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ANTIBIOGRAM OF BIOFILM FORMING METHICILLIN-RESISTANT *Staphylococcus aureus* ISOLATED FROM ACCIDENT AND BURN WOUND PATIENTS IN TERTIARY HOSPITALS WITHIN ENUGU METROPOLIS

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Abstract:

Biofilm forming Methicillin-resistant Staphylococcus aureus (MRSA) strain remains a leading cause of infections with high frequency of morbidity and mortality in wound patient, but little information exists regarding the prevalence and characterization of biofilm forming MRSA from different wound sources. Thus, this study was aimed at determining the biofilm forming potentials of MRSA isolated from patient wound in National Orthopedic Hospital Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) Enugu State. A total of 100 samples were collected from NOHE (76 from accident wound and 24 from burn wound) while 100 samples were collected from ESUTH (69 from accident wound and 31 from burn wound). The collected samples were analyzed and isolates identified using standard microbiological techniques. Antimicrobial susceptibility testing was carried out using Kirby–Bauer disk diffusion method and S. aureus isolates were screened for MRSA strain using cefoxitin and oxacillin. MRSA strains were studied for biofilm forming potential using Quantitative Assay. Multiple antibiotic resistance indices (MARI) were also determined. A total of 76(76.0 %) and 69(69.0 %) of S. aureus isolates were obtained from patients wound in NOHE and ESUTH respectively. Various degrees of resistance were observed among the S. aureus isolated to the tested antibiotics which ranged between 33.3 to 100 percentages. Exactly, 62(62.0 %) and 48(48.0 %) MRSA were found from patients wound in NOHE and ESUTH respectively. The prevalence of biofilm forming MRSA recorded 20(20.0 %) and 14(14.0 %) from patients wound in NOHE and ESUTH respectively. The isolates displayed average MARI value of ≥0.5. This study observed that biofilm forming MRSA can be treated with ciprofloxacin and imipenem. Thus, proper drug usage in the treatments of infection is recommended.

Keywords: MRSA - Methicillin Resistance Staphylococcus aureus
MSSA - Methicillin Susceptible Staphylococcus Aureus
NOHE - National Orthopedic Hospital Enugu
ESUTH - Enugu State University Teaching Hospital
MARI - Multiple Antibiotics Resistance Index

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INTRODUCTION:

Staphylococcus aureus is a Gram-positive, coagulase-positive pathogen belonging to the family Staphylococcaceae. It is a spherical bacterium of approximately 1μm in diameter which forms a grape-like cluster. It is a commensal that is often present asymptomatically on some parts of the human body like skin, skin glands, mucous membranes, noses and guts of healthy individuals (Gould and Chamberlaine, 1995). However, along with similar bacterial species that can colonize and act symbiotically, they can cause disease if they begin to take over the tissues they have colonized, or invade other tissues which results to an infection called "pathobiont". Studies show that about 20% of individuals are persistent nasal carriers of *S. aureus* and around 30% are intermittent carriers, whereas about 50% are non-carriers (Wertheim *et al.*, 2005). This pathogen affects both immune-competent and immuno-compromised individuals frequently resulting in high morbidity and mortality with complications which constitute problem to infected individuals. *S. aureus* has been reported by several studies as the causative agent of wide variety of diseases of supportive infections such as boils, wound infections, pustule, subcutaneous and sub-mucosa abscesses, osteomyelitis, mastitis, impetigo, septicemia, meningitis, bronchopneumonia, food poisoning (a common cause of vomiting and diarrhea) and urinary tract infections. It is also the most common cause of infections in hospitals with high causality among newborn babies, surgical patients, malnourished persons, patients with diabetes and chronic diseases (*Tong et al., 2015*).

Historically, *S. aureus* resistance emerged within 2 years of the introduction of penicillin (Chambers and Deleo, 2009). Benzyl penicillin was no longer effective for treatment of most *S. aureus* infections within 10 years after its introduction for use because of the acquisition of plasmid-encoded β-lactamase. Penicillin resistant *S. aureus* became pandemic throughout the late 1950s and early 1960s. *Staphylococcus aureus* isolates that are resistant to the isoxazoyl-penicillins such as methicillin, oxacillin and flucloxacillin are regarded as methicillin resistant *Staphylococcus aureus* (MRSA). Methicillin resistant *S. aureus* (MRSA) is any strain of *S. aureus* bacterium that are resistant to a large group of antibiotics called the beta-lactams mostly methicillin and oxacillin. Beta-lactam (β-lactam) antibiotics are broad spectrum group that include some penams (penicillin derivatives such as methicillin and oxacillin) and cephems such as the cephalosporins (*Gurusamy et al., 2013*). They can also be referred to as multidrug-resistant *S. aureus* or Oxacillin resistant *S. aureus* (ORSA). MRSA strains produce an altered penicillin-binding protein (PBP) associated with decreased affinity for most semi-synthetic penicillins. In addition to resistance, the pathogenicity of MRSA is an extremely important feature to be understood. Virulence is multi-factorial process and requires the use of a variety of components which are coordinately regulated to allow the organisms to adapt to the host environment and become successful pathogens. These virulence determinants promote tissue colonization, tissue damage and distant diseases. The pathogenesis of this bacterium depends on a combination of extracellular factors and biofilm forming ability (Dinges *et al.*, 2000). The adherence of *S. aureus* to biotic and abiotic surfaces is mediated by a protein family of staphylococcal Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs). Whereas the cell aggregation is led by the synthesis of polysaccharide intercellular adhesin (PIA) molecule encoded by the intracellular adhesion (ica) (Dobinsky *et al.*, 2003).

In general, two biofilm phenotypes have been identified (Polysaccharide intercellular adhesion (PIA) dependent and Polysaccharide intercellular adhesion (PIA) independent). Polysaccharide intercellular adhesion (PIA-) dependent biofilms are composed of poly-*β*-1, 6-N-acetylglucosamine (PNAG-) based matrices. PIA is synthesized from the products of genes located at the ica locus (Dobinsky *et al.*, 2003). Polysaccharide intercellular adhesion (PIA)-independent biofilm are composed of cell surface components such as teichoic acid, fibronectin binding proteins FnBpA and FnBpB, and autolysin extracellular DNA (eDNA) (Rice *et al.*, 2007). The prevalence and the epidemiology of MRSA are constantly changing, with novel MRSA clones appearing in different geographical regions. Continuous vigilance for MRSA through monitoring the characteristics, host specificity and transmission routes of newer strains in each setting is required. Understanding the molecular epidemiology of MRSA is therefore crucial in assessing existing precautionary measures and planning appropriate protective treatment for the infection.

MATERIALS AND METHODS:

Study area

Samples were collected from two study area; (i) Enugu State university teaching hospital, Parklane located at latitude 6°27'41.8"N and longitude 7°29'37.5"E. (ii) National Orthopedic Hospital Abakaliki Rd, Thinkers Corner, Enugu and is located at latitude 6°27'59.4"N and longitude 7°31'30.7"E. They hospitals have approximately over 200 available beds across seven major hospital wards (internal medicine, surgery, orthopedics, psychiatric,

gynecology, obstetrics and pediatrics). They hospitals serve the city of Enugu in Enugu State and are major referral hospitals in South Eastern Nigeria. Demographic and clinical information was collected by interviews and review of clinical records.

