Prevalence and Antibiogram of Healthcare-Associated Methicillin-Resistant
Staphylococcus aureus (HA-MRSA) in Ebonyi State, Nigeria

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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 Ebonyi State, Nigeria. A total of 315 clinical samples were obtained from government-owned and private-owned healthcare facilities (HCFs) in Ebonyi State. S. aureus were characterized and identified based on standard microbiological procedures. MRSA isolates were detected using Kirby-Bauer disc diffusion technique with oxacillin (1µg) and cefoxitin (30µg) antibiotic discs. HA-MRSA prevalence frequency of 41.5% was recorded in the HCFs in Ebonyi State. Frequency of HA-MRSA isolation was highest in urine samples (51.1 %). Furthermore, a higher HA-MRSA isolation rate (63.6%) was observed in clinical samples from private HCFs than the government HCFs (28.4%). HA-MRSA were highly resistant to penicillin (99.4 %), ceftazidime (96.4 %), and erythromycin (90.6 %), but highly susceptible to ciprofloxacin and gentamycin. A mean MARI index of 0.71 was observed in this study with 96.5 % > 0.2. In conclusion, prevalence of HA-MRSA was quite alarming in our study area. Therefore, more proactive measures must be taken to curb this public health menace before it escalates beyond control. The use of therapeutics such as ciprofloxacin and gentamycin is strongly recommended for the treatment of MRSA infections in our study area.

Keywords: MRSA, HA-MRSA, antibiotic resistance, antibiotics, clinical samples, healthcare facilities

INTRODUCTION

Staphylococcus aureus is one of the major organisms that cause hospital and community-acquired infections, resulting in serious consequences. It can affect the bloodstream, skin and soft tissues, lower respiratory tract, and can cause infections related to medical instrumentation, such as central-line associated bloodstream infection (CLABSI), as well as some serious deep-seated infections such as endocarditis and osteomyelitis.1,2 S. aureus is also a commensal organism that is often present asymptptomatically in parts of the human body such as skin, skin glands, and mucous membranes, including noses and guts of healthy individuals.3,4 Studies have shown that about 20 % of individuals are persistent nasal carriers of S. aureus and around 30 % are intermittent carriers, whereas about 50 % are non-carriers. This colonization therefore significantly increases the chances of infections by providing a reservoir of the pathogen.2

S. aureus is equipped with a repertoire of virulence factors and toxins, often making it responsible for many toxin-mediated diseases, including toxic shock syndrome, staphylococcal food-borne diseases (SFD), and scalded skin syndrome. However, S. aureus maintains a fine control of its virulence factors and for the most part, rarely causes severe life-threatening infections in otherwise-healthy individuals.5 Strains of S. aureus with an altered penicillin-binding protein soon countered the semi-synthetic beta-lactam antibiotics that had replaced penicillin, with methicillin-resistant Staphylococcus aureus (MRSA).5

Emergence and spread of S. aureus strains which are resistant to methicillin, referred to as MRSA resulted in high morbidity, high mortality, high treatment costs and increased lengths of hospital stays.6 MRSA strains produce an altered penicillin-binding protein (PBP) associated with decreased affinity for most semi-synthetic penicillins. The protein is encoded by an acquired gene, mecA.7 This methicillin-resistant genetic component is carried on a mobile genetic element (MGE) designated staphylococcal cassette chromosome mec (SCCmec).8 Hence, the
emergence of methicillin-resistant strains of staphylococci is due to the acquisition and insertion of these mobile; genetic elements into the chromosomes of susceptible strains. This acquisition of antimicrobial resistance has presented a challenge to the medical world in terms of treatment and control of staphylococcal infections.9 MRSA in most cases accounts for at least 25 to 50 % of S. aureus infections in hospital settings. They are of major concern due to the high morbidity and mortality as well as their resistance to all available penicillins and most of the other beta-lactam drugs, except cefaroline and cefotiboprole.10

The transmission of MRSA infections may be limited by universal infection control measures, patient education, screening and decolonization of asymptomatic MRSA carriers in both healthcare and community settings.11 Therefore, considering the menace caused by HA-MRSA globally, this study was aimed at evaluating the prevalence and antibiogram of healthcare-associated methicillin resistant Staphylococcus aureus (HA-MRSA) in selected healthcare facilities in Ebonyi State, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 315 clinical samples [urine, sputum, wound swabs, semen, urethral swab, endocervical swab (ECS), bone tissue aspirate, high vaginal swab (HVS), throat/nasal swabs] were collected from government hospitals (Alex Ekwueme Federal Teaching Hospital, Abakaliki (AEFUTHA) and Mile 4 Gen Hospital, Abakaliki) and private diagnostic laboratories (Eston Medical Laboratory and JEM Medical Laboratory) between March 2017 and August 2019 in Ebonyi state, Nigeria for this study. Urine, sputum, and semen 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.

