

Prevalence and Antibigram of Healthcare-Associated Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) in a Public Tertiary Healthcare Facility (NH) in Enugu, Enugu State, Nigeria.

Ukpai, Ekenem Grace¹, Chukwurah, Ifeoma Edna², Oshilonyah, Lucy Unoma³, Ukwuonu, Chukwudi Aloysius⁴

¹Department of Microbiology and Parasitology, David Umahi Federal University of Health Sciences (DUFUHS), Uburu, Ebonyi State.

¹International Institute for Health Policy, Systems and Knowledge Translation, DUFUHS, Uburu, Ebonyi State.

¹International Institute for Infectious Diseases, Biosafety and Biosecurity Research, DUFUHS, Uburu, Ebonyi State.

²Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

³Department of Biology, University of Delta Agbor, Delta State, Nigeria.

⁴ Department of Medical Microbiology, College of Medicine, Godffery Okoye University, Ugwuomu Nike Enugu, Enugu State.

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*Corresponding Author:
Ukpai, Ekenem Grace¹
Email: ukpaieg@dufuhs.edu.ng
Tel: +2348038804738

ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) remains a major cause of both community and healthcare-associated infections. This study was designed to determine the prevalence and antibiogram of healthcare-associated MRSA (HA-MRSA) in a public tertiary healthcare facility (NH) in Enugu, Enugu State, Nigeria. A total of 62 {(male-33; female- 29) clinical samples were obtained from NH in Enugu State. *S. aureus* was isolated, characterized and identified based on standard microbiological procedures. Antibiograms of isolated MRSA isolates were determined by the Kirby-Bauer disc diffusion technique according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The prevalence of MRSA was higher amongst the isolates obtained from males (48.5%) than females (41.4%). The highest prevalence of MRSA in relation to age and sample source were obtained from {(31-45) and (46-60)} years and urine as (66.7%) and (42.6%), respectively. HA-MRSA were highly resistant to penicillin (100%), tetracycline (95.6%), and erythromycin (95.6%), but moderately susceptible to gentamycin and ciprofloxacin. A mean multiple antibiotic resistance index (MARI) of 0.8 was observed in this study with 96% > 0.2. In conclusion, prevalence of HA-MRSA was high in the study area and there is a need for more proactive measures to curb this public health menace before it escalates beyond control.

INTRODUCTION

S. aureus is a commensal microorganism that is known to be inhabited by a variety of human body parts, including the pores of the skin, skin glands, and the noses and guts of healthy people^{1,2}. Approximately 20%, 30%, and 50% of people are consistent carriers, irregular carriers, and non-carriers of *S. aureus*, according to earlier research. Notably, the likelihood of infection is increased by the pathogen's pattern of colonization on the human body. According to the majority of findings, *S. aureus* strains that infect humans end up becoming commensals³.

Severe health issues culminating from hospital stays and community-acquired infections have been linked to *S. aureus* infections. Staphylococcal infections are capable of damaging the lower respiratory tract, lymphatic system, and skin and soft tissues. They have been further linked to infections that affect medical equipment, including deep-seated illnesses like endocarditis and osteomyelitis, as well as central-line affiliated bloodstream infections (CLABSI)³. *S. aureus* is endowed with several virulence factors and toxins, often making it a culprit for many toxin-mediated diseases, as well as scalded skin syndrome, staphylococcal food-borne diseases (SFD), and toxic shock syndrome^{4,5}. These virulence factors and toxins also permit *S. aureus* to evade the oppositions presented by the human immune system. Following such complicated mechanisms, one might think that humans would be highly prone to severe infections by *S. aureus*. It is worthy to note that despite the mastery of *S. aureus* virulence factors, severe life-threatening infections in otherwise-healthy individuals are not common^{4,5}.

Strains of *S. aureus* which has an altered penicillin-binding protein within a short period opposed the semi-synthetic beta-lactam antibiotics that had substituted penicillin, with methicillin-resistant *Staphylococcus aureus* (MRSA) first described in 1961^{3,6}. High death and illness rates as well as increased treatment costs resulted from inception and proliferation of *S. aureus* strains which are resistant to methicillin, referred to as **MRSA**^{7,8}.

