WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 244-255. Research Article ISSN 2277– 7105

METHICILIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN CLINICAL SAMPLES AND FOMITES

Abinokhauno Solomon*[1], Iyevhobu Kenneth O.[1], Oghena Marcus[1], Obodo Basil Nnaemeka[1] and Ebadan Maxwel[2]

[1]Department of Medical Laboratory Science, Faculty of Basic Medical Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

[2]Quality Assurance Clina-Lancet Laboratories Limited, Victoria Island, Lagos State, Nigeria.

Article Received on
15 Oct. 2018,
Revised on 05 Nov. 2018,
Accepted on 26 Nov. 2018
DOI: 10.20959/wjpr201819-13819

***Corresponding Author**
Abinokhauno Solomon
Department of Medical Laboratory Science, Faculty of Basic Medical Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

ABSTRACT

A total of 200 swabs from clinical samples and fomites which comprised of 105 swabs from the skin, 56 swabs from nasal and 38 swabs from fomites i.e laboratory coats, each were collected and examined. From this study, 30(15.0%) were identified as *Staphylococcus aureus*, and 170(85.5%) showed no growth or other species of bacteria and analysis show that it was statistically significant P<0.05, the prevalence of Methicillin/ Oxacillin Resistant *Staphylococcus aureus* (MRSA/ORSA) was 13(6.5%) with skin having the highest occurrences of 9(4.5%) and nasal passage and fomites having 4(2.0%) and 0(0.0%) respectively and the Methicillin/ Oxacillin Sensitive *Staphylococcus aureus* (MSSA/OSSA) having prevalence of 15(7.5%), 2(1.0%) and 0(0.0%) for isolates from the skin, nasal passage and formites respectively and when the number of MRSA isolated was compare with MSSA statistically it was found not to be statistically significant p-value=0.193, p>0.05. When mean diameter of Oxacillin was used to compared the mean diameter of Vacomycin it was 5.3±2.83 vs 17.4±18.47; t_{cal}=2.072,p-val=0.068, and df=9, respectively, it was not statistically significant i.e P>=0.05, at α=95% or 0.05, the mean, standard deviation, F-value calculated, degree of freedom, p-values of the diameters of zones of inhibitions of Oxacillin, Vacomycin, and sulbatam, when mean diameter of Oxacillin was used to compared the mean diameter of Vacomycin and Sulbatam is was 5.3±2.83 vs 17.4±18.47 and 1.11±0.281; F_{cal} Between groups=6.1.47, p-val=0.06, and df=29 respectively, it was not statistically significant i.e P>=0.05, at α=95% or 0.05. However, there seems to be a steady rise of MRSA isolates

resistance to commonly used antibiotics like Sulbatam and Vacomycin but the present rate is still low in comparison to values in some other studies.

KEYWORDS: *Staphylococcus*, Vacomycin, Methicillin, Fomites, Resistant.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) are strains of *Staphylococcus aureus* which are resistant to methicillin and related penicillins and are particularly difficult to treat because they are also resistant to most other common antibiotics (Cheesbrough, 2000).

Although *Staphylococcus aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern. MRSA was endemic in hospitals by the late 1960s, but it appeared rapidly and unexpectedly in communities in the 1990s and is now prevalent worldwide (Deleo, 2009; Liebowitz, 2009). Staphylococci are gram positive cocci of uniform size, occurring characteristically in groups but also singly and in pairs. They are non-motile and non-capsulated (Cheesbrough, 2000). *Staphylococcus aureus* is the most medically important member in terms of pathogenicity of the group (Ochei and Kolhatkar, 2000).

Staphylococcus is present in the nose of 30% of healthy people and may be found on the skin. It causes infection most commonly at sites of lowered host resistance, such as damaged skin or mucous membrane (Humphrey, 2007). Although 50 – 60% of patients with MRSA are merely colonised (i.e. they carry the bacteria but do not have symptoms or an illness), serious infections such as those involving the blood stream, respiratory tract and bones or joints do occur (Humphrey, 2007). *S. aureus* causes boils, pustules, styes, impetigo, infections of wounds (cross-infections), ulcers and burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. Also, toxic food poisoning (rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation (Chessbrough, 2000).

