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Mupirocin Susceptibility of Methicillin-Resistant *Staphylococcus aureus* isolated from Nasal Carriers among Health Care Workers in Critical Units at The University of Ilorin Teaching Hospital, Ilorin, North Central Nigeria

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Abstract

Mupirocin is a Topical Antimicrobial Agent with excellent anti-staphylococcal and anti-streptococcal activity. Mupirocin resistance of Methicillin Resistant S. aureus (MRSA) is a reported in some parts of the world. The aim of the study was to determine the susceptibility of Methicillin Resistant Staphylococcus aureus from nasal carriers among Health Care Workers (HCWs) at the University of Ilorin Teaching Hospital to Mupirocin. One hundred and twenty Health Care Workers were selected. The demographic data of each of participants was collected using a standardized questionnaire. Two anterior nares swabs were taken from each of the 120 HCWs selected. Altogether, 240 anterior nares swab samples were collected. Identification of S. areus was done microscopically. Detection of MRSA was done using cefoxitin disc (30µg) diffusion method. Antimicrobial susceptibility Test was done by Kirby-Bauer disc diffusion method using Mupirocin (5 µg), Mupirocin (200µg) and other antibiotics. The E-Test was used to determine the Minimum Inhibitory Concentration (MIC) of Mupirocin. Interpretations was based on Clinical Laboratory Standards Institute. All the MRSA isolates in this study were sensitive to Mupirocin. Of the 240 nasal swabs collected from 120 HCWs, 150 swabs were isolated with S.

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aureus, 75 (50.0%) were MRSA. Of the 120 HCWs, 46 (38.3%) were carriers of MRSA. Potters (71.4%), Nurses (41.9%) and Doctors (37.5%) were the predominant carriers. All antibiotics used, except Mupirocin had some resistance to MRSA. The 100% susceptibility of MRSA to Mupirocin suggests it could be a drug of choice for nasal decolonization of MRSA in at risk populations.

Key Words*:* Methicillin-Resistant *Staphylococcus aureus* (MRSA); Nasal Carriers; Mupirocin Susceptiblity; Health Care Workers.

Abrreviations

AST-Antimicrobial Susceptibility Testing

CLSI -Clinical and Laboratory Standards Institute

DRU-Dialysis/ Renal Unit

E-Test- Epsilometer Test

HAI- Hospital Acquired Infections

HCWs - Health Care Workers

ICU-Intensive Care Unit

MDR-Multi Drug Resistant

MHA-Mueller-Hinton agar

MIC-Minimum Inhibitory Concentration

MRSA- Methicillin Resistant *S. aureus*

MSSA- Methicillin Susceptible *S. aureus*

NICU-Neonatal Intensive Care Unit

UITH- University of Ilorin Teaching Hospital

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most common etiological agent of Hospital Acquired Infections (HAIs). *S. aureus* has emerged as one of the most important pathogen because of the burden of diseases it causes and its Multi Drug Resistant (MDR) nature (Chaudhary *et al*., 2012). The spectrum of diseases caused by

S. aureus varies from mild skin infections to serious and invasive diseases which includes septicemia, pneumonia, endocarditis, deep-seated abscesses and toxinoses including food poisoning and toxic shock syndrome (Obajuluwa *et al*., 2014).

Methicillin Resistant *S. aureus* (MRSA) has been widely identified as the etiological agent for a wide variety of infections. It has accounted for one third of all *S. aureus* infections globally since 1990s (Samanta *et al*., 2014). Studies worldwide have documented the prevalence of nasal carriage of *S. aureus* strains, varying from 16.8% to 90% (Creech *et al*., 2005; Morange-Saussier *et al*., 2006; Heininger *et al*., 2007).

Nasal carriage of *S. aureus* is a major risk factor for the spread and transmission of both Community and Hospital Acquired *S. aureus* infections. HCWs who are carriers of MRSA could be important reservoir of transmission through human activities such as coughing, sneezing and personal contact. The infection is more complicated in patients with immunocompromised or surpressed immune system such as patients undergoing dialysis, and patients admitted in the intensive care unit (Levy *et al*., 2013; Sollid *et al*., 2014; Totté *et al*; 2016).

Mupirocin is a naturally occurring antibiotic produced by *Pseudomonas fluorescens* that interrupt protein synthesis in *S. areus* by binding competitively to bacterial isoleucyl-tRNA synthetase (IRS). It became known for its potency in eliminating *S. aureus* nasal carriage in the mid1990s (Upton *et al*., 2003).

