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Prevalence of Methicillin Resistant *Staphylococcus aureus* Infections among Hospitalized Wound Patients from Selected Tertiary Hospitals within Enugu Metropolis

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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

The incidence of *Staphylococcus aureus* infection especially in wound infections continues to rise. This study was aimed to evaluate the prevalence of methicillin resistant *Staphylococcus aureus* in hospitalized patients with wound infections in selected tertiary hospitals within Enugu metropolis. A total of 385 samples comprising mainly of wound swabs were collected using Levine's technique and cultured on blood agar, MacConkey agar and Mannitol salt agar. *Staphylococcus aureus* was identified based on their conventional cultural characteristics, gram staining reactions and

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biochemical tests. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) 25.0 package. A total of 188 (48.8%) *Staphylococcus aureus* were isolated from 385 patients with wound infections. Of 188 *Staphylococcus aureus* isolates, 86 (22.3%) were identified as methicillin resistant *Staphylococcus aureus* (MRSA) and 102(26.5%), methicillin susceptible *Staphylococcus aureus* (MSSA) respectively. The prevalence rate of MRSA according to the gender of the participants was 37(9.6%) for males and 49(12.7%) for the females with a mean age of 31.77. Generally, the isolates showed the highest resistance to Ampicillin (100%), Penicillin (100%) and Cefoxitin (100%) and were moderately sensitive to Clindamycin (52.3%) and Gentamycin (54.7%). No statistical significance was established (P>0.05) among these factors. This study highlighted high prevalence of MRSA among hospitalized wound patients and as such, the need for infection control strengthening in hospitals cannot be overemphasized.

Keywords: Staphylococcus aureus; wound infections; Methicillin resistant Staphylococcus aureus (MRSA); prevalence; hospitalized patients.

1. INTRODUCTION

In the microscopic world, *Staphylococcus aureus* is one of the most ubiquitous organisms. It is found worldwide and is a leading cause of several diseases. Even though it is classified as an opportunistic pathogen, it causes a wide range of possible infections [1]. Humans are natural reservoirs for *Staphylococcus aureus* [2]. They cause a variety of clinical diseases both in community-acquired and hospital-acquired settings [3]. This pathogen affects both immune competent and immunocompromised individuals and this is a problem in the healthcare settings because of the high morbidity associated with *S. aureus* infections. *S. aureus* is well characterized and known to have a broad arsenal of virulence factors that causes prominent inflammatory response [4,5].

Staphylococcus aureus has an increasing notoriety in acquisition of resistance to new antimicrobials. Among *S. aureus* resistant strains, one that is of increasing concern to humans is methicillin-resistant *S. aureus* (MRSA). The initial acquisition of methicillin resistance in hospitals and later in the community has shown success and obstinacy of this organism as a pathogen [6]. This strain is characterized by the presence of a single mutation that renders it resistant to methicillin, a semisynthetic penicillin used to treat Staphylococcal infections that are resistant to mold-derived penicillin. This strain of *S. aureus* was first isolated in the early 1960s, shortly after methicillin came into wide use as an antibiotic [7]. On the other hand, infections caused by *Staphylococcus aureus* that can be treated with penicillins and cephalosporins are referred to as Methicillin sensitive *Staphylococcus aureus* (MSSA). Methicillin resistant *Staphylococcus aureus* (MRSA) are strains that are resistant to multiple antibiotics, especially penicillin derivatives. It is notable that MRSA causes similar infections as the MSSA but its resistance to most antibiotics makes it very challenging and difficult to manage [8]. In Staphylococci, methicillin resistance produces a penicillin binding protein 2a (PBP2a), and this is encoded by the *mec*A gene [9].

About 50 million people worldwide are believed to carry MRSA [10]. It has become a global pathogen which is readily passed by skin contact but rarely causes infection in healthy individuals. Hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) often infects individuals with health care risk factors, such as surgery or residence in long term care facilities or similar institutional settings [11,12]. When cases of MRSA infection have been identified in the community, a thorough investigation usually reveals a history of recent hospitalization, close contact with a person who has been hospitalized or other risk factors such as previous antimicrobial drug therapy [13]. Many Community acquired - methicillin resistant *Staphylococcus aureus* (CA-MRSA) and methicillin susceptible *Staphylococcus aureus* (MSSA) often infect healthy persons who do not have the listed risk factors [7].