Mannitol salt agar is a useful selective medium for recovering *S. aureus* from faecal specimens when investigating staphylococcal food poisoning. It can also be used to screen for nasal carriers. *S. aureus* ferments mannitol and is able to grow on agar containing 70 – 100g/l sodium chloride. Mannitol salt agar containing 75g/l sodium is recommended particularly for isolating MRSA strains (Cheessbrough, 2000).

On mannitol salt agar, *S. aureus* produces yellow colonies (Ochei and Kolhatkar, 2000). The MRSAs are usually sensitive to vancomycin (Ochei and Kolhatkar, 2000). Flucloxacillin and chloxacillin are used to treat β-lactamase (penicilinase) producing staphylococci. Vacomycin is often needed to treat MRSA infections. Antibacterial resistance to penicillin may occur due to the β-lactamase production, cell membrane alterations reducing antibiotic uptake (gram negative bacteria), or changes in the penicillin-binding protein as occurs with MRSA (Cheesbrough, 2000).

There is no effective immunisation with toxoids or bacterial vaccines for preventing the spread of *S. aureus* (Levinson and Jawetz, 2002). The control and prevention of MRSA involves early and reliable detection in the laboratory through surveillance, patient isolation when admitted to hospital, good professional practice by all healthcare workers (including compliance with hand hygiene guidelines), effective hospital hygiene programmes and sensible use of antibiotics (Humphrey, 2007).

MRSA are potential source for the spread of nosocomial infections in patients and healthcare works, cause patients that are hospitalized to over stay in the hospital and spend financial resources up to 400% that is, four times up to what they would have spent without Hospital acquired nosocomial infections (Ahlstrom, 2011), CDC reported that MRSA or ORSA are primary source of nosocomial infections, which could be transferred from patients to patients, patients to health workers or health workers to health workers or health workers to patients. Then, this project determines the prevalence of MRSA in clinical samples and fomites to validated the claims of CDC and other researchers that had worked on such work else were. To the best of our ability this research work has been done in the area of study**.**

MATERIALS AND MEHODS

This project work was carried out in both Ekpoma, Esan West Local Government Area, Edo State, Nigeria with longitude 6.13^{o}E and latitude 6.73^{o}N having a population of about 61,870 people (Population of Cities, 2007) and in Irrua Specialist Teaching Hospital (formerly Otibhor Okhae Teaching Hospital), Irrua was established by decree 92 of 1993 to provide tertiary health care delivery services to the people of Edo State. The hospital is located in Irrua, Edo central senatorial district, along the Benin-Abuja Highway at about 87 kilometers north of Benin City, the Edo State capital.

Subject Sector

A target of 200 clinical samples and fomites served as the test subject for this project work.

Research Design

This project work was carried out within a period of two months. 200 swab samples from clinical samples and fomites (laboratory coat) were collected randomly and used for this project work.

Materials Used

The following materials and apparatus were used for the bacteriological analysis: mannitol salt agar, Nutrient agar, swab sticks, Petri dishes, conical flask, distilled water, autoclave, Bunsen burner, inoculating wire loop, binocular microscope, weighing balance, measuring cylinder, glass slides, human plasma, hydrogen peroxide, crystal violet Lugol's iodine, acetone, neutral red, sensitivity discs (Oxacillin, Vacomycin and Sulbatam) and other reagents.

Media Used

The media used were mannitol salt agar and Nutrient agar.

Sample Collection

Two hundred (200) swab specimen used were collected randomly from apparently clinical samples of skin, nasal and fomites i.e laboratory coat from irrua specialist teaching hospital, Irrua, Edo State. Nasal swabs were collected in good light vision from subjects bending their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick, Both right and left nostrils were used, swab were also taken from skin and fomites (laboratory coat), were swabbed bearing labels as swabs, code number and date of collection. The swab sticks were carefully returned to their sterile containers, sealed with adhesive tape and labelled accordingly. Collected specimens were taken to the laboratory where bacteriological analysis was carried out immediately.

