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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN BURNS AND PRESSURE ULCER PATIENTS

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ABSTRACT

Methicillin resistant Staphylococcus aureus (MRSA) is a multidrug resistant bacterium that threatens the continued effectiveness of antibiotics worldwide. The objective of this study was to investigate the prevalence of MRSA and its antibiotic susceptibility pattern in patients with burns and bedsore. This was a cross- sectional study that was carried out at National Orthopaedic Hospital, Enugu, Nigeria. A structured questionnaire was used to obtain information on demographic and source of wounds. Pus from the wound was collected with swab sticks or 2ml syringe and analyzed bacteriologically, using mannitol salt agar sheep red cell blood agar. Isolates of Staphyloccus aureus were subjected to oxacillin and cefoxitin disc-diffusion assay and confirmed by chromogenic Brilliance MRSA 2 Agar; for identification of MRSA and MSSA. The MRSA and MSSA strains were tested for antimicrobial susceptibility patterns and multiple antibiotic index calculated. Of 104 wound swabs analyzed, 52 (50%) were Staphylococcus aureus isolates, while 21 (20.2%) were MRSA and 31 (29.8%) were MSSA. No significant differences were observed in the prevalence of MRSA among gender, duration of wounds, wound dressing interval and source of wound. There was an association between age, prolonged hospital admission MRSA infection. Methicillin-resistant Staphylococcus aureus isolates showed high resistance to ampicillin 90.5% followed by erytromycin 81% and ciprofloxacillin 71.4%. All the MRSA isolates were susceptible to vancomycin. All isolates of MRSA were resistant to β-lactams, aminoglycosides and quinolones group of antibiotic used. Minimum Inhibitory Concentration of vancomycin showed that the break point was between 0.5-2µg/ml and that of ampicillin was ranges from 4 µg/ml-128 µg/ml. MAR Index was >0.2 which indicates the resistance emanates from hospital. The high prevalence of MRSA and antibiotics resistance may increase the disease burden amongst these patients. It is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in the hospitals and community settings, to prevent the spread of MRSA.

Keywords: MRSA, Brillience ChromAgar, ampicillin, vancomycin, multiple antibioticindex,

LA PREVALENCE ET LA MODELE DE SENSIBILITE AUX ANTIBIOTIQUES DE *STAPHYLOCOCCUS AUREUS* RESISTANT A LA METHICILLINE CHEZ LES PATIENTS BRULES ET LES PATIENTS ATTEINTS D'ULCERE DE PRESSION.

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RESUME:

Staphylococcus aureusrésistant à la Méthicilline (MRSA) est une bactériemulti résistant qui présente une menace pour l'efficacité des antibiotiques dans le monde entier. Le but de cette étudeétait d'enquêter sur la prévalence de MRSA et sa modèle de sensibilité aux antibiotiques chez les patients brulés escarres. Ce fut une étude transversale qui a étéréalisée à l'hôpitalorthopédique national, Enugu, Nigeria. Un questionnaire structuréàétéutilisé pour obtenir l'informations sur démographique et source de blessures. Le pus de plaies a été recueilli avec bâtons d'écouvillons ou une seringue de 2ml et analysébacteriologiquement, utilisant Mannitol agar moutons de sel globules rouges gélose au sang. Isolats de Staphylococcus aureus ont été exposés au oxacilline et cefoxitine test de diffusion sur disque et a confirmé par Brillance MPSA 2 Agar chromogène; pour l'identification de MRSA et MSSA. Les souches de MRSA et MSSA ont été analysées pour la modèle de

sensibilité antimicrobien et l'indice multiples antibiotiques calculés. Sur 104 des tampons enroulésanalysés, 52 (50%) étaient souches de *Staphylococcus aureus*, alors que 21 (20,2%) étaient MRSA et 31 (29,8%)étaient MSSA. Aucunesdifférencesconsidérables n'ont été observées dans la prévalence de MRSA parmi le sexe, la durée des plaies, l'intervalle de pansement et la source de plaie. Il y avait une association entre l'âge, l'hospitalisationprolongée d'infection MRSA. Les souches de *Staphylococcus aureus*résistants à la Méthicilline montraient une résistance plus élevéeà l'ampicilline 90,5% suivi par érythromycine 81% et ciprofloxacilline 71,4%. Tous les isolats de MRSA étaientprédisposés à vancomycine. Toutes les isolats de MRSA étaientrésistants au β – lactamines, les Aminoglycosides et le groupe des antibiotique quinolones utilisées. L'inhibitrice minimale de la concentration de vancomycine a montré que le point de rupture était 0,5 - 2μg/ml et celle d'ampicilline variait de 4μg/ml à 128μg/ml. L'indice MAR était >0,2 qui indique que la résistanceémane de l'hôpital. La haute prévalence de MRSA et la résistance des antibiotiques peut augmenter le fardeau de la maladie chez les patients. Il est nécessaire d'établir le système de surveillance de la sensibilité aux antimicrobiens et d'améliorer les programmes courants pour contrôler l'infection aux hôpitaux et milieux communautaires pour éviter la propagation de MRSA.

