Original Research Article

PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN ABEOKUTA, NIGERIA

Abstract
This study examines the prevalence of Methicillin-resistant *Staphylococcus aureus* in clinical samples of patients in Abeokuta, Ogun State, Nigeria using standard recommended procedures. A total of 338 clinical specimens of Pus, Aspirate, Ear and Wound swabs were collected from three major health facilities in Abeokuta, Nigeria. Each sample was cultured for bacteria isolates and examined for colonial and cellular morphology while biochemical characterization was performed. Of the clinical samples collected, 50.3% were collected from male and highest rate of 37.9% was from age group 0-9 years and least rate of 7.6% was from age 10-19 and 40-49years. Only 32.9% samples were ear swabs, wound swabs (29.2%) and lowest rate of 16.8% aspirate. Of the 161 *Staphylococcus aureus* (32.2%) isolated; there was no significant disparity found in relation to the study sites (χ^2=7.145, p-value = 0.308). The present study indicates high prevalence rate of MRSA that require empirical and urgent intervention to prevent staphylococcal infection among the hospital patients and its outbreak.

Key words: *Staphylococcus aureus*, methicillin, resistance

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium belonging to the family Staphylococcaceae and is often found as a commensal on the skin and mucous membranes particularly in the nose of healthy individuals (Plata *et al.*, 2009). It is a versatile human pathogen causing infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumonia, osteomyelitis, endocarditis as well as toxin mediated syndromes such as toxic shock syndrome (Shittu *et al.*, 2011).

Staphylococcus aureus is able to cause a large diversity of both benign and lethal infections in humans and animals because of a wide range of virulence factors that include various toxins and enzymes (Bal and Gould, 2005). It has emerged as one of the most important human pathogens and has become a leading cause of hospital and community acquired infections (Shittu and Lin, 2006).

Prior to the introduction of penicillin for the treatment of *S. aureus* infections in the 1940s, the mortality rate of individuals with staphylococcal infections was about 80% (Skinner and Keefer, 1941). However within two years of the introduction of penicillin to medical use, penicillin-resistant strains were discovered. By 1960, about 80% of all *S. aureus* strains were found to be resistant to penicillin (Deurenberg and Stobberingh, 2008). Methicillin was introduced in 1959 to treat infections caused by penicillin resistant *S. aureus* (Enright *et al.*, 2002) but by 1961 there were reports of methicillin-resistant *S. aureus* from hospitals (Barett *et al.*, 1968).

Methicillin resistant *Staphylococcus aureus* has been severally shown to cause variety of diseases ranging from mild, superficial dermatological diseases to severe and potentially fatal systemic debilitations (Moran, 2005). In spite of the availability of considerable number of effective antimicrobial chemotherapeutic agents, MRSA still remains an increasing cause of post-surgical wound infections (Gorttilebs *et al.*, 2000; Graffunder and Venezia, 2002) and some

invasive infections such as nosocomial sepsis, acute endocarditis and osteomyelitis, pneumonia and other soft tissue infections (Rello and Diaz, 2003).

Methicillin resistant *Staphylococcus aureus* prevalence varies greatly with geographical location, type of hospital and studied population (Adetayo *et al.,* 2014). High prevalence has been recorded in tertiary hospitals in US, Southern European countries, Asia and South America (Diekema, *et al.,* 2001). In Africa, MRSA prevalence varies with different countries (Bell and Turnidge, 2002). There are several studies on methicillin-resistant *Staphylococcus aureus* in Nigeria, particularly in Southwestern zone (Adesida, *et al.,* 2005; Shittu and Lin, 2006; Ghebremedhin *et al.,* 2009; Adeleke and Asani, 2009; Onipede *et al.,* 2009). This observation coupled with the threat posed by MRSA has led to the investigation of the prevalence in Abeokuta, Nigeria.

