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Antibiotic-Resistant Pattern and Multiple Antibiotic Index of Multidrug-Resistant *Staphylococcus aureus* from Wound Infection Patients in a Tertiary Institution in South Eastern Nigeria

Joan Miracle James-Onyekwere [a *] | **Immaculate Ugochi Nwankwo** [a] | **Ebubechi Uloma Okey-kalu** [a]

a. Department of Microbiology, College Of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

***Corresponding author:** Michael Okpara University of Agriculture, Umudike. P. M. B 7267, Umuahia , Abia State, Nigeria.
E-mail address: joanjamesonyekwere@gmail.com

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A B S T R A C T

Background: This study evaluated the Antibiotic Resistant Pattern and Multiple Antibiotic Index of multidrug-resistant *Staphylococcus aureus* from wound infection patients in Federal Medical Center Umuahia, Abia State, Nigeria.
Methods: A total of 300 wound swab samples from diabetic leg ulcers, accident wounds, burn wounds and surgical site wounds were collected from different wards and cultured on Blood agar and Mannitol Salt agar.
Results: *S. aureus* was isolated from 50 (16.7%) samples, of which, 14 (28%) were diabetic leg ulcers, 11 (22%) accident wounds, 16 (32%) burn wounds, and 9 (18%) surgical site wounds. There was no significant difference (P>0.05) in the different wards and sites of collection and between age and those with highly resistant *S. aureus*. The antimicrobial resistance profile of the isolates in the study samples from wound swabs and surgical site wounds was highest in amoxicillin (100%), erythromycin (100%), and Norfloxacin (100%). The overall prevalence of methicillin-resistant *S. aureus* was 14.0%, while 66% of the *S. aureus* was multidrug-resistant. Multiple antibiotic resistance index from the study was between 0.4 - 0.6.
Conclusion: Antibiotic susceptibility testing should be performed before treatment and adequate measures should be taken.

1. Introduction

Staphylococcus aureus is a Gram-positive and spherical coccus with diameters of 1µm –1.3 µm. It appears in clusters like bunches of grapes on microscopic examination. Some of the strains produce toxins while growing in food. The toxins can cause gastrointestinal disease which is generally referred to as Staphylococcal food poisoning. The produced enterotoxin is a heat-stable protein that resists heating at 100 °C for 30–70 min. *S. aureus* is also responsible for food-borne infections [1]. In addition, this organism causes various diseases such as toxic shock syndrome, septicemia, and wound infections. Besides, skin pustules, impetigo, osteomyelitis, renal abscess, pneumonia, endocarditis, meningitis, gastroenteritis, and sometimes serious conditions in patients undergoing hemodialysis, diabetic Mellitus, etc. may also be caused by *S. aureus* [2]. Several methods can identify *S. aureus* including Gram's staining, cell morphology, production of catalase and coagulase enzymes, pigment production, susceptibility to lysostaphin and lysozyme, and anaerobic production of acid from glucose [3]. Several other commercially available systems that allow strains to be biochemically characterized have also been developed. This organism can cause a wide range of infections, from minor infections such as skin and eye infections to major ones like bloodstream infections and pneumonia [4, 5, 6]. Multi-drug-resistant *S. aureus* is one of the main organisms that causes bloodstream infections which cause morbidity and mortality in the world. [7].

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Studies on bloodstream infections have shown that neonatal septicemia is commonly caused by this organism [8]. Epidemiological studies found that there is a significant difference in bloodstream infection causing pathogens between developed and developing countries [9]. A report from a Finnish Hospital Infection Program conducted between 1999-2001 and 2005-2010, found that *S. aureus* ranked among the top three organisms that cause bloodstream infections [10]. Moreover, a nationwide observational study conducted in Switzerland on all intravascular catheter tip culture cases showed *S. aureus* as one of the most prevalent organisms causing bloodstream infections in non-intensive and intensive care unit patients [11]. *S. aureus* has also been isolated from lower respiratory tract infections such as pneumonia. Several studies have also listed its role as the predominant organism causing ventilator-associated pneumonia [12, 13, 14] which is the single most common healthcare-associated infection in intensive care units across the world [14]. *S. aureus* has also been reported as the major causative agent of community-acquired pneumonia [15]. The emergence of infections caused by drug-resistant bacteria is a serious and growing global health concern. Significant efforts are made in developing new antimicrobial compounds with improved efficacy [16, 17]. The main aim of this study was to determine the Antibiotic-resistant Pattern of Multidrug-Resistant *S. aureus* isolates and the Multiple Antibiotic Index.