Mots - clés: MRSA,Brillance chrom Agar, l'ampicilline, vancomycine, multiples antibiotiques, l'indice.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that have acquired the ability to grow in the presence of methyl penicillin derivatives, including methicillin, oxacillin and nafcillin (1). The first report of methicillin-resistant Staphylococcus aureus (MRSA) was in 1961 after the introduction of methicillin in clinical settings (2). Subsequently, the spread was observed globally both as hospital acquired (HA-MRSA) and community acquired (CA-MRSA) infection within the population without any apparent risk factor (3). MRSA have proven particularly difficult to treat because they possess antimicrobial resistance gene known as mecA (4). Staphylococcal cassette chromosome mec (SCCmec) is a genomic island of unknown origin containing the antibiotic resistance gene mecA which is responsible for resistance to methicillin and other β lactam antibiotics (5,6)

The reasons for the emergence of MRSA are multifactorial and can be attributed to host factors, infection control practices and antimicrobial pressures (7). Eileen and Venezia (7) attributed the use of levofloxacin and macrolides as promoters of MRSA spread within hospital environment. Hospital associated MRSA isolates often show multiple resistances to other commonly used antimicrobial agents, including quinolones, aminoglycosides, erythromycin, clindamycin, co-trimaxole and tetracycline (8,9).

Burn wound infection is a major complication in burn patients after initial period of shock and the chance of infection persist until complete wound healing (10). Thermal injury destroys the skin barriers that normally prevent invasion by microorganisms (4,11,12). The removal of epithelial layer of the skin in burns enhances a suitable site for bacterial colonization and multiplication and presents a more persistent and richer source of infection than surgical wound (13).

Pressure ulcers are more common among patients who are immobilized because of injury, acute illness or sedation. Their immobile state does not have to occur for long for bed sores to develop and the prevalence is particularly high in hospital setting especially in critical care unit (14). The nature of burn or bedsore involves removal of epithelial barrier. Patients with extensive burn injuries or sore are especially susceptible to infections with MRSA due to loss of the skin barrier prolonged antibiotic therapy and reduced immunological capacity such that the T cells cannot reach sites of infection (15). MRSA infections in burn or bedsore patients are classified as a secondary infection because of the traumatized skin (16).

Infections of these wounds may lead to systemic conditions such as septicaemia, pneumonia, endocarditis, deep-seated abscesses or multiple organ dysfunction syndrome (4). Transmission of organisms can be from the patient's own skin, gut and respiratory flora. Healthcare provider-to-patient transfer is common, especially when healthcare providers move from patient to patient without performing necessary hand-washing techniques inbetween patients (17). The objective of this study were in two fold; to assess the prevalence of methicillin resistant *staphylococcus aureus* in burns/bed sore patients and determine the patterns of antibiotic resistance.

MATERIALS AND METHODS

Study Area: The patients were recruited from National Orthopedic Hospital Enugu. This is a tertiary health care institution with 701 bed spaces that is dedicated to handling of trauma, burns and accident victims. The hospital is a regional center for burns and serves the whole of south east and south-south geographical region of Nigeria.

Study population: The subjects for this study were patients that had several degrees of burns and

patients that had developed bedsore due to prolonged illness. These patients were chosen because of long stay in hospital and have been administered with several antibiotics both topically, orally or injection. Those patients with burns and have not stayed a minimum of 30 days in the hospital were not selected. Those that cannot afford the hospital expenses but comes from their homes for treatment and dressing were included in the study. The participants were administered with a structured questionnaire to obtain information on demographic characteristics and risk factors that gave rise to the initial injury. Information was obtained either orally or via their medical records.

Ethical Consideration: Ethical Commitee of National Orthopaedic Hospital Enugu, Nigeria reviewed and approved the study protocol. The patients endorsed Informed Consent Forms and participation was voluntary.