The medical practice worldwide especially in Nigeria has faced methicillin-resistant *S. aureus* as a consequential health challenge worldwide sequel to the upsurge in the development and display of antibiotic resistance to almost all last line antibiotics. High proportion of antibiotic resistance especially among the antibacterial agents have been observed and recorded from several studies⁷. Despite numerous efforts to control the matter of antibiotic resistance in MRSA, the evolution of increasing

antimicrobial resistance has been on the rise. Therefore, considering the menace caused by **HA-MRSA** globally, this study aimed at studying the prevalence and antibiogram of healthcare associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in a government-owned healthcare facility – National Orthopaedic Hospital Enugu State.

MATERIALS AND METHODS

Ethics

Ethical approval for the collection of the clinical samples was duly obtained from the management of the healthcare institution (IRB/HEC NUMBER- S.313/IV). This research was carried out in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects and identifiable human material or data⁹.

Sample collection

A total of 62 clinical samples [urine-47, wound swabs-10, sputum- 1, U/S -1, bone tissue aspirate-3] were collected from a public tertiary healthcare facility (NH) in Enugu, Enugu State, Nigeria for this study. Clinical samples were collected using sterile specimen bottles. The collected samples were immediately transported to the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki for bacteriological analysis.

Culturing, isolation, characterization and identification of the isolates

Using a sterile wireloop, the clinical samples were aseptically inoculated on mannitol salt broth (Oxoid, UK) and incubated at 37 °C for 48 hours. A loopful of the inoculated mannitol salt broth was later streaked on mannitol salt agar (MSA, Oxoid, UK) and incubated at 37°C for 24 h. The plates were observed for creamy golden colonies typical of *S. aureus*. Purified colonies were later cultured on sheep blood agar. Colonies displaying beta-haemolysis on sheep blood agar were presumptively identified as *S. aureus*. These suspected *S. aureus* isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test and other biochemical tests which include sugar fermentation test, and coagulase test^{10,11}.

Detection of methicillin resistant *Staphylococcus aureus* (MRSA)

This was done using Kirby-Bauer disc diffusion method

according to Clinical and Laboratory Standard Institute (CLSI) guidelines¹². Mueller-Hinton agar plates were prepared according to its manufacturers' specification. Two (2) colonies of the isolated bacteria were suspended in 5mL of nutrient broth. The turbidity of the broth culture was adjusted to 0.5 McFarland standard, which approximately equals 1.5×10^8 CFU/mL. Standardized inoculum was swabbed onto the prepared Mueller-Hinton agar plate. After 3 min, antibiotic discs impregnated with cefoxitin (FOX) (30µg), oxacillin (OX) (1µg), were placed on Mueller-Hinton agar plate for MRSA detection. The plate was then incubated at 37°C for 24 hours. Inhibition zone diameter was measured to nearest millimeter and interpreted according to CLSI guidelines^{11,12}.

Antibiotic Susceptibility Test

The susceptibility patterns of isolated *S. aureus* isolates were determined by the Kirby-Bauer disc diffusion technique according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014; Ariom *et al.*, 2019). Each of the isolate was standardized to 0.5 McFarland equivalent and aseptically spread on prepared Muller-Hinton agar (Oxoid, UK) plates using sterile swab stick. The inoculated plates were allowed to stand for 10 minutes. Antibiotic impregnated discs namely ceftazidime (CAZ) 30µg, ciprofloxacin (CIP) 5µg, clindamycin (DA) 2µg, penicillin (P) 10µg, erythromycin (E) 15µg, nitrofurantoin (F₂₀₀) 300µg, gentamicin (CN) 5µg, sulphamethaxazole (RL) 25µg, vancomycin (VA) 20µg, and tetracycline (TE) 30µg (Oxoid, UK) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37°C for 24 h after which the zones of inhibition around each disc were measured to the nearest millimeter with a ruler, recorded and interpreted according

to the Clinical Laboratory Standard Institute (CLSI) guidelines^{11,12}.