2. Materials and Method

2.1 Study Location

The Federal Medical Centre (FMC) Umuahia, Abia State, Nigeria was used for this research. Ethical approval was obtained by the ethical committee of the hospital. FMC Umuahia is located along Aba road. It is one of the FMCs located in the South-Eastern geopolitical region in Nigeria. It serves at least all the 17 Local Government Areas in the State. The study was conducted in the wards and clinics of this institution. The clinic opens for services on weekdays between 8.00 am to 5.00 pm. The staff includes consultants, postgraduate resident doctors, medical officers, nurses, departmental clerks, and ward attendants. The hospital has four hundred bed spaces.

2.2 Study Design

The study was a hospital-based cross-sectional analytical study.

2.3 Sample Collection and Processing

A total of 300 samples were obtained using sterile swab sticks. These samples were comprised of 93 diabetic leg

ulcers, 75 accident wounds, 69 burn wounds, and 63 surgical site wounds. The samples were strictly from the male surgical, female surgical, wound dressing room, female medical, plastic surgery, accident, and emergency wards. The collected samples were inoculated onto Blood agar and Mannitol Salt agar by streaking method and incubated for 24 h at 37°C. Typical colonies were Gram-stained and standard biochemical tests were performed to identify the isolates [18].

2.4 Isolation and identification of bacteria

The pure culture of *S. aureus* was obtained by aseptically streaking representative colony of the isolates on culture plates into freshly prepared nutrient agar, and incubated for 24 h at 37°C.

2.5 Antibiotic Susceptibility and Resistance Test

The antibiotic susceptibility and resistance testing of *S. aureus* were done using the disk diffusion method by Kirby-Bauer procedure [19] and was interpreted by the standards of the Clinical Laboratory Standards Institute (CLSI) [18] on Muller Hinton agar (Hardy Diagnostics USA). Mueller Hinton culture plates were inoculated by dipping a sterile cotton wool swab into a suspension of the overnight growth of the organism prepared to the density of an Mc Farland no 0.5 capacity standard; excess liquid from the swab was expressed before inoculation by spread plate method. The antibiotic discs that were used are of the following concentrations: Streptomycin 30ug: Norfloxacin 10ug: Chloramphenicol 30ug: Gentamicin 10ug: Amoxicillin 30ug: Ciprofloxacin 10ug: Erythromycin 30ug: Rifampicin 10ug: Ampicillin: Levofloxacin 10ug. After overnight incubation, the control and test plates were examined to check the growth for confluent or near confluent. Inhibition zone size was measured using a ruler and then interpreted using the standard recommendation of CLSI [20]. The sensitivity and resistance were recorded as (S) and (R) respectively.

2.6 Detection of Methicillin Resistance

Methicillin resistance determination was done using the cefoxitin disc diffusion method following the CLSI guidelines [21]. All the isolates were subjected to a cefoxitin disk diffusion test using a 30ug disk. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on Mueller- Hinton agar plate. Plates were incubated at 37°C for 24 h and zone diameters were measured. Results were interpreted according to CLSI guidelines [22]. An inhibition zone diameter of less than 19 mm was reported as resistant and greater than 20 mm was considered sensitive.

2.7 Multiple Antibiotic Resistance Index (MARI)

The MARI was calculated as proposed by [23]. MAR index is defined as a/b.

Where: a is the aggregate antibiotics resistance score of all the isolates. b=Total number of antibiotics tested. MAR index values greater than 0.2 indicate the high-risk source of contamination where antibiotics are often used.