Collection of samples: The surface of wound was first cleaned with a disinfectant to eliminate surface contaminants. The sides of the tissue surrounding the wound were carefully pressed with hand until pus comes out. This was then collected with a sterile swab or a 2ml syringe. The pus samples were collected in batches and were immediately transported to the laboratory for bacteriological analysis.

Bacteriological Techniques: Swab samples and/or pus were aseptically inoculated into Blood agar (enriched with 10% sheep red blood) and Mannitol Salt Agar plates and then incubated aerobically at 37°C for 24 hours. The cultures were examined and those growth were identified using typical morphological characteristics biochemical and methods. Isolates that were Gram positive cocci in clusters, catalase positive and coagulase positive were confirmed as S. aureus.

Identification of MRSA: The *S aureus* isolates were subjected to secondary culture and antibiotic sensitivity to identify the MRSA and methicilin sensitive *Staphyloccus aureus* (MSSA) strains.The confirmatory antibiotics used were: Oxacillin disk (1 μg) and Cefoxitin disk (30μg),and culturing on Brilliance MRSA 2 Agar plate (oxoid, UK).

Antibiotic susceptibility test: All the MRSA and MSSA isolates were tested for antibiotic susceptibility by the disk diffusion method according to the Clinical Laboratory Standard Institute (CLSI) guidelines (2012). The tested antibiotics were gentamicin (10μg), erythromycin (15μg), ciprofloxacin (5μg), levofloxacin (5μg), Rifampicin (2μg) clindamycin (2μg),

Ceftriaxone (30 μ g), Ampicillin (10 μ g), and vancomycin (30 μ g).

Minimum inhibitory concentrations (MIC): The MIC was done using ampicillin and vancomycin. Preparation of antibiotic stock solutions was according to CLSI and antibiotic dilution range of 0.06-32 mg/L for vancomycin and 0.03-128 mg/L for ampicillin was prepared using sterile Muller Hinton broth in serial dilution. Macrodilution method was used for the MIC.

Multiple Antibiotic Resistance index (MAR): Isolates resistant to the tested antibiotics in at least three of the following classes: β -lactams, aminoglycosides and quinolones group of antibiotics were considered multi drug resistant. The MAR index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistance and b represents the number of antibiotics to which the isolates was subjected to.

RESULTS

Patients with burns and bedsore wounds recruited for this study, comprised of 104 subjects. The males were 53(50.9%) and female were 51(49.0%); with a mean age of 36.6 ± 20.7 (range1-86 year-olds).Out of 104 wound swab/pus, 52 (50%) *S. aureus* isolates were obtained, and 21(20.2%) were identified as MRSA while 31(29.8%) were MSSA.

Characteristics of patients: The MRSA isolates were more in females than the males with 11(10.6%) and 10(9.6%) respectively. The presence of MRSA was independent of sex. The MRSA were obtained from all age groups with the highest 7(6.7%) from age group of 21-40 and 61 and above respectively. The age group of ≤20 and 41-60 years had 3(2.9%) and 4(3.8%) MRSA isolates respectively. The duration of wound though not statistically significant shows that patients that had suffered the infection within 1-2 vears had the highest MRSA isolates, 12(11.5%). Of the 70 patients with burns, 14 (13.5%) had MRSA isolates while 34 patients with bedsore, 7(6.7%) had MRSA. The analysis of wound dressing indicates that the interval for routine dressing of wound as chosen by individuals or family was not statistical significant. p=0.504.To assess whether the MRSA was hospital acquired or community acquired, the status of the patients in respect to hospital admission were analysed. Those that were on admission for 6months or more were 51 of which 15 (14.4%) were positive for MRSA while those that were on admission for less than 6months were 53 of which 6(5.8%) were MRSA. There was an association between hospitalization and MRSA infection (p=0.022)(Table 1)

