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Distribution of resistance genes and resistance profile of methicillin-resistant *Staphylococcus aureus* among animal handlers in Animal Market, Jos

Olukayode Olugbenga Orole [1]✉, Foluke Grace Olawyui [2], and Lillian Yami Adogo [3]

[1]*Department of Microbiology, Federal University of Lafia, Nigeria.*
[2]*Department of Microbiology, Federal College of Animal Health and Production Technology, Nigeria.*
[3]*Department of Biological Sciences, Bingham University, Karu, Nigeria.*

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Abstract: The problems associated with bacteria resistance to antibiotics are increasing despite spirited efforts to overcome the menace and its many attendants' negative implications on human health and the related burden of failure by health institutions. The study characterized and determined the prevalence of resistance genes among animal handlers in livestock markets within Jos, Nigeria. Nose lining secretions and skin surface samples (384) were collected and bacteria were isolated from them. Isolates were characterized for methicillin and multidrug resistance, after which *mecA* and *mecC* presence was determined using molecular method. Seven bacterial species were isolated with the genus *Staphylococcus* having two species had 52.0% prevalence on skin surface and in nasal secretions. Thirty-three *Staphylococcus aureus* isolates were methicillin-resistant, and 37 skin surface and 34 nose secretions isolates were multidrug-resistant. *mecA* genes were present in 18 methicillin-resistant *Staphylococcus aureus* isolates obtained from the skin surface and 12 isolates from the nose lining. The study confirmed the presence of a high number of methicillin-resistant *Staphylococcus aureus* (MRSA) with *mecA* resistance regulatory gene.

Keywords: Antibiotics; Methicillin; *Staphylococcus*; MRSA; *mecA*.

1. Introduction

Drug-resistant bacteria and their associated infection outcomes are a worldwide health concern [1]. *Staphylococcus aureus* is a gram-positive bacterium inhabiting the mucosa linings of the body organs of humans and animals and can translate to being a facultative opportunistic pathogen when the host becomes susceptible. methicillin-resistant *Staphylococcus aureus* (MRSA) causes sickness in humans and animals regardless of age [2-4], and the bacterium on evading the host defenses exerts its virulence by synthesizing toxic metabolites that visit the host unexpectedly with sicknesses [5, 6]. The clones of MRSA in several regions cause hospital-acquired MRSA (HA-MRSA) infections. Until late, there are rare incidences of MRSA causing infections among community members known as community-associated MRSA (CA-MRSA) without prior exposure to healthcare settings [7], and wherever applicable, cases are usually fatal [8]. Some associated symptoms include pneumonia, skin infection, soft-tissue infection, and pulmonary sepsis.

CA-MRSA peculiar to healthy people started to spread in the 1990s and later it was found in animals that were labeled as reservoirs for the bacterium. The pathogen is zoonotic and some strains from animal sources have been reported to possess genes from hospital-acquired MRSA (HA-MRSA) strains [9]. Earlier reports explained that animals in intensive care (ruminants and birds) are colonized by *S. aureus*, especially the livestock-associated MRSA (LA-MRSA) strain [10]. Transmission is by direct contact with carriers or through vectors, while the bacterium can survive outside the host for weeks [11]. Transmission is affected by the type and age of animals, size of herd, intensity, and duration of contact with the carrier, type of vector, number of animals, proximity to animals and animal intensive care system, and animal fecal waste [12-14]. CA-MRSA infections are increasing lately and the causal strains with methicillin-resistant encoding genes have been isolated from hospital settings [15]. These resistant strains carry small SCCmec (iv-v) that confer resistance to non–β-lactam antibiotics [16], while multidrug-resistant HA-MRSA strains carry larger SCCmec types (i-iii) [17]. The spread and transmission of CA-MRSA are promoted by its

Dr. Olukayode Olugbenga Orole
Department of Microbiology,
Federal University of Lafia, Nigeria.
E-mail: olukayode.orole@science.fulafia.edu.ng

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increased fitness, host immune evasion capability, and toxin production.

