



Prevalence of Methicillin Resistant *Staphylococcus aureus* Isolated from Skin and Nasal Cavity of Microbiology Students of Federal University Birnin Kebbi, Kebbi State, North-Western Nigeria

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* constitutes part of the growing health problem associated with chemotherapy because they are more difficult to treat and could transfer drug resistance to other bacteria. The aim of this study is to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among Microbiology students in federal university Birnin Kebbi (FUBK). A cross-sectional study of the prevalence of MRSA among microbiology students of FUBK was conducted. A total of 50 nasals and 50 skin swabs were collected from the participants. The samples were inoculated directly on to mannitol salt agar and incubated for 24 hours at 37°C. The positive plates were sub cultured on nutrient agar. The bacterial species were identified using staining techniques and biochemical tests. *Staphylococcus aureus* isolates were screened for methicillin resistance using 5µg/ml Oxacillin disc on Mueller Hinton agar plate. Out of the 100 swab samples analyzed, 56 (56%) *Staphylococci* were recovered from the culture, 16 of which (16%) were identified by biochemical tests and gram stain to be *Staphylococcus aureus*. However, the remaining 45 (45%) were coagulase-negative staphylococci (CNS). Out of 16 *S. aureus* isolates, 15 (94%) strains of MRSA were identified using sensitivity tests, 14 (93%) were recovered from anterior nares, and only 1 (7%) from skin. Among the *S. aureus* strains, 14 strains (87.5%) were sensitive to Ciprofloxacin and Ofloxacin, 12 strains (75%) to Nitrofurantoin and 3 strains (19%) to Gentamicin. The isolated MRSA strains showed multidrug resistance. They were all resistant to Ceftazidime, Cefuroxime, Augmentin and Cefoxime. The result of this study shows the prevalence of MRSA. Ciprofloxacin, Ofloxacin and Nitrofurantoin were the most sensitive antibiotics. This study suggests that indiscriminate use of antibiotics is among the predisposing factors to these multi-drug resistant bacteria.

Keywords: Methicillin-resistance, MRSA, Prevalence, Oxacillin.

INTRODUCTION

The family *Staphylococcaceae* contains five genera, the most important of which is *Staphylococcus* (Willey *et al.*, 2014). *Staphylococci* were first seen by Robert Koch in the year 1878; cultivated in liquid medium by Pasteur in 1880 and named so by Alexander Ogston in 1881. The name *Staphylococcus* was derived from the greek "*Staphyle*" (a bunch of grapes) and *coccus* (a grain or berry) (Rajesh and Rattan, 2008). They are gram-positive spherical cells, usually arranged in grapelike irregular clusters, aerobic and facultative anaerobic non motile cocci. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow (Brooks *et al.*, 2013).

The genus *Staphylococcus* has at least 40 species. The four most frequently encountered species of clinical importance are; *S. aureus*, *S. epidermidis*, *S. lugdenensis* and *S. saprophyticus*. *Staphylococcus aureus* is the most important human pathogen, it is a coagulase positive which differentiates it from the other species (Brooks *et al.*, 2013). It is a normal inhabitant of the skin and anterior nares, Although a harmless colonizer, *S. aureus* can cause severe infections (Victor and John, 2011). The infections range from skin infections such as impetigo, folliculitis, furuncle,

carbuncle, paronychia and wound infections. Systematic infections; include bacteremia, osteomyelitis, septic arthritis, endocarditis, pneumonia, meningitis and deep-seated abscess. The bacterium also causes toxin-mediated diseases such as food poisoning, toxic shock syndrome and Staphylococcal scalded skin syndrome (Brooks *et al.*, 2013).

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first discovered in 1961 in united kingdom, it is a strain of *Staphylococcus aureus* that has developed resistant to multiple antibiotics, it is sometime referred to as superbug, meaning "multi drug resistant organism" (Geofrey *et al.*, 2015). The epidemiology of methicillin-resistant *Staphylococcus aureus* is constantly changing and both circulating clones and their antibiotics resistance profiles differ considerably throughout regions and countries (Reveles *et al.*, 2016). Human health professionals have long been concerned with microorganisms gaining resistance to antimicrobials and antibiotics, which are used to treat and prevent common infections. Those antibiotic resistant bacteria have a genetic make-up different than antibiotics susceptible organisms which allow them to survive antibiotic treatments and other potential stressors in their environment (Jonathan, 2012).