TABLE 1.CHARACTERISTIC OF PATIENTS WITH BURNS AND BEDSORE WOUNDS

VARIABLES	Total no. of subjects (n)	MRSA (%)	MSSA(%)	P-Value
SEX:				0.732
Male	53	10 (9.6)	17 (16.3)	
Female	51	11 (10.6)	14 (13.5)	
AGE:				0.023
≤ 20	27	3 (2.9)	6 (5.8)	
21-40	32	7 (6.7)	10 (9.6)	
41-60	26	4 (3.8)	6 (5.8)	
61-above	19	7 (6.7)	9 (8.7)	
DURATION OF WOUND (MONTHS):				0.078
<1	38	4 (3.8)	7 (6.7)	0.070
1-2	53	12 (11.5)	20 (19.2)	
>2	13	5 (4.8)	4 (3.8)	
TYPE OF WOUND:				0.297
Burns	70	14 (13.5)	20 (19.2)	
Bedsore	34	7 (6.7)	11 (10.6)	
INTERVAL FOR WOUND DRESSING:				0.504
Daily	22	5 (4.8)	0	
2days	31	5 (4.8)	9 (8.7)	
3days	28	8 (7.7)	12 (11.5)	
4days	23	3 (2.9)	10 (9.6)	
HOSPITAL ADMISSION:				
≥ 6months				0.022
< 6months	51	15 (14.4)	19 (18.3)	
	53	6 (5.8)	12 (11.5)	

Sources of wound: The sources of injury through which the patients got the wound varied from patients to patients. The patients with home accidents such as hot water, home fire were 19 and 14 of which 6(5.8%) and 3(2.9%) had MRSA isolates respectively. Twenty-five patients that had injury due to car-(road) accident, 2(1.9%) with burns had MRSA while 3(2.9%) with bedsore had MRSA. Source of wound was not statistical significant, p=0.904 (Table 2)

Prior antibiotic use: The antibiotic usage among the patients was assessed to know the type of antibiotics

consistently used as prescribed by the physicians. Ceftriazone with frequency of 98(16.3%) followed by Augmentin 84(14.5%) and Metronidazole 80(13.8%) were mostly prescribed. The least prescribed was Imipenem 3(0.5%). Patients are usually encouraged to combine drugs with metronidazole in very severe cases and as first line therapy. Antibiotics gels like gentamicin, penicillin ointment and other gels containing antibiotics were consistently used on the patients, especially those with burns (Table 3).

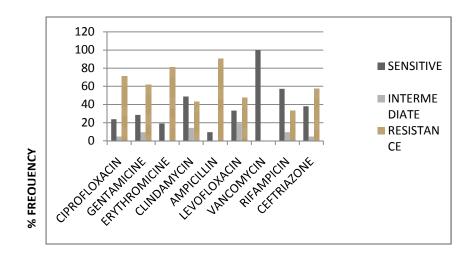
TABLE 2: SOURCES OF WOUNDS AMONG THE SUBJECTS

Variables	Total no. of subjects (n)	BURNS (n = 70)		BEDSORE (BEDSORE (= 34)	
		MRSA (%)	MSSA (%)	MRSA (%)	MSSA (%)	
Accident (road)	25	2 (1.9)	3 (3.9)	3 (2.9)	5 (4.8)	
Home fire	14	3 (2.9)	4 (3.8)	0 (0)	0 (0)	
Acid bath	4	0 (0)	0 (0)	0 (0)	0 (0)	
Industrial fire	9	2 (1.9)	3 (2.9)	0 (0)	0 (0)	
Hot water	19	6 (5.8)	5 (4.8)	0 (0)	0 (0)	
Fuel/gas burn	8	1 (1.9)	3 (2.9)	0 (0)	0 (0)	
Hot metal	19	0 (0)	0 (0)	0 (0)	0 (0)	
Eletric burn	2	0 (0)	2 (1.9)	0 (0)	0 (0)	
Other chemical than acid	3	0 (0)	0 (0)	0 (0)	0 (0)	
Paralysis (spinal injury/stroke)	5	0 (0)	0 (0)	2 (1.9)	2 (1.9)	
Other sickness (chronic diabetes)	14	0 (0)	0 (0)	2 (1.9)	4 (3.8)	
	104	14 (13.5)	20 (19.5)	7 (6.7)	11 (10.6)	

TABLE 3: FREQUENCY OF PRIOR ANTIBIOTICS THERAPY BY THE PATIENTS

Antibiotics therapy	Frequency	Percentage
Augmentin	84	14.5
Ceftriatriazone	98	16.9
Ciprofloxacillin	61	10.6
Clindamycin	65	11.2
Gentamicin	67	11.6
Ampiclox	40	6.9
Levofloxacillin	50	8.7
Cotrimaxole	10	1.7
Metronidazole	80	13.8
Erythromycin	20	3.5
Imipenem	3	0.5
Total	578	100

Susceptibility pattern: The MRSA isolates exhibited multi resistance pattern as the 9 selected antibiotic showed different variation of resistance to the MRSA isolates. The antibiotic vancomycin was sensitive to all MRSA isolates. The highest resistance was Ampicillin with 19(90.5%) MRSA isolates. This was followed by Erythromycin, Ciprofloxacillin and Ceftriazone with 17(81.0%), 15(71.4%) and 12(57.2%) respectively (Fig 1a). The antibiotic susceptibility pattern was compared to MSSA and almost the same pattern were observed on MSSA with ampicillin having highest resistance 11(35.5%) (Fig 1b).