Ethics

Ethical approval for the collection of the clinical samples were duly obtained from the respective healthcare institutions. 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 data.12

Culturing, isolation, characterization and identification of the isolates

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 hours. 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 oxidase test, indole test, H₂S production test, Voges-Proskauer test, methyl red test, sugar fermentation test, and coagulase test.13,14

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.13,15 Each of the isolate was standardized to 0.5 McFarland equivalent and aseptically inoculated on prepared Muller-Hinton agar (Oxoid, UK) plates using sterile swab stick. The inoculated plates were allowed to stand for 10-15 min. Antibiotic impregnated discs namely cefoxitin (FOX) 30 µg, oxacillin (OX) 1 µg, ceftazidime (CAZ) 30 µg, ciprofloxacino (CIP) 5 µg, clindamycin (DA) 2 µg, penicillin (P) 10 µg, erythromycin (E) 15 µg, nitrofurantoin (F₂₀₀) 300 µg, gentamicin (CN) 5 µg, sulphamethaxazole (RL) 25 µg, vancomycin (VA) 20 µg, and tetracycline (TE) 30 µg (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 mm with a metre rule, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2015; Ariori et al., 2019).

Detection of methicillin resistant Staphylococcus aureus (MRSA)

This was done using Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2015). A Mueller-Hinton agar plate was prepared according to its manufacturer’s specification. Colonies of the isolated bacteria were suspended in 5 ml of nutrient broth. The turbidity of the broth culture was adjusted to 0.5 McFarland standard, which approximately equals 1.5 x 10⁸ CFU/ml. Standardized inoculum was swabbed onto the prepared Mueller-Hinton agar plate. After at least 3 min, antibiotic discs impregnated with oxacillin and cefoxitin 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.13,15

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.16 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). MARI = Number of antibiotics to which the tested isolate was resistant to (a) / Total number of antibiotics tested (b).
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 females than males. A prevalence of 23.5 % (12/51) versus (vs) 22.0 % (9/41) and 90 % (27/30) versus 86.7 % (13/15) for females vs males in Alex Ekwueme Federal Teaching Hospital Abakaliki (AEFUTHA) and Eston Medical Laboratory (EML) were observed respectively. However, the reverse was the case in Mile Four General Hospital (MFGH): 40.5 % (17/42) vs 28.6 % (20/70) and JEM medical laboratory (JML): 55.2 % (16/29) vs 44.7 % (21/47) where prevalence was higher among males than females (Table 6). The prevalence of MRSA in relation to age presented the patient visiting EML { (0-15) years and (46-60) years } and JML { (61-75) years to have the highest frequency. Cumulatively in the age range among the total population studied, the highest prevalence values of 53.0 % and 51.1 % were observed in the age range (16 – 30) years and the lowest (20 %) and (3 %) within the age range (0-15) years and (>76) years. In addition, highest cumulative prevalence of MRSA relative to the source of samples and the total population studied isolated obtained from semen were (62.5 %) and (51.1 %) respectively and zero percent was obtained in urethral swabs as presented in Table 6.

Out of the total 325 (males = 127, females = 198) samples collected from patients attending government and private health facilities in Ebonyi, 55 (43.3%) and 80 (40.4%) were found to be MRSA positive amongst the males and females respectively. HA-MRSA isolates in this study had 100 % resistance to penicillin (P) – [(MFGH, EML, JML); erythromycin (E) - (EML); ceftazidine (CAZ) - (EML, JML)]. For HA-MRSA, only 7.3 %, 14 %, 19.2 % and 25.1 % of the HA-MRSA isolate recorded MARI values greater than 0.3, 0.4, 0.5 and 0.6 respectively. The highest percentage of 27.4 % was recorded for MARI values of 0.8 followed by 20.0 % for MARI value of 0.9. There was an absolute resistance (MARI = 1.0) to antibiotics observed in 10.4 % of the HA-MRSA isolates.

