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Prevalence and Antibiotic Resistance of *Staphylococcus Aureus* Isolated from Hospital Environments Located in Benin City, Edo State, Nigeria

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ABSTRACT

Methicillin-resistant Staphylococcus aureusand Methicillin-sensitive Staphylococcus aureus are prevalentin hospitalized patients as well as tertiary healthcare facilities globally. In this study, we investigated the prevalence and antibiotic susceptibility of Staphylococcus aureus isolated from 3 healthcare facilities located in Benin City, Edo State, Nigeria. The isolation of Staphylococcus aureus, their antibiotic susceptibility pattern and distribution of pathogenic and toxigenic genes by Polymerase chain reaction were carried out by established methods. The total heterotrophic and staphylococcal counts ranged from 163.33 × 10^3 ± 12.1 to 300 × 10^3 ± 0.00 cfu and 0.00 × 10^3 ± 0 cfu respectively for hospital 1; 1.0 × 10^3 ± 01 to 195.5 × 10^3 ± 14.7 cfu and 0.00 × 10^3 ± 0.0 to 49 × 10^3 ± 6.9 cfu for hospital 2; 2.0 × 10^3 to 300 × 10^3 cfu and 0.0 × 10^3 to 26 × 10^3 cfu for hospital 3. All isolates were further observed to be methicillin-sensitive, with varying degree of resistance and susceptibility to 10 commonly used antibiotics. Finally, none of the MSSA strains possessed the mecA, entA and SCCmec1 genes. The result of this study indicated that MSSA is distributed in tertiary healthcare facilities located in Benin City, Edo State, Nigeria, and effort should be made at improving the sanitary conditions in these hospitals, to avoid outbreaks of MSSA infections.

Keywords: *Methicillin, Antibiotics susceptibility, Infectious diseases, Public Health, Hospital*

INTRODUCTION

Staphylococcus aureus is a well-known human pathogen,and continues to represent a significant public health challenge globally. It is a Gram positive coccus commonly found colonizing the skin and nasal cavities of healthy individuals [1]. *S. aureus* is known to produce a variety of virulence factors that are responsible for specific acute staphylococcal toxemia syndromes, septic shock, infective endocarditis, arthritis, and necrotizing pneumonia [2]. The prevalence of *S. aureus* is a major challenge in health institutions and healthcare facilities, especially with the emergence of methicillin-resistant strains of *S. aureus* (MRSA) MRSA is known to have evolved from methicillin-susceptible *S. aureus* (MSSA), and its evolution is attributed to it acquiring the staphylococcal chromosome cassette. Since its emergence, MRSA has consistently evolved resistance mechanisms, leading to the emergence of hospital acquired multi-resistant clones. The majority of these clones are reported as a major cause of nosocomial infections globally[3].

The prevalence and molecular characterization of hospital-acquired MRSA and hospital-acquired MSSA have previously been reported[4]. In Africa, a number of pandemic MRSA clones have been identified with some clones limited to specific countries or regions [5]. However, there are limited data on the prevalence of hospital acquired MRSA and MSSA in Nigerian hospital environment. The current study was therefore aimed at investigating the prevalence of MRSA and MSSA in tertiary healthcare facilities located in Benin City, Edo State, Nigeria, their antibiotic susceptibility pattern and the distribution of toxigenic and pathogenic genes.

MATERIALS AND METHODS

Sample Collection

A total of 30 samples from 3 different hospitals all located in Benin City, Edo State, Nigeria

were collected and used for this study. The samples were collected using sterile swab sticks, pre-immersed in 2ml of normal saline. The samples were collected in a bio-hazard bag and transported to the lab in cold pack for immediate analysis.

Microbiological Analysis

Three-fold serial dilutions were done for each of the collected samples. 1ml of the third-dilution was cultured on the appropriate medium by the spread plate method in triplicates. All microbiological analysis was done within one hour following collection. The cultured plates were incubated at 37^0 C for 24hrs.