Susceptibility of MSSA. *aureus* and MRSA) to Mupirocin has been reported in previous studies. Kaur *et al*., 2014, reported 99% susceptibility. Other studies have also shown 98%, 93%, 88% and 81% susceptibility of MRSA to Mupirocin (Singh Amit et al., 2013; Daiji *et al*., 2017; Agarwal *et al*., 2018). Thus, it is used to eliminate nasal carriage of *S. aureus*, especially MRSA strains in patients and HCWs (Seah *et al*., 2012). However, other studies have reported mupirocin resistant MRSA (Rudresh *et al*., 2015).

The study determined the susceptibility of MRSA isolated from nostrils of HCWs at UITH, to Mupirocin using Epsilometer Test (E-Test). Findings from this study will provide baseline information on the susceptibility profile of MRSA to Mupirocin at the UITH.

Materials And Methods

Study Area

The study was carried out at the University of Ilorin Teaching Hospital, Ilorin, Kwara State, North Central Nigeria. It lies between latitude $8^{0}30^{1}$N and longitude $4^{0}\ 30^{1}$ E.

Study Design

The study is a cross sectional hospital-based study.

Study Population

The participants recruited for this study were all consenting staff (Doctors, Nurses, Laboratory Technologists, Porters and Cleaners) working in the hospital units involved in the treatment and management of critically ill patients; Intensive Care Unit (ICU), Dialysis/ Renal Unit (DRU), Trauma and Neonatal Intensive Care Unit (NICU). Staff with evidence of infection, antibiotic treatment or prophylaxis were excluded from the study.

Sample Size

All the 120 HCWs in the Critical Units of the University of Ilorin Teaching Hospital (UITH) were sampled

Ethical clearance

Ethical clearance was obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital, Ilorin, (NHREC/02/05/2010) prior to the study.

Sampling Technique

Non-random sampling, whereby all the consenting staff in the selected units was recruited into the study.

Data Collection

A standardized and pretested questionnaire was used to obtain demographic data such as age, gender, cadre, number of years in service and number of years in the units from each enrolled participant.

Sample Collection

Two nasal swabs were collected from each participant using a sterile cotton swab. The swab was rotated in the anterior nares for 3 seconds. In case of sneezing, resampling was done. After collection, swabs were re-inserted in the transport tube (Tween 80), labelled accordingly and transported to the laboratory for further processing.

Isolation and Identification of *S. areus*

All the swabs collected were inoculated on Blood Agar and Mannitol Salt Agar and incubated at 35°C for 15 hours. Staphylococcal isolates were identified phenotypically using gram staining, biochemical tests and yellow colonies on Mannitol Salt Agar Medium.

Detection of MRSA

Detection of Methicillin Resistance was done using cefoxitin disc (30µg) diffusion method. A lawn culture was made on Mueller-Hinton agar (MHA) with 4% NaCl from suspension of turbidity equivalent to 0.5 MacFarland Standards from overnight growth in nutrient agar and incubated aerobically at 35°C for 24 hours. After incubation, the plates were examined for zone of inhibition. An inhibition zone diameter ≤21 mm was reported as Methicillin Resistance and ≥22 mm was reported as Methicillin susceptibility according to Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2014).

Antimicrobial Susceptibility Testing (AST)

Antimicrobial Susceptibility Testing of MRSA isolates was done by Kirby-Bauer disc diffusion method using Gentamycin (10µg),

Erythromycin (15µg), Ciprofloxacin (5µg), Cefuroxime (30µg), Ceftriaxone (30µg), Mupirocin (5 µg) and Mupirocin (200µg) (Mast, UK). Interpretation was based on Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2014).

Four to five morphological identical colonies picked from overnight growth in nutrient agar, were inoculated into 5 ml of peptone water and incubated at 37°C until turbid. The turbidity was compared with 0.5 McFarland Standards. After standardization of turbidity, a sterile cotton swab was used to make a lawn culture on the surface of Mueller Hinton agar. Antibiotic discs were applied by pressing gently using sterile forceps on the surface of media. The antibiotic discs were placed at least 20 mm apart from each other.

Determination of Minimum Inhibitory Concentration (MIC) of Mupirocin

E-test was performed by Kirby Bauer disc-diffusion method as per CLSI guidelines using mupirocin strip (Clinical and Laboratory Standards Institute, 2014). Lawn culture was made on the surface of Mueller Hinton agar medium. The strip with mupirocin antibiotic ranges from 0.1-240 µg/ml was applied by gently pressing using sterile forceps. The plates were then incubated aerobically at 35°C for 24 hours and examined for the minimum inhibitory concentration (MIC) thereafter. Isolates with MICs > 512 µg/ml were considered as high level resistant, those with MICs 8-256 µg/ml were considered as low level resistant. Isolates with <4 µg/ml were considered as mupirocin sensitive (Clinical and Laboratory Standards Institute, 2014).