![Figure 1: Antibiotic resistance pattern of HA-MRSA isolated from health facilities in Ebonyi State.](image)

**Key:** FT = Alex Ekwueme Fed Teaching Hosp. Abak. MG = Mile 4 General Hospital, Abakaliki ES = Eston Medical laboratory, Abakaliki. JM = JEM Medical laboratory, Abakaliki
### Table 1: Demographic distribution of MRSA Isolated from Patients attending Public and Private Health Facilities in Ebonyi State

| AEFUTHA | MFGH | EML | JML | No. of MRSA isolated (%) | Cumulative total No of samples examined | Cumulative No of S.A. isolated (%) | No. of MRSA isolated (N = 135) |
|---------|------|-----|-----|--------------------------|----------------------------------------|-----------------------------------|-------------------------------|
| Gender  |      |     |     |                          |                                        |                                   |                               |
| M       | 41   | 12  | 21  | 29.3                     |                                        |                                   |                               |
| F       | 51   | 13  | 27  | 25.5                     |                                        |                                   |                               |
| Age     |      |     |     |                          |                                        |                                   |                               |
| 0-15    | 21   | 2   | 19  | 9.5                      |                                        |                                   |                               |
| 16-30   | 23   | 6   | 34  | 26.1                     |                                        |                                   |                               |
| 31-75   | 21   | 3   | 22  | 14.3                     |                                        |                                   |                               |
| 46-60   | 16   | 7   | 17  | 43.8                     |                                        |                                   |                               |
| 61-75   | 7    | 4   | 14  | 57.2                     |                                        |                                   |                               |
| >75     | 4    | 1   | 6   | 25.0                     |                                        |                                   |                               |
| Sources |      |     |     |                          |                                        |                                   |                               |
| Urine   | 24   | 8   | 32  | 33.3                     |                                        |                                   |                               |
| HVS     | 3    | 0   | 20  | 45.0                     |                                        |                                   |                               |
| Sputum  | 5    | 4   | 33  | 60.0                     |                                        |                                   |                               |
| Wound   | 29   | 11  | 3  | 31.9                     |                                        |                                   |                               |
| Semen   | 0    | 0   | 0   | 0.0                      |                                        |                                   |                               |
| Throat/na sal | 3 | 1  | 0   | 33.3                     |                                        |                                   |                               |
| Aspirate/ BT | 4 | 0   | 13  | 46.2                     |                                        |                                   |                               |
| U/S     | 2    | 0   | 0   | 0.0                      |                                        |                                   |                               |
| ECS/    | 9    | 0   | 12  | 33.3                     |                                        |                                   |                               |

**Key:** U/S - Urethral swab; ECS - Endocervical swab; BT - Bone tissue; HVS - High vaginal swab

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MRSA (\%)

Prevalence of MRSA in relation to S. aureus isolated

Table 2: Overall prevalence of HA-MRSA in Ebonyi States

| No. of Samples Examined | No. of S. aureus isolated in (%) | MRSA (%) | Total Prevalence of MRSA in (%) | Prevalence of MRSA in relation to S. aureus isolated |
|-------------------------|---------------------------------|----------|---------------------------------|---------------------------------------------------|
| M                       | Female                          | Male     | M and F                         | M F                                               |
| 127                     | 198                             | 65 (51.2)| 95 (48.0)                       | 55 (43.3)                                         |
|                         |                                 | 80 (40.4)| 23 (17.0)                       | 1 (0.7)                                           |
|                         |                                 | 14 (10.4)|                                |                                                   |
|                         |                                 | TOTAL    | 135 (100)                       |                                                   |

Key: M=Male; F=Female

Table 3: Multiple Antibiotic Resistance Index (MARI) values for HA-MRSA isolates

| MARI VALUE | Number in (%) |
|------------|---------------|
| 0.1        | 1 (0.7)       |
| 0.2        | 1 (0.7)       |
| 0.3        | 8 (5.9)       |
| 0.4        | 9 (6.7)       |
| 0.5        | 7 (5.2)       |
| 0.6        | 8 (5.9)       |
| 0.7        | 23 (17.0)     |
| 0.8        | 37 (27.4)     |
| 0.9        | 27 (20.0)     |
| 1.0        | 14 (10.4)     |
| TOTAL      | 135 (100)     |