MRSA is a common organism isolated from infected wounds, often involving significant areas of skin or deeper soft tissues. Wound infections result from the active interactions between a host, a potential pathogen and other surrounding extrinsic factors. The increasing drug resistance exhibited by MRSA has posed a serious challenge for the remedy and management of wound infections. This is because the antimicrobial resistance further delays wound

healing and the infection becomes worst which prolongs hospital stay and increases hospital cost. In Enugu, there is scanty data on the prevalence of MRSA in wound infections. This study was aimed to determine the prevalence of MRSA in wound infections among hospitalized patients at 2 tertiary hospitals in Enugu – National Orthopedic Hospital, Enugu and Enugu State University Teaching Hospital, Parklane.

2. MATERIALS AND METHODS

2.1 Study Area and Design

A cross-sectional study design was adopted to evaluate the prevalence of MRSA in wound infections among hospitalized patients. A simple random sampling technique was used to select two tertiary hospitals within Enugu metropolis as well as the subjects that were enrolled into the study. The selected tertiary hospitals were-National Orthopedic Hospital (NOHE), Enugu and Enugu State University Teaching Hospital, Paklane (ESUTH), Enugu. Both hospitals are tertiary hospitals in Enugu metropolis.

2.2 Sample Collection

A total of 385 clinical specimens from burns, surgical wounds, foot ulcers (in diabetic patients), cancers, accident wounds (automobile) and pressure ulcers were collected from the various patients at National Orthopedic Hospital (NOHE) and Enugu State University Teaching Hospital, Parklane (ESUTH), according to standard methods of sample collection using sterile swabs [14]. After adequate counselling, each patient voluntarily signed the informed consent form and filled his/her questionnaire. The wound sample was obtained using sterile cotton swabs. The specimens were collected using Levine's technique [15].

2.3 Isolation and Identification of *Staphylococcus aureus* Strains

The samples collected were cultured on 5% Sheep Blood agar, MacConkey agar and Mannitol salt agar and incubated at 37^0C for 24 hours. Colonies suggestive of *Staphylococcus aureus* were identified by their characteristic features including hemolysis on Blood agar, yellow colony surrounded by yellow zone on Mannitol salt agar, gram staining, catalase and coagulase testing according to standard biological procedures. For microscopic Observation of a pure culture was selected and subjected to gram staining. Then the shape, arrangement and gram reactions of the isolated were observed under a microscope at amagnification of x100. Other biochemical tests including DNase, Indole and Oxidase tests were performed according to the methods described by Cheesbrough [16]. All isolates were identified based on Bergey's Manual of Determinative Bacteriology [17].

2.4 Antimicrobial Susceptibility Testing

The susceptibility of isolates to various antibiotics was determined by Kirby-Bauer disc diffusion method [16]. Mueller-Hinton agar plates were prepared following the Manufacturer's instructions. Using sterile swab sticks for each isolate, the plates were seeded with 0.5 McFarland turbidity equivalent of the bacterial suspension made on sterile normal saline, they were then allowed to stand for 20 minutes. Thereafter, the antimicrobial discs were placed on the surface of the media, each disk being well spaced from the other. These antibiotics discs from Oxoid UK include Erythromycin (macrolides) (15µg), Gentamycin (aminoglycosides) (10µg), Ciprofloxacin (fluoroquinolones) (5µg), Ampicillin (30µg), Penicillin (10 µg), Clindamycin (Lincosamides) (2µg), Ceftriaxone (cephalosporins) (30µg), Ofloxacin (fluoroquinolones) (5µg) and Levofloxacin (fluoroquinolones) (5µg). The plates were subsequently incubated in ambient air at 37^oC for 18 hours. Results were read and interpreted according to Clinical and Laboratory Standards Institute recommendation [18] guidelines.

2.4.1 Screening for *Methicillin resistant Staphylococcus aureus* isolates

All isolates that were identified as *Staphylococcus aureus* were screened for methicillin resistance using Cefoxitin (beta lactam) (30µg) and Oxacillin (beta lactam) (30µg) (Oxoid, UK). A bacterial suspension was prepared in sterile saline and the turbidity adjusted to 0.5 McFarland standards. The suspensions were inculated onto MHA plates. The discs were placed on the Mueller Hinton agar plates against the growth of *S. aureus*. Inhibition zones were interpreted according to CLSI guidelines [18]. Isolates with inhibition zones of ≤21mm (Cefoxitin) and ≤21mm (Oxacillin) were recorded as resistant (MRSA isolates). Cefoxitin is used as a surrogate in

MRSA detection because it is more reliable in the detection of *mecC*-positive MRSA [19]. This was used to infer *mecA*-mediated methicillin resistance which was later confirmed with molecular analysis (PCR).

