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Isolation and Antibiotic Resistance of *Staphylococcus aureus* Isolated from Nosocomial Sources

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Methicillin resistance *Staphylococcus aureus* have been reported worldwide to emerge mostly in developing and developed countries. This study aimed at isolated and antibiotic resistance from nosocomial sources in Dalhatu Araf specialist Hospital, Lafia, Nigeria. A total of (200) samples were collected from February 2021 to May 2021 from different Nosocomial sources such as door handles, seat handles, surgical equipment and stretchers and *Staphylococcus aureus* was isolated and identified using standard microbiological method. The Antibiotic susceptibility test for the isolates were carried out and interpreted in accordance with Clinical and Laboratory Standard

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Institute (CLSI) protocol. The occurrence of *Staphylococcus aureus* from the samples was 50 (25%). The highest occurrence of Staphylococcus aureus is from seat handle swab with (32%) and the lowest occurrence is (18%) from surgical equipments. The Antibiotic resistance of *Staphylococcus aureus* showed that the isolates were more resistant to oxacillin.

Keywords: Staphylococcus aureus; antibiotics; nosocomial infection.

1. INTRODUCTION

"Staphylococci are Gram-positive cocci, usually commensal organisms that are found occurring on the skin and mucosa of humans and animals. *Staphylococcus aureus* (*S. aureus*) is an important pathogen of clinical significance, causing variety of illnesses in both humans and animals worldwide" (Chakraborty et al., 2011). "It causes superficial skin infections and life-threatening diseases including endocarditis, sepsis and soft tissues, urinary tract, respiratory, intestinal tract, and bloodstream infections" (Rallapalli et al., 2008). "Close association has been observed to enhance spread of staphylococcal strains among livestock and veterinary care-givers and animal handlers through contact or aerosol" (Ajuwape et al., 2001). "*S. aureus* is a major food borne pathogen due to its production of enterotoxins that cause serious intoxications" (Wu et al., 2011; Liu et al.,2014). "Fast identification of *S. aureus* and its toxins in food is crucial to determine microbial risk and assure food quality. A variety of selective or differential culture media have been used to isolate and identify the organism" (Thakar et al., 2013). "The use of culture media for *S. aureus* isolation in combination with coagulase activity and haemolysis determination as secondary tests have improved the accuracy of identification, and was in consonance with gene sequence analysis compared with the use of the culture media alone" (Trujillo et al., 2013). "The management of *S. aureus* infections especially methicillin resistant ones is often difficult because methicillin resistant *S. aureus* (MRSA) is usually resistant to multiple antibiotics" [1-5]. "Vancomycin is commonly used to treat such infections and occasionally, Macrolide-Lincosamide Streptogramin B (MLS_B) family of antibiotics are used as substitute" (Adhikari et al., 2017). "Due to the rising incidences of methicillin resistance, glycopeptides such as vancomycin have been recommended as therapeutic agents for serious staphylococcal infections" (Nunes et al., 2006). "However, the extensive use of glycopeptides has decreased the susceptibility of staphylococcal species to these agents" [6-9]. "Inducible vancomycin resistance is due to a sophisticated mechanism that combines synthesis of cell wall peptidoglycan precursors with low affinity for glycopeptides and elimination of the normal target precursors" (Foucault *et al*., 2010). "*Staphylococcus aureus* develops resistance to antimicrobials by employing different mechanisms" [10-15]. "These mechanisms include limiting uptake of the drug, modification of the drug target, enzymatic inactivation of the drug, and active efflux of the drug" [16-19]. "The bacteria may use one or several of these mechanisms depending on the antimicrobial. In particular, the localization of resistance genes on transferable genetic elements such as plasmids and transposons facilitates horizontal transfer of resistance between bacteria" (Van Hoek et al., 2011). "The development of such resistance does not cause the organism to be more intrinsically virulent than strains of *Staphylococcus aureus* that have no antibiotic resistance, but resistance does make MRSA infections more difficult to treat with standard types of antibiotics and thus more dangerous" (Jenson and Lyon, 2009). "Methicillin-resistant *Staphylococcus aureus* (MRSA) is a gram-positive bacterium that is resistant to methicillin (a member of the penicillin family) and many other beta-lactam antimicrobials (beta-lactam antimicrobials include penicillins and cephalosporins), and are resistant to macrolides and aminoglycosides [20-29]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an isolate of *Staphylococcus aureus* which has acquired genes encoding antibiotic resistance to all penicillins including methicillin [30-33]. This resistance is mediated by an altered penicillin binding protein (PBP2a) which is encoded by the MecA gene that is carried on a large mobile genetic element, the staphylococcal cassette chromosome (Palavecino, 2007; Ahmed et al., 2012).