Procedure for Culture

(i) The swab sticks were used to make wells of inoculum on each nutrient agar surface.

(ii) Spreading was done by streaking from the primary inoculum using inoculation wire loop to obtain discrete colonies.

(iii)The plates were then incubated at 37^{o}C for 24 hours.

(iv) Growths were observed after incubation.

(v) Suspected colonies were confirmed by other methods which include gram staining.

Gram Staining Procedure

(i) A smear was made and allowed to air dry and then fixed with gentle heat by passing the slide three times over a Bunsen flame.

(ii) It was stained with crystal violet for one minute.

(iii) It was washed with tap water.

(iv) Lugol's iodine was applied and left for one minute.

(v) It was washed with tap water.

(vi) Decolourisation was done with acetone until no more colour appeared to ooze out of the smear for 2 seconds.

(vii) It was washed immediately with tap water.

(viii) It was counterstained with neutral red for 2 minutes.

(ix) It was washed with tap water.

(x) It was blotted with blotting paper and dried.

(xi) It was examined microscopically using 100X objective with immersion.

Biochemical Test

The biochemical tests that were used for this work include:

(i) Catalase test

(ii) Coagulase test

Catalase Test: This test helps to differentiate staphylococci from streptococci.

Method

(i) 3ml of H_2O_2 was placed in a test tube

(ii) With sterile plastic rod, some colonies of the organism were picked and immersed in the H_2O_2 solution.

(iii) Immediate gas bubbling was observed.

Coagulase Test

Procedure

(i) Two separate drops of saline were placed on a slide

(ii) Colonies of the organism were emulsified in each of the drops to make thick suspensions.

(iii)The tip of straight wire loop was dipped into the undiluted plasma and the adhering traces of plasma was mixed into one of the bacterial suspensions.

(iv)Immediate coarse clumping of the mixture was looked for within 10 seconds.

— Tube test was performed to confirm all slide test coagulase negative staphylococcus, were truly negative for coagulase.

Antibiotic Sensitive Test

2µg/disc Oxacilin discs and other antibiotic discs such as 8µg/disc Vacomycin and 8µg/disc Sulbatam (manufactured by Abtek Biologicals Ltd) were used to test the susceptibility of staphylococcal isolates obtained. The test isolates were inoculated into peptone water broth and the inoculum was used to seed Nutrient agar agar plate. The antibiotic discs were placed aseptically on the seeded plate. This was incubated at 37^{o}C for 24 hours and examined for zones of inhibition. The zones of inhibition were measured in millimetres and recorded.

Statistical Analysis

Chi-square was used to determine the difference between MRSA and MSSA, t- student test and Anova statistical analysis was used to determine the differences in the level of sensitive to Oxacilin and Vacomycin, and Oxacilin, Vacomycin and Sulbatam by Methicillin Sensitive *Staphylococcus aureus*.

RESULTS

A total of 200 swabs from clinical samples and fomites which comprised of 106(53%) swabs from the skin, 56(28%) swabs from nasal and 38(19%) swabs from fomites i.e laboratory coats, each were collected and examined. With the help of biochemical characterisation, 30(15.0%) were identified as *Staphylococcus aureus*, other species of bacteria 58(29%) and 112(56%) showed no growth (Table 1). *Staphylococcus aureus* was more isolated from swabs from the skin 24(12.0%), followed by nasal swab with 6(3.0%) and swab from fomites i.e laboratory coat with 0(0.0%) when the number of isolates was compared within *th*e samples statistically it was found to be statistically significant, $p<0.05$.

Among the various *Staphylococcus aureus* isolated from the various samples, the prevalence of Methicillin/ Oxacillin Resistant *Staphylococcus aureus* (MRSA/ORSA) was 13(6.5%) with skin having the highest occurrences of 9(4.5%) and nasal passage and fomites having 4(2.0%) and 0(0.0%) respectively and the Methicillin/ Oxacillin Sensitive *Staphylococcus aureus* (MSSA/OSSA) having a prevalences of 15(7.5%), 2(1.0%) and 0(0.0%) for isolates from the skin, nasal passage and formites respectively and when the number of MRSA isolated was compare with MSSA statistically it was found not to be statistically significant**, p>0.05.**(Table 2).