CA-MRSA and HA-MRSA are two types of acquired infections defined by their genetic characteristics [18]. The conditions and agents promoting CA-MRSA amongst others are intravenous medication use, close contact with infected people, gay people, swarming, ongoing anti-microbial use, and past hospitalization [19]. *mecA*, a conserved gene exclusive to multidrug-resistant strains is an important marker for β-lactam resistant bacteria [20]. Antimicrobial use has not substantially altered the prevalence of MRSA due to the challenge of out-competing other bacteria. The explanation attributed was that antibiotic use imposes selective pressure on MRSA, which encourages its survival. MRSA strains with low fitness costs still out-compete other bacteria even when specific antibiotics have been discontinued in hospital settings [9]. Factors such as the topography of an area set up of health facilities, and population all modulate the prevalence of MRSA [21]. African nations have diverse MRSA prevalence promoted by the level of community and hospital infections and hygienic conditions [22, 23].

Diseases caused by MRSA prolong the length of stay at health institutions thus making patients pay more for treatment, which could trigger newer cases and increase the death rate, which all constitute problems for practically all medical facilities concerning the management of MRSA disease [24, 25]. Illnesses that are caused by MRSA are hard to treat adversely affecting localities and particularly helpless countries where drug accessibility and other essentials are lacking which all culminate to necessitate the study. The study will provide information and up-to-date data on the bacterium prevalence and resistant profile of zoonotic MRSA, aimed at the better management of associated diseases and infections.

The discovery of MRSA transmission from animals to humans has led to the alarming realization that animals not only serve as reservoirs for MRSA but also act as potent carriers, posing a significant threat to human well-being. As one of the most prevalent opportunistic pathogens in humans, the excessive use of antibiotics in livestock production is believed to be the primary factor contributing to the emergence of LA-MRSA. While *Staphylococcus aureus* is typically found in the nasal cavity and skin of humans as part of their normal microflora, it can migrate to the skin and soft tissues, causing infections such as bacteremia, pneumonia, and various skin conditions. The study is aimed at determining the distribution of resistance genes and resistance profile of MRSA among animal handlers in a livestock market in Jos, Nigeria.

2. Experimental

2.1 Study subjects and ethical approval

Investigation of 384 nasal secretions and skin surfaces was carried out from samples collected from participants between January and February 2019. The subjects were community members (traders) with regular contact with animals and birds in the livestock markets in Jos Plateau State, Nigeria. A prevalence of 46.9% based on the methods of Adeiza et al. [26] was adopted to obtain the sample size. The Ethics Committee of the Jos University Teaching Hospital (JUTH) approved the study. Consent of participants aged 18 to 65 years (male and female) with prior antibiotics intake was sought. Community members without a history of prolonged antibiotic intake and those who do not consent were excluded from the study.

2.2 Isolation and identification of bacterial isolates and MRSA

The nose lining and the skin surface were swabbed and inoculated on blood agar medium and incubated at 37°C for 18–24 h. Identification of bacterial isolates was done by marking colonial appearance, hemolysis, pigmentation, and biochemical. MRSA was identified using the disc diffusion technique. Already prepared Mueller Hinton agar was inoculated with test isolates and the antibiotics discs containing 1 µg of oxacillin, 5 µg of cloxacillin, and 30 µg of cefoxitin (Oxoid, UK) were placed on the inoculated plates, allowing for pre-dispersion to take place then kept at 35°C for 18 to 24 h according to the method of Ugwu et al. [23]. The inhibition zones were determined based on the guidelines set by the Clinical and Laboratory Standard Institute (CLSI).

2.3 Determination of the antibiotic profile of isolated *Staphylococcus aureus* strain

The technique of Kirby-Bauer disc diffusion was employed to determine the antibiotic sensitivity of *Staphylococcus aureus* isolated. Isolate's suspension corresponding to 0.5 MCF standard was prepared and inoculated onto the surface of 20 mL sterile molten agar. Paper disks with ceftriaxone (30 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (25 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), and chloramphenicol (30 µg) were carefully placed over the surface of the agar and left to incubate for 24 h at 37°C. The inhibition zones are recorded accordingly. Minimum inhibitory concentrations of gentamicin, oxacillin sodium salt, and vancomycin hydrochloride (Oxoid UK) were determined against the isolates from a stock (128 µg/mL) of the antibacterial drug. The MIC for each isolate was taken to be the concentration at which no growth was visible.