"In Africa, countries show different MRSA prevalence" (Bell and Turnidge, 2002). "MRSA is one of the major causes of infections in humans, occurring in both the community and the hospital" (Ugwu et al., 2016). Akerele et al. (2016) reported that "acquisition of MRSA has been associated with two different environments; Community-associated MRSA (CA-MRSA) and

healthcare-associated MRSA (HA-MRSA)". "They are usually differentiated by their structural and functional genomic traits" (Otto, 2013). MRSA infections in the community can also be caused by livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA). It is initially associated with livestock (Lewis et al., 2008; Layer et al., 2012), and differs from genotypic HA-MRSA and genotypic CA-MRSA in its genomic traits. "The risk factors for community acquired infection include intravenous drug use, close contact with persons who have MRSA, men who have sex with men, crowding, poor hygiene, recent antibiotic use, and previous hospitalization" (Moran et al., 2006; King et al., 2006; Hota et al., 2007; Boucher & Corey, 2008),

2. MATERIALS AND METHODS

2.1 Study area

This study was carried out at Dalhatu Araf Specialist Hospital (DASH) in Lafia, Nasarawa State, Nigeria. DASH was established in 2003 by the Nasarawa State Government to cater for the health needs of the people of the state at the tertiary level.

2.2 Sample Size Determination

A prevalence of 16% was used. This is based on studies carried out in Northern Nigeria (Okon et al., 2011; 2014). The sample was then determined using the formula;

$$n = \frac{pqz^2}{d^2}$$

n= minimum sample size required
p = proportion of the target population estimated to have particular problem
q = 1 – p
z = level of precision (1.96) which corresponds to 95 % confidence level
d = degree of accuracy desired set at 0.05

$$n = \frac{0.16\,(0.84)(1.96^2)}{0.05^2} = 200$$

2.3 Sample Collection

A total of two hundred (200) samples were collected from different nosocomial sources such as catheters, bed handles, door handles, dishes, forceps and toilet seats within the hospital. Aseptic procedures was used for the collection, surfaces was swabbed using sterile cotton swabs immersed in normal saline solution.

2.4 Isolation and Identification of *Staphylococcus aureus*

The swab-sticks containing the specimen was inoculated in nutrient broth and incubated at 37ºC for 18 h. It was then sub-cultured on Mannitol Salt Agar (MSA) by streaking, then incubated at 37ºC for 24 h, and the cultural characteristics of colonies on the MSA was observed. Golden-yellow colonies were indicative of *S. aureus* (Owaku et al., 2017).Presumptive *S.aureus* was identified by microscopy (Gram staining), biochemical tests and commercial kit identification.

2.5 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the isolates was carried out using Kirby-Baeur disk diffusion method as described in Clinical and Laboratory Standards Institute (CLSI) Guidelines [34].

3. RESULTS

The cultural, morphological and biochemical characteristics of *S.aureus* isolated from fomites are as shown in Table 1.

The occurrence of *S. aureus* in the fomites is as shown in Table 2. From the 200 samples, 50 (20.0%) *S. aureus* were isolated, with the highest occurrence (32.0%) from swabs taken from seat handles and the lowest (18.0%) from surgical equipment.

Streptomycin had 80% which was the highest, Penicillin had 76%, Rifampicin had 72%, Ampicilcin/sulbactam had 64%, Clindamycin had 64%, Oxacillin had 62%, Ceftriaxone had 58%, Vancomycin had 28%, Levofloxacin had 26% and Gentamycin had 22% which is the lowest as shown in Table 3.

4. DISCUSSION

Staphylococcus aureus has been identified as one of the most common pathogens associated with both hospital and community-acquired infections worldwide, with various infections that are devastating and life-threatening (Peterson et al., 2013; Yaw et al., 2014). "The propensity for staphylococci to develop antimicrobial resistance is a cause for great concern in both human medicine" [35]. Antimicrobial resistance was high-lightened as an urgency issue [36].

