agar was inoculated with the isolate using sterile swab stick. Rapid labs multiple antibiotic discs were placed on the inoculated plates and the plates were incubated at 37^{o}C for 24 hours. Vancomycin antibiotic sensitivity test was performed using Oxoid single vancomycin disc in separate plates of Mueller Hinton agar. The diameter of zone of inhibition of the isolate to each antibiotic was interpreted according to CLSI (2013).

2.7 Multiple antibiotic resistance (MAR) index

This was obtained by dividing the number of antibiotics the isolate exhibited resistance by the total number of antibiotics tested (Sule and Bello, 2021).

2.8 Extraction of bacterial DNA

The pure culture of each isolate was grown in nutrient broth and the DNA was extracted using Zymogen DNA extraction kit and following the manufacturer's instructions.

Table (1): Primer sequences for detection of spa, mecA and mecC genes

Primer name	Sequences (5'→3')	Amplicon size	References
spa1113(F) spa1514(R)	TAAAGACGATCCTTCGGTGAGC CAGCAGTAGTGCCGTTTGCTT	180 – 600bp	Stegger *et al*., 2011; DTU Food (2012); Paterson *et al*., 2013; Khademi *et al*., 2016.
mecA(F) mecA (R)	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162bp	Stegger *et al*., 2011; DTU Food (2012); Cikman *et al*., 2019.
mecC (F) mecC (R)	GAAAAAAAGGCTTAGAACGCCTC GAAGATCTTTTCCGTTTTCAGC	138bp	Stegger *et al*., 2011; DTU Food (2012); Cikman *et al*., 2019.

2.9 Synthesis of primers

The primer sequences were synthesized at Inqaba Biotechnical Industry (Pty) Ltd., Pretoria, South Africa. The quotation order was NG2019/19366. The details of the primers used are given in Table 1.

2.10 PCR amplification of spa, mecA, and mecC genes

The extracted DNA of the isolates were used for PCR. The PCR cocktail consist of 2.5ul of 10x PCR buffer, 1ul of 25mM $MgCl_2$, 1ul each of forward primer and reverse primer (as listed in Table 1), 1ul of DMSO, 2ul of 2.5mM DNTPs, 0.1ul of 5u/ul Taq DNA polymerase, and 3ul of 10ng/ul DNA. The total reaction volume was made up to 25ul using 13.4ul Nuclease free water. The PCR cycling parameters is given as follows. Initial denaturation at 94^{o}C for 5 minutes, followed by 36 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds and elongation at 72^{o}C for 45 seconds, followed by a final elongation step at 72^{o}C for 7 minutes and hold at 10^{o}C. The amplified fragments were visualized on safe view stained 1.5% agarose electrophoresis gels. The ladder used for loading was 50bp from NEB and the hyper ladder from Bioline.

3. RESULTS

3.1 Physicochemical and bacteriological characteristics of the soils and wooden table surfaces from the abattoirs

The abattoirs' soils pH ranged from 7.3 to 8.8 while the soil temperature ranged from 30.2 to 32.4^{o}C. The heterotrophic bacterial count of the soils and wooden table ranged from $1.0 \times 10^5 – 3.4 \times 10^7$ and $2.1 \times 10^5 – 1.0 \times 10^8$ cfu/g respectively. The counts of *S. aureus* of the soils and wooden table surfaces ranged from $1.0 \times 10^3 – 3.0 \times 10^7$ and $1.0 \times 10^3 - 3.1 \times 10^6$ cfu/g respectively (Table 2).

3.2 Characterization and identification of *Staphylococcus aureus*

The 28 colonies that gave yellow colour on mannitol salt agar were taken tentatively as *S. aureus*. Eleven out of the 28 colonies produced the typical characteristics of *S. aureus*. They were Gram positive cocci, arranged in clusters, positive for catalase, tube coagulase, oxidase and mannitol fermentation.

3.3 Antibiotic resistance patterns of *S. aureus* isolates and MAR index

All the *S. aureus* were resistant to ampicillin, and ceftazidime, followed by augmentin (82%), vancomycin, cefuroxime, and nitrofurantoin (55% each); gentamicin and ciprofloxacin (36% each) and ofloxacin (18%). All the isolates were resistant to at least 2 of the antibiotics. The MAR index ranged from 0.22 to 0.89 (Table 3).