3. Results and Discussion

The demographic distribution of *S. aureus* is shown in Table 1. Isolation of S. aureus from the wound dressing room was highest at 16 (17.7%) followed by those from the male surgical ward. Isolation of *S. aureus* from plastic surgery unit 4 (16.0%) was the least followed by those from accident and emergency unit 5 (33.3%) and female surgical ward 5(11.1%). The antibiotic resistance profile of the test organism is shown in Table 2. Resistance of the various wound types to the antibiotics showed 82% resistance to norfloxacin, 80% to ampiclox, and 76% to amoxil while the least resistance was seen in levofloxacin (26%) and streptomycin (34%). Isolates from accident wounds also showed 100% resistance to amoxil, while those from surgical site wounds showed 100% resistance to Erythromycin and Norfloxacin. There was no resistance in isolates from burn wounds to levofloxacin (0%). The sensitivity and resistance of the antibiotics by sex and age are shown in Table 3. There was no significant difference ($P>0.05$) in resistance and also in sensitivity to the antibiotics used to sex and age. Table 3b shows the sensitivity and resistance of the antibiotics according to the wards and sites of collection. There was also no significant difference ($P>0.05$) seen in wards and sites of collection to the resistance of the antibiotics. Table 4 shows the multiple antibiotic indexes of the isolate. Isolates from accident wounds were seen to be highest at 0.68 (6.1%). The MARI was lowest in isolates from burn wounds at 0.42 (2.6%) . Table 5 shows the incidence of methicillin resistance. Isolates from accident wounds and surgical site wounds were the least 1(9.1%) and 1(11.1%) respectively, it was highest in diabetic ulcers 3(21.4%).

This study presented the Antibiotic Resistant Pattern and Multiple Antibiotic Index of Multidrug-Resistant *S. aureus* from wound infection patients in a tertiary institution in South Eastern Nigeria. Wound infection due to MRSA is a major concern in resource-limited countries where there are poor infection prevention and control measures [24]. In this study, the overall prevalence of *S. aureus* from wound infections was 16.6%. This finding is similar to a study conducted by Awka (17.46%) [25] and by Akinkunmi et al. (18.3%) [26]. However, the prevalence reported in the current study is lower than a study by the National orthopedic hospital Kano (36%) [27]. The variation in prevalence might be due to variation in the study subjects and the method employed in the detection of *S. aureus*. Infection with *S. aureus* may also occur due to contamination of hospital or surgical instruments. In circumstances where there is the disruption of the natural skin barrier, *S. aureus* which is a common bacterium on surfaces easily finds its way into wounds [28]. Prevalence of *S. aureus* from the study concerning wound types showed burn wounds have the highest prevalence of 23.1%. This is however lower than studies reported in Delta (33.3%), [29], Enugu (50%) [30] and Kano 64.4% [31]. The variations seen in the different studies may be a result of the difference in locations. Burn wounds are usually characterized by a large surface area which can be a potential site for colonization by the organism. The prevalence of methicillin-resistant *S. aureus* in the study was 14.0%. This result is closely in line with the study reported from Cameroon (13.16%) [32]. However, the result is lower than reports from studies done in Enugu (20.1%) [29] and other African countries like Libya (31.0%) [33]. Although methicillin-resistant Staphylococcus aureus prevalence is known to vary with geographical location, study population, and the detection methods employed. It is obvious however that MRSA has become a global nosocomial pathogen with attendant therapeutic problems. The observed high prevalence of MRSA in the study may be due to the high rate of certain antibiotics use either due to availability or cost-effectiveness issues [30]. Concerning the antimicrobial resistance profile of the isolates in the study from the various wound types, *S. aureus* isolates showed resistance to norfloxacin (82%), ampiclox (80%), amoxicillin (76%), chloramphenicol (62%), rifampicin (52%), erythromycin (44%), ciprofloxacin (42%), gentamycin (42%), streptomycin (34%) and levofloxacin (26%). This pattern however is not in keeping with a study done at the orthopedic ward of Ahmadu Bello University Teaching Hospital Zaria- Nigeria where ampicillin showed the highest resistance of (100%), gentamycin (54.5%), and ciprofloxacin (51.5%) [34] and from a cross-sectional study reported from Jimma university Southwest Ethiopia where they got ampicillin (95.7%), ciprofloxacin (96%), norfloxacin (96%) and gentamycin (96%) [28]. The findings were higher than that reported in a retrospective study at Ndola teaching hospital where norfloxacin (69%), chloramphenicol (43%), and ciprofloxacin (8.6%) were reported. This increased resistance in the various studies may be due to the antibiotics were used for a long time. Overuse of antibiotics contributes to organisms developing resistance [28]. MARI from the study was between 0.4 - 0.6. A similar trend was observed in a study in the Maiduguri metropolis (0.4-0.6) [35]. However, lower values were obtained from studies in Akwa Ibom State (0.1-0.3) [36]. Variations in the different areas of study may be due to the source of the isolates.

4. Conclusion

In areas or hospitals where multidrug resistance strains of *S. aureus* are highly endemic, encounters with other patients or health care personnel harboring the strain during the previous hospitalization may result in transmission and spread. From the study, the overall prevalence of multidrug-resistant *S. aureus* from wound infection patients was 33 (66%), 14% was methicillin-resistant.