Total Heterotrophic and Staphylococcal counts

One millimeter (1ml) of each sample was collected using a sterile pipette and diluted into 9ml of sterile distilled water in a test tube. Three further dilutions were made, and 1ml each of the third dilution was cultured on Nutrient agar (Titan Biotech Limited, India) and mannitol salt agar respectively, in triplicates. All plates were then incubated at 37^0 C for 24hrs. The total number of colonies was counted from each plate using a colony counter (Labtech, England).

Subculturing of Staphylococcus aureus *Isolates*

Discrete colonies characteristic of yellowish colour indicative of Mannitol fermentation were picked from Mannitol Salt Agar plates using a sterile wire loop, and streaked on Nutrient Agar plates, following which the plates were incubated at 37^0 C for 24hrs. Pure isolates were kept on a slant at 4^0 C for further studies. Only one Staphylococcus aureus was picked per sample.

Presumptive Identification of *Staphylococcus aureus*

Bacterial isolates with the characteristic yellowish colour on Mannitol salt agar were confirmed as *Staphylococcus aureus* by their Gram reaction, catalase test, coagulase test and citrate test as described by Fooladi et al.[6].

Phenotypic identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA)

Organisms presumptively identified as *Staphylococcus aureus* from the biochemical test were further screened for their resistance and/or sensitivity to the antibiotics methicillin as described by Fooladi et al. [6]. Briefly, mannitol salt agar (1L) was supplemented with 0.4g of oxacillin and the isolates introduced to the medium before being incubated at 37^0 C for 24hrs. Growth of *Staphylococcus aureus* on the medium (Mannitol-oxacillin medium) was indicative of Methicillin-resistance while inhibition of *Staphylococcus aureus* growth indicated Methicillin-sensitive strains of *Staphylococcus aureus*.

Antibiotic Susceptibility Test

Selected *Staphylococcus aureus* strains isolated (MRSA and/or MSSA) were tested for their resistance and sensitivity against 10 different antibiotics, which included; Septrin (10µg), Pefloxacin (10µg), Gentamicin (30µg), Ampiclox (20µg), Zinnacef (10µg), Amoxacillin (10µg), Rocephin (25µg), Ciprofloxacin (10µg), Streptomycin (30µg), and Erythromycin (10µg) as described by the Clinical Laboratory Science Institute [7]. ≤12mm were considered resistant, 12mm to 17mm were considered intermediate while ≥18mm were considered sensitive.

DNA Extraction

Pure DNA was extracted from all *Staphylococcus aureus* (MRSA and/or MSSA) isolates using the commercial DNA extraction kit (ZR Fungal/Bactrerial DNA MiniPrep™, Zymo Research Corporation, USA). The DNA extraction was carried out according to the manufacturer's instruction. Pure DNA was kept at 4^0 C before use.

Gene Amplification using Polymerase Chain Reaction

Polymerase chain reaction (PCR) was used to amplify target genes in the templates DNA using primers (forward and reverse) specific for each gene (mecA, entA and SCCmec1). The PCR conditions were done as described by Fooladi et al. [6]. Each reaction contained 0.5µl of the forward primers, 0.5µl of reverse primers, template DNA, 1.5µl, 12.5µl of One Taq Quick-load Purple (New England Biolab, UK). The volumes were then made up to 25µl using nuclease-free water (AMRESCO, USA). The PCR tubes were loaded into the PCR machine using an initial denaturation temperature of 94^0 C for 5min, a denaturation step of 1min at 94^0 C, an annealing step of 2min, with varying temperature depending on the gene of interest (Table 1), extension step of 1 min at 72^0 C and a final extension step of 5min at 72^0 C. The denaturation, annealing and extension were done over 35 cycles, while the holding temperature for all PCR runs were 4^0 C. The primers used, targeted gene and their base pair as well as the annealing temperatures are presented in Table 1.