Sample collection

All sampling procedures were in accordance with guidelines of the Clinical Laboratory Standard Institute (CLSI, 2017). The sample size was determined according to methods described by Kadam and Bhalerao, (2010) and 200 patients on admission in the National Orthopedic Hospital Enugu (N.O.H.E) and Enugu State University Teaching Hospital (E.S.U.T.H) Parklane were screened. The gender and age of each patient was recorded. Wound swab (WS) was collected from each patient and transported in sterile plastic bags (ZIPLOC) containing ice packs to the Microbiology Laboratory unit of Applied Microbiology Department, Faculty of Sciences, Ebonyi State University Abakaliki, for bacteriological analysis. A total of 200 samples were collected for the purpose of this analysis. The parameters that were considered includes

a. wound producing pus
b. dry wound
c. duration of the wound
d. age of the patient
e. sex of the patient
f. type of wound (Accident and Burn)

A total of 100 samples were collected from National Orthopedic Hospital Enugu (comprising of 76 and 24 from accident and burn wound patient respectively) while100 samples from Enugu State University Teaching Hospital (comprising of 69 and 31 samples from accident and burn wound patient respectively).

Antibiotic susceptibility studies of biofilm forming MRSA

The Clinical Laboratory Standard Institute (CLSI) modified disc agar diffusion techniques were used. Discrete colonies of confirmed biofilm forming MRSA isolates growing on nutrient agar plates were emulsified in 3mL of phosphate buffered solution (PBS) and the turbidity were adjusted to 0.5McFarland standard. Using a sterile swab stick, the surface of Mueller Hinton agar in a 90 mm diameter plate was inoculated with the bacterial suspension by streaking the surface of the agar in three directions, rotating the plate approximately 60^0 to ensure even distribution. The plates were allowed to dry for 10 minutes before antibiotic discs were placed aseptically on the surface of the agar. The following antibiotics and concentrations were used to determine the antibiogram of the isolates: Chloramphenicol (10 µg), Erythromycin (15 µg), Imipenem (10 µg), Vancomycin (30 µg), Ciprofloxacin (5µg), Ceftazidime (10 µg), Clindamycin (2 µg), and Lincomycin (2 µg). They were allowed a further period of 30 minutes to dry and then incubated at 35^0C for 24 hrs. After overnight incubation, isolates were classified as susceptible, intermediate, or resistant to each antibiotic. Strains classified as resistant and intermediate were included in the same group (non-susceptible) (CLSI, 2019).

Determination of Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Paul *et al.*, 1997).

RESULT:

Table 1: Morphology and Biochemical characteristics of *Staphylococcus aureus* isolated from wound of patients in National Orthopaedic Hospital Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH).

Key: Positive **(+),** Negative **(-)**

							Sugar Fermentation							**Probable organism**
Shape	**Colour**	**Gram Staining**	**Catalase**	**Coagulase**	**Dnase test**	**Mannitol**	**Glucose**	**Lactose**	**Arabinose**	**Sucrose**	**Galactose**	**Xylose**	**Dextrose**	
Cocci	Golden-yellow	+	+	+	+	+	+	+	-	+	+	-	-	*S. aureus*

The proportion of *S. aureus* was 76 (76.0 %) and 69 (69.0 %) from wound patient in National Orthopaedic Hospital Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH) respectively. *S. aureus* was highly predominant in accident wounds at 61 (79.2 %) and 37 (63.8 %) when compared with burn wound at 15 (65.2 %) and 32 (76.2 %) from NOHE and ESUTH respectively while the overall prevalence of *S. aureus* was 145 (72.5 %). Prevalence of *S. aureus* was significantly associated with wound samples *p* value (<0.05) as presented in Table 2.

Table 2: Distribution of *S. aureus* in different wound samples isolated from patients in National Orthopaedic Hospital Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH).

Location	**Wound Sample**	**No. of sample**	**No. of *S. aureus* (%)**	**p-*value****
NOHE	Accident	76	61(79.2)	.0007
	Burn	24	15(65.2)	
		100	**76(76.0)**	
ESUTH				
	Accident	69	37(63.8)	
	Burn	31	32(76.2)	
		100	**69(69.0)**	
Total		**200**	**145(72.5)**	

Key: **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State University Teaching Hospital.

In NOHE, gender occurrence of *S. aureus* revealed that 21(72.4 %) and 10(55.6 %) were from accident wound female and burn wound female patient respectively while accident wound male and burn wound male patient recorded 29(90.6 %) and 16(76.2 %) respectively. In ESUTH, the occurrence of *S. aureus* revealed 15(71.4 %) and 7(53.8 %) in accident wound female and burn wound female patient while 34(69.4 %) and 13(76.5 %) was recorded against accident wound male and burn wound male patient as shown in Table 3.

Table 3: Distribution of *S. aureus* in different wound samples isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to Gender.

Location	Wound Sample	Gender	No. of sample	No. of *S. aureus* isolated in (%)
NOHE	Accident	Female	29	21(72.4)
	Burn	Female	18	10(55.6)
	Accident	Male	32	29(90.6)
	Burn	Male	21	16(76.2)
			100	**76(76.0)**
ESUTH	Accident	Female	21	15(71.4)
	Burn	Female	13	7(53.8)
	Accident	Male	49	34(69.4)
	Burn	Male	17	13(76.5)
			100	**69(69.0)**
	Total		**200**	**145(72.5)**

Key: **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State University Teaching Hospital.

Accident wound patient from NOHE with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed *S. aureus* prevalence rate of 30(73.2 %), 19(86.4 %) and 9(69.2 %) respectively and burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed *S. aureus* prevalence rate of 11(78.6 %), 5(71.4 %) and 2(66.7 %) respectively. In ESUTH, accident wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed *S. aureus* prevalence rate of 16(69.6 %), 24(77.4 %), and 7(46.7 %) respectively while burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed *S. aureus* prevalence rate of 14(82.4 %), 3(60.0 %) and 5(55.6 %) respectively as shown in Table 4.

Table 4: Distribution of *S. aureus* in different wound sample isolated from patient in National Orthopaedic Hospital Enugu (NOHE) and Enugu State university teaching hospital, Parklane according to wound duration.

Location	Wound Sample	Duration	No. of sample	*S. aureus* (%)
NOHE	**Accident**	**1-4weeks**	41	30(73.2)
		5-8weeks	22	19(86.4)
		9weeks and above	13	9(69.2)
			76	**58(76.3)**
	Burn	**1-4weeks**	14	11(78.6)
		5-8weeks	7	5(71.4)
		9weeks and above	3	2(66.7)
			24	**18(75.0)**
	Total		**100**	**76(76.0)**
ESUTH	**Accident**	**1-4weeks**	23	16(69.6)
		5-8weeks	31	24(77.4)
		9weeks and above	15	7(46.7)
			69	**47(68.1)**
	Burn	**1-4weeks**	17	14(82.4)
		5-8weeks	5	3(60.0)
		9weeks and above	9	5(55.6)
			31	**22(70.9)**
			100	**69(69.0)**

Distribution of *S. aureus* according to wound characteristic among accident wound patient from NOHE revealed 45 (76.3%) and 13 (76.5%) from pus and dry wound respectively and burn wound patient recorded 14 (77.8%) and 4 (50.0%) *S. aureus* from pus and dry wound respectively. While the proportion of *S. aureus* according to wound characteristic among accident wound patient from ESUTH revealed 42 (70.0%) and 5 (55.6%) from Pus and dry wound respectively and burn wound patient recorded 15 (83.3%) and 7 (53.8%) *S. aureus* from pus and dry wound respectively as presented in Table 5.