Determination of Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance indices (MARI) of the *S. aureus* isolates were calculated using the technique described by Moses *et al.* (2020)¹³. This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).

$$\text{MARI} = a/b \text{ ----- (1)}$$

Results

The distribution of HA-MRSA isolated in relation to gender, age, and sample source indicates that prevalence of MRSA was higher amongst the isolates obtained from males compared to females. A prevalence of 48.5% (16/33) versus (vs) 41.4% (12/29) for males vs females in NH (Table1). The prevalence of MRSA in relation to age shows that patients visiting NH {(31-45) years and (46-60)} years both have the highest frequency (66.7%). In addition, highest prevalence of MRSA relative to the source of samples and the total MRSA isolated were obtained from urine (71.4 %) and zero percent (0.0%) was obtained in sputum as presented in Table 1.

HA-MRSA isolates in this study showed varying degrees of resistance to the different antibiotics they were subjected to thus: penicillin (P) - 100%, erythromycin (E) 95.6 %, tetracycline (TE)- 95.6%, clindamycin (DA) -91.3%, ceftazidime (CAZ) 87.0%, nitrofurantoin (F₂₀₀) - 72.8%, sulphamethaxazole (RL) - 72.8%, vancomycin (VA) - 72.8%.

Table 1: Demographic Distribution of MRSA isolated from patients attending a Public Tertiary Healthcare facility (NH) in Enugu, Enugu State.

Gender				
M	33	21(63.7)	16(48.5)	57.1(16/28)
F	29	12(41.4)	12(41.4)	42.8(12/28)
Age				
0-15	10	4(40.0)	4(40.0)	14.2 (4/28)
16-30	13	7(53.8)	5(38.5)	17.9 (5/28)
31-45	15	11(73.3)	10(66.7)	35.7 (4/28)
46-60	9	6(66.7)	6(66.7)	21.4(4/28)
61-75	13	4(30.8)	2(15.4)	7.1 (4/28)
>76	2	1(50.0)	1(50.0)	3.6 (1/28)

Sources				
Urine	47	24(51.1)	20(42.6)	71.4(20/28)
Sputum	1	0 (0.0)	0 (0.0)	0.0(0/28)
Wounds	10	6(60.0)	5 (50.0)	17.9(5/28)
BTAsp	3	2(66.7)	2 (66.7)	7.1(2/28)
U/S	1	1(100.0)	1(100.0)	3.6(1/28)

M=Male; F= Female; BTAsp= Bone tissue aspirate; U/S= Urethral swab

Table 2: The participants' perceptions of pharmacy curriculum and mental healthThe least resistance observed were to ciprofloxacin (CIP) - 52.2% and gentamicin (CN) - 47.8%, which indicated moderate susceptibility (Fig. 1). From the analyses of the MARI values HA-MRSA isolates in the studied population, only 7.1 %, 14.3%, and 17.9 % of the isolates recorded MARI values less than 0.3, 0.5 and 0.6, respectively. The highest percentage of 32 % was recorded for MARI value of 0.8. There was an absolute resistance (MARI = 1.0) to antibiotics observed in 25 % of the HA-MRSA isolates (Table 2).