The Minimum inhibition concentration to Oxacillin, Vacomycin and Sulbatam is shown on table 3.

The mean, standard deviation, t student statistic calculated, and p values of the diameters of zones of inhibitions of Oxacillin and Vacomycin, when mean diameter of Oxacillin was used to compared the mean diameter of Vacomycin it was 5.3±2.83 vs 17.4±18.47; t_{cal}=2.072, p-val=0.068, and df=9 respectivey, it was not statistically significant i.e P>=0.05, at α=95% or 0.05 as shown on table 4.

The mean, standard deviation, F-value calculated, degree of freedom, p-values of the diameters of zones of inhibitions of Oxacillin, Vacomycin, and sulbatam, when mean diameter of Oxacillin was used to compared the mean diameter of Vacomycin and Sulbatam is was 5.3±2.83 vs 17.4±18.47 and 1.11±0.281; F_{cal} Between groups=6.1.47, p-val=0.006, and df=29 respectivey, it was not statistically significant i.e P>=0.05, at α=95% or 0.05 and using LSD Post Hoc Test to do multiple comparisons between Oxacillin against Vacomycin and Sulbatam; Vacomycin against Oxacillin and Sulbatam and Sulbatam against Oxacillin and Vacomycin with p-values of 0.018 and 0.393; 0.018 and 0.02; and 0.393 and 0.02 showing that mean diameter of Oxacillin was significantly different from Vacomycin while the mean diameter of Oxacillin is not differ from that of Sulbatam, while the mean diameter of Sulbatam differs from that of Vacomycin as shown on table 5.

Table 1: Distribution of *Staphylococcus aureus* among studied clinical samples and fomites (%).

Sample type	No. examined	No Growth	No of *S.aureus*	other bacteria
Skin	106	38 (19%)	24(12%)	44(22%)
Nasal	56	36(36%)	6(3%)	14(7%)
Lab coat	38	38 (19%)	0	0
Total	**200**	**112(56%)**	**30(15%)**	**58(29%)**
X^2=31.2, Degree of freedom=2, p-value=1.7x10^{-7}, p<0.05				

N = Number

Table 2: Distribution of MRSA and MSSA among studied clinical samples and fomites(%).

Sample type	N of *S.aureus* isolates	N of MRSA	N of MSSA
Skin	24	9(4.5%)	15(7.5%)
Nasal	6	4(2.0%)	2(1.0%)
Lab coat	0	0	0
Total	**30**	**13(6.5%)**	
17(7.5%)			

X^2=1.663, Degree of freedom=1, p-value=10.193, p>0.05.

N= Number

Table 3: Minimum Inhibition Concentration toVacomycin, Oxacillin and Sulbatam.

Plate no	Oxacillin Mm	Vacomycin mm	Sulbatam mm
160	8	16	0.9
82	4	8	1.0
139	6	8	1.1
85	4	32	1.7
127	12	3	1.5
81	2	64	0.8
157	4	16	1.1
91	4	3	0.9
141	5	8	1.1
106	4	16	1.0
Mean	**5.3**	**17.4**	**1.1**

SENSITIVITY STANDARD

Oxacillin: 2µg/ml

Vancomycin: 8µg/ml

Sulbatam: 8µg/ml

Table 4: Mean, standard deviation, t-student statistic calculated, p-values of the diameters of zones of inhibition of Oxacillin and Vacomycin on MSSA.

	Test value		Comparison (test value=5.3)		
	Oxacillin	Vacomycin	t value	p-value	Significant
MeanZone diameter	5.30	17.40	2.072	0.068	p>0.05
Standard deviation	2.83	18.34			

Table 5a: Anova of multiple comparison of diameter of Oxacillin, Vacomycin and Sulbatam.

(a) ANOVA					
	Sum of Squares	**df**	**Mean Square**	**F**	**Non Sig.**
Between Groups	1431.101	2	715.550	6.147	.006
Within Groups	3143.209	27	116.415		
Total	**4574.310**	**29**			

Table 5b: Post hoc test multiple comparison of diameter of Oxacillin, Vacomycin and Sulbatam.