2.4.2 Screening for *Methicillin sensitive Staphylococcus aureus* isolates

Methicillin sensitive *Staphylococcus aureus* isolates were screened using Penicillin (10µg) and Ceftriaxone discs (30µg) (Oxoid, UK). Inhibition zones of ≥29mm for Penicillin and ≥21mm for Ceftriaxone were recorded as sensitive/susceptible.

2.5 Statistical Analysis

Statistical analysis was performed with SPSS 25.0 (SPSS Inc., Chicago, IL) software. Comparative resistant strains of methicillin resistant *Staphylococcus aureus* was statistically analyzed using T tests and results were considered significant at 95% confidence level (P-value <0.05).

3. RESULTS

In this study, a total of 385 study participants were recruited. Of these, 162(42.1%) were males and 223(57.9%) were females. The age of the study participants was from 15 to 65 years with a mean age of (33.66 ± 10.51). The age range mostly affected with wounds were 25 to 29 (20.8%) with the least age involvement seen in ages 60 to 65 (1.3%). Slightly less than half of the study participants were married (48.3%) with a majority of the population residing in the urban (77.7%). The number of participants with tertiary level of education were highest (81.3%), while the least number of participants was recorded for those with primary level of education (2.6%). With respect to occupation type, civil servants were the highest (30.6%), traders made up one-fourth of the population (24.4%), followed by students (19.5%). Home workers were the least according to occupation types (2.3%) (Table 1).

Out of the 385 study participants, the prevalence of *Staphylococcus aureus* isolates were 188 (48.8%). In this, the overall prevalence of MRSA was 86 (22.3%) and MSSA was 102 (26.5%) (Table 2).

Out of the 188 *Staphylococcus aureus* isolated from wound swabs, MRSA showed a high level of resistance to Ampicillin, Cefoxitin and Penicillin (100%) respectively. This was followed by Erythromycin (90.7%) and Ofloxacin (87.2%). *S. aureus* isolates showed moderate sensitivity to Clindamycin (52.3%) and Gentamycin (54.7%). 97.7% resistance was recorded against Oxacillin. Other levels of drug sensitivity recorded include Levofloxacin (36.0%), Ciprofloxacin (43.0%) and Ceftriaxone (32.6%) (Table 3).

Table 1. Demographic characteristics of the study participants

Variable	Frequency (%)
Sex	
Male	162 (42.1%)
Female	223(57.9%)
Total	385(100%)
Age	
15-19	18 (4.7%)
20-24	60 (15.6%)
25-29	80 (20.8%)
30-34	76 (19.7%)
35-39	41 (10.6%)
40-44	46 (11.9%)
45-49	32 (8.3%)
50-54	13 (3.4%)
55-59	14 (3.6%)
60-65	5 (1.3%)
Total	385 (100%)
Marital status	
Single	141 (36.6%)
Married	186 (48.3%)
Separated	6 (1.6%)
Divorced	2 (0.5%)
Widowed	50 (12.9%)
Total	385 (99.9%)
Area of residence	
Urban	299 (77.7%)
Rural	86 (22.3%)
Total	385(100%)
Level of Education	
Primary	10 (2.6%)
Secondary	37 (9.6%)
Tertiary	313 (81.3%)
None	25 (6.5%)
Total	385 (100%)
Occupation	
Health worker	18 (4.7%)
Home worker	9 (2.3%)
Artisan	52 (13.5%)
Farming	19 (4.9%)
Civil Servant	118(30.6%)
Trader	94 (24.4%)
Student	75 (19.5%)
Total	385 (99.9%)

Table 2. Prevalence of *Staphylococcus aureus* isolates

Variable	Frequency
S. aureus	188 (48.8%)
MRSA	86 (22.3%)
MSSA	102 (26.5%)

Table 3. Antibiotic susceptibility pattern of MRSA isolates

Antibiotics	Sensitive to	Resistant to
Levofloxacin	31 (36.0%)	55 (64%)
Ciprofloxacin	3.7(43.0)	49 (57.0%)
Gentamycin	47 (54.7%)	39 (45.3%)
Ofloxacin	11 (12.8%)	75 (87.2%)
Clindamycin	45 (52.3%)	41 (47.7%)
Erythromycin	8 (9.3%)	78 (90.7%)
Ceftriaxone	28 (32.6%)	58 (67.4%)
Ampicillin	0 (0%)	86 (100%)
Oxacillin	2 (2.3%)	84 (97.7%)
Penicillin	0 (0%)	86 (100%)
Cefoxitin	0 (0%)	86 (100%)
Vancomycin	66 (74.7%)	20 (23.2%)