2.4 DNA extraction and analysis

The boiling method described by Goering et al. [27] was adopted for the extraction of DNA from bacterial cells. Suspected *Staphylococcus aureus* isolates were confirmed using primers with amplicon size 270 bp, F (5′-GCGATTGATGGTGATACGGTT-3′) and R (5′-

AGCCAAGCCTTGACGAACTAAAGC-3′), which amplifies the *mecC* gene with the PCR. The gene encodes a nuclease enzyme specific to S. aureus. For the MRSA cells, *mecA*-P1-5'- TCCAGATTACAACTTCACCAGG-3'0 and *mecA*-P2-5'- CCACTTCATATCTTGTAACG-3' [162bs] and *mecC*-P1-5'- GAAAAAAAGGCTTAGAACGCCTC-3 and *mecC2*-P2-5'- GAAGATCTTTTCCGTTTTCAGC-3' [138bs] were employed as primers for the target genes.

Cells were cultivated in 200 µL of PBS, vortexed, and spurned for 1 min at 13,000 rpm. 200 µL of 10% chelex suspension was added, incubated for 15 min at 57°C, centrifuged once more for 10 s, and then incubated at 100°C for 8 min. The supernatant was then discarded. The mixture was once more centrifuged for 3 min at 13000 rpm before being frozen at -80°C. The deoxynucleotide triphosphates (dATP, dCTP, dGTP, and dTTP) are each 0.25 mM in the PCR mixture of 25 mL with 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, and 1 U of Taq DNA polymerase (Promega Corporation, USA). The cycling profile used to amplify the DNA was an inertial denaturation at 95°C for 10 min and a 35x cycle of amplification (denaturation: 95°C for 45 s, annealing; 55°C for 45 s, extension: 72°C for 1 min, and final extension: 72°C for 10 min) in a thermal cycler (Mastercycler gradient, Eppendorf AG, Germany). On a 1.5% agarose gel, the amplification product was examined using 0.53 tris-borate-EDTA buffer using a molecular size marker of 100-bp DNA ladder (Promega Corporation). UV imaging was done after the gels were stained with 1% ethidium bromide.

3. Results & Discussion

3.1 Bacterial species isolated from patients with infections

Bacteria from the genera *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, and *Pseudomonas* were the isolated microorganisms from the participants (Figure 1). *Staphylococcus aureus* had the highest percentage from the skin surface and the nose lining with 45.35% and 36.59% prevalence respectively, while *Streptococcus pyogenes* was the least isolated (2.44%) in the nose lining (Supplementary Table 1).

3.2 Antibiotic susceptibility test of *Staphylococcus aureus* isolates

The antibiotic susceptibility test showed that oxacillin was the most resisted antibiotic with 34 (43.59%) *Staphylococcus aureus* isolates resisting it among the skin surface isolates, while norfloxacin and amoxicillin with 60.00% were the most resisted by the isolates in the nose lining isolates (Table 1). All tested antibiotics were at varying percentages resisted by the *Staphylococcus aureus* isolates with norfloxacin being the most resisted by 58 (47.15%) isolates.

3.3 Multidrug-resistant *Staphylococcus aureus* and MAR index of isolates

Forty-one isolates of *Staphylococcus aureus* from the skin surface and 11 isolates from the nose lining were not multidrug-resistant. Other isolates mentioned in Table 2, were all resistant to more than two classes of antibiotics. Isolates obtained from the nose lining had 10 of them resisting all the 10 antibiotics tested in the study (Table 2).