The resistance is mediated by an altered penicillin binding protein (PBP2a) which is encoded by the *mecA* gene (Udobi *et al.*, 2013). Resistance to an antibiotic by bacteria generally occurs through a change in the genetic make-up of the organism. Resistant gene can be translated vertically or horizontally. Vertical resistance results from a mutation to the genetic code responsible for, in the case of *S. aureus*, penicillin-binding protein. This genetic mutation is in response to the antimicrobial agent being introduced into the organism's environment. Horizontal mutation occurs when genes carried by a plasmid are introduced from outside of the cell resulting in the bacteria incorporating the genetic code for resistance (Shopsin *et al.*, 2000). Resistant strains of *S. aureus* possess the *Staphylococcal* chromosomal cassette (SCC) *mec* gene. The *mecA* gene encodes for a variant penicillin binding protein (PBP-2a) which is conformationally different from PBP of *S. aureus* strains sensitive to β -lactam antibiotics. PBP-2a has a lower affinity for penicillin antibiotics than that of regular PBP and is therefore "refractory to the action of all available β -lactam antibiotics" (Stapleton and Taylor, 2002).

Methicillin-resistant *Staphylococcus aureus* as an established pathogen causes hospital and community acquired infections worldwide, i.e. two strains of MRSA have been identified, which are; Hospital-acquired MRSA and community-acquired MRSA. Hospital-acquired is associated with infections that are contracted in medical facilities such as hospitals or nursing home. Community acquired MRSA is associated with infections that are transmitted through close personal contact with an infected person or through direct contact with an infected wound. This type of MRSA infection may also develop as a result of poor hygiene (Grema *et al.*, 2015).

MRSA have now become a major clinical problem worldwide, i.e., it is not restricted to any geographical location, and it is more prevalent in hospitals, schools, prisons, nursing homes, as well as congested unhygienic areas (Sit *et al.*, 2017). There are several studies on MRSA in this country, but the prevalence of this organism has never been investigated in this University, so therefore, there is need to conduct a research to investigate the prevalence of MRSA and its antibiotics susceptibility pattern among students in Federal University Birnin Kebbi (FUBK).

MATERIALS AND METHODS

Study Area

This study was carried out in Federal University Birnin Kebbi, Kebbi State of Nigeria. It is located in Birnin Kebbi, the capital of Kebbi State in the North-West Geo-political zone of Nigeria, it lies at latitude $12^{\circ} 24'50.2''N$ and longitude $4^{\circ} 11'32.3''E$.

Sample Collection

Prior to sample collection, Questionnaires were administered to all consented students from whom the

samples were obtained. The respondents were asked about their demographic characteristics; whether they apply cosmetics, experience runny nose, use handkerchief, and whether they have profuse sweat before collection of the sample. A total of 50 nasals and 50 skin swabs were collected from students of microbiology department FUBK. The nasal samples were collected from anterior nares of students using a sterile cotton swab stick which was moistened with sterile normal saline, the swab stick was introduced 2-3 cm in to the nasal cavity and rotated 4-5 times both clockwise and anti-clockwise, however, the skin swabs was obtained by swabbing the skin surface using moistened swab stick.

Isolation and Identification of *Staphylococcus aureus*

Both skin and nasal swabs were inoculated into mannitol-salt agar (Liofilchen, Roseto degli Abruzzi, Italy) and incubate at $37^{\circ}C$ for 24 hours. The genus *Staphylococcus* was confirm using Gram staining and catalase reaction. The identification of *Staphylococcus aureus* strains was performed with latex agglutination test (Pastorex Staph-Plus, Biorad, Marnes-la-coquette, France) and DNase test. All *Staphylococcal* strains, negative for latex agglutination and DNase tests, were considered as coagulase negative staphylococci (CNS).