Table 5: Distribution of *S. aureus* in different wound samples isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to wound characteristic.

Location	Wound Sample	Wound characteristic	No. of sample	*S. aureus* (%)
NOHE	**Accident**	Pus	59	45(76.3)
		Dry	17	13(76.5)
			76	**58(76.3)**
	Burn	Pus	18	14(77.8)
		Dry	6	4(50.0)
			24	**18(75.0)**
			100	**76(76.0)**
ESUTH	**Accident**	**Pus**	60	42(70.0)
		Dry	9	5(55.6)
			69	**47(68.1)**
	Burn	**Pus**	18	15(83.3)
		Dry	13	7(53.8)
			31	**22(71.0)**
			100	**69(69.0)**

The proportion of Methicillin Resistant *S. Aureus* was 62(62.0 %) and 48(48.0 %) from patient's wound in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) respectively. MRSA was highly predominant in accident wounds 53(68.8 %) and burn wound 27(62.3 %) from NOHE and ESUTH respectively while the overall prevalence of MRSA was 110(55.0 %). Prevalence of MRSA was significantly association with wound samples *p* value (<0.05) as presented in Table 6.

Table 6: Distribution of Methicillin Resistant *Staphylococcus aureus* (MRSA) in different wound sample isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH).

Location	Wound Sample	No. of sample	No. of *S. aureus* (%)	MRSA	MSSA	p-*value**
NOHE	Accident	77	61(79.2)	53(68.8)	8(10.4)	.00001
	Burn	23	15(65.2)	9(39.1)	6(26.1)	
		100	**76(76.0)**	**62(62.0)**	**14(14.0)**	
ESUTH	Accident	58	37(63.8)	21(36.2)	16(27.6)	
	Burn	42	32(76.2)	27(62.3)	5(15.6)	
		100	**69(69.0)**	**48(48.0)**	**21(21.0)**	
Total		**200**	**145(72.5)**	**110(55.0)**	**35(17.5)**	

Key: **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State Teaching Hospital, **MRSA**- Methicillin Resistant *S. aureus*, **MSSA**- Methicillin Susceptible *S. aureus.*

In NOHE, gender occurrence of MRSA revealed 19(65.5 %) and 6(33.3 %) in accident wound female and burn wound female patient while accident wound male and burn wound male patient recorded 29(90.6 %) and 8(38.1 %) respectively. In ESUTH, MRSA revealed 11(52.4 %) and 7(53.8 %) in accident wound female and burn wound female patient while 25(51.0 %) and 5(29.4 %) was recorded against accident wound male and burn wound male patient as shown in Table 7.

Table 7: Distribution of MRSA in different wound samples isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to Gender.

Location	Wound Sample	Gender	No. of sample	*S. aureus* (%)	MRSA	MSSA
NOHE	Accident	Female	29	21(72.4)	19(65.5)	2(6.9)
	Burn	Female	18	10(55.6)	6(33.3)	4(22.2)
	Accident	Male	32	29(90.6)	29(90.6)	0(0.0)
	Burn	Male	21	16(76.2)	8(38.1)	8(38.1)
			100	**76(76.0)**	**62(62.0)**	**14(14.0)**
ESUTH	Accident	Female	21	15(71.4)	11(52.4)	4(19.0)
	Burn	Female	13	7(53.8)	7(53.8)	0(0.0)
	Accident	Male	49	34(69.4)	25(51.0)	9(18.4)
	Burn	Male	17	13(76.5)	5(29.4)	8(47.1)
			100	**69(69.0)**	**48(48.0)**	**21(21.0)**
	Total		**200**	**145(72.5)**	**110(55.0)**	**35(17.5)**

Accident wound patient from NOHE with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed MRSA prevalence rate of 23(56.1 %), 15(68.1 %) and 9(69.2 %) respectively and burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed MRSA prevalence rate of 8(57.1 %), 5(71.4 %) and 2(66.7 %) respectively. While in ESUTH, accident wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed MRSA prevalence rate of 13(56.5 %), 19(61.3 %) and 5(33.3 %) respectively and burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed MRSA prevalence rate of 7(41.2 %), 1(20.0 %) and 3(33.3 %) respectively as shown in Table 8.

Table 8: Distribution of MRSA in different wound sample isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to wound duration.

Location	Wound Sample	Duration	No. of sample	*S. aureus* (%)	MRSA	MSSA
NOHE	**Accident**	**1-4weeks**	41	30(73.2)	23(56.1)	7(17.1)
		5-8weeks	22	19(86.4)	15(68.1)	4(18.2)
		9weeks and above	13	9(69.2)	9(69.2)	0(0.0)
			76	**58(76.3)**	**47(61.8)**	**11(14.5)**
	Burn	**1-4weeks**	14	11(78.6)	8(57.1)	3(21.4)
		5-8weeks	7	5(71.4)	5(71.4)	0(0.0)
		9weeks and above	3	2(66.7)	2(66.7)	0(0.0)
			24	**18(75.0)**	**15(62.5)**	**3(12.5)**
	Total		**100**	**76(76.0)**	**62(62.0)**	**14(14.0)**
ESUTH	**Accident**	**1-4weeks**	23	16(69.6)	13(56.5)	3(13.1)
		5-8weeks	31	24(77.4)	19(61.3)	5(16.1)

	9weeks and above	15	7(46.7)	5(33.3)	2(13.3)
		69	**47(68.1)**	**37(53.6)**	**10(14.5)**
Burn	**1-4weeks**	17	14(82.4)	7(41.2)	7(41.2)
	5-8weeks	5	3(60.0)	1(20.0)	2(40.0)
	9weeks and above	9	5(55.6)	3(33.3)	2(22.2)
		31	**22(71.0)**	**11(35.5)**	**11(35.5)**
Total		**100**	**69(69.0)**	**48(48.0)**	**21(21.0)**

Key: **NOHE-**National Orthopaedic Hospital Enugu, **ESUTH**-Enugu State Teaching Hospital, **MRSA**- Methicillin Resistant *S. aureus*, **MSSA-** Methicillin Susceptible *S. Aureu*

Distribution of MRSA according to wound characteristic among accident wound patient from NOHE revealed 38(64.4 %) and 9(52.9 %) from pus and dry wound respectively and burn wound patient recorded 12(66.7 %) and 3(50.0 %) MRSA from pus and dry wound respectively. While the proportion of MRSA according to wound characteristic among accident wound patient from ESUTH revealed 37(61.7 %) and 0(0.0 %) from pus and dry wound respectively and burn wound patient recorded 8(44.4 %) and 3 (23.1 %) MRSA from pus and dry wound respectively as presented in Table 9.

Table 9: Distribution of MRSA in different wound sample isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to wound characteristic.