Table 1: Demographic distribution of *S. aureus* from different units and sites of sample collection

Site	No of samples (no of isolate, %)						
	MSW	FSW	WDR	FMW	PSU	AEU	TOTAL
Diabetic ulcer	30(4, 13.3%)	20(3, 15.0%)	25(4, 16.0%)	15(2, 13.3%)	1(1, 100%)	2(0, 0%)	93(14, 15.0%)
Accident wound	20(3, 15.0%)	5(0, 0%)	25(4, 16.0%)	15(2, 13.3%)	8(1, 12.5%)	2(1, 50.0%)	75(11, 14.6%)
Burn wound	15(4, 26.6%)	10(1, 10.0%)	20(6, 30.0%)	10(1, 10.0%)	9(2, 22.2%)	5(2, 40.0%)	69(16, 23.1%)
Surgicalsite wound	15(3, 20.0%)	10(1, 10.0%)	20(2, 10.0%)	5(1, 20.0%)	7(0, 0%)	6(2, 33.3%)	63(9, 14.2%)
TOTAL	80(14, 17.5%)	45(5, 11.1%)	90(16, 17.7%)	45(6, 13.3%)	25(4, 16.0%)	15(5, 33.3%)	300(50, 16.7%)

* KEY: MSW- Male Surgical Ward , FSW- Female Surgical Ward , WDR- Wound Dressing Room , FMW- Female Medical Ward , PSU- Plastic surgery Unit , AEU- Accident and Emergency Unit.

Table **2:** Antibiotic resistance profile of the test organisms.

Sample type	No tested	CHL	CN	AMP	AMX	CPX	ERY	LEV	S	RD	NB
		Number of resistant isolates (%):									
Diabetic ulcer	14	9(64.2)	8(57.1)	11(78.5)	12(85.7)	5(35.7)	2(14.2)	3(21.4)	4(28.5)	3(21.4)	11(78.5)
Accident wounds	11	7(63.6)	4(36.3)	10(90.9)	11(100)	7(63.6)	8(72.7)	6(54.5)	7(63.6)	6(54.5)	9(81.8)
Burn wounds	16	9(56.2)	7(43.7)	11(68.7)	9(56.2)	4(25.0)	3(18.7)	0(0.00)	3(18.7)	10(62.5)	12(75.0)
Surgical site wounds	9	6(66.6)	2(22.2)	8(88.8)	6(66.6)	5(55.5)	9(100)	4(44.4)	3(33.3)	7(77.7)	9(100.0)
Total	50	31(62%)	21(42%)	40(80)	38(76)	21(42)	22(44)	13(26)	17(34)	26(52)	41(82)

* KEYS: Lev- levofloxacin 10ug, NB-Norfoxacin10ug, CHL- Chloramphenicol 30ug, CN- Gentamicin10ug, AMP- Ampiclox 10ug, AMX- Amoxicillin 30ug, CPX-Ciprofloxacin 10ug, E- Erythromycin 30ug, S- Streptomycin 30ug, RD- Rifampicin 10ug.

Table 3: Sensitivity and resistance of antibiotics inwards and wound types

Wards	N	Sensitivity	Resistance
		Mean ± SD	Mean ± SD
Males Surgical Ward	14	3.64 ± 2.59	6.34 ± 2.59
Female Surgical Ward	5	4.60 ± 2.07	5.40 ± 2.07
Wound Dressing room	16	4.56 ± 2.47	5.43 ± 2.47
Female Medical Ward	6	6.17 ± 2.64	3.83 ± 2.63
Plastic Surgery unit	4	4.00 ± 0.82	6.00 ± 0.81
Accident and Emergency	5	5.80 ± 2.59	4.20 ± 2.58
Total	50	4.59 ± 2.46	5.42 ± 2.46
F-value (P-value) P>0.05=Not Significant		1.23 (.313)	1.23 (.313)
Wound types: Accident	11	3.16 ± 1.73	3.16 ± 1.73
Diabetic wound	14	4.90 ± 2.34	5.09 ± 2.34
Burn wound	16	3.67 ± 1.91	6.33 ± 1.91
Surgical site wound	9	1.94 ± 1.00	1.94 ± 1.00
Total	50	4.58 ± 2.46	5.42 ± 2.46
F- value (P- value) P>0.05= Not Significant		1.52 (0.23)	1.52 (0.23)