Antibiotic Sensitivity Tests

The antimicrobial susceptibility testing was carried out by using Kirby Bauer's disk diffusion method as per Clinical and Laboratory Standard institute (CLSI, 2008) guidelines. The following standard antibiotic disks (Abtex Biological Ltd, Liverpool, UK) were used: Cefazidime (30 μ g), Cefuroxime (30 μ g), Cefotaxime (30 μ g), Gentamicin (10 μ g), Ciprofloxacin (5 μ g), Ofloxacin (5 μ g), Augmentin (30 μ g), Nitrofurantion (300 μ g). A standard inoculum was prepared by direct colony suspension in saline and compared with 0.5 McFarland standard turbidity and inoculated on the Mueller Hinton agar plate (Abtex Biological Ltd, Liverpool, UK). The plates were incubated at $35^{\circ}C$ for 18 hours. The results were interpreted according to the CLSI guidelines. MRSA detection was done using Oxacillin disk (5 μ g) (Abtex Biological Ltd, Liverpool, UK) according to CLSI, 2008 guidelines all *S. aureus* isolates resistant to Oxacillin were considered as MRSA. *S. aureus* was used as quality control.

RESULTS

A total of 50 nasal and 50 skin swab samples were analyzed in this study, which were obtained from 50 students of the department of Microbiology, federal university Birnin Kebbi (Table 1). Of the 100 swab samples analyzed, 56 (56%) *Staphylococci* were recovered from the culture, 16 of these isolates (16%) were identified by biochemical reactions and gram stain to be *Staphylococcus aureus* (Table 2). Out of 16 *S. aureus* isolates, 15 (15%) strains of MRSA were identified using sensitivity testing, 14 (28%) of these MRSA were recovered from nasal cavity, and only 1 (2%) was recovered from skin (Table 1).

Table 1: The Percentage Prevalence of Methicillin-Resistant *Staphylococcus aureus* Isolated from Microbiology Students of FUBK according to the sample site

Site	No. positive (%)	No. negative (%)	Total sample collected (%)
Nasal	14(28)	36(72)	50(100)
Skin	1(2)	49(98)	50(100)
Total	15(15)	85(85)	100(100)

Fifty-six (56) staphylococci were isolated during the period of current study. Sixteen (16) strains of which were *S. aureus* and forty-six (45) strains were CNS (Table 2). Sensitivity test was carried out only on *S. aureus* strains which demonstrated good sensitivity patterns to Nitrofurantoin, Ciprofloxacin, and Gentamicin. However, most were resistant to Ceftazidime, Cefuroxime, Augmentin, and Ofloxacin. Out of the 16 *S. aureus* strains isolated, 15 (93.7%) were MRSA. Twelve (12) of these strains were sensitive to nitrofurantoin, 8 strains to

ciprofloxacin, 3 strains to gentamicin and 2 strains to Ofloxacin. All of the *S. aureus* strains isolated were multidrug resistant. However, they were all fully resistant to Ceftazidime, Cefuroxime, Augmentin and Cefoxime. It revealed that 15 (93.7%) of the *S. aureus* isolates were resistant to oxacillin (Figure 1). Multidrug resistant profile of *S. aureus* conducted in this study reveals that 2 (12.5%) out of the 16 strains were resistant to antibiotics while, the remaining 14 (87.5%) strains were resistant to 5 or more antibiotics (Table 3).

Table 2: Identification of Bacteria using Biochemical Tests

Tests	No. Positive (%)	No. Negative (%)	Total
MSA	56(56) <i>Staphylococci</i>	44(44)	100
Catalase	56(100) <i>Staphylococci</i>	0(0)	56
Coagulase	16(29) <i>Staphylococcus aureus</i>	45(71) CNS	56
DNase	16(71) <i>Staphylococcus aureus</i>	45(71) CNS	56

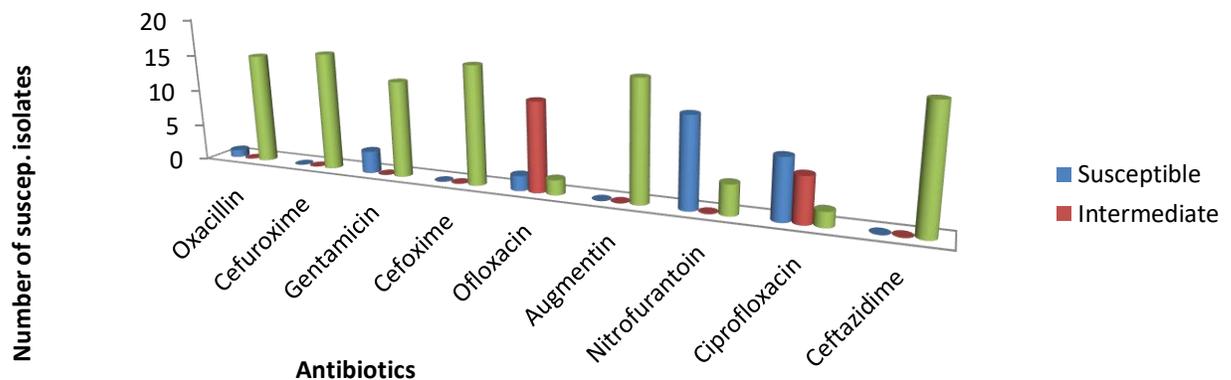
**Figure 1:** Antibiotic Susceptibility Pattern of MRSA