Location	Wound Sample	Wound characteristic	No. of sample	*S. aureus* (%)	MRSA	MSSA
NOHE	**Accident**	Pus	59	45(76.3)	38(64.4)	7(11.9)
		Dry	17	13(76.5)	9(52.9)	4(23.5)
			76	**58(76.3)**	**47(61.8)**	**11(14.5)**
	Burn	Pus	18	14(77.8)	12(66.7)	2(11.1)
		Dry	6	4(50.0)	3(50.0)	1(16.7)
			24	**18(75.0)**	**15(62.5)**	**3(12.5)**
	Total		**100**	**76(76.0)**	**62(62.0)**	**14(14.0)**
ESUTH	**Accident**	**Pus**	60	42(70.0)	37(61.7)	5(8.3)
		Dry	9	5(55.6)	0(0.0)	5(55.6)
			69	**47(68.1)**	**37(53.6)**	**10(14.5)**
	Burn	**Pus**	18	15(83.3)	8(44.4)	7(38.9)
		Dry	13	7(53.8)	3(23.1)	4(30.7)
			31	**22(71.0)**	**11(35.5)**	**11(35.5)**
	Total		**100**	**69(69.0)**	**48(48.0)**	**21(21.0)**

Key: **MRSA**- Methicillin Resistant *S. aureus*, **MSSA-** Methicillin Susceptible *S. aureus*

The proportion of biofilm forming MRSA was 20(20.0%) and 14(14.0%) from wound patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH) respectively. MRSA was highly predominant in accident wounds patient 17(22.1%) and 9(15.5%) from NOHE and ESUTH respectively over burn wounds patient 3(13.0 %) and 5(11.9 %) from NOHE and ESUTH respectively while the overall prevalence of biofilm forming MRSA accounted for 34(17.0 %). Prevalence of biofilm forming MRSA had no significantly different among wound samples *p* value <0.05 as presented in Table 10.

Table 10: Distribution of biofilm forming Methicillin Resistant *Staphylococcus aureus* (MRSA) in different wound sample isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH).

Location	Wound Sample	No. of sample	No. of *S. aureus* (%)	MRSA (%)	Biofilm (%)	Non-biofilm (%)	p-*value**
NOHE	Accident	77	61(79.2)	53(68.8)	17(22.1)	36(46.8)	.2278
	Burn	23	15(65.2)	9(39.1)	3(13.0)	6(26.1)	
		100	**76(76.0)**	**62(62.0)**	**20(20.0)**	**42(42.0)**	
ESUTH							
	Accident	58	37(63.8)	21(36.2)	9(15.5)	12(20.6)	
	Burn	42	32(76.2)	27(62.3)	5(11.9)	22(52.4)	
		100	**69(69.0)**	**48(48.0)**	**14(14.0)**	**34(34.0)**	
Total		**200**	**145(72.5)**	**110(55.0)**	**34(17.0)**	**76(38.0)**	

Key: **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State Teaching Hospital, **MRSA**- Methicillin Resistant *S. aureus*

In NOHE, gender occurrence of biofilm forming MRSA revealed 8(27.6 %) and 4(22.2 %) in accident wound female and burn wound female patient while accident wound male and burn wound male patient recorded 6(18.8 %) and 2(9.5 %) respectively. In ESUTH, biofilm forming MRSA revealed 4(19.0 %) and 2(15.4 %) in accident wound female and burn wound female patient while 5(10.2 %) and 3(17.6 %) was recorded against accident wound male and burn wound male patient as shown in Table 11.

Table 11: Distribution of biofilm forming MRSA in different wound sample isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to Gender.

Location	Wound Sample	Gender	No. of sample	*S. aureus* (%)	MRSA (%)	Biofilm (%)	Non-biofilm (%)
NOHE	Accident	Female	29	21(72.4)	19(65.5)	8(27.6)	11(37.9)
	Burn	Female	18	10(55.6)	6(33.3)	4(22.2)	2(11.1)
	Accident	Male	32	29(90.6)	29(90.6)	6(18.8)	23(71.9)
	Burn	Male	21	16(76.2)	8(38.1)	2(9.5)	6(28.6)
			100	**76(76.0)**	**62(62.0)**	**20(20.0)**	**42(42.0)**
ESUTH	Accident	Female	21	15(71.4)	11(52.4)	4(19.0)	7(33.3)
	Burn	Female	13	7(53.8)	7(53.8)	2(15.4)	5(38.5)
	Accident	Male	49	34(69.4)	25(51.0)	5(10.2)	20(40.8)
	Burn	Male	17	13(76.5)	5(29.4)	3(17.6)	2(11.8)
			100	**69(69.0)**	**48(48.0)**	**14(14.0)**	**34(34.0)**
	Total		**200**	**145(72.5)**	**110(55.0)**	**34(17.0)**	**76(38.0)**

Key: **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State Teaching Hospital, **MRSA**- Methicillin Resistant *S. aureus.*

Accident wound patient from NOHE with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed biofilm forming MRSA prevalence rate of 6(14.6 %), 3(13.6 %) and 5(38.5 %) respectively and burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed biofilm forming MRSA prevalence rate of 2(14.3 %), 3(42.9 %) and 1(33.3 %) respectively. In ESUTH, accident wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed biofilm forming MRSA prevalence rate of 3(13.0 %), 4(12.9 %) and 1(6.6 %) respectively while burn wound patient with wound duration of 1-4weeks, 5-8weeks and 9weeks and above revealed biofilm forming MRSA prevalence rate of 2(11.8 %), 1(20.0 %) and 3(33.3 %) respectively as shown in Table 12.

Table 12: Distribution of biofilm forming MRSA in different wound samples isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to wound duration.

Location	Wound Sample	Duration	No. of sample	*S. aureus* (%)	MRSA (%)	Biofilm (%)	Non-biofilm (%)
NOHE	**Accident**	**1-4weeks**	41	30(73.2)	23(56.1)	6(14.6)	17(41.5)
		5-8weeks	22	19(86.4)	15(68.1)	3(13.6)	12(54.5)
		9weeks and above	13	9(69.2)	9(69.2)	5(38.5)	4(30.8)
			76	**58(76.3)**	**47(61.8)**	**14(18.4)**	**33(43.4)**
	Burn	**1-4weeks**	14	11(78.6)	8(57.1)	2(14.3)	6(42.9)
		5-8weeks	7	5(71.4)	5(71.4)	3(42.9)	2(28.6)
		9weeks and above	3	2(66.7)	2(66.7)	1(33.3)	1(33.3)
			24	**18(75.0)**	**15(62.5)**	**6(25.0)**	**9(37.5)**
			100	**76(76.0)**	**62(62.0)**	**20(20.0)**	**42(42.0)**
ESUTH	**Accident**	**1-4weeks**	23	16(69.6)	13(56.5)	3(13.0)	10(43.5)
		5-8weeks	31	24(77.4)	19(61.3)	4(12.9)	15(48.4)
		9weeks and above	15	7(46.7)	5(33.3)	1(6.6)	4(26.7)
			69	**47(68.1)**	**37(53.6)**	**8(11.6)**	**29(42.0)**
	Burn	**1-4weeks**	17	14(82.4)	7(41.2)	2(11.8)	5(29.4)
		5-8weeks	5	3(60.0)	1(20.0)	1(20.0)	0(0.0)
		9weeks and above	9	5(55.6)	3(33.3)	3(33.3)	0(0.0)
			31	**22(71.0)**	**11(35.5)**	**6(19.4)**	**5(16.1)**
			100	**69(69.0)**	**48(48.0)**	**14(14.0)**	**34(34.0)**

MRSA- Methicillin Resistant *S. aureus*

Distribution of biofilm forming MRSA according to wound characteristic among accident wound patient from NOHE revealed 10(16.9 %) and 3(17.6 %) from pus and dry wound respectively and burn wound patient recorded 4(22.2 %) and 3(50.0 %) MRSA from pus and dry wound respectively. The proportion of biofilm forming MRSA according to wound characteristic among accident wound patient from ESUTH revealed 9 (15.0 %) and 0 (0.0 %) from Pus and dry wound respectively and burn wound patient recorded 3(16.7 %) and 2(15.4 %) biofilm forming MRSA from Pus and dry wound respectively as presented in table 13.