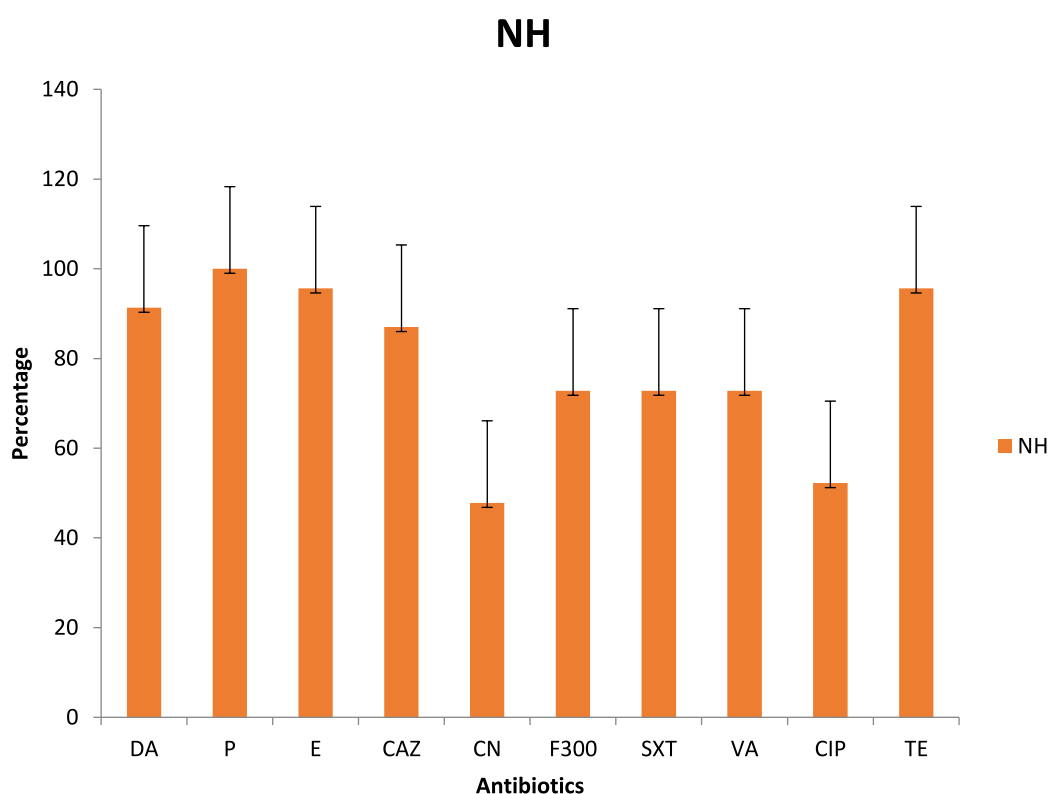


Figure 1: Antibiotic resistance pattern of HA-MRSA isolated from a Public Tertiary Healthcare facility (NH) in Enugu, Enugu State.

Key:

DA- Clindamycin P- Penicillin E - Erythromycin CAZ- Ceftazidine
 CN- Gentamicin F₃₀₀- Nitrofurantion RL- Sulphamethaxazole
 VA- Vancomycin CIP- Ciprofloxacin TE - Tetracycline

Table 2: Multiple Antibiotic Resistance Index (MARI) values for HA-MRSA isolates

MARI VALUE	HA-MRSA Number in (%)
0.1	0 (0.0)
0.2	1 (3.6)
0.3	1 (3.6)
0.4	0(0.0)
0.5	2 (7.1)
0.6	1 (3.6)
0.7	2 (7.1)
0.8	9 (32.0)
0.9	5 (17.9)
1.0	7 (25.0)
TOTAL	28 (100)

DISCUSSION

In this study, the colonization by healthcare-associated *S. aureus* (HA-SA) in NH, Enugu State was higher amongst the males (63.7%) than females (41.4%). This observation was similar to the previous findings of higher frequency in males than females^{14,15,16}. It calls for proper and improved advocacy for the general public especially the male folks on the importance of cultivating good personal hygiene as a way of curtailing antibiotic resistant organisms. Analyses of the isolation rates of HA-*S. aureus* from the healthcare facility (NH) in relation to the different age ranges were quite high. For example, 73.3% isolation rate amongst participants within the age groups (31-45) in the studied area was observed. These findings align with previous studies^{16,17}. This is in contrast with the previous reports of 30.7% as the highest prevalence from wounds^{15,18}. However, this study also reported a relatively higher prevalence of 60.0% HA-*S. aureus* from wounds.

The strength of the surveillance system in this healthcare facility as well as the social economic status of the patients attending NH could influence such differences.

This study also revealed the prevalence of HA-MRSA to be 46.5% and 41.4% amongst the males and females, respectively. Furthermore, out of the total MRSA isolated in NH, gender distribution of HA-MRSA was higher amongst the males (57.1%; 16/28) than females (42.8%; 12/28). This agrees with the reports of a higher prevalence in males than females^{8,14} and disagrees with the reports of a higher prevalence in females^{16,19}. The higher frequency in females could be attributed to the physiology of the female

anatomy²⁰. The relevance of good personal hygiene as a way of reducing antibiotic resistant organisms such as MRSA is therefore highly recommended for the general public to imbibe. In this study, both age groups 31-45 and 46-60 recorded the highest prevalence of MRSA with 66.7% and the least from 61-75 with 15.4% out of the total population examined amongst the different age groups. These findings align with previous studies with reports of highest prevalence among ≥ 25 yrs = 68% and 20-30 yrs = 61.5%, respectively^{16,21}. This could be explained by supporting the view that individuals within such age groups are active and are most likely to get infected.