1 a: Antibiotic susceptibility pattern of MRSA Isolates

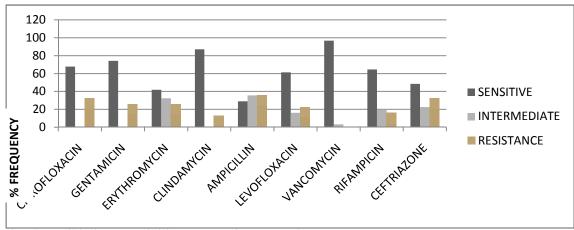


Fig 1 b: Antibiotic susceptibility pattern of MSSA Isolates

MIC of MRSA: The Minimum Inhibitory Concentration (MIC) of Vancomycin and Ampicillin to MRSA isolates were determined to assess the level of their resistance and sensitivity (Table 4). MIC of Ampicillin indicates that 10 MRSA isolates had >128μg/ml while 5 had MIC of 64μg/ml. Low MIC values of 4μg/ml was exhibited by 2 MRSA isolates to ampicillin. The MIC of Vancomycin shows that all the isolates were between $\leq 0.5μg/ml$ and 2μg/ml.

MAR Index analysis of the isolates: Multiple Antibiotic Resistance index of the isolates ranged from 0.33 to 0.77. It indicates that all MRSA isolates were hospital acquired.

DISCUSSION

Staphylococcus aureus is the predominant bacteria responsible for burn and pressure sore infection and it is perhaps the most common cause of health care associated infections worldwide (19). In this study, the MRSA prevalence of 20.1% was considered to be high against the study in Maiduguri, Northeastern Nigeria with a prevalence level of 12.5% (20) and was however lower than the report in Ibadan (30.4%), Ilorin (34.7%) and Jos (43%) all in Nigeria(21,22,23). Although MRSA prevalence is known to vary with geographical location, type of health institution, studied population and method of detection employed (20). It is clear that MRSA has become a global nosocomial pathogen with attendant therapeutic problems.

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TABLE 4: ESTIMATION OF MIC VALUE IN AMPICILLIN AND VANCOMYCIN

AMPICILLIN AND	VANCOMYCIN				
MRSA Isolate	Ampicillin (μg/ml)	Vancomycii	11 n (μg/ml)	16	1
1	32	1	12	128	0.5
2	128	1	13	128	0.5
3	64	0.5	14	16	1
			15	64	1
4	4	2	16	128	1
5	128	1	17	4	1
6	64	1	18	128	2
7	128	1			
8	128	0.5	19	64	1
9	128	1	20	128	1
			21	64	1

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TABLE 5: MAR INDEX ANALYSIS OF THE ISOLATES

Groups of isolates	Total no. of isolate	No. of antibiotics to which the isolates where resistance (a)	MAR Index
1	2	3	0.33
2	4	4	0.44
3	11	5	0.55
4	3	6	0.66
5	1	7	0.77

The prevalence rate of 13.5% and 6.7% of burns and pressure ulcer respectively were relatively high which in a similar study by Nery Silver Pireet *et al* (24), identified a rate of 43.5% MRSA in patients with pressure ulcer and concluded that the rate of MRSA colonization in pressure ulcer is a risk factor which lead to prolonged hospitalization or poor prognosis. In a study at Ibadan, Nigeria, Adetoye *et al* (21) identified 30.4% colonization of MRSA in wound patients but fail to show whether they are burns or bedsore.

There was no association between sex and infection though prevalence of MRSA was 9.6% in males and 10.6% in females. This was similar to other findings where there is no significant different (25). There was association when the age of patients were compared. MRSA infection affected participants of all ages with highest in those age groups of 21-40 and 60+ with the prevalence of 6.7%. This was agreed with the report of Bodh et al (26) who showed that MRSA was high in 10-40 year and those above 60 years but the difference was not statistically significant. Madani (27) reported that MRSA affected all age groups, but almost half (45.9%) of the patients were in the "extremes of age" group (< 1 or > 60 years). The problem with this outcome is that MRSA will induce severe disease irrespective of age which will eventually worsen their condition.