Table 4: Multiple antibiotics resistance index (MARI) of the isolates

S/no	Sample type	Sample size	MARI	Percentage (%)
1	Diabetic ulcers	14	0.48	3.4
2	Accident wounds	11	0.68	6.1
3	Burn wounds	16	0.42	2.6
4	Surgical site wounds	9	0.65	7.2

Table 5: Prevalence of methicillin resistance of the test isolate

Sample type	No tested	No positive for methicillin resistance	Percentage (%)
Diabetic leg ulcers	14	3	21.4
Accident wounds	11	1	9.1
Burn wounds	16	2	12.5
Surgical site wounds	9	1	11.1

From the demographic distribution of *S. aureus*, the male surgical ward had the highest number of isolates 17.5% followed by the wound dressing room 17.7%. Antibiotic resistance profile showed the highest resistance to amoxicillin 100%from accident wound samples and 100% resistance to erythromycin and norfloxacin from surgical site wounds. Measures should be put in place by the hospital management to prevent the transfer of this multidrug-resistant *S. aureus* from patient to patient.

Authors' Contributions

Joan Miracle James-Onyekwere: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Visualization; Writing- review and editing. Immaculate Ugochi Nwankwo: Supervision; Methodology. Ebubechi Uloma Okey-Kalu: Investigation; Writing-original draft, Writing - Review and Editing.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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References

1. Center for Disease Control and Prevention. Staphylococcal Food Poisoning. US Department of Health and Human Services. August 9, 2018.

2. Steven YC, Tong JS, Davis EE ,Thomas L, Holland VG, Fowler J. Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clinical Microbiology Reviews.* 2015; 28(3): 603-61.

3. Paul D, George G, Dorothy J, Noel RK, Wolfgang L, Fred A, Rainey KS, William BW. *Bergey's Manual of Systematic Bacteriology :The Firmicutes* .2009.

4. Kazimoto T, Abdulla S, Bategereza L. Causative Agents and Antimicrobial Resistance Patterns of Human Skin and Soft Tissue Infections in Bagamoyo, Tanzania. *Acta Trop.* 2018;186:102-6.

5. Deguchi H, Kitazawa K, Kayukawa K. The Trend of Resistance to Antibiotics for Ocular Infection of *Staphylococcus aureus*, Coagulase Negative Staphylococcus and Cornybacterium Compared with 10-years Previous: A Retrospective Observational Study. *PLoS one.* 2018; 13(9):e0203705.

6. Belyhun Y, Moges F, Endris M. Ocular Bacterial Infections and Antibiotics Resistance Patterns in Patients Attending Gondar Teaching Hospital, Northwest Ethiopia. *BMC Res Notes.* 2018; 11:597.

7. Keihanian F, Saeidinia A, Abbasi K, Keihanian F. Epidemiology of Antibiotic Resistance of Blood Culture in Educational Hospitals in Rasht, North of Iran. *Infection and Drug Resistance* . 2018; 11:1723-8.

8. Yadav NS, Sharma S, Chaudhary DK. Bacteriological Profile of Neonatal Sepsis and Antibiotic Susceptibility Pattern of Isolates Admitted at Kanti Children's Hospital, Kathmandu, Nepal. *BMC Research Notes.* 2018; 11:301.

9. Dat VQ, Vu HN, Nguyen HT. Bacterial Bloodstream Infections in a Tertiary Infectious Disease Hospital in Northern Vietnam: Aetiology, Drug Resistance, and Treatment Outcome. *BMC Infectious Diseases.* 2017; 17(1):493.

10. Huttunen R, Attman E, Aittonieme J. Nosocomial Bloodstream Infections in a Finnish Tertiary Care Hospital: A Retrospective Cohort Study of 2175 Episodes During the Years 1999-2001 and 2005-2010. *Infectious Disease.* 2015;47(1):20-6.

11. Buetti N, Lo Priore E, Atkinson A. Low Incidence of Subsequent Bacteraemia or Fungaemia after Removal of a Colonized Intravascular Catheter Tip. *Clinical Microbiology and* Infection. 2018;24:548e1-e3.

12. Walaszek M, Rozanska A, Walaszek MZ, Wojkwoska-Mach J. Epidemiology of Ventilator-Associated Pneumonia, Microbiological Diagnostics and the Length of Antimicrobial Treatment in the Polish Intensive Care Units in the Years 2013-2015. *BMC Infectious Dis*ease. 2018;18(1):1-9.