Table 1. Cultural, morphological and biochemical characteristics of *Staphylococcus aureus*

Cultural characteristics	Morphological characteristics		Biochemical Characteristics														Inference
	Gram stain	Morphology	Cat	Coa	Vp	Akp	ONPG	Ur	Arg	Man	Su	Lac	Ar	Rf	Tr	Mal	
Golden yellow colonies on MSA	+	Cocci in cluster	+	+	+	+	-	+w	+w	+	+	+	-	-	+	+	*S. aureus*

MSA= Mannitol Salt Agar; Cat= Catalase; Coa= Caogulase; Vp= Voges-Proskauer; Akp= Alkaline phosphatase; ONPG= Ortho-nitrophenyl-β-galactoside; Ur= Urease; Arg= Arginine Utilization; Man= Mannitol; Su= Sucrose; Lac= Lactose; Ar= Arabinose; Rf= Raffinose; Tr= Trehalose; Mal= Maltose; + =Positive; +w= Positive to weak reaction; − =Negative

Table 2. Isolation rates of *Staphylococcus aureus* in relation to the fomites

Source	No. of samples	No. (%) of *S. Aureus*
Door handles	50	12 (24.0)
Seat handles	50	16 (32.0)
Surgical equipment	50	9 (18.0)
Stretchers	50	13 (26.0)
Total	200	50 (25.0)

Table 3. Antibiotic resistance profile of *Staphylococcus aureus* isolated from fomites

Antibiotics	Disc content (µg)	No. (%) of resistance (n=50)
Rifampicin	5	26 (72.0)
Clindamycin	2	22 (64.0)
Vancomycin	15	14 (28.0)
Levofloxacin	5	13 (26.0)
Oxacillin	1	31 (62.0)
Ceftriaxone	30	29 (58.0)
Ampicilcin/sulbactam	25	32 (64.0)
Streptomycin	25	40 (80.0)
Gentamycin	30	11 (22.0
Penicillin	5	38 (76.0)

Decades now, multidrug resistance in *S. aureus* has spread throughout the world, evident with many studies across the world. However, the occurrence rate of multidrug resistant *Staphylococcus aureus* infections can vary from country to country and between hospitals; and it also varies between different units of the same hospital and also varies in prevalence depending on geographical area and the socio-demographic characteristics of the populations [37].

From this study, we observed that the occurrence of *S. aureus* isolated from selected fomites was 20.0% and less than 33.6% reported by Onanuga and Awhowho (2016) isolated from fomites in Yenagoa. The occurrence of *S. aureus* isolates from some formite samples in this study were observed to be higher 32.0%, 26.0% and 24.0% from Seat handles, Stretchers and door handles swabs respectively than 1.8%, 21.1%, 0.9% for the different specimens respectively as reported by Oyepola et al. [38] from fomites in Southwest Nigeria. The isolation of *S. aureus* from the fomite samples of door handles, stretchers and seat handles swabs of fomites suggested that the organism may likely be responsible for most hospital acquired infections, since *S. aureus* has been reported as one of the bacteria associated with hospital infections [39]. However, the occurrence of *S. aureus* 20.0% from fomite swabs was in close agreement with 22.1% from studies reported by Oyepola et al. [38],[40-42] and also in agreement that *S. aureus* is also a pathogen associated with wounds infections [39].

The antimicrobial susceptibility testing of the isolates was carried out using Kirby-Bauer disk diffusion method as described in Clinical and Laboratory Standards Institute (CLSI) Guidelines [34,43-47]. The following antibiotic resistance was recorded; Streptomycin had 80% which was the highest, Penicillin had 76%, Rifampicin had 72%, Ampicilcin/sulbactam had 64%, Clindamycin had 64%, Oxacillin had 62%, Ceftriaxone had 58%, Vancomycin had 28%**,** Levofloxacin had 26% and Gentamycin had 22% which is the lowest.

5. CONCLUSION

The occurrence of *S. aureus* isolates in the selected fomites in this study was high. The *S.aureus* isolates were more resistant to antibiotics such as streptomycin, clindamycin, rifampicin, oxacillin, ceftriaxone, penicillin and ampicillin/ sulbactam, but less resistant to antibiotics such as levofloxacin and gentamicin. These antibiotics of higher susceptibility will be useful in treatment of infections caused by *S. aureus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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