The duration of wound plays an important role in the outcome of infectious agents colonizing both burns and pressure ulcer. For instance, pressure ulcers result from long periods of uninterrupted pressure exerted on the skin, muscle and bone. In this study, MRSA was isolated more from the patients that had suffered from wound infection within 1-2 years, with prevalence of 11.5%. Earlier studies suggested that duration and intensity of predisposing illness leads to

development of pressure ulcer (28). Therefore, conditions of associated with prolonged and impaired healing may enhance the colonization of MRSA in these group of patients. However, the intervals of wound dressing as chosen by individual or their family members as a result of their socioeconomic condition, where most of them cannot afford daily dressing expenses were not statistically significant likewise the source of wound. There was a significant association between length of admission in the hospital and MRSA infection as those that were on hospital admission for≥ 6months had the highest prevalence of 14.4%. This might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure (29).

It is a common practice in health sector that allows changes in antibiotic usage by physician to achieve immediate healing. Looking at the previous antibiotic usage, some of these prescription by physicians showed that ceftriaxone had the highest frequency of 16.3% followed by Augmentin with 14.5%. Some of these prescription may in turn be disadvantageous due to the induction of resistance to the microorganism and since MRSA are resistance to all β-lactams including cephalosporin (30). The overuse and misuse of antibiotics are major contributing factors for bacterial resistance; therefore antibiotics must be prescribed only when indicated and the drug chosen should have the narrowest spectrum of activity and be given at an appropriate dose and duration. High multi antibiotic resistance rate were observed in the MRSA isolates. The most culprits were Ampicillin, Erytromycin and Ciprofloxacin. Similar facts have been demonstrated in the study by some reports where Ampicillin was highly resistance (31,32,33). This high resistance may be due to the expression of chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein (PBP) which has low affinity for binding βlactam antibiotics. All MRSA isolates were susceptible to Vancomycin. This may be due to its efficacy against bacteria resistant to β - lactamase antibiotics. Crandon et al (34) stated that vancomycin inhibits peptidoglycans biosynthesis, binding to the D-alanyl-D alanine peptide subunit and is unaffected by bacterial β -lactamases. The antibiotic susceptibility pattern was compared to MSSA and almost the same pattern were observed on MSSA with ampicillin having highest resistance. Furthermore, a high level of multiple drug resistance was observed in both MRSA and MSSA isolates.

The gold standard for antimicrobial susceptibility testing has been the Minimum Inhibitory Concentration (MIC). The MIC was done using ampicillin and vancomycin because of their

extremities in their resistance and susceptibility pattern. The MIC value of vancomycin was 0.5 - $2\mu g/ml$ and that of Ampicillin was 4 - $128\mu g/ml$. This indicates that Ampicillin has high resistance and less of vancomycin is required to inhibit the growth of MRSA. MIC values lower than $4 \mu g/mL$ indicate that the staphylococcus is susceptible to vancomycin (35,36) and is more effective antimicrobial agent. Therefore continuous monitoring of antibiotic usage on burns and bedsore patients should be encouraged to know the best antibiotic to use to avoid therapy failure.

Multi Antibiotic Resistance index analysis reveals that all the 21 MRSA isolates had a high MAR index value greater than 0.2. The antibiotic susceptibility reveal that all the isolates were resistant to all the β -lactams, aminoglycosides and quinolones group of antibiotic tested fulfilling the criteria to be designated as Multi Drug Resistance (37). From the study, MAR index ranges from 0.33 to 0.77 as compared to work done by Subramani and Vinesh (37). Therefore bacteria having MAR index >0.2 originates from an environment where several antibiotics are used(38), indicating that all MRSA were hospital acquired. This may be due to prolonged use of antibiotics either topical or systematically followed by poor infection control mechanism and hygiene.