MATERIALS AND METHODS

Collection of Samples

A total of 338 clinical specimens consisting of wound swab, ear swab, aspirate and pus were collected from Medical Microbiology unit of Federal Medical Centre, Idi-Aba, Sacred Heart Hospital, Lantoro and Ogun State Management Board, Ijaiye, all in Abeokuta, Ogun State for the period of 6 months. The swab samples were collected using commercially prepared sterile swab sticks (Oxoid U.K.). They were kept refrigerated at 4^{o}C until delivery, to the laboratory. All the samples where cultured immediately on appropriate media, within 12 hours of collection.

Isolation and Identification

The sterile culture media plates of Mannitol Salt agar (MSA), Mac Conkey agar (MCA), and Blood agar (BA) were dried in the oven to remove water of condensation in the plates as well as on the surface of the culture media. The swab samples were rubbed over one-quarter of each of the different agar plates (i.e. MSA, MCA and BA), the rest parts of the plates were streaked with a sterile wire loop to obtain discrete colonies. The inoculated culture media were incubated at 37^{0}C in an incubator for 24-48 hours. Suspected discrete colonies of *Staphylococcus aureus* were sub-cultured on Nutrient agar plates to obtain pure culture and for further analyses. Each organism was identified according to Cowan and Steel (2003) method of Bacteria identification, by their colonial appearance such as size, shape, consistency, colour, elevation and its differential characteristics such as pigmentation, lactose fermentation on Mac Conkey and Gram staining were done to further identify the isolates.

Gram Staining Technique

A smear of the suspected colony from the culture plate was made on clean, grease-free glass slide. The smear was heat-fixed on slide by passing the slide over Bunsen burner flame briefly. The slide was then covered with Crystal violet stain and allowed to stain for one minute. The stain was decanted, rinsed with tap water and stained with Lugol's iodine for one minute. The stain was decanted and the film (smear) decolourized with acetone for few seconds. The slide was quickly washed with distilled water and counter stained with Safranin for one minute. The slide was finally washed with water, dried and examined under the microscope using the oil-immersion objective lens. Suspected *Staphylococcus aureus* isolates were Gram positive cocci (appearing purples) and were arranged in clusters.

Biochemical Identification

Catalase test: A drop of 3% hydrogen peroxide solution was placed on a clean, grease-free glass slide; the edge of another clean slide was used to pick the test organisms and was dipped into the hydrogen peroxide. Observed bubble formation was regarded positive.

Coagulase Test: Part of the colony was emulsified on a clean grease free glass slide. 10µl of citrated human plasma was added and was observed for the presence of agglutination which indicates a positive reaction.

Tube Coagulase test: Isolates to be tested for Coagulase production were incubated in Mueller Hinton broth at 37oC for 24hrs. 0.2ml of the overnight broth culture was added to 0.5ml citrated human plasma in a sterile glass test tube with gentle mixing; the test tubes were incubated at 37oC and observed for coagulation at the 1hr of 2hrs, 4hrs and 24hrs. Formation of a firm opaque clot which remains in the place when the tube is tilted on its side was considered positive.

Mannitol fermentation: Plates of Mannitol salt agar were prepared according to the manufacturer's directions allowed to cool at 50oC and poured in a sterile disposable plate to set. The plates were labeled against the samples to be tested. The organism to be identified was inoculated on the set dried cooled plate with the aid of a sterile wire loop (streaking), Incubated at 37oC for 18hrs and was checked for evidence of growth on the agar surface and as well for colour change from red to golden yellow. Mannitol salt agar enables us to determine the different characteristics. The first is the organism ability to tolerate a high salt concentration environment which was indicated by the evidence of growth on the mannitol salt plate. Secondly, fermentation of the sugar mannitol by producing an acids an end product which changes the red pH indicator in the media to yellow. Change from red to yellow colour was considered positive.