DISCUSSION

In this study, the cumulative colonization of healthcare-associated S. aureus (HA-SA) in Ebonyi State was higher amongst the males (61.2 \%) than females (48.0 \%) from the different healthcare facilities (HCFs) in Ebonyi State. This observation was similar to the findings of Onwubiko & Sadiq\(^7\), Abdullahi & Iregbu\(^8\) who reported higher frequency in males than females. It calls for proper advocacy for the general public to imbibe good personal hygiene as a way of curtailing antibiotic resistant organisms. Analyses of the isolation rates of HA- S. aureus from the healthcare facilities in relation to the different age ranges are quite high. Thus, 100 \% isolation rate amongst participants within the age groups, \{(0 - 15) yrs, (46-60)yrs\} in Eston Medical laboratory JEM medical laboratory were observed. Interestingly, the highest overall prevalence of 61.5 \% was detected amongst patients within (16-30) years. These findings align with previous studies by Udeani et al.\(^9\) and Abdullahi and Iregbu.\(^8\) In relation to the anatomical site, this study recorded the highest overall isolation rate (sample level) of HA-S. aureus from semen samples. This is in contrast with the reports of 30.7 \% by Udobi et al.\(^10\) and Onwubiko and Sadiq\(^7\) who reported the highest prevalence from wounds. However, this study also reported a relatively similar prevalence of 44.1 \% HA -S. aureus from wounds. The prevalence of HA- S. aureus (HA-SA) was higher in samples obtained from private healthcare facilities (HCF) than from government HCF with 71.9 \% and 35.9 \% respectively. The strength of the surveillance system in the respective healthcare facilities as well as the social economic status of the patients attending such facilities could determine such differences. Findings of this study also revealed the overall prevalence of HA-MRSA to be 43.3 \% and 40.4 \% amongst the males and females respectively. Furthermore, out of the HA -S. aureus isolated in Ebonyi state, gender distribution of HA-MRSA was higher amongst the males (84.6 \%; 80/95) than females (84.2 \%; 55/65). This agrees with the work of Garoy et al.\(^2\) who reported a higher prevalence in males than females and disagrees with the reports of Ugbo et al.\(^2\) Abdullahi and Iregbu\(^8\) who reported a higher prevalence in females. In this study, the age group 16-30 recorded the highest prevalence with 51.1 \% and the least from ≥ 76 with 3 \% out of the total MRSA isolated. These findings align with previous studies by Abdullahi and Iregbu\(^8\), Ike et al.\(^2\), and Udeani et al.\(^9\) who reported highest prevalence among ≥ 25yrs = 68 \%; 20-30yrs = 61.5 \% and 21-40yrs = 6.7 \%. This could be explained by supporting the view that individuals within such age groups are active are most likely to get infected. Similarly, the highest prevalence of MRSA was recorded from semen (62.5 \%). This was however followed by urine (53.4 \%) and ECS (45.3 \%). Interestingly, the highest frequency of MRSA detection was from urine (51.1 \%) and the lowest from urethral swab (0.0 \%). This is in accordance with the report of Ugbo et al.\(^2\) who reported highest frequency of MRSA in patient’s urine at Abakaliki in Ebonyi State. Terry et al.\(^2\) and OgbonUde et al.\(^4\) similarly reported highest distribution of MRSA from urine. Unlike contrary reports from Okon et al.\(^2\), Shittu et al.\(^4\), and Udeani et al.\(^9\) who reported highest isolation rate from wounds and lowest rate from HVS/ECS, semen, ECS, nasal and nil respectively. However, a higher isolation rate of HA-MRSA was observed from the private HCFs (63.6 \%) than the government HCFs (28.4 \%) in this study. Independent samples t-test indicated that there was no statistically significant difference in the distribution of HA-MRSA between government hospitals (Mean = 59.00, S.D. = 1.41) and private hospitals (p= 0.98 (at p < 0.05)). A very remarkable resistance index > 60 \% was observed on most of the antibiotics used in all the study centres within the states. Worthy of note is the high resistance of HA-MRSA isolates to more than two (2) classes of antibiotics (Fig 1). The respective mean percentage resistance profile for the HA-MRSA isolates to the different antibiotics used were as follows: penicillin (99.4 \%), cefazidime (96.4 \%), sulphamethoxazole (80.0 \%), erythromycin (90.6 \%), clindamycin (88.0 \%), respectively.