REFERENCES

- Rahimi .F, Bouzari .M, Maleki .Z and Rahimi .F (2009). Antibiotic susceptibility pattern among Staphylococcus spp. With emphasis on detection of mecA gene in methicillin resistant Staphylococcus aureus isolates. Iranian J Clin Infect Dis 4:143-150
- Brown D.F.J. (2001). Detection of methicillin/oxacillin resistance in Staphylococci. J Antimicrob Chemother. 48, 65-70.
- 3. Pramodhini .S, Thenmozhivalli P.R, Selvi .R ,Dillirani .V, Vasumathi .A and Agatha D.(2011).Comparison of Various Phenotypic Methods and mecA Based PCR for the Detection of MRSA. J Clin Diag Res 5(S2):1359-1362.
- 4. Church .D, Elsayed .S, Reid .O, Winston .B, and Lindsay .R (2006).Burn Wound infection. Clin Microbiol Rev 19:403-434
- Pantosti .A, Sanchini .A and Monaco.M (2007). Mechanism of antibiotic resistance in staphylococcus aureus Future Microbiol 2:323-334
- Katayama.Y,Zhang.H and Chamber.H (2004).PBP2a mutation producing very high level resistance to beta-lactams. Antimicrob Agents Chemother 48:453-459.
- Eileen M.G and Venezia R.A (2002).Risk Factor associated with nosocomial methicillin resistance staphylococcus aureus infection including previous use of antimicrobials. J Antimicrob Chemother 49: 999-1005

Limitations of the study: In this study, characterization of MRSA into Hospital acquired and Community acquired was difficult, because the patients have been hospitalized during the course of injury. Secondly, the number of times the patients changed antibiotics were not ascertained due persistent buying of these antibiotics outside prescriptions by the physician which makes it difficult to control the antibiotic usage among the patients. Secondly, antibiotic genetic characterization of MRSA isolates was not carried out due to unavailability of reagents in our environment.

CONCLUSSION: The prevalence of MRSA isolates is increasing in hospital settings and showed multiple drug resistance to the beta-lactams and commonly prescribed antibiotics. Vancomycin is the most effective agent against isolated MRSA and MSSA strains. These data show that antimicrobial resistance is increasing among *Staphylococcus aureus* strains in our locality. This increase highlights the value of prudent prescribing of antibiotics (including vancomycin) and avoiding their irrational use. It is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in our hospitals to prevent the spread of resistant microorganisms including MRSA especially with patients with burns and bedsore.

- 8. Bou.G (2007).Minimum inhibitory concentration analysis and susceptibility testing of MRSA. Methods in Molecular Biology: MRSA protocol pg 29-49
- 9. Tacconelli.E, De Angelis .G, Cataldo.M, Pozzi.E and Cauda.R (2008). Does antibiotics exposure increase the risk of Methicillin Resistance Staphylococcus aureus isolation: A Systemic review and meta-analysis. J Antimicrob Chemother Antimicrob 61:26-38
- 10. Kaushik R., Kumar S., Sharma R. and Lal P. (2001). Bacteriology of burn wounds, the first three years in a new burn unit at Medical College Chandigarh. Burns 27(6): 595-597.
- 11. Nasser S., Mabrouk A. and Maher A. (2003).Colonization of burn wounds in Ain Shams University Burn Unit. Burns 29: 229-233.
- 12. Singh N.P., Goyal R., Manchanda V., Das S., Kaur I. and Talwar V. (2003). Changing trends in bacteriology of burns in the burns unit, Delhi, India. Burns 29:129-132.
- Ozumba U.C and Jiburum B.C (2000).Bacteriology of burn wounds in Enugu, Nigeria. Burns 26,178-180
- 14. Daniel .B and Ashkan J (2008). Pressure ulcer: Prevention, evaluation and management. Amer Family Phys 78:1186-1194
- 15. Alireza .É, Alireza.S, Mohammad.A, Enayat.k and Farhad.H (2010).Prevalence of methicillin resistant *Staphylococcus species* isolated from burn patients in a burn center, Ahvaz,Iran. Jundishapur J Microbiol 3:84-91