3.4 Distribution of MRSA and *mecA* resistance gene in the isolates

Thirteen (28.9%) of the isolates were methicillin-resistant from the nose lining, while 19 (24.4%) from the skin surface were resistant to methicillin (p-value = 0.004) as shown in Figure 2. *mecA* gene was found in 12 isolates (N43, N45, N47, N50, N53, N66, N70, N71, N86, N76, N79, N84) from the nose lining and 18 isolates (S3-S5, S7, S9, S10, S13, S14, S22, S23, S25, S31, S35-S38, S40, S41) from the skin surface. *mecC* gene was not present in any of the analyzed isolates in the study.

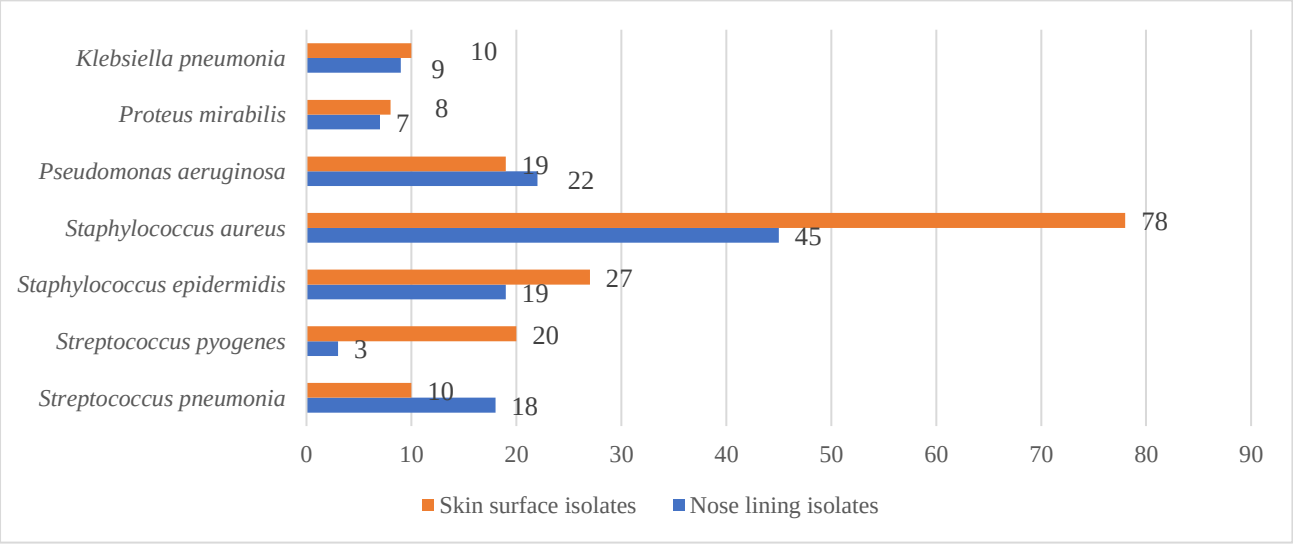

Figure 1. Prevalence of isolated bacterial species from consenting participants.

Table 1. Antibiotic resistance of isolated *Staphylococcus aureus* in different seasons.

Antibiotics	Skin surface isolates N=78 (%)	Nose lining isolates N=45 (%)	Total N=123 (%)
Oxacillin (1µg)	34 (43.59)	15 (33.33)	49 (39.84)
Ciprofloxacin (5µg)	14 (17.95)	12 (26.67)	26 (21.14)
Norfloxacin (10mcg)	31 (39.74)	27 (60.00)	58 (47.15)
Chloramphenicol (30mcg)	23 (29.49)	20 (44.44)	43 (34.96)
Erythromycin (30mcg)	20 (25.64)	16 (35.56)	36 (29.27)
Gentamycin (10mcg)	14 (17.95)	14 (31.11)	28 (22.76)
Amoxicillin (20mcg)	19 (24.36)	27 (60.00)	46 (37.40)
Cefoxitin (30µg)	19 (24.36)	13 (28.89)	33 (26.83)
Ampliclox (20mcg)	21 (26.92)	14 (31.11)	34 (27.64)
Augmentin (30mcg)	26 (33.33)	19 (42.22)	(36.59)

Table 2. Antibiotic resistance profile and MAR index of resistant *Staphylococcus aureus*.