3.4 PCR amplification of spa, mecA and mecC genes

Isolates S1, S2, S6, S10 and S11 showed the presence of Staphylococcal protein A (spa). They produced at least 2 bands (Figure 1). None of the isolates possessed mecA gene (Figure 2). However, isolates S1, S2, S5, S6, S9, and S10 possessed mecC genes (Figure 3).

Table 2: Physicochemical and bacteriological characteristics of soils and wooden table surfaces at the abattoirs.

SN	Sampling site	Soil pH	Soil temp. (°C)	Bacterial count (cfu/g) S	Bacterial count (cfu/g) W	*S. aureus* (cfu/g) S	*S. aureus* (cfu/g) W
1	Ajanaku	7.3	30.3	3.0×10^7	1.0×10^5	2.2×10^7	1.5×10^3
2	Tipper-Garage	7.9	32.4	2.8×10^7	2.0×10^6	10×10^3	2.0×10^5
3	Oja- Tuntun	8.8	30.6	1.0×10^5	2.1×10^5	9.0×10^4	1.0×10^3
4	Akerebiata I	8.5	30.5	1.4×10^6	1.4×10^6	3.5×10^5	3.2×10^4
5	Akerebiata II	7.4	30.2	2.7×10^6	1.9×10^6	1.0×10^6	1.0×10^3
6	Akerebiata III	7.9	31.5	1.6×10^7	2.5×10^6	3.2×10^6	1.0×10^3
7	Mandate	8.3	30.7	6.0×10^5	1.9×10^7	1.0×10^3	3.1×10^6
8	Oloje	7.7	31.5	3.4×10^7	1.0×10^8	3.0×10^7	3.0×10^6

S= Soil sample; W= wooden table surface

Table 3: Antibiotic susceptibility patterns of the *Staphylococcus aureus* isolates

Isolates	Sample	Zones of inhibition (mm) Van	Nit	Amp	Caz	Crx	Gen	Cpr	Ofl	Aug	MAR Index
S1	OL*	R	17(S)	R	R	R	R	15(R)	17(I)	10(R)	0.78
S2	OL**	16(S)	R	R	R	R	R	23(S)	20(S)	R	0.67
S3	OL*	15(S)	22(S)	23(R)	R	25(S)	22(S)	25(S)	24(S)	22(S)	0.22
S4	OL**	R	20(S)	25(R)	R	25(S)	22(S)	25(S)	25(S)	10(R)	0.44
S5	OT*	R	R	R	R	R	17(S)	R	R	R	0.89
S6	TG*	R	7(R)	25(R)	R	22(S)	17(S)	22(S)	18(I)	R	0.56
S7	AI*	21(S)	10(R)	22(R)	R	26(S)	15(S)	25(S)	20(I)	12(R)	0.44
S8	AII*	16(S)	10(R)	R	10(R)	R	R	25(S)	22(S)	R	0.67
S9	AIII*	19(S)	22(S)	26(R)	10(R)	22(S)	22(S)	24(S)	24(S)	22(S)	0.22
S10	AJ*	R	19(S)	R	R	R	R	14(R)	16(I)	12(R)	0.78
S11	MA*	R	R	R	R	R	18(S)	R	R	R	0.89

Van = Vancomycin 30µg, Nit = Nitrofurantoin 300µg, Amp = Ampicillin 10µg, Caz = Ceftazidime 30µg, Crx = Cefuroxime 30µg, Gen = Gentamycin 10µg, Cpr = Ciprofloxacin 5µg, Ofl = Ofloxacin 5µg, Aug = Augmentin 30µg, R = Resistant, S = Susceptible, I= Intermediately susceptible, OL = Oloje, OT = Oja-Tuntun, TG = Tipper-Garage, AI = Akerebiata I, AII = Akerebiata II, AIII = Akerebiata III, AJ = Ajanaku, MA = Mandate, * = Soil, ** = Swab