Table1. *Table showing the primers used, their annealing temperature and product size*

Gene name	Forward sequence	Reverse sequence	AT (°C)	PS (bp)
EntA	TTGGAACGGTTAAAACGAA	GAACCTTCCCATCAAAAACA	50	121
mecA	TGAGTTGAACCTGGTGAAGTT	TGGTATGTGGAAGTTAGATTGG	57	857
SCCmec 1	TTTAGGAGGTAATCTCCTTGATG	TTTTGCGTTTGCATCTCTACC	52	154

Key: AT: *Annealing Temperature; PS: Product Size; bp: Base Pair*

Gel Electrophoresis

Gel electrophoresis was used to separate DNA fragments following PCR, using 1.5% agarose in 100ml of 1×TAE buffer. The agarose was stained with 3µl of ethidium bromide and poured into the gel container using 10 wells. 10µl of each PCR product mixed with loading dye (New England Biolab, UK) was lowered into each wells with the DNA ladder (100-1000bp) being added into the first well. The Amplified DNA was kept in the electrophoretic tank for 30-45min at 100Amp. Following electrophoresis, gels were viewed using a UV transilluminator.

Results And Discussion

The prevalence of *S. aureus* in healthcare facilities have been reported quite extensively especially in developed countries. However, many of the studies in Nigeria rarely distinguish the *S. aureus* isolates into MRSA and MSSA, as well as investigate the presence of pathogenic and toxigenic genes. The current study was therefore aimed at providing the existing gap in current knowledge in Nigeria.

The result of the current study shows that *Staphylococcus aureus* and other bacteria are present in hospital environment in Benin City, Edo State, Nigeria. The total heterotrophic counts reported ranged from 163.3 ± 12.1 to 300.0 ± 0.0cfu in hospital 1, 1.0 ± 0.0 to 195.5 ± 14.7cfu in hospital 2 and 2.0 ± 0.0 to 300.0 ± 0.0cfu in hospital 3, while the total staphylococcal counts ranged from 0.0 ± 0.0 to 0.0 ± 0.0cfu, 0.0 ± 0.0 to 49.0 ± 6.9cfu and 0.0 ± 0.0 to 26.0 ± 1.2cfu in hospital 1, hospital 2 and hospital 3 respectively (Table 2). The sampling sites in the 3 hospitals and the number of isolates are presented in supplementary Tables 1, 2 and 3.

Table2. *Mean heterotrophic and mean staphylococcal counts obtained from the different hospitals*

Sample	THC (X 10^3)		TSAC (X10^3)	
	Minimum	Maximum	Minimum	Maximum
Hospital 1	163.3 ± 12.1	300.0 ± 0.0*	0.0 ± 0.0	0.0 ± 0.0^a
Hospital 2	1.0 ± 0.0	195.5 ± 14.7$^{\#}$	0.0 ± 0.0	49.0 ± 6.9^b
Hospital 3	2.0 ± 0.0	300.0 ± 0.0$^{\#}$	0.0 ± 0.0	26.0 ± 1.2^c
			P = 0.018	

Key: THC: *Total Heterotrophic Counts; TSAC: Total Staphylococcal Counts Different symbols signify significant difference*

The presence of these isolate are particularly worrisome, owing to their potential to cause pathogenic and nosocomial diseases. No *S. aureus* was reported in any of the samples examined in hospital 1, and the absence of *S. aureus* could be attributed to proper disinfection of surfaces prior to sample collection.Although, scientific literature on the removal of *S. aureus* from surfaces by disinfectants are generally lacking [8], recently reported the prevention and removal of biofilm formation of *S. aureus* strains from raw milk samples by citric acid treatments. This was also the case reported by Steuden and Szymaniec [9]. Meanwhile, hospitals 2 and 3 were heavily loaded with *S. aureus.*

Staphylococcus aureus have previously been isolated from air and environment surfaces in Iranian hospital [10], surfaces in a tertiary healthcare facility in Libya [11], from door handles and other points of contact in a public hospital in Ghana [12], Cameroon [13], environmental surfaces in 4 hospitals in Kaduna Metropolis, Nigeria [14].

The *S. aureus* isolated from surfaces such as door handles, bed etc are transmitted through the transient colonization of the hands of hospital personnel who have acquired the organism via direct contact with patients or following the handling of contaminated materials [10].