Similarly, the highest prevalence of MRSA was recorded from urethral swab (100.0%). This was however followed by bone tissue aspirate (66.7%) and wounds (60.0%). Interestingly, the highest frequency of MRSA detection with respect to the total MRSA isolated was from urine (71.4%) and the lowest from sputum (0.0%). This is in accordance with the report of^{17,22,23} who reported highest isolation rate from wounds and lowest rate from HVS/ECS, semen, ECS and nasal. These findings are in accordance with reports of highest frequency of MRSA in patients' urine at Abakaliki in Ebonyi State¹⁹ and others who reported highest distribution of MRSA from urine in other regions of Nigeria^{24,25}.

A very remarkable resistance index $> 60\%$ was observed on most of the antibiotics used in studied healthcare facility. There was a high resistance of HA-MRSA isolates to more than six (6) classes of antibiotics (Fig 1). This study therefore reveals a high resistance profile of HA-MRSA to penicillin, ceftazidime and erythromycin and tetracycline

in the studied area. However, the least resistance observed were to ciprofloxacin (CIP) - 52.2% and gentamicin (CN) - 47.8%, which indicated moderate susceptibility and is in agreement with the research that reported gentamicin to have effect on MRSA¹⁴. High resistance of HA-MRSA to these antibiotics has been reported in previous researches in Abakaliki, Nigeria¹⁴. Other similar previous reports were by^{15,16,26,27} in Nigeria and beyond. Dilnessa and Bitew (2016)²⁸ reported 100% resistance of HA-MRSA to penicillin, erythromycin and trimethoprim- sulphamethoxazole in Ethiopia. Also, high susceptibility of HA-MRSA to gentamicin (93.9%) has been reported previously^{24,25}. The high resistance observed in this study could be associated with previous exposure of these isolates to drugs which may have encouraged development of resistance. There is also the possibility of a high level of antibiotic abuse in this environment stemming from self-medication which is often associated with inadequate or compromised dosage and failure to comply with treatment, and availability of antibiotics to consumers over the counters with or without prescription.

It is not surprising from the antibiotic profile obtained from the clinical isolates in NH indicating that the highest resistance was observed in penicillin. This is supported by other studies in different geographical zones as well because, being a beta-lactam (β -lactam), more MRSA are resistant to it. Resistance to the above class of antibiotic is normally mediated when there is disruption in the beta-lactam ring by the enzyme (beta-lactamase) which inactivates the molecules antibacterial property⁶. Furthermore, penicillin is very cheap and could easily be assessed by consumers with or without prescription. The relative moderately low resistances observed from the HA-MRSA isolates to gentamicin and ciprofloxacin from this study are not unusual.

These aminoglycoside and flouroquinolone are agents that produce their antimicrobial effect through the inhibition of protein synthesis and DNA gyrase. Gentamicin comes in parenteral forms, thus it is not misused or abused like tablets owing to difficulty in administration and its invasive nature³⁰. Moreover, from research, ciprofloxacin and fluoroquinolones as a group cause arthropathy in weight bearing joints of juvenile animals, thus the use of ciprofloxacin in paediatrics has been limited due to the possibility of arthropathy³⁰.

A mean MARI index of 0.8 was observed in this study with 96.4% recording a MARI greater than 0.2. This implies that the strains of MRSA originate from an environment where several antibiotics are used³¹. The MARI values obtained in

this study conform to those obtained in the previously published study by¹¹ who had very high values above 0.2. This shows that a very greater proportion of the bacterial isolates have been exposed to several antibiotics.

CONCLUSION AND RECOMMENDATION

Prevalence of HA-MRSA is high with obvious multidrug resistance strains of MRSA in the studied facility in Enugu State. Further testing of other classes of antibiotics should be carried out to improve treatment of MRSA infections as ciprofloxacin and gentamycin were only moderately effective for the treatment of MRSA infections in the area.

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