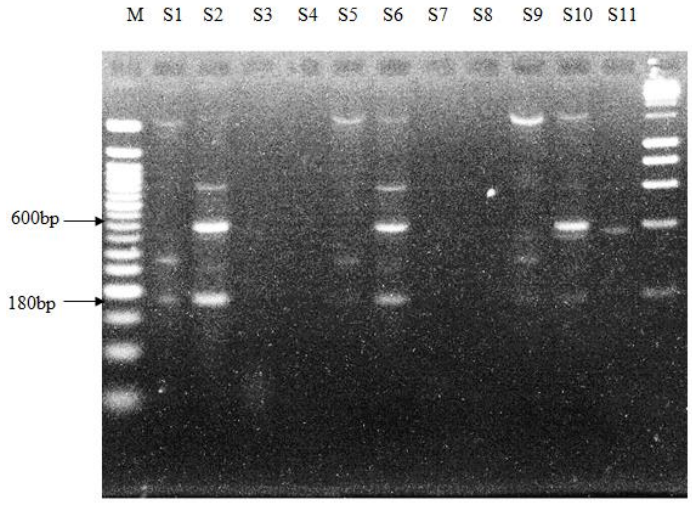


Figure 1: PCR amplification of spa genes of *S. aureus* isolates

S1-S11= *S.aureus* isolates

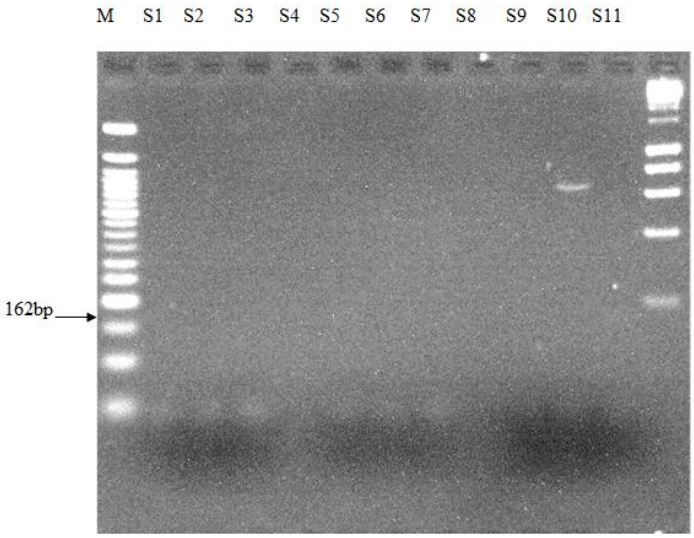


Figure 2: PCR amplification of mecA genes of *S. aureus* isolates

S1-S11= *S. aureus* isolates

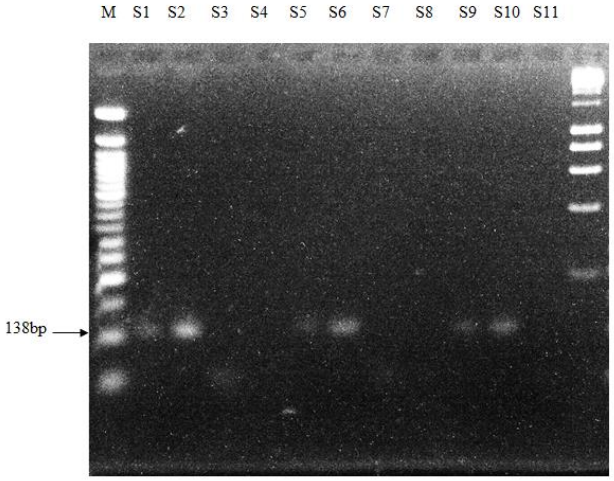


Figure 3: PCR amplification of mecC genes of *S. aureus* isolates

S1-S11= *S. aureus* isolates

4. DISCUSSION

The high heterotrophic bacterial counts obtained in this study is supported by the abattoir soil pH which was between slightly to moderately alkaline (7.3 – 8.8). The soil temperature favoured the growth of bacteria since majority of them are mesophiles (Pelczar *et al*., 2005). The temperature obtained in this study ranged from 30.2 to 32.4ºC. Generally, the *S. aureus* counts were less than the heterotrophic bacterial counts for both the soils and wooden table surfaces.

Only 11 of the 28 tentative yellow colonies on mannitol salt agar displayed the typical *S. aureus* characteristics. They were positive for Gram staining, catalase, oxidase, mannitol fermentation and tube coagulase (Ali, 2016; and Khademi *et al*., 2016).