Table 13: Distribution of Biofilm forming MRSA in different wound samples isolated from patient in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to wound characteristic.

Location	Wound Sample	Wound characteristic	No. of sample	*S. aureus* (%)	MRSA (%)	Biofilm (%)	Non-biofilm (%)
NOHE	**Accident**	Pus	59	45(76.3)	38(64.4)	10(16.9)	28(47.5)
		Dry	17	13(76.5)	9(52.9)	3(17.6)	6(35.3)
			76	**58(76.3)**	**47(61.8)**	**13(17.1)**	**34(44.7)**
	Burn	Pus	18	14(77.8)	12(66.7)	4(22.2)	8(44.4)
		Dry	6	4(50.0)	3(50.0)	3(50.0)	0(0.0)
			24	**18(75.0)**	**15(62.5)**	**7(29.2)**	**8(33.3)**
			100	**76(76.0)**	**62(62.0)**	**20(20.0)**	**42(42.0)**
ESUTH	**Accident**	**Pus**	60	42(70.0)	37(61.7)	9(15.0)	28(46.7)
		Dry	9	5(55.6)	0(0.0)	0(0.0)	0(0.0)
			69	**47(68.1)**	**37(53.6)**	**9(13.0)**	**28(40.6)**
	Burn	**Pus**	18	15(83.3)	8(44.4)	3(16.7)	5(27.8)
		Dry	13	7(53.8)	3(23.1)	2(15.4)	1(7.7)
			31	**22(71.0)**	**11(35.5)**	**5(16.1)**	**6(19.4)**
			100	**69(69.0)**	**48(48.0)**	**14(14.0)**	**34(34.0)**

Key: **MRSA**- Methicillin Resistant *S. aureus*

Biofilm forming MRSA from accident wound patients at NOHE demonstrated increase level of resistant to Chloramphenicol 82.4%, Erythromycin 70.6%, Ceftazidime 76.5%, Lincomycin 76.5% but were sensitive to Ciprofloxacin 76.5% and Imipenem 100%. Biofilm forming MRSA from burn wound patients at NOHE were highly resistant to Lincomycin 100%, Clindamycin 100%, Ceftazidime 66.7% but were susceptible to Chloramphenicol 66.7%, Imipenem 100% and Ciprofloxacin 100%. Isolate recovered from accident wound samples at ESUTH exhibit 66.7%, 77.8%, 55.6% resistance to Ceftazidime, Erythromycin and Clindamycin but were susceptible to Vancomycin 44.4%, Ciprofloxacin 66.7% and Imipenem 100%. Biofilm forming MRSA from burn wound patients at ESUTH were extremely resistant to Ceftazidime 80%, Lincomycin 60%, Clindamycin 60% and were susceptible to Imipenem 100%, Ciprofloxacin 100%, Vancomycin 40% as shown in table 14.

Table 14: Antibiotic susceptibility profile of biofilm forming MRSA isolated from different patients wound in National Orthopedic Hospital, Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH).

	NOHE				ESUTH			
	Accident (n=17)		Burn (n=3)		Accident (n=9)		Burn (n=5)	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	13(76.5)	4(23.5)	2(66.7)	1(33.3)	6(66.7)	3(33.3)	4(80.0)	1(20.0)
Imipenem (10)	0(0.0)	17(100)	0(0.0)	3(100)	0(0.0)	9(100)	0(0.0)	5(100)
Erythromycin (15)	12(70.6)	5(29.4)	2(66.7)	1(33.3)	7(77.8)	2(22.2)	3(60.0)	2(40.0)
Lincomycin (15)	13(76.5)	4(23.5)	3(100)	0(0.0)	6(66.7)	3(33.3)	3(60.0)	2(40.0)
Clindamycin (15)	11(64.7)	6(35.3)	3(100)	0(0.0)	5(55.6)	4(44.4)	3(60.0)	2(40.0)
Chloramphenicol (10)	14(82.4)	3(17.6)	1(33.3)	2(66.7)	7(77.8)	2(22.2)	4(80.0)	1(20.0)
Ciprofloxacin (5)	4(23.5)	13(76.5)	0(0.0)	3(100)	3(33.3)	6(66.7)	0(0.0)	5(100)
Vancomycin (30)	10(58.8)	7(41.2)	2(66.7)	1(33.3)	5(55.6)	4(44.4)	3(60.0)	2(40.0)

Key: n-number of isolates**, R-** Resistance**, S**-Susceptible, **NOHE**-National Orthopedic Hospital, **ESUTH**-Enugu State Teaching Hospital.

From NOHE, biofilm forming MRSA from accident wound female patient exhibit 87.5% resistant to Chloramphenicol, Erythromycin 75. 0%, and Vancomycin 50.0% but were susceptible to Lincomycin 50.0%, Ciprofloxacin 62.5% and Imipenem 100% respectively. From burn wound female patient at NOHE majority of the isolate demonstrated 75.0%, 75.0%, 75.0 %, and 25.0 % resistant to Vancomycin, Ceftazidime, Erythromycin and Clindamycin respectively but were sensitive to Imipenem recording 100%. From accident wound male patient, the isolates were susceptible to Ceftazidime 50.0%, Vancomycin 50.0% and Imipenem 100% but were83.3%, 66.7% and 33.3% resistant to Chloramphenicol, Clindamycin and Ciprofloxacin respectively. Among burn wound male patient, the isolates were 100% resistant to Erythromycin, Clindamycin and Lincomycin but were susceptible to Ceftazidime 50.0%, Ciprofloxacin 100% and Imipenem 100%. From ESUTH, biofilm forming MRSA from accident wound female patient exhibit 50.0% resistant to Chloramphenicol, Erythromycin 75.0% and Vancomycin 50.0% but were susceptible to Ciprofloxacin 25.0% and Imipenem 100% respectively. From burn wound female patient at ESUTH majority of the isolate demonstrated 100% resistant to Clindamycin, Erythromycin, Ceftazidime and Lincomycin but were sensitive to Imipenem, Vancomycin and Ciprofloxacin recording 100% respectively. From accident wound male patient, the isolates were susceptible to Ciprofloxacin 80.0%, Clindamycin 40.0% and Imipenem 100% but were 80.0%, resistant to Chloramphenicol and Erythromycin respectively. From burn wound male patient, biofilm forming MRSA isolates were resistant to Ceftazidime 100%, Chloramphenicol 33.3%, Clindamycin 66.7% and Lincomycin66.7% but were susceptible to Ciprofloxacin 100% and Imipenem 100% as shown in Table 15.

Table 15: Antibiotic susceptibility profile of biofilm forming MRSA isolated from different wound patient in National Orthopedic Hospital Enugu (NOHE) and Enugu State University Teaching Hospital (ESUTH) according to Gender.