4. DISCUSSION

In this study, the overall isolation rate of *Staphylococcus aureus* was 48.8% among the wound specimens examined. This is similar to other previous studies; 36%, 48% and 50% within different locations in Nigeria [20, 21, 22]. The pattern of prevalence in the present study may have to do with the level of *Staphylococcus aureus* infection in the study locality. This high prevalence of *S. aureus* observed in wound specimens shows the notoriety of this organism, amongst other bacteria in causing infections and this has contributed to the endemicity of this pathogen in hospital environments. Same high incidence of *S. aureus* in wound specimens is also consistent with the works of Obiazi *et al.* [23] (48%) and Nwoire *et al.* [24] (60.4%). However, our findings are in contrast to other studies conducted in Cameroon (28.9%) [25], Jimma (23.6%) [26] and Tanzania (26.7%) [27] where the prevalence are lower. This lower level of incidence may be attributed to variations and attitudes in different locations. The high incidence of *S. aureus* in wound specimens observed in this study could further be attributed to exposure of the wounds, which might have made it more prone to contamination and infection. Furthermore, it was observed that a high number of participants in this study engage in self-medication with antibiotics and other drugs and only seek proper medical attention when the infection gets out of hand. A higher prevalence was reported by Mofolorunsho *et al* [28] and Ibe *et al.* [29] where a prevalence of 84% in Ayingba and 82.1% in Abia were respectively documented. This difference in pattern of prevalence shows that *S. aureus* infection may be influenced by the geographical location of patients and physical activity. In other parts of the world, studies have shown prevalence rates of 56.9% in Nepal, 51.5% in Brazil, and 34.5% in Ethiopia, [30,31,32] which is similar to the prevalence in the present study. In this study overall wound specimens were collected from 162 (42.1%) males as against 223 (57.9%) females and the result was statistically insignificant (P> 0.05). Female patients outnumbered male patients and this agrees with the studies carried out by some previous researchers [30, 33, 34]. However, some other studies have shown wound infections to be higher in males [35,36].

The observed prevalence of MRSA in our study was 22.3%. This is comparable to a previous study done in Enugu by Udeani *et al.* [22] (20.1%) and in Southwestern Nigeria by Ghebremedhin *et al.* [37] (20.2%). Similarly, Nwankwo reported 28.6% prevalence in Kano, Nigeria [38], and Ike reported 22.6% in Anambra, Nigeria [33]. Ayeni also recorded a prevalence similar to the one in this study in Marrakech (22%) [39]. A higher prevalence of MRSA was recorded in Ilorin (34.7%) [40], Sokoto metropolis (36.6%) [41] and Jos Nigeria (43.5%) [42]. In addition, Onemu and Ophori [43] have reported MRSA prevalence rate of 79% in Benin City indicating that various locations have varying distributions of the organism. The high antibiotic pressure among overcrowded inhabitants in this region may create an enabling environment that is suitable for the rapid development, efficient and effective spread of MRSA and other multi drug resistant pathogens. Another high prevalence of 36.1% frequency of MRSA was reported in Pakistan by Ullah *et al.* [44]. This confirms the high regional variations in the findings from different countries and cities. However, these do not agree with the works of Tsige *et al* where MRSA prevalence was as low as 9.8% in Ethiopia [32], 12.5% prevalence recorded in Maiduguri, Northeastern, Nigeria (Okon *et al*., 2013), Eretria (9%) [45] and Brazil (5.6%) (Almeida *et al*., 2014). These lower prevalence may be attributed to low rate of antibiotic abuse in these areas. The incidence of MRSA in the hospitalized patients studied is relatively low when compared to some studies and this may be because of the enlightenment

status of the people herein. Many of the patients reside in the urban area of Enugu metropolis where the people are enlightened to an extent. So that if this factor is considered, there will be an explanation for this prevalence. In a different context, a study in Japan showed the prevalence of MRSA in skin and soft tissue infections as 24.4% which is similar to the prevalence in the present study (Shinichi *et al*., 2017). Even higher prevalence of MRSA has been recorded in other parts of the world; an average MRSA rate of 73% was recorded in a survey in Korea in 2011 (Mendes *et al*., 2011) and 46.7%% in Singapore (Hsu *et al*., 2007). The contrasting trend of MRSA infection in different locations according to various studies may be due to naturally occurring fluctuations. Also, this variance may be explained by difference in hospital and community characteristics such as infection control and antibiotic utilization, the source of MRSA isolates and methods used for MRSA detection. On a general note, the prevalence observed in this study strongly indicate that MRSA continues to be a challenge in Nigerian hospitals. This is largely attributable to lack of a relevant antibiotic policy so that patients as well as some physicians (who prescribe antibiotics in the absence of culture tests) continue to abuse these drugs, thereby encouraging the emergence of drug resistant strains.