Table 4: Multi drug resistant profile of *S. aureus* isolated from Microbiology Students of FUBK

Number of drug resisted	Number percentages (%)
1	0 (0)
2	0 (0)
3	0 (0)
4	2 (12.5)
≥ 5	14 (87.5)

DISCUSSION

The findings of this study reveals that the prevalence of MRSA among Microbiology students in FUBK was 15%, this is in contrast and disagreement with studies carried out by Osinupebi *et al.* (2018), in Abeokuta, Udobi *et al.* (2013) in Zaria,

Nigeria, that recorded a prevalence rate of 41% and 85.25% respectively. This study also indicated lower prevalence than the works of Jean-marie *et al.* (2014) on surgical site infection in Kinshasa who recorded a prevalence of 63.5%, Barkin *et al.* (2009) who reported prevalence of 53.4% from Florida, and the work of a group of Indian researchers (INSAR) where they reported 42% in 2008 and 40% in 2009. The difference in sample size, study sites and geographical variations may be the causes of this variation.

However, George *et al.* (2017) reported prevalence of 0.9% from his study which is lower than the result obtained from this study. This variation might exist due to difference in study site and geographical location. The result of this study however, is in concordance with a report of the study carried out by Geoffrey *et al.* (2015) from Tanzania, and Islam *et al.* (2008) from Bangladesh who reported 13.9% and 25% respectively.

This relatively high prevalence obtained from this part (North-west) of the country where no reasonable report of MRSA prevalence was previously established, is certainly a matter of concern. Results obtained showed the highest resistance of MRSA strains to Cefuroxime (100%), Cefoxime (100%), and Ceftazidime (100%). The very high resistance to these second generation cephalosporins was not surprising since all MRSA strains have been variously reported to be resistant to all β -Lactam antibiotics of which cephalosporins are part (Udobi *et al.*, 2013). The isolated strains were also, resistant to Augmentin (100%), and Gentamicin (81%). This result is comparable with the result obtained by Osinupebi *et al.* (2018) where most of the MRSA isolates were resistant to many antibiotics. In the current study about 87.5%, 87.5% and 75% of MRSA isolates were sensitive to Ciprofloxacin, Ofloxacin

and Nitrofurantoin respectively. This is indicative that there is no absolute cross-resistance between penicillinase resistant penicillin (e.g. Oxacillin) and other classes of antibiotics (Jean-marie *et al.*, 2014). There is high level of self-medication in Nigeria which is associated with incomplete dosing regimen of antibiotics and failure to comply with treatment and availability to consumers with or without prescription. The indiscriminate use of antibiotics in developing countries like Nigeria renders them completely ineffective in the treatment of different infections.

However, the multi-drug resistant profile of the bacteria in this study reveals that, the bacteria were resistant to about 56% of the antibiotic used, this also correlate with the work of Osinupebi *et al.* (2018) Igbinsosa *et al.* (2016) and George *et al.* (2017) where they also reported higher resistance of the isolates to the antibiotics used in their studies.

CONCLUSION

The prevalence of MRSA among microbiology students in FUBK to be was reported to be 15%, with 14 (28%) from nasal cavity and only 1 (2%) from the skin. The results also, indicate that ciprofloxacin, ofloxacin and nitrofurantoin are active against the MRSA isolated. Many of the antibiotics used were resisted by the *Staphylococcus* species isolated.

Competing Interest

The authors declare no competing interest

Authors' contributions

Aliyu B. contributed to the conception of the study, the analysis of the result and preparation of manuscript. Bazata A.Y. and Bashar M.D. contributed to the analysis of results and preparation of manuscript. Ajibola O. contributed to the conception of the study. Gumah M.S. contributed to the processing of samples, analysis of result and manuscript preparation. Bashar H.G. and Jega B.G. contributed to the manuscript preparation. All the authors have read and approved the final version of the manuscript.

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