Antimicrobial Sensitivity Testing

The susceptibility of isolates to various antibiotics was determined according to Bauer *et al.*(1996) and Clinical laboratory Standard (NCCLS) modified disc diffusion technique (Cheesbrough, 2000). Inoculum was prepared by touching a colony of the test organism with a sterile wire loop and the growth transferred into a Bijou bottle containing Mueller Hinton Broth. The cell suspensions were incubated for 2 hours and the bacterial suspension adjusted to 0.5 Mc Farland's standard using Mueller Hinton Broth. Mueller Hinton agar plates were dried in the hot-air oven by inverting petri dishes containing the media to remove surface moisture. Plates were then inoculated within 15minutes of preparation of the suspension. A sterile cotton swab was dipped into the suspension of isolate and excess fluid was removed by rotation of the swab against the side of the bottles above the fluid level. The media were then inoculated by even streaking of the swab over the entire surface of the plate in three directions at 60^{o} to each other. The plate was left for 10 to 15 minutes on the laboratory bench before single antibiotic discs were aseptically placed using the Antibiotic disc dispenser. Plates were incubated at 37^{o}C for 18 to 24 hours. Growth inhibition was shown as circular zone of no growth around disc. The diameter of zone of inhibition was measured using a graduated ruler and result interpreted according Clinical Laboratory Standard Institute (2015).

Methicillin-Resistance Screening

Isolates were screened for methicillin resistance using the method of Markowitz *et al.*, (1983). *Staphylococcus aureus* was inoculated into Mueller Hinton Broth (MHB) and was incubated at 37^{o}C overnight. A suspension of the inoculum which is equivalent to 0.5 Mc Farland Standard was streaked on Mueller Hinton agar (MHA) with 4% NaCl. Cefoxitin (30µg) was aseptically placed on the inoculated plate and the plate was incubated for 18 hours at 35 ^{o}C. The isolate with inhibition zone of ≤21mm was considered Methicillin resistant *Staphylococcus aureus* (MRSA) Strain. *Staphylococcus aureus* ATCC 25923 was used as a Control.

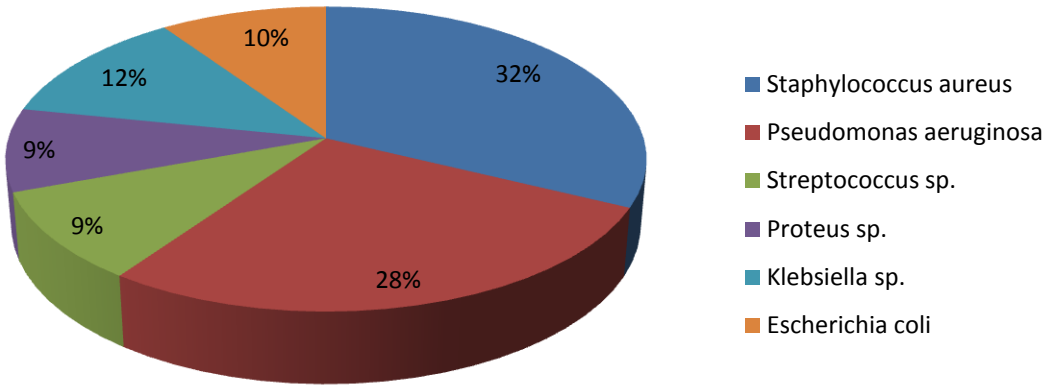
RESULTS

Out of the 500 bacterial isolates obtained from 338 clinical specimens from Federal Medical Centre, Idi-Aba, Sacred Heart Hospital, Lantoro and Ogun State Hospital, Ijaiye; 161 (32.2%) were identified by biochemical reactions to be *Staphylococcus aureus* as shown in Figure 1. Out of these samples, 14.9% were recovered from Aspirate, 20.5% Pus, 29.8% and 34.8% from Ear and wound swabs respectively (Figure 2).

Table 1 shows that there was no significant disparity found in the distribution of *Staphylococcus aureus* in relation to the study sites (χ^2=7.145, p-value = 0.308). Also, out of the 161 *Staphylococcus aureus*, 81 (50.3%) were males while 80 (49.7%) females were observed in the study.