The increasing antimicrobial resistance by *S. aureus* is still a huge challenge because of their frequent associations with hospital and community acquired infections. The organism exhibit remarkable versatility in their behavior towards antibiotics with some strains like MRSA becoming even more resistant to commonly used antibiotics. In this study, a high level of resistance was observed to Penicillin (100%), Cefoxitin (100%) and Ampicillin (100%). This is similar to a study done in Owerri by Nsofor *et al.* [46] and the reports of Tiwari in India [47]. The resistance profile of MRSA to penicillin in our study is also similar to the results obtained in Ethiopia [48,32] Phillipines (100%) [49], Awka [33]. Also, the high resistance to Ampicillin as seen in this study agrees with previous studies in Nigeria [50, 51, 5]. Ampicillin (like penicillin) is a beta-lctam antibiotic and its resistance is mediated by the disruption in the beta lactam ring by the enzyme beta lactamase and this deactivates the supposed effect of the antibiotic [5]. Some causes of this level of resistance to some of the antibiotics used in this study as is common in the area of study may include non-compliance to prescription and other regional habits such as patronizing patent medicine stores without prescription and dosage [33]. On the other hand, the resistance to Clindamycin and Gentamycin in the present study is similar to other studies in Nigeria where resistance is less than 50%. Some of the isolates were considerably sensitive to Clindamycin (52.3%), Gentamycin (54.7%) and Ciprofloxacin (43.0%). This is similar to the reports of other researchers both within and outside Nigeria [5, 52, 45]. The susceptibility to Gentamycin may largely be because of their route of administration which is intravenous, thereby making it somehow difficult to abuse. This showed that the drug is still effective and can be considered as alternative options in the empirical treatment of *S. aureus* infections in the study area. This frequency of resistance observed in these antibiotics may be attributed to their use in the treatment of animals [53] and humans and to earlier exposure of the isolates to these drugs which may have enhanced the development of resistance. This assertion can further be strengthened by the high level of antibiotic abuse in our locality, arising from several forms of self medication and indiscriminate use of antibiotics by both clinicians and patients and poor antibiotic sale behavior such as sale of under dose or substituting brands. In Enugu state of Nigeria, people purchase antimicrobials without Physician's prescription and without culture tests and this is a common occurrence. In contrast, the resistance to Erythromycin (90.7%) and Ofloxacin (87.2%) is higher compared to some previous studies [54,55]. The main variation in drug resistance patterns among different studies might be due to indiscriminate use and availability of these antibiotics in certain areas. The high resistance documented in the present study has been corroborated in studies done in Germany [56] and Australia [57]. However, on a general note, the pattern of resistance shown by *S. aureus* is a thing of major concern in available therapeutic options and this further confirms the organism as a multi-drug resistant bacterium. The main variation in drug resistance pattern in previous studies might be attributed to the indiscriminate use of antibiotics and ready availability of these antibiotics in some areas or strain variation on sensitivity profile of *S. aureus*. The variation of resistance rates among different locations indicates that the susceptibility pattern of antibiotics differ from location to location and also changes from time to time and this may also be linked to differences in antibiotic prescription practices expressed in different geographic locations. These varying resistance to antibiotics

as seen in the present study remains a huge global challenge. They often result to longer hospital stays, higher medical costs and increased mortality. This is because when infections can no longer be treated with first-line antibiotics (as is mostly seen in resistant isolates), more expensive and higher ones are employed and this will result invariably in longer hospital stays thereby increasing health care costs and economic burdens on families and societies at large.

5. CONCLUSION

Staphylococcus aureus infections are creating serious problems in wound treatment in various locations in this country. This challenge calls for proper preventive and control measures to reduce transmission and infection. The most important way to control MRSA infection is by regularly checking the antibiotic resistance profile so as to formulate antibiotic policies and effective infection control measures. The regular surveillance of this highly resistant organism is imperative at this time. It is in fact a prerequisite to designing and implementing effective interventions. This study also highlights the need for antibiotic susceptibility testing so as to prevent treatment failures and increase in resistance. The knowledge of the prevalence and trend of MRSA infection can be used as a guide to identify priority areas for intervention and serve as a baseline for measuring the impact of interventions. Finally, wounds are a great source of MRSA infections and should be the focus of prevention and control strategies to avoid spread, assist in patient treatment, speed up wound healing time and minimize the economic burden of prolonged hospitalization.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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