MAR Index	Skin surface-resistant isolates	Nose lining-resistant isolates
0.30 – 0.39	S2, S12, S18, S26, S28, S30, S33, S38, S39, S40	N46, N50, N53, N56, N57, N61, N63, N75, N77, N78, N88
0.40 – 0.49	S31	-
0.50 – 0.59	S1, S5, S7, S8, S11, S16, S17, S25, S27, S37	N49, N58, N62, N65, N81, N83, N86, N90
0.60 – 0.69	S4, S6, S10, S19, S20, S21, S36	N43, N54, N76,
0.70 – 0.79	S15, S24	-
0.80 – 0.89	S3, S13, S14, S22	N84, N89
1.00	S9, S23, S35	N45, N47, N48, N51, N52, N66, N68, N70, N71, N74

Bacterial species isolated in the study are pathogenic to humans and responsible for diseases such as bacteremia, bacteruia, pneumonia, urinary-associated diseases, and nosocomial infections [28]. Poverty and unhygienic conditions are contributors to the incidence recorded in the study [29]. This presents a danger to the well-being of healthy individuals in the communities where such people and infected marketers are residents. The high incidence of pathogenic bacteria reported in the study emanated from the occupational hazard faced by the participants in caring for or trading with animals. The bacterial strains have been reported to cause invasive blood, bones, and joint infections among animal handlers [30]. MRSA colonization was higher in the study due to the contact the people had with livestock animals. The finding agreed with the earlier report by Pirolo et al. [30]. Staphylococcus aureus was the most common isolate in the study. The prevalence calls for concern as its presence is associated with the poor hygienic condition of the market. The prevalence obtained in this study agrees with Bukhari et al. [31] (41.9%) and Askari et al. [32] (52.7%) but contradicted the prevalence of 63.2% by Shahkarami et al. [33] and 80.5% by Mohajeri et al. [34] The disparity in values recorded by the authors might be as a result of the different methods employed in collating prevalence values. Other parameters, that could adversely affect the result obtained, include the use of different antibiotics and marketing management methods.

The other antibiotics tested presented lower outcomes. Resistance reported by the bacterial isolates was due to some of the factors of drug abuse, poverty, ignorance, and wrong prescription by physicians. Isolates from the skin surface and nose lining were multidrug-resistant (MDR), with similar resistance profiles to the antibiotics tested. It has been proposed that these resistant strains can evolve

into more pathogenic strains because their virulence genes are located on the mobile genetic element [35]. Obtained MRSA strains from the skin surface were more resistant to antibiotics compared to those from the nose linings which might be attributed to the size of the Staphylococcal Cassette Chromosome [36]. Resistance by isolates to all classes of antibiotics reported in this study raises the alarm for serious attention, implying that no antibiotic is effective against the pathogens in the sampling communities. Concern for the high prevalence of MRSA reported is a reason for worry as resistance drives mortality and dismalness in old patients, and individuals with organ dysfunction combined with a high associated monetary burden [37, 38]. In contrast to the findings of Kateete et al. [18] who got 5.7% (42/742) MRSA from which 95.2% (40/42) had multidrug-resistant activities, our analysis revealed a larger frequency of MRSA cases compared to isolates with multidrug-resistant activity. More isolates from the skin surface were resistant compared to those from the nasal secretion.

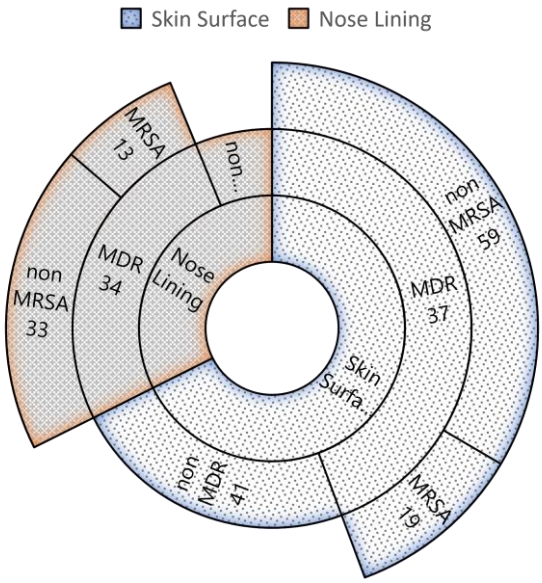

Figure 2. Distribution of methicillin-resistant S. aureus and resistance genes among isolates.