(TEST VALUE)	**GROUP**	**Sig.**
OXACILIN	Vacomycin	.018
	Sulbatam	.393
VACOMYCIN	Oxacilin	.018
	Sulbatam	.002
SULBATAM	Oxacilin	.393
	Vacomycin	.002
***. The mean difference is significant at the 0.05 level.**		

DISCUSSION

Despite recognising *Staphylococcus* species as regional flora of the skin and mucus membrane, certain species have been found frequently as aetiological agent of a variety of human infections. The most common among these infections are the superficial supportive infection caused by *Staphylococcus aureus*. Under minding the introduction of chemotherapy and recent improvement in medical services, methicillin resistant *Staphylococcus aureus* (MRSA) strain emerge and has posed a major threat to public health in treatment and management of *Staphylococcus aureus* infection. The increasing prevalence of MRSA among *Staphylococcus aureus* strains resulted in a significant increase in the utilization of vancomycin. Methicillin resistant *Staphylococcus aureus* (MRSA) have been recognized as a major strain of *Staphylococcus aureus* prevalent as cause of nosocomial infection which often results to increase in morbidity and mortality (Schaumacher-Perdreau, 1999).

A total of 200 swabs from clinical samples and fomites which comprised of 106(53%) swabs from the skin, 56(28%) swabs from nasal and 38(19%) swabs from fomites i.e laboratory coats, each were collected and examined. With the help of biochemical characterisation, 30(15.0%) were identified as *Staphylococcus aureus*, and 112(56%) showed no growth or other species of bacteria and analysis show that it was statistically significant $P<0.05$. the overall prevalence of MRSA was 13(6.5%) it was noticed that the highest number isolates incident of MRSA on the skin 9(4.5%) while Nasal passage had 4(2.0%) and lab coat had 0(0.0%), the difference noticed between MRSA on the skin and Nasal passage could be the difference in the number of samples used from skin and nasal passage and also due to geographical distribution of micro-organism, and this work in line with work of Ayliffe *et al.*, (1988); Majumdar *et al* (2009); who reported 9.0%, and 7.5% but was in constraint to Akpaka *et al* (2006) who reported 23.0%. and Okodua *et al.*, (2013) of 29.7% although samples differences, samples size and Techniques has effect on the overall prevalence of MRSA.

Methicillin/Resistance *Staphylococcus aureus* (MRSA) and Methicillin/Sensitive *Staphylococcus aureus* (MSSA)] among studied population and fomites had 43.3% prevalence which is in variation with previous work done in different region of Nigeria where Taiwo *et al.*, (2004) that, reported a prevalence of 34.7% in Illorin, Kwara State. Comparatively, study area, sample size, sample type, implementation of standard operating procedures in work place might account for the observed differences. The highest occurrence of MRSA was seen in skin 30% and 13.3% from nasal nares while 56.6%of the *Staphylococcus aureus* isolated in our study population were methicillin sensitive *Staphylococcus aureus* (MSSA).

It was noticed that the diameter of zone of inhibition of Vacomycin was greater than those of Oxacillin and Sulbatam, with the mean diameter and standard deviation of 5.30±2.83, 17.4±18.47 and 1.11±0.28 respectively, Vacomycin was the most active antibiotic which shows a greater mean and standard compare to those of Oxacillin and Sulbatam. This is in agreement with report of CDC, (2010), and with all available literature, but the Sulbatam was resistant to all the MRSA as shown from its mean diameter and standard deviation of 1.11±0.28, but to the MRSA, Oxacillin was in the boarderline between sensitive and intermediate with mean diameter and standard deviation of 5.30±2.83.

There was no significant difference between mean diameter and standard deviation of the susceptible MRSA to Vacomycin and Oxacillin with a t_{cal} value of 2.072 and P_{value} of 0.068 and this also agreed on available literature.