Statistical Analysis

Data generated in this study was entered into the computer and analysed using the Statistical Package for Social Sciences (SPSS) software version 20.0 (IBM SPSS, 2015). Statistical significance of the Mupirocin susceptible *Staphylococcus aureus* isolate in association with the nasal carriage was determined using the Chi-square. P-value of <0.05 was considered significant.

Results

A total of 120 HCWs workers with the age range of 20-59 years (mean = 2.14, SD = ±1.13) were sampled. Twenty five (21.0 %) were Males and 95 (79.0%) Females. The mean number of years in service and duration of stay in the present units of the participants were 1.47 and 1.28 respectively. The Profession of the participants were as follows: Doctors (32); Nurses (62); Cleaners (14); Potters (7) and Technologists (5) (Table 1).

Of 120 HCWs 78 (65.0%) were carriers of all forms of *S. aureus,* while MRSA alone was 38.3% prevalent (Table 2).

A total of 240 samples were collected from the 120 HCWs. Of the 240 samples, 150 were isolated with *S. aureus*. Of the 150 *S. aureus* isolates, 75 (50.0%) were Methicillin Susceptible *Staphylococcus aureus* (MSSA) and 75 (50.0%) MRSA) (Table 3).

All MRSA isolated showed some resistance to all antibiotics used except to Mupirocin which recorded 100% sensitivity (Table 4)

Table1: Distribution of participants by Age, Gender, Years in the unit and Profession

Parameter	Frequency (%)
Age years (mean = 2.24, SD = ± 1.12)	
20-29	47 (39.2)
30-39	33 (27.5)
40-49	19 (15.8)
50-59	21 (17.5)
Gender	
Males	25 (21.0)
Females	95 (79.0)
Number of years in Unit (Means =1.28, SD = ± 0.45)	
≤5	87 (72.5)
>5	33 (27.5)
Category of workers	
Doctors	32 (26.6)
Nurses	62 (51.7)
Potters	7 (5.8)
Lab. Technologist	5 (4.2)
Cleaners	14 (11.7)
Total	**120**

Table 2. Distribution of Nasal Carriage by Gender, Hospital Units and Profession

Variable	Number Sampled	No. (%) of *S. aureus* Carrier	No. (%) of MRSA Carrier	P-value (χ^2)	
				S. aureus Carrier	MRSA Carrier
Gender				0.816	1.008
Male	25	17 (21.8)	10 (21.7)		
Female	95	61 (78.2)	36 (78.3)		
Total	**120**	**78 (100)**	**46 (38.3)**		
Hospital Unit				0.455	0.097
NICU	47	34 (72.3)	21 (44.7)		
ICU	38	22 (57.9)	10 (26.3)		
DRU	26	16 (61.5)	9 (34.6)		
Trauma	9	6 (66.7)	6 (66.7)		
Total	**120**	**78 (65.0)**	**46 (38.3)**		
Profession				0.593	1.27
Doctor	32	18 (56.3)	12 (37.5)		
Nurse	62	43 (69.4)	26 (41.9)		
Potter	7	6 (85.7)	5 (71.4)		
Technologist	5	3 (60.0)	0 (0.0)		
Cleaner	14	8 (57.1)	3 (21.4)		
Total	**120**	**78 (65.0)**	**46 (38.3)**		

Legend: **NICU**: Neonatal Intensive Care Unit; **ICU**: Intensive Care Unit (for adults); **DRU**: Dialysis/Renal Unit

Table 3 Antibiotics Susceptibility Profile of MSSA and MRSA Isolates

Antibiotics	MSSA, N=75 (%)		MRSA, N=75 (%)		P-value
(µg)	S (%)	R(%)	S (%)	R (%)	(χ^2)
Ciprofloxacin (5)	64 (85.3)	11 (14.7)	20 (26.3)	55 (73.3)	0.000
Gentamycin (10)	68 (90.7)	7 (9.3)	36 (47.4)	39 (52.0)	0.003
Cefuroxine (30)	55 (73.3)	20 (26.7)	50 (65.9)	25 (33.3)	0.858
Erythromycin (15)	62 (82.7)	13 (17.3)	31 (40.8)	44 (58.7)	0.000
Cefriaxone (30)	55 (73.3)	20 (26.7)	11 (14.5)	64 (85.3)	0.000
Mupirocin (5)	75 (100.0)	0 (0.0)	75 (100.0)	0 (0.0)	-
Mupirocin (200)	**75 (100.0)**	**0 (0.0)**	**75 (100.0)**	**0 (0.0)**	**-**

Legend: **MSSA**: Methicillin-sensitive *S. aureus*; **MRSA**: Methicillin-resistant *S. aureus;* **S:** Sensitive; **R:** Resistant.