- 16. Giudice P.D, Blanc. V and Durupt. F (2006).Emergence of two populations of Methicillin Resistance Staphylococcus aureus with distinct epidemiological, clinical and biological feature, isolated form patients with community acquired skin infections. Brit J Dermatol 154:118-124
- 17. Muto.C.A, Jernigan.J.A,Oshowsky.B.E, Richet.H.M, Jarvis.W.R, Boyce.J.M, and Farr.B.M(2003).SHEA guideline for preventing nosocomial transmission of multidrug-resistant strain of *Staphylococcus aureus* and *Enterococcus*. Infect Contr Hosp Epidemiol 24:362-386
- 18. Clinical and Laboratory Standards Institute/NCCLS (2012). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard CLSI/NCCLS document M07-A9
- 19. McDonald L.C (2006).Trends in antimicrobial resistance in health care associated pathogens and effect on treatment. Clin Infect Dis 42:65-71
- Okon, K.O, Shittu A.O, Usman H, Adamu N, Balogun S.T, Adesina, O.O (2013). Epidemiology and Antibiotic Susceptibility Pattern of Methicillin-Resistant Staphylococcus aureus Recovered from Tertiary Hospitals in Northeastern. Nig J Med Medl Sci. 4: 214-220,
- 21. Adetayo, T. O., Deji-Agboola A. M., Popoola M.Y., Atoyebi T.J and Egberongbe K. J. (2014).Prevalence of Methicillin Resistant *Staphylococcus Aureus* from Clinical Specimens in Ibadan, Nigeria. The Inter J Engr Sci 3:1-11
- Ikeh, E. I. (2003). Methicillin Resistant Staphylococcus aureus (MRSA) at Jos Teaching Hospital. Afr J Clin Exptl Microbiol 4: 52-55.
- Taiwo S.S, Onile B.A, and Akanbi A.A (2004). Methicilin Resistant Staphylococcus Aureus (MRSA) isolates in Ilorin, Nigeria. Afr J Clin Exptl Microbiol 5: 189-197.
- 24. NerySilver-Pireet C.C,Bragar I.A,Ribas R.N, Guntip-Filor P.P and Filor A.D(2012).Presure Ulcer colonization by MRSA as a reservoir and risk for MRSA bacteria in patients at Brazillian Hospital. Wounds 24:67-75.
- Okwu M.U, Okorie T.G, Mitsan .O and Osakue .E.O (2014). Prevalence and comparison of three methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in tertiary health institutions in Nigeria. Can Open Biol Sci J. 1:1 12
- Bodh R.P,Anil K.S and Abdulrahman S.A (2005).Prevalence of Methicillin Resistance and Methicillin Sensitive Staphylococcus aureus nasal colonization among patients at the time of admission to the hospital. Ann Saudi Med.25:304-308

- 27. Madani TA (2002). Epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* (MRSA) at the University Hospital, Jeddah, Saudi Arabia. J King Abdulaziz Univer Medl Sci 10: 3-12
- 28. Bergstrom N; Breden BJ; Laguzza A; Holman V (1987): The Braden Scale for predicting sore risk. Nurs Res 36:205-210
- 29. Gajbhiye P S and Damle A S (2015). Study of MRSA Isolates from Patients of Tertiary Care Hospital. J Denta Medl Sci 14:59-62.
- 30. Centers for Disease Control and Prevention (2013).Methicillin resitant Staphylococcus aureus infection:Laboratory Testing for MRSA.
- 31. Onanuga .A, Oyi. A.R and Onaolapo. J.A (2005). Prevalence and susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. Afr J Biotech 4:1321-1324.
- 32. Onemu.O.S, and Ophori.E.A (2013).Prevalence Of Multi-Drug Resistant Staphylocccus aureus In Clinical Specimens Obtained From Patients Attending The University Of Benin Teaching Hospital, Benin City, Nigeria. J Natl Sci Res 3: 154
- 33. Ibe.C, Onyeagba.R.A, Charles.S.U, Onuabuchi.I.A, Jacobs.C, Nduka.C.J, Nnadozie. J and Osuocha.K.U (2013). Prevalence and Antibiotic Susceptibility Patterns of Methicillin-Resistant Staphylococcus Aureus (MRSA) Isolated from Healthy Inhabitants of Uturu Rural Communities, Abia State, Nigeria. J Natl Sci Res 3: 85
- 34. Crandon.J.L, Kuti.J.L and Nicolau.D.P (2010).Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin resistant Staphylococcus aureus with a range of vancomycin MICs in a murine pneumonia model. Antimicrob Agents Chemother 54:5115-5119
- 35. Clinical and Laboratory Standards Institute/NCCLS (2006). Performance Standards for antimicrobial susceptibility testing. Sixteenth informational supplement. CLSI document M100-S16.
- 36. Tenover F.C and Moellering R.C. (2007). The rationale for revising the clinical and laboratory standards institute vancomycin minimal inhibitory concentration interpretive criteria for Staphylococcus aureus. Clinical Infectious Disease. 2007;44(9):1208–1215
- 37. Subramani .S and Vignesh.S (2012).MAR Index Study and MDR Character Analysis of a few Golden Staph Isolates. Asian J Pharm Life Sci. 2(2):151-154.
- 38. Tamberkar D.H, Dhanorkar D.V, Gulhane S.R, Khandelval V.K and Dudhane M.N (2006).Antibacterial Susceptibility of some Urinary Tract Pathogens to commonly used antibiotics. Afri J Biotech 5:1562-1565.