The *mecA* genes in MRSA isolates encode transpeptidase PB2a, which confers resistance to the bacterium [33]. While other genes harbored by Staphylococcus aureus can confer resistance against macrolide, aminoglycoside, and penicillin, and they are located in plasmids and transposon, *mecA* that is located inside SCCmec and acquired through horizontal gene transfer [7]. The MRSA isolates can inhibit the activity of antibodies by evasion through the production of an antiphagocytic zwitterionic capsule that prevents opsonization. The detection of a *mecA* in the study is not surprising because the gene that encodes methicillin resistance is peculiar to MRSA clonal lineages circulating in hospitals, communities, and animals that are affected by such risk factors as industrialization, geographical area, host diversity, and water supply [39]. The presence of the *mecA* gene in the isolates confirmed that they were MRSA isolates [40]. The study determined the prevalence of *mecA* in nasal mucosa and skin to be over 90%, indicating a high *mecA* gene burden in the study area. This may be the reason for the high resistance index of the isolates. Other MRSA strains lacking *mecA* and *mecC* but with high MDRI levels display resistance utilizing mechanisms based on overproduction of β-lactamases and specific changes in various amino acids in the protein-binding protein cascade [40].

The results of this study are consistent with previous research conducted by Khairalla et al. [41]and Cikman et al. [42]. These authors, through their investigations in Egypt and Turkey, also observed the absence of the *mecC* gene in MRSA strains collected from individuals across various regions. LA-MRSA in pigs and cattle is the source of the *mecC* gene, which is responsible for infections in both humans and animals. Although this gene is typically associated with specific hosts, there have been instances where it has crossed over to other animal species and strains, leading to infections in humans as well [39]. The lack of *mecC* genes in the study area indicates that the presence of CA-MRSA carrying the *mecC* gene is not widespread. These results support previous research demonstrating that LA-MRSA (*mecC* positive) is specific to certain regions, thereby reducing the likelihood of zoonotic transmission to traders and community members. It is worth noting that our findings contradict the results of a study conducted in Malaysia [43], which reported a high percentage of *mecC*-positive LA-MRSA strains. According to Bietrix et al. [44], *mecC*-positive strains have been found to readily spread in environmental samples and maintain their presence for an extended duration following introduction to a new location. Research findings suggest that the colonization of MRSA in cattle could pose an occupational risk to local communities.

4. Conclusion

The investigation verified the existence of *mecA*-positive, methicillin- and other antibiotic-resistant virulent MRSA. The study's findings indicated that the settings serve as reservoirs for the regulatory gene *mecA*, peculiar to MRSA associated with community and hospital-acquired infections. The study also emphasizes the issue of microbial resistance at the heart of our healthcare facilities, where people are expected to receive treatments for a range of illnesses. According to the study, there is a very high prevalence of MRSA. Since the hospital environment is a crucial component of the community, a concerted effort must be made to improve personal and community hygiene, significantly lowering patient carriage. The public should be made aware of the possibility of developing any of the diseases linked to methicillin-resistant Staphylococcus aureus to reduce the risk of MRSA infections, which are avoidable.

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Declarations

Author Contribution: OOO designed the research, analyzed samples, and compiled the first draft of the manuscript, FGO did a sample collection, carried out analysis of samples, and read the draft, while LYA performed statistical analysis of the results obtained.

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Conflict of Interest: The authors declare that there is no conflict of interest among them.

Ethical Approval: Obtained approval is included in supplementary materials.

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