Table 4: Minimum Inhibitory Concentration Range of MRSA Isolates to Mupirocin

N=75

Number of Isolates (%)	MIC Range (µg/ml)	Interpretation
20 (27)	0.064-0.094	Sensitive
25 (33)	0.095-0.25	Sensitive
20 (27)	0.26-0.75	Sensitive
10 (13)	0.76-1.50	Sensitive

Discussion

There is prevalence of MRSA among Health Care workers (HCWs) at University of Ilorin Teaching Hospital (38.3%). However, all the isolated MRSA were susceptible to Mupirocin. Therefore, mupirocin can be used to decolonized MSRA among Health Care workers at the University of Ilorin Teaching Hospital (UITH).

The 38.3% prevalence reported in this study is similar to previous reports where there was 33% and 25.5% prevalence (Nonika *et al*., 2014; Kulshrestha *et al*., 2019). A higher prevalence (46.4%) has been reported in Uganda (Nabil *et al*., 2017). Also, lower (3.4%-15.6%) prevalence has been reported in other studies (Khanal *et al*., 2015; Agawal *et al*., 2018; Agricola *et al*., 2018; Justus *et al*., 2019). The difference in prevalence of MRSA in various hospitals may be attributed to the inter-laboratory variation in the methods of detection as well as the effectiveness of hospital infection control interventions or abuse of rational antibiotic policy.

All MRSA isolates in this study were susceptible to Mupirocin. This could be due to the fact that there is no intrinsic resistance in the study area because Mupiroin is not in routine use for treatment of MRSA infections at the UITH, where the study was conducted. Whereas Mupicorin has been used in the treatment of skin and soft tissue infections caused by *S. aureus*, and also used in healthcare institute for nasal decolonization of HCWs and patients (Srivalli *et al.*, 2018), there has been report of possible transfer of muPA, a Mupiciron Resistance encoding Gene, from normal flora of the skin to MRSA when Mupiciron is used in therapy (Chase *et al*., 2011).

A high prevalence (65.0%) of *S. aureus* nasal carriage among HCWs is recorded in this study. The prevalence of 65.0% reported in this study is comparable to that documented in other studies; 64% (Hurdle *et al*., 2005) and 56.7% (Akujobi *et al*., 2013). The prevalence is lower than the 85% reported in Yemen (Ayepola *et al*., 2018). However, the prevalence of nasal carriage rate of *S. aureus* in the present study is higher than the recently reported in the studies conducted by Nabil *et al*., 2017 and Agarwal *et al*., 2018 where the *S. aureus* nasal carriage rates were 28.8% and 41.4% respectively. These variations in prevalence may be associated with the variations

in hygiene practices at the different study areas.

Findings have revealed a diverse variation in the different prevalence values for *S. aureus* carriage in different countries; 30% in Argentina (Abdelmonem *et al*., 2012), 26% in Indian (Boncompain *et al*., 2017) 31% in Iran and Palestine (Kulshrestha *et al*., 2019). Prevalence of 18.3 and 28.8% have been reported in Kenya and Ethiopia, (Omuse *et al*., 2012; Shibabaw *et al*., 2013).

Differences in the prevalence of nasal carriage of *S. aureus* strains between countries and hospitals could be explained in part by differences in relative abundance of *S. aureus* in the respective study sites, the quality and size of samples and the use of different Microbiological Methods.

Conclusion

The 100% susceptibility of the MRSA to Mupirocin suggests it could be considered for treatment and nasal decolonization of HCWs with MRSA. Also, the high prevalence of MRSA and other *S. areus* among HCWs has a significant implication on public health. Infected HCWs could be a reservoir and link in the transmission of both Community and Hospital Acquired *S. areus* infections.

The high prevalence of MRSA among HCWs in Critical Units as recorded in this study calls for sustained hygiene and safety practices by the HCWs and possible routine decolonization of MRSA and other *S. areus* from the nasal passage of HCWs.

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