NOHE	**Female**				**Male**			
	Accident (n=8)		**Burn (n=4)**		**Accident (n=6)**		**Burn (n=2)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	5(62.5)	3(37.5)	3(75.0)	1(25.0)	3(50.0)	3(50.0)	1(50.0)	1(50.0)
Imipenem (10)	0(0.0)	8(100)	0(0.0)	4(100)	0(0.0)	6(100)	0(0.0)	2(100)
Erythromycin (15)	6(75.0)	2(25.0)	3(75.0)	1(25.0)	4(66.7)	2(33.3)	2(100)	0(0.0)
Lincomycin (15)	4(50.0)	4(50.0)	4(100)	0(0.0)	5(83.3)	1(16.7)	2(100)	0(0.0)
Clindamycin (15)	5(62.5)	3(37.5)	4(100)	0(0.0)	4(66.7)	2(33.3)	2(100)	0(0.0)
Chloramphenicol (10)	7(87.5)	1(12.5)	2(50.0)	2(50.0)	5(83.3)	1(16.7)	2(100)	0(0.0)
Ciprofloxacin (5)	3(37.5)	5(62.5)	1(25.0)	3(75.0)	2(33.3)	4(66.7)	0(0.0)	2(100)
Vancomycin (30)	4(50.0)	4(50.0)	3(75.0)	1(25.0)	3(50.0)	3(50.0)	2(100)	0(0.0)

ESUTH	**Female**				**Male**			
	Accident (n=4)		**Burn (n=2)**		**Accident (n=5)**		**Burn (n=3)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	3(75.0)	1(25.0)	2(100)	0(0.0)	3(60.0)	2(40.0)	3(100)	0(0.0)
Imipenem (10)	0(0.0)	4(100)	0(0.0)	2(100)	0(0.0)	5(100)	0(0.0)	3(100)
Erythromycin (15)	3(75.0)	1(25.0)	2(100)	0(0.0)	4(80.0)	1(20.0)	2(66.7)	1(33.3)
Lincomycin (15)	3(75.0)	1(25.0)	2(100)	0(0.0)	3(60.0)	2(40.0)	2(66.7)	1(33.3)
Clindamycin (15)	3(75.0)	1(25.0)	1(50.0)	1(50.0)	3(60.0)	2(40.0)	2(66.7)	1(33.3)
Chloramphenicol (10)	2(50.0)	2(50.0)	2(100)	0(0.0)	4(80.0)	1(20.0)	1(33.3)	2(66.7)
Ciprofloxacin (5)	3(75.0)	1(25.0)	0(0.0)	2(100)	1(20.0)	4(80.0)	0(0.0)	3(100)
Vancomycin (30)	2(50.0)	2(50.0)	0(0.0)	2(100)	4(80.0)	1(20.0)	2(66.7)	1(33.3)

In NOHE, biofilm forming MRSA from patients with wound duration of 1-4weeks demonstrated resistant to Chloramphenicol 83.3 %, Clindamycin 83.3 % and Vancomycin 66.7 % but were sensitive to Ceftazidime 50.0 %, Ciprofloxacin 66.7 %, and Imipenem 100 %. Among 5-8weeks wound duration, the isolates were highly resistant to Lincomycin 100%, Ceftazidime 66.7 %, and Vancomycin 66.7 % but were susceptible to Erythromycin 33.3 % Imipenem 100 % and Ciprofloxacin 66.7%. Isolate recovered from wound duration 9weeks and above exhibited 80.0 % resistance to Erythromycin, Ceftazidime and Chloramphenicol while Ciprofloxacin and Imipenem were 80.0 % and 100 % effective against the isolates. While for burn wounds, biofilm forming MRSA from patients in National Orthopaedic Hospital Enugu (NOHE) with wound duration of 1-4weeks demonstrated resistant to Chloramphenicol 100 %, Erythromycin 100 %, Clindamycin 50.0 % and Vancomycin 50. 0 % but were sensitive to Ciprofloxacin 100 %, and Imipenem 100 %. Among 5-8weeks duration, the isolates were resistant to Clindamycin 100 %, Lincomycin 100%, Ceftazidime 66.7% and Vancomycin 66.7% but were susceptible to Ciprofloxacin

66.7%, Erythromycin 100 % and Imipenem 100 %. Biofilm forming MRSA recovered from wound duration of 9weeks and above were extremely resistance to Vancomycin, Erythromycin, Ceftazidime, Clindamycin and Chloramphenicol recording 100 % but were sensitive to Ciprofloxacin and Imipenem recording 100 % as shown in Table 16.

Table 16: Antibiotic susceptibility profile of biofilm forming MRSA isolated from accident and burn wound patient in National Orthopaedic Hospital, Enugu (NOHE) according to wound duration.

NOHE (ACCIDENT)	**1-4weeks (n=6)**		**5-8weeks (n=3)**		**9weeks and above (n=5)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	3(50.0)	3(50.0)	2(66.7)	1(33.3)	4(80.0)	1(20.0)
Imipenem (10)	0(0.0)	6(100)	0(0.0)	3(100)	0(0.0)	5(100)
Erythromycin (15)	4(66.7)	2(33.3)	2(66.7)	1(33.3)	4(80.0)	1(20.0)
Lincomycin (15)	4(66.7)	2(33.3)	3(100)	0(0.0)	4(80.0)	1(20.0)
Clindamycin (15)	5(83.3)	1(16.7)	2(66.7)	1(33.3)	3(60.0)	2(40.0)
Chloramphenicol (10)	5(83.3)	1(16.7)	1(33.3)	2(66.7)	4(80.0)	1(20.0)
Ciprofloxacin (5)	2(33.3)	4(66.7)	1(33.3)	2(66.7)	1(20.0)	4(80.0)
Vancomycin (30)	4(66.7)	2(33.3)	2(66.7)	1(33.3)	3(60.0)	2(40.0)

NOHE (BURN)	**1-4weeks (n=2)**		**5-8weeks (n=3)**		**9weeks and above (n=1)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	1(50.0)	1(50.0)	2(66.7)	1(33.3)	1(100)	0(0.0)
Imipenem (10)	0(0.0)	2(100)	0(0.0)	3(100)	0(0.0)	1(100)
Erythromycin (15)	2(100)	0(0.0)	0(0.0)	3(100)	1(100)	0(0.0)
Lincomycin (15)	2(100)	0(0.0)	3(100)	0(0.0)	1(100)	0(0.0)
Clindamycin (15)	1(50.0)	1(50.0)	3(100)	0(0.0)	1(100)	0(0.0)
Chloramphenicol (10)	2(100)	0(0.0)	1(33.3)	2(66.7)	1(100)	0(0.0)
Ciprofloxacin (5)	0(0.0)	2(100)	1(33.3)	2(66.7)	0(0.0)	1(100)
Vancomycin (30)	1(50.0)	1(50.0)	2(66.7)	1(33.3)	1(100)	0(0.0)

Key: n-number of isolates**, R-** Resistance**, S**-Susceptible, **NOHE**-National Orthopedic Hospital.

In Table 17, Biofilm forming MRSA from accident patients in Enugu State University Teaching Hospital (ESUTH) with wound duration of 1-4weeks showed resistant to Erythromycin 66.7 %, Chloramphenicol 33.3 %, Lincomycin 100 % and Clindamycin 66.7 % but were sensitive to Ciprofloxacin 100 %, and Imipenem 100 % while among 5-8weeks wound duration, the isolates were resistant to Erythromycin 75.0 %, Clindamycin 100 %, Lincomycin 100%, Ceftazidime 50.0 % and Vancomycin 75.0 % but were susceptible to Ciprofloxacin 75.0 % and Imipenem 100 %. Biofilm forming MRSA strain isolated from wound duration 9weeks and above were resistance to Clindamycin, Ceftazidime, Vancomycin, Erythromycin and Chloramphenicol recording 100 % but were sensitive to Ciprofloxacin and Imipenem recording 100 %. While for burn wound ESUTH, biofilm forming MRSA from burn patients with wound duration of 1-4weeks showed resistant to Ceftazidime 50.0 %, Clindamycin 50.0 %, Erythromycin 100% and Lincomycin 100 %, but were sensitive to Chloramphenicol 50. 0 %, Vancomycin 100 %, Ciprofloxacin 100 %, and Imipenem 100 % while among 5-8weeks wound duration, the isolate were resistant to Chloramphenicol 100%, Erythromycin100%, Clindamycin 100 % and Lincomycin 100%, but were susceptible to Vancomycin 100 %, Ciprofloxacin 100 % and Imipenem 100 %. Biofilm forming MRSA strain isolated from wound duration 9weeks and above were resistance to Vancomycin 66.7 %, Ceftazidime 66.7 %, Clindamycin 100 %, and Erythromycin 66.7 % but were sensitive to Ciprofloxacin 100 % and Imipenem 100 %.