Table 3 shows the antibiotic susceptibility pattern of selected *Staphylococcus aureus* isolates to the following antibiotics: Pefloxacin (10µg), Zinnacef (10µg), Rocephin (25µg), Ciprofloxacin (10µg), Septrin (30µg), Erythromycin (10µg), Gentamin (30µg), Ampiclox (20µg), Amoxacillin (10µg) and Streptomycin (30µg). The Staphylococcus aureus isolated from microscope

had low susceptibility (40%) to the antibiotics. Meanwhile, *Staphylococcus aureus* isolate from fridge door, lab door handle, ward toilet door, ward bed hand rest and ward table had high susceptibility (100%) to the antibiotics used. Surprisingly, all *S. aureus* isolated from the 3 hospital facilities were observed to be methicillin-sensitive strains. The report of this finding is contrary to the report of other studies, for example, Mirzaii et al. [10] reported 94% of *S. aureus* isolated from both hospital patients and surfaces to be methicillin-resistant strains. In addition, Saba et al. [12] reported 39% of *S. aureus* isolated for a public hospital in Ghana to be methicillin resistant. In an Orthopedic hospital in Ahmadu Bello University Teaching Hospital (ABUTH), 73% of *S. aureus* isolated were reported to be MRSA [15].

Table3. *Antibiotic susceptibility pattern of selected S. aureus isolates and their phenotypic identification into MRSA and MSSA*

Sample Code	SXT	E	PEF	CN	APX	Z	AM	R	CPX	S	MRSA/MSSA
FD03	20	20	20	20	20	20	20	20	20	20	MSSA
M04	11	00	20	10	15	20	12	20	20	11	MSSA
LDH05	20	20	20	20	20	20	20	20	20	20	MSSA
WTD15	20	20	20	20	20	20	20	20	20	20	MSSA
WBHR13	20	20	20	20	20	20	20	20	20	20	MSSA
WT15	20	18	20	20	16	20	12	20	20	20	MSSA

Key: SXT: *Septrin; PEF: Pefloxacin; CN: Gentamin; APX: Ampiclox; Z: Zinnacef; AM: Amoxacillin; R: Rocephin; CPX: Ciprofloxacin; S: Streptomycin; E: Erythromycin; MRSA: Methicillin-resistant S. aureus; MSSA: Methicillin-sensitive S. aureus.*

In a similar study, Brunel et al. [16] reported strains of methicillin-sensitive *S. aureus* especially the emerging clonal complex 398, as the most frequent clone isolated from patients, healthcare workers and environmental sites in an intensive care unit in France. Person-to-person transmission of MSSA has been reported within community households [17] and more recently in a hospital [18] and an urban jail [19] in which a high proportion of detainees sharing a holding tank were colonized with MSSA. Blomfeldt et al. [20] also reported MSSA isolates from bacteremic patients in Norwegian University Hospital. The isolation of different spa types of MSSA from patients in both studies above suggests that MSSA is also capable of causing life threatening infections. However, its management is expected to be easier, when compared to MRSA strains. David et al. [21] reported MSSA as a predominant health-care associated pathogen. They hypothesized in that studies, using University of Chicago Medical Center that MSSA patients were more likely to have bacteremia, endocarditis or sepsis. Finally, they concluded that patients with MSSA have characteristics consistent with a health-care associated infection more often, compared to infections caused by MRSA.

The absence of mecA, entA and sccmecI genes in all MSSA strains isolated from this study was however not surprising as these genes have only been previously reported in MRSA strains. The mecA gene is found in bacterial cells which allow a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics, with MRSA being the most commonly known carrier of the mecA gene. Similarly, the entA and sccmec1 genes have also been reported to be mostly common in MRSA strains, with non, reported in MSSA strains

Conclusion/Recommendation

Methicillin-sensitive *S. aureus* (MSSA) were the most frequently isolated *S. aureus* isolates isolated from 3 hospital environments located in Benin City, Edo State, Nigeria, with varying levels of resistance and sensitivity to the commonly used antibiotics. They were also observed to lack the mecA, entA and sccmec1 genes commonly found in MRSA strains.

Considering the potential of MSSA to also cause bacteremia, nosocomial infections and other healthcare-associated infections, it is therefore recommended that effort should be made at removing this isolate from surfaces in healthcare facilities.

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