CONCLUSION

Evidence from the results obtained has shown that the 13(6.5%) of the studied samples were Methicillin Resistant *Staphylococcus aureus* and that the level of resistance shown by MRSA isolates to other antibiotics when compared with that of methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates, is by far higher.

However, there seems to be a steady rise of MRSA isolates resistance to commonly used antibiotics like Sulbatam and Vacomycin but the present rate is still low in comparison to values in some other studies.

From this study, haven established the prevalence of MRSA in skin and nasal nares, it is necessary for medical Personnel, especially those involved in routine care, monitoring and prescription of commonly used antibiotics to pay attention to the prevalence of methicillin/ resistance *Staphylococcus aureus* (MRSA), methicillin/ sensitive *Staphylococcus aureus* (MSSA) in treatment of *Staphylococcus aureus* infections. These outbreaks were associated with certain higher risk groups, including individuals who used IV drugs, participants in close contact sports, and residents living together in crowded conditions, such as inmates, military recruits, and disabled individuals in group homes. A regular surveillance of both nosocomial, Hospital Acquired methicillin/ resistance *Staphylococcus aureus* (HA-MRSA) and Community Acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) infection is necessary to succumb it prevalence in the hospital settings and community at large. Regular monitoring of antibiotic sensitivity pattern of *Staphylococcus aureus* must be made mandatory, to control further spread of its infections. Proper hygiene should be maintained at all times by all health workers to reduce its spread and infection by contact. Hand –washing, particularly is very important by all health workers after each procedure with a patient before touching the next patient. This will stop the transmission of MRSA. The use of disposable aprons and gloves by hospital staff should be practice thereby reducing the risk of transmission in the hospital.

From this study, MRSA are on the continual rising in comparison with other studies done. With this increase prevalence rate of MRSA, within the next two decades there might not be any reliable treatment left for most *Staph. aureus* infections.

It is hereby recommended that combination therapy with good therapeutic effect should be considered for the treatment of *Staph. aureus*

REFERENCES

1. Ayliffe GAJ, Babb JR, Davies, JG, Lilly HA. Hand disinfection: a comparison of various agents in laboratory and ward studies. *J Hosp Infect.*, 1988; 11: 226–243.
2. Ahlstrom, D. (2011). *"New strain of MRSA superbug discovered in Dublin hospitals"*. The Irish Times. http://www.irishtimes.com, Retrieved May 3, 2011.
3. CDC (2006): CDC Guideline "Management of Multidrug-Resistant Organisms in Healthcare Settings, 2006".
4. CDC, "Guidelines for Infection Control in Health Care Personnel,". Centers for Disease Control and Prevention. http://www.cdc.gov, Retrieved, December, 2010; 18: 2013.
5. Cheesbrough M. *Staphylococcus aureus* In: *District Laboratory Practice in Tropical Countries, Part 2.* Cambridge University Press, UK, 2000; 133: 155-158.
6. Deleo FR, Chambers HF. Re-emergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J.Clin Invest.* 2009; 2464-2474.
7. Humphreys H. Staphylococcus In: *Medical Microbiology* (17th edition). Churchill Livingstone. 2007; 172: 174-175.
8. Levinson W, Jawetz E. Staphylococcus In: *Medical Microbiology and Immunology* (7th edition). Lange Medical Books/ McGraw-Hill. 2002; 95.
9. Liebowitz LD. MRSA burden and interventions. *Int. Antimicrobial Agents*, 2009; *34:* 11–13.
10. Ochei J, Kolhatkar A. *Methicillin-Resistant Staphylococcus aureus In: Medical Laboratory Science Theory and Practice.* Tata McGraw-Hill Publishing Company Limited, New Delhi, 2000; 813, 665, 805-806.
11. Schumacher-Perdreau F. Clinical significance and laboratory diagnosis of coagulase – negative staphylococci. *Clinical Microbiology Newsletter*, 1999; 13: 97-101.
12. Taiwo DA, Ikeh EI, Olayemi A. Prevalence of Methicillin-resistance *Staphylococcus aureus* in Illorin Nigeria. *African Journal of Clinical and Experimental Microbiology*, 2004; 5: 342-14.