Sixty-six (41%) were Methicillin resistant *Staphylococcus aureus* (MRSA) as indicated by their resistance to Cefoxitin and ninety-five (59%) were Methicillin Susceptible *Staphylococcus aureus* (MSSA) as indicated by their sensitivity to Cefoxitin as shown in Table 2. The result of the association between MRSA and MSSA with gender vividly reveals that there was no significant statistical difference as observed in Table 3 (χ^2 = 0.004, p-value = 0.538).

Distribution of MRSA in relation to the age of patients revealed that age group 0-9 (36.4%) had the highest prevalence of MRSA when compared with age group 70-79 (3.0%) having the least as shown in Table 6. But the result of the association of the MRSA and MSSA with age groups state that there was no statistical significant association as revealed in Table 5 (χ^2 = 7.833, p-value = 0.348). Similarly, Table 4 unveils statistical significant disparity between MRSA and MSSA in relation to the study sites (χ^2 = 15.226, p-value = 0.000).



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167 **Figure 1: Distribution of bacterial isolates from clinical samples in Abeokuta.**

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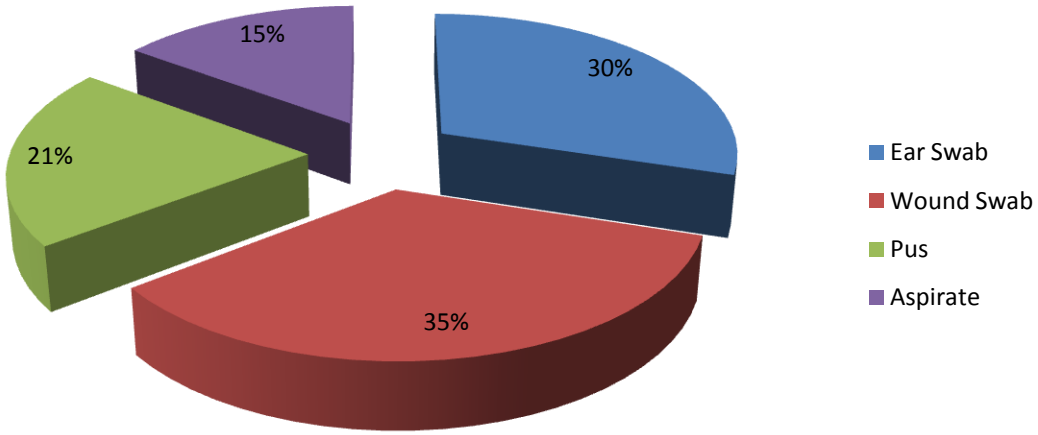

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170 **Figure 2: Distribution of *Staphylococcus aureus* in Clinical specimens obtained from**

171 **different health facilities in Abeokuta**

Table 1: Distribution of *Staphylococcus aureus* in clinical specimens in relation to Study sites

CLINICAL SPECIMENS	Health facilities (%)	χ^2	P-value
Ear Swab	48 (29.8)		
Wound Swab	56 (34.8)	7.145	0.308
Pus	33 (20.5)		
Aspirate	24 (14.9)		

Table 2: Percentage Distribution of Methicillin resistant *Staphylococcus aureus* and Methicillin Susceptible *Staphylococcus aureus*

***S. aureus* Isolates**	**Number (%)**
MRSA	66 (41.0)
MSSA	95 (59.0)
TOTAL	161 (100)

Key:

MRSA=Methicillin resistant *S. aureus*;

MSSA= Methicillin susceptible *S. aureus*

Table 3: Association of Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) in relation to gender

	FEMALE	MALE	χ^2	P-value
MSSA	47 (49.5)	48 (50.5)		
			0.004	0.538
MRSA	33 (50.0)	33 (50.0)		

Table 4: Association of Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) in relation to study sites

	Health facilities (%)	χ^2	P-value
MSSA	95 (100)		
		15.226	0.000
MRSA	66 (100)		