Table 17: Antibiotic susceptibility profile of biofilm forming MRSA isolated from accident and burn wound patient in Enugu State University Teaching Hospital (ESUTH) according to wound duration.

ESUTH (ACCIDENT)	**1-4weeks (n=3)**		**5-8weeks (n=4)**		**9weeks and above (n=1)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	2(66.7)	1(33.3)	2(50.0)	2(50.0)	1(100)	0(0.0)
Imipenem (10)	0(0.0)	3(100)	0(0.0)	4(100)	0(0.0)	1(100)
Erythromycin (15)	2(66.7)	1(33.3)	3(75.0)	1(25.0)	1(100)	0(0.0)
Lincomycin (15)	3(100)	0(0.0)	4(100)	0(0.0)	1(100)	0(0.0)
Clindamycin (15)	2(66.7)	1(33.3)	4(100)	0(0.0)	1(100)	0(0.0)
Chloramphenicol (10)	1(33.3)	2(66.7)	3(75.0)	1(25.0)	1(100)	0(0.0)
Ciprofloxacin (5)	0(0.0)	3(100)	1(25.0)	3(75.0)	0(0.0)	1(100)
Vancomycin (30)	2(66.7)	1(33.3)	3(75.0)	1(25.0)	1(100)	0(0.0)
ESUTH (BURN)	**1-4weeks (n=2)**		**5-8weeks (n=1)**		**9weeks and above (n=3)**	
Antibiotics (µg)	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ceftazidime (30)	1(50.0)	1(50.0)	1(100)	0(0.0)	2(66.7)	1(33.3)
Imipenem (10)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)	3(100)
Erythromycin (15)	2(100)	0(0.0)	1(100)	0(0.0)	2(66.7)	1(33.3)
Lincomycin (15)	2(100)	0(0.0)	1(100)	0(0.0)	2(66.7)	1(33.3)
Clindamycin (15)	1(50.0)	1(50.0)	1(100)	0(0.0)	3(100)	0(0.0)
Chloramphenicol (10)	1(50.0)	1(50.0)	1(100)	0(0.0)	1(33.3)	2(66.7)
Ciprofloxacin (5)	0(0.0)	2(100)	0(0.0)	1(100)	0(0.0)	3(100)
Vancomycin (30)	0(0.0)	2(100)	0(0.0)	1(100)	1(33.3)	2(66.7)

Key: n-number of isolate**, R-** Resistance**, S-**Susceptible, **ESUTH**-Enugu State Teaching Hospital.

Multiple Antibiotic Resistant Index (MARI) of Biofilm forming Methicillin Resistant *Staphylococcus aureus* (MRSA) is presented below in Table 18. All the strains of biofilm forming MRSA from different sample sources demonstrated multidrug resistant with MARI value ranging between 0.3 - 0.7.

Table 18: Multiple Antibiotic Resistance Index of biofilm forming Methicillin Resistant *Staphylococcus aureus* (MRSA) in different wound samples isolated from patients in National Orthopaedic Hospital, Enugu (NOHE) and Enugu State Teaching Hospital (ESUTH).

Location	**Wound Sample**	**Gender**	**No of Samples**	**MARI**
NOHE	Accident	Male	6	0.7
		Female	8	0.4
	Burn	Male	5	0.3
		Female	6	0.6
ESUTH	Accident	Male	5	0.5
		Female	9	0.3
	Burn	Male	4	0.7
		Female	7	0.5

Key: **MARI-** Multiple Antibiotic Resistance Index, **NOHE**-National Orthopaedic Hospital, Enugu, **ESUTH**-Enugu State Teaching Hospital.

DISCUSSION:

In this study, *S. aureus* was identified in 72.5% of wound samples from NOHE and ESUTH; the high rate of the *S. aureus* colonization substantiates the report from earlier studies in Sudan 76.0% (Yousif *et al.*, 2021), Nepal 56.9% (Upreti *et al.*, 2018), Egypt 45.1% (Rasmi *et al.*, 2022), Italy 79.4% (Puca *et al.*, 2021), Adamaoua region 52.9% and far northern Cameroon47.1% (Mohamadou *et al.*, 2022) and also Kano in Nigeria with 36% and 41.9% (Abdulrahim *et al.*, 2019; Oche *et al.*, 2020). They observed high frequency of *S. aureus* which connotes that it is a major pathogen of wound infection. In addition, the colonization of this strain could be linked to poor level of personal hygiene and susceptibility to infection among most patient whose immune system are compromised.

Accident wounds in NOHE and burn wound in ESUT were more prevalence with *S. aureus*, similar to other studies (Shittu *et al.*, 2002; Kihla *et al.*, 2014; Rasmi *et al.*, 2022). Such heterogeneity could be due to direct topical application of antimicrobials to the infection site which might have affected growth of bacteria from this site noted in the study area.

In NOHE, *S. aureus* accounted for 79.2% in wounds from accident than wounds from burn. The variation could be attributed to the number of samples collected during the period of the study. On the other hand, wounds from burn samples gotten from ESUTH had 76.2 % of *S. aureus* isolates over wounds from accident. Hence, most frequent isolation of *S. aureus* from burn wounds might also be due to contamination of collected specimens with skin normal flora due to burnt disruption of the skin's protective layer, allowing successful proliferation of these opportunistic bacteria.

The incidence of *S. aureus* in male patient from NOHE (90.6%, 72.6%) and ESUTH (69.4 %, 76.5 %) was higher than Female. Similar trend was noted in earlier study where *S. aureus* was much higher in males [n = 36 (70.6%)] than in females [n = 15 (29.4%)] (Rasmi *et al.*, 2022), in yet another study male accounted for 71% of total cases (Yousif *et al.*, 2021). Muluye *et al.* (2014) stated that the prevalence of *S. aureus* in male and female was 38.1% and 28.7%, respectively. From our knowledge, male gender has been considered to be a risk factor for infection following trauma (Offner *et al.*, 1999; Yousif *et al.*, 2021) and for SSIs (Langelotz *et al.*, 2014; Al-Qurayshi *et al.*, 2018). Also, Zhang *et al.* (2018) emphasized that differences in infection rate between male and female can occur due to anatomical sites, health behaviors, environmental experiences, stress and exposure to risk. In this study, lower number of female patients might be due to small sample size as compared to other studies (Rajput *et al.*, 2008; Upreti *et al.*, 2018) were female patients outnumbered the male patients.