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tetracycline (81.0%), vancomycin (86.4%), nitrofurantoin (61.8 %), ciprofloxacin (57.5 %) and gentamycin (31.3%) (Fig 1). This study therefore reveals a high resistance profile of HA-MRSA to penicillin, ceftazidime and erythromycin in the healthcare facilities studied. However, least resistance of HA-MRSA was observed on gentamicin only and is in agreement with the research that reported gentamicin to present a great effect on MRSA. In spite of this observation, no statistical significant difference between the percentage antibiotic resistance patterns of the isolates obtained from schools and healthcare facilities was recorded (p < 0.05). Very high resistance of HA-MRSA to these antibiotics have been reported by Arior et al., in a previous study in Abakaliki, Nigeria where they observed 100 %, 100 %, 100 %, 89.5 % and 78.9 % percentage resistance to penicillin, tetracycline, ceftazidime, clindamycin and erythromycin. Despite the relative differences in resistance to ciprofloxacin (94.7 %) and nitrofurantoin (89.5 %) reported by Arior et al., they observed the highest susceptibility of HA-MRSA to gentamicin. Other similar previous reports were by Abdullahi and Iregbu, Aminu et al., Wang et al., Onwubiko and Sadiq in Nigeria and other parts of the globe. Dilnessa and Bitew reported 100 % resistance of HA-MRSA to penicillin, erythromycin and trimethoprim-sulphamethoxazole in Ethiopia. Also, high susceptibility of HA-MRSA to Gentamín (93.9 %) has been reported previously by Garoy et al., although with high susceptibility to vancomycin (15.9 %) and erythromycin (11 %). Several other works have also supported HA-MRSA susceptibility to vancomycin in different parts of the world. The high-level resistance as observed in this study could be associated with earlier exposure of these drugs to isolates which may have enhanced development of resistance. There is also the possibility of a high level of antibiotic abuse in this environment arising from self-medication which is often associated with inadequate dosage and failure to comply to treatment, and availability of antibiotics to consumers across the counters with or without prescription. The indiscriminate use of antibiotics without prescriptions in the developing countries such as Nigeria where there are no regulatory policies in this respect has rendered the commonly used antibiotics completely ineffective in the treatment of Staphylococcus aureus and subsequently MRSA infections. The relative low resistances observed from the HA-MRSA isolates to gentamicin from this study are not surprising. These aminoglycoside and fluoroquinolone are agents that produce their antimicrobial effect through inhibition of protein synthesis and DNA gyrase. This also strengthens the point that antibiotic misuse can actually encourage the development of resistance to as these agents are rarely used in children. According to Ugwu, gentamicin comes in parenteral forms, thus it is not misused or abused like tablets owing to difficulty in administration and its invasive nature. Moreso, 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 arthropathy. It is not surprising from the antibiotic profile obtained from the clinical isolates in Ebonyi State indicate that the highest resistance was observed in penicillin and supported by other studies in different geographical zones as well because being a beta-lactam (ß-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 deactivate the molecule’s antibacterial property. Penicillin is very cheap and could easily be assessed by consumers with or without prescription. The variations observed in MRSA antimicrobial resistance pattern observed at different places around the world is one of the major reasons for the successful spread of the pathogen. It therefore demands periodic study of MRSA resistance to know the new and emerging resistance pattern. A mean MAR index of 0.71 was observed in this study with 96.5 % recording a MAR index greater than 0.2. This implies that the strains of MRSA originate from an environment where several antibiotics are used. The MAR indices obtained in this study is in conformity to the value obtained in previously published study by Arior et al., 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. Moreover, the differences insensitivity profile of the bacteria isolates among the sources may be attributed to practices of self-medication, drug abuse and indiscriminate use of antibiotics.

CONCLUSION AND RECOMMENDATION

From the findings of this research, prevalence of HA-MRSA is quite high in Ebonyi states. It is crystal clear that the challenges of MRSA both in private and government healthcare settings are becoming a real threat to humans. So much effort have been employed to curb this health menace and antibiotic resistant Staphylococci, especially MRSA. Notwithstanding the conscientious efforts employed to control this antibiotic resistant bacteria by aggressive infection control methods, MRSA has become one of the most frequent cause of hospital acquired infections worldwide. It is clear that more efforts are required to as much as possible ameliorate this situation that poses a worldwide threat. The use of therapeutics such as ciprofloxacin, gentamycin and vancomycin are strongly recommended for the treatment of MRSA infections.

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