Table 5: Association of Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin susceptible *Staphylococcus aureus* (MSSA) in relation to age groups

AGE GROUPS	MSSA	MRSA	χ^2	P-value
0-9	25 (26.3)	24 (36.4)		
10-19	14 (14.7)	5 (7.6)		
20-29	14 (14.7)	14 (21.2)		
30-39	6 (6.3)	7 (10.6)	7.833	0.348
40-49	14 (14.7)	5 (7.6)		
50-59	13 (13.7)	6 (9.1)		
60-69	7 (7.4)	3 (4.5)		
70-79	2 (2.2)	2 (3.0)		
TOTAL	95 (100)	66 (100)		

Table 6: Distribution of MRSA in relation to Age of Patients in Abeokuta

AGE GROUPS (Years)	Number (%)
0-9	24 (36.4)
10-19	5 (7.6)
20-29	14 (21.2)
30-39	7 (10.6)
40-49	5 (7.6)
50-59	6 (9.1)
60-69	3 (4.5)
70-79	2 (3.0)
TOTAL	66 (100)

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious threat to hospitalized patients globally and it now represents a challenge for public health; as community associated infections appear to be on the increase in both adults and children in various regions and countries (Layton *et al.,* 1995). A number of investigations have reported *Staphylococcus aureus* among the most frequently encountered bacteria species in Microbiology laboratory in Nigeria (Ambe *et al.,* 2007).

This study was aimed at determining the prevalence of MRSA infections in Abeokuta as well as determining the antibiotic resistance indices of MRSA from different clinical specimens in Abeokuta. In this study, prevalence of MRSA was 41%. This is in agreement with a report carried out by Ikeh (2003) in Jos, Nigeria that reported a rate of 43.0% prevalence of MRSA; likewise, the result obtained is in concordance with a report from Oshogbo, Nigeria that recorded a prevalence rate of 47.8% (Olowe *et al.,* 2007). Although this prevalence was higher than 20.3% reported by Ghebremedhin *et al*. (2009) in Ibadan; 12.5% reported by Okon *et al*., (2011) in Maiduguri, and Taiwo *et al.* (2004) in Ilorin (34.7%).

The different Prevalence results obtained from several studies indicated that the prevalence of the MRSA varies from one Geographical location to another. It is clear that MRSA has

become a Global nosocomial pathogen with attendant therapeutic problems and warrant urgent infection awareness, considering the common practice of unregulated sale of antimicrobial agent (Holloway, 2000; Okon *et al.*, 2011; Akinduti *et al.*, 2011).

Though MRSA does not show predilection for any particular age or sex, no age is exempt from these infections. In the present study, it was found that maximum number of MRSA was observed from age group of 0-9 years comprising 36.4% of the total isolates while age group of 70-79 had the least prevalence of MRSA with 3.0% (Table 6). Higher incidence of MRSA infections among males has also been reported by Chua *et al*. (2008) which do not correlate with our findings that show 50% for both male and female.

CONCLUSION

In this study, the prevalence of MRSA is considerably high to warrant urgent infection awareness. The high resistance to the β-lactam antibiotics used in this study shows that these agents are becoming ineffective in the treatment. Also, there is need to reassess policies on antibiotic use within the hospital environment. However, a major limitation to this study was the inability to identify Panton-Valentine Leukocidin positive isolates, as this would have revealed the level of potential public health *Staphylococcus aureus* poses to the general population of Abeokuta, Nigeria.

RECOMMENDATIONS

Since complete eradication of MRSA may not be possible, control of transmission seems to be the appropriate goal. The efficacy of some controlling methods are widely recognized and recommended by most authors. The first and the most effective way among these are to avoid transmission through hand contamination from personnel even to patients.

The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant bacteria. Therefore chemotherapy should be guided by sensitivity of the probable causative organism. Accurate detection of MRSA by clinical laboratories is of great importance; also awareness should be created about the route of its transmission in the community and the risk factors for infection such as antimicrobial and parental drug use.

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