According to wound duration, similar incidence of *S. aureus* was noted in NOHE (accident 5-8weeks, burn 1-4weeks) and ESUT (accident 5-8weeks, burn 1-4weeks). Notably with 1-4weeks the explanation for increased incidence of *S. aureus* may be as follows; in the initial phase of infections, within the first week, Gram-positive bacteria, especially *S. aureus* strains, appear to be the most frequent colonizers (Huszczynski *et al.*, 2019; Puca *et al.*, 2021). From the beginning of the second week, Gram-negative bacteria, such as *P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *A. baumannii*, start to colonize the wound, provoking sepsis if they enter the lymphatic system and blood vessels (Puca *et al.*, 2021; Hassan *et al.*, 2019, Zhou *et al.*, 2019). After 5-8weeks, the action of antibiotic, lack of nutrients and moisture in the skin may be pivotal factors in diminishing the proliferation of fastidious bacteria, thereby increasing the incidence of *S. aureus* that are a typical component of normal skin microbial flora which may get access into the wound easily. Regardless of the kind of wound, wound infections are commonly related to morbidity and 0–80% of patient's mortality in developing countries (Upreti *et al.*, 2018; Hassan *et al.*, 2022).

S. aureus found in wound producing pus was high over dry wound surface and it is in tandem with report from other researchers (Bowler *et al.*, 2001; Parikh *et al.*, 2007; Upreti *et al.*, 2018). Generally, pus samples are considered as sample of choice from deep seated and closed wound infections. Nevertheless, wound exudates put damaged skin at risk of colonization of pathogenic microorganisms compared to dry wound surface which may be deficient in moisture and nutrient availability.

In this study, the overall prevalence of MRSA was 55.0% and was similar to a study from Kano, Nigeria that reported high prevalence of MRSA (67.9%) in orthopedic patients, (61%) in Iran (Ghaznavi-Rad, and Ekrami, 2018), Egypt 91.5% (Rasmi *et al.*, 2022), but disagree with low prevalence reported in Northeast Ethiopia 9.8% (Tsige *et al.*, 2020), Eritrea 35.6% (Garoy *et al.*, 2019), and Cameroon (13.16%) (Bissong *et al.*, 2016). The noted high occurrence MRSA in this study may be due to the high rate of certain antibiotics use either due to availability or cost-effectiveness issues. Regarding the possible associated risk factors, MRSA wound infections,

their occupation may expose patients to wound infection and make them use antibiotics without prescription.

High prevalence of MRSA may be attributed by resistant strain bacterial cross-contamination in health institutions. Also, healthy people may carry MRSA asymptomatically for long periods of time, but patients with compromised immune system are at a significantly greater risk of symptomatic infections (Holmes *et al.,* 2005; Zakour *et al.,* 2008; Tsige *et al.,* 2020). The increase of MRSA in wound infections among this patient has contributed to high treatment costs and longer hospital stays, which have major implications for infection management, particularly in study setting.
Phenotypic detection of biofilm formation using Tube method accounted 17.0% in MRSA. This was lower than those reported by earlier studies were 50%, 52.7% and 76.02% of MRSA strain were biofilm producers (Abdulrahim *et al.,* 2019; Coraça-Huber *et al.,* 2020; Omidi *et al.,* 2020). The variation may be due to different method of biofilm assay employed by most researchers. Nevertheless, no biofilm assay method has well been established to be the best or more sensitive. Biofilm production noted in this study could have been the mainstay of prolong hospitalization and wound chronicity among these patients.

The incidence of biofilm forming MRSA in accident wound from both NOHE 22.1 % and ESUTH 15. 5% outnumber the burn wound patient. It could be the cause of prolong hospitalization noted among accident wound patient during this study.

The notable strain sensitivity to chloramphenicol 66.7 % among wound patient could be linked to a lower use of these antibiotics due to their shortage availability in the market, high costs, and toxic side effects. The results obtained showed a high resistance rates of the species isolated to vancomycin 50.0-100% and it substantiate with data from earlier studies (Garoy*et al*., 2019; Chelkeba and Melaku , 2021; Tania *et al.,* 2021) but were enough difference when compared to the sensitivity data in literature reported elsewhere (Mohammed *et al.*. 2017; Tsige *et al.,* 2020; Yousif *et al.,* 2021; Rasmi *et al.* 2022). The variation of resistance and susceptible rate among studies indicates that resistance susceptibility pattern of antibiotics varies according to regional and geographical location and also changes over time.

Importantly, this study reported high resistance to third generation cephalosporine; ceftazidime as similar pattern of resistance was noted among wound patient in Egypt, Mexico, Tehran and Nigeria (Yousif *et al.,* 2021; Uribe-García *et al.,* 2020; Mohammadi *et al.,* 2020; Ariom *et al.,* 2019). Cephalosporines prophylaxis is commonly used in surgery practices in most hospitals in Sudan, in addition to the usage for treatment of sepsis, acute pneumonia and post-operative situations. Therefore, continuous exposure of bacteria such as *S. aureus* to cephalosporin's and hospital over-prescription may be enrolled as prediction for failure of treatment (Abbas *et al.,* 2017; Ahmed *et al.,* 2019; Hamid *et al.,* 2020).

Frequent use of certain antibiotic such as Erythromycin, Lincomycin, Clindamycin could be an indication of high rates of resistance noted among the aforementioned antimicrobial agent. It is not surprising that in recent time's resistance to these agents are common in biofilm forming MRSA strain (Garoy *et al*., 2019; Tsige *et al*., 2020; Uribe-García *et al*., 2020; Gaire *et al*., 2021) and may be due to increased consumption of a particular class of antibiotics, resulting expression of inducible *erm* gene due to low inhibition of ribosomal translocation. As a result, they are no longer effective in treating wound infections.

Among the multi-drug resistant strains this study found some resistant to two or more different antimicrobials agent with MARI of 0.5-0.7 was observed. In this context, similar trend of MDR results has been found elsewhere (Azmi *et al.,* 2019; Ali and Seiffein, 2022). Therefore, in addition to being characterized by a multi-drug resistance, the capability of these strains to produce biofilm makes the therapeutic treatment more difficult. The rate of similar MDR bacteria in wounds sample in NOHE and ESUT might be explained by a number of variables, including demographics, age differences, gender, hospitalization length, and prior antibiotic treatment. Furthermore, hospitalization may substantially impact the prevalence and kind of MDR bacteria since patients are at risk of cross-infection with nosocomial infections that withstand some prescription antibiotics.

The presence of multi-drug resistant strains, the variability of the biofilm composition, its tolerance towards the antibiotics, as well as the possible wound polymicrobial nature of biofilms disseminating different resistant determinant, suggest the need for multi-target or combinational approaches in biofilm treatment.

Surprisingly, biofilm forming MRSA was sensitive to ciprofloxacin and imipenem. Unlike most of the studies in literature, ciprofloxacin and imipenem was

not detected among the most resisted antibiotics 47.9–100% (Ariom *et al.*, 2019; Upreti *et al.*, 2018; Abdullahi and Iregbu, 2019; Puca *et al.*, 2021). Their good susceptibility to ciprofloxacin and imipenem makes it effective agent for biofilm forming MRSA in wounds.

CONCLUSION:

In the current research, biofilm forming MRSA was reported in accident and burn types of wounds 17.0 %. The prevalence of biofilm forming MRSA was high among NOHE patients 20.0 % with various wound infection. MRSA resistance profile was high against vancomycin, Ceftazidime and clindamycin. Multiple factors may contribute to rapid development of antimicrobial resistance by pathogens including misuse, overuse, and underuse of antimicrobials by both clinicians and patients. But as well noted, all isolates (100%) of biofilm forming MRSA were sensitive to ciprofloxacin and imipenem. This study emphasizes the importance of strict nosocomial infection control strategies and careful prescription of antimicrobials by clinicians in the health care centers.

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