Isolation of *S. aureus* from the abattoir soils and wooden table surfaces was an indication of the poor level of hygiene at these abattoirs and is of public health concern. Bhandare *et al*. (2007) reported that most of the abattoir workers and meat retailers are untrained and they pay little or no attention to the hygienic standards and contribute immensely to bacterial contamination especially in developing countries like Nigeria. Waste products from the animals such as faeces and urine when not properly disposed increases the occurrence of pathogenic bacteria such as *S. aureus* in the abattoirs (Adeyemi and Adeyemo, 2007). Activities of the meat sellers, butchers and other handlers may lead to contamination of the meat and the abattoirs' environments (Nevry *et al.*, 2011). Wooden meat slabs are commonly used in most of the abattoirs in

Nigeria and could harbour microorganisms due to its absorptive nature (Fasanmi and Sansi, 2008).

The *S. aureus* isolated in this study were all resistant to ceftazidime and ampicillin, followed by augmentin (82%); vancomycin, cefuroxime and nitrofurantoin (55% each); gentamicin and ciprofloxacin (36% each), and ofloxacin (18%). The multiple antibiotic resistance (MAR) index of the isolates ranged from 0.22 to 0.89.

Protein A, spa gene bands were seen in 5 of the isolates used in this study representing 45.5%. All the spa positive isolates produced at least 2 bands. Fatemeh *et al*. (2014) reported that spa genes can produced multiple bands when amplified. Spa typing is based on the sequencing of only a single locus, the polymorphic X region ranging from 200 to 600 bp in size (Khademi *et al*., 2016). Staphylococcal protein A (spa) is one of the virulent factors of *S. aureus* and is useful in molecular epidemiological studies (Ali, 2016). Kareem *et al*. (2020) detected spa genes in 67.2% and 46.15% of their methicillin resistant and methicillin susceptible *S. aureus* isolates respectively. Furthermore, Shakeri *et al*. (2010) had 3.8% of their *S. aureus* lacking spa gene band. Similar to this study, they got *S. aureus* with single and double spa gene bands in their study.

The band for mecA is expected to be seen at 162bp according to Stegger *et al*. (2011); DTU food (2012); and Cikman *et al*. (2019). mecA gene did not amplify for all the isolates used in this study. Using the same primer used in this study, Cikman *et al*. (2019) obtained mecA positivity of 63.8%. Kareem *et al*. (2020) had 82.43% of their *S. aureus* harboured mecA gene. Ayeni *et al*. (2018) using a different primer for mecA in their study detected low level of mecA in their study. Furthermore, Al-Ruwaili (2018) confirmed 21 of their *S. aureus* isolates to be methicillin resistant due to the presence of mecA gene. Bale *et al*. (2019) have reported the detection of mecA genes in *S. aureus* isolated from individuals at Malete, Kwara State, Nigeria.

In this study mecC gene is expected to produce band at 138bp. A single band was produced by each of the isolates. This is the first study that detected mecC gene in *S. aureus* in Nigeria. Six of the isolates possessed mecC gene representing 54.5%. Stegger *et al*. (2011) using the same primer sequence was able to obtain mecC gene amplification in 12 of their isolates. However, using primers different from the one used in this study, Paterson *et al*. (2013) and Loncaric *et al*. (2013) were able to obtain mecC genes in 0.45% and 3.29% of their isolates respectively.

Larsen *et al.* (2013) reported that mecC gene in MRSA can be transferred from animals (cow and sheep) to humans. The consequences of building up antibiotic resistance *S. aureus* isolates in the abattoir soils and wooden table surfaces are that these antibiotic resistant isolates may colonize the human population via contaminated meat and meat products, waste runoff from slaughter houses and even direct contamination from the soil (Gomez *et al*., 2004).

5. CONCLUSION AND RECOMMENDATION

It is concluded that the abattoir soils and wooden table surfaces have been contaminated by *S. aureus* isolates possessing mecC genes as well as the virulent factor, spa genes. Public awareness on the effect of misuse of antimicrobial agents should be encouraged. Abattoir effluent should be properly treated before being discharged into the environment. Indiscriminate use of antibiotics in livestock farming should be discouraged. Livestock feed such as silage, fodder, hay should be prepared and kept under hygienic conditions rather than introducing antibiotics to them in order to suppress microorganisms. Good sanitary conditions should be maintained in the abattoirs at all times in order to avoid contamination of the animals and their meat.

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