13. Huang Y, Jiao Y, Zhang J. Microbial Etiology and Prognostic Factors of Ventilator-Associated Pneumonia: A Multicenter Retrospective Study in Shanghai. *Clinical Infectious Disease.* 2018;18:308.

14. Bonell A, Azarrafiy R, Huong V T L. A Systematic Review and Meta-Analysis of Ventilator-Associated Pneumonia in Adults in Asia: An Analysis of National Income Level on Incidence and Etiology. *Clinical Infectious Disease.* 2018; 68(3):511-8.

15. El-Sokkary RH, Ramadan RA, El-Shabrawy M. Community Acquired Pneumonia Among Adult Patients at an Egyptian University Hospital: Bacterial Etiology, Susceptibility Profile and Evaluation of the Response to Initial Empiric Antibiotic Therapy. *Infection and Drug Resistance.* 2018; 11:2141-50.

16. Inaci F, Pezzotti P, Panrosti A. Antimicrobial Resistance: A Global Multifaceted Phenomenon. *Pathogens and Global Health.* 2015:109-309.

17. Mannaa D, Mandal AK, Sen IK. Antibacterial and DNA Degradation Potential of Silver Nanoparticles Synthesized via Green Route. *International Journal of Biological Macromolecules.* 2015; 80:455-9.

18. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement, in CLSI Document M100-S21. Clinical and Laboratory Standards Institute. Wayne, PA. 2011.

19. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology.* 1996;36: 493-6.

20. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. Document No. M02-A10. Clinical and Laboratory Standards Institute. Wayne, PA USA. 2009.

21. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Fifteenth Informational Supplement Document. M 100-515. *CLSI.* Wayne, Pa USA. 2005.

22. Clinical Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard. M7-A7.Wayne, PA. 2006.

23. Kruperman PH. Multiple Antibiotics Resistance Indexing of *Escherichia coli* to Identify High Risk Sources of Fecal Contamination of Foods. *Applied Environmental Microbiology. 1983*;46:165-70.

24. Wangai FK, Masika MM, Maritim MC, Seaton RA. Methicillin-Resistant S. aureus (MRSA) in East Africa: Red Alert or Red Herring. *Biomedical Journal of Infectious Diseases.* 2019;19(1):596.

25. Angus N, Eze DE, Gugu TH, Ezeobi I, Maduagwu UN, Ihekwereme CP. Multiple Antibiotic Resistant Extended Spectrum Beta Lactamase Producing Bac Teria Pose a Challenge to the Effective Treatment of Wound and Skin Infection. *Pan African Medical Journal* . 2016; 27:66.

26. Akinkunmi EO, Adesunkanmi AR, Lamikanra A. Pattern of Pathogens from Surgical Wound Infections in a Nigerian Hospital and Their Antimicrobial Susceptibility Profiles. *African Health Science.* 2014;14 (4): 802-9.

27. Abdulrahim U, Kachallah M, Rabiu N, Usman NA, Adeshina GO, Olayinka BO. Molecular Detection of Biofilm- Producing S.aureus Isolates from National Orthopedic Hospital Dala, Kano State, Nigeria. *Open Journal of Medical Microbiology.* 2019;9(3).

28. Byrd A, Belkaid Y, Segre J. The Human Skin Microbiome. *National Review of Micrbiology.* 2018; 16: 143-55.

29. Ojo SKS, Oyechi TO, Esumeh FI. Antibiotic Resistance Profile of *Staphylococci* Isolated from Hospital Out Patients in Accident and Emergency Unit. *Journal of Pharmacy and Biological Sciences.* 2013;7(3):70-4.

30. Udeani TK, Onyebuchi CJ, Ikpenwa MC, Ezenwaka UR. Prevalence and Antibiotic Susceptibility Pattern of Methicillin Resistant *Staphylococcus aureus* in Burns and Pressure Ulcer Patients. *African Journal of Clinical and Experimental Microbiology.* 2016;17(2):130-9.

31. Kumurya AS, Sule H, Muje AU. *Staphylococcus aureus* from the Microbial Load of Open Wound from Patients Attending Aminu Kano Teaching Hospital Kano, Nigeria. *Journal of Bacteriology and Mycology Open Access.* 2017;5(7):434-7.

32. Bissong M, Wirgham M, Enekegbe M, Niba P, Foka F. Prevalence and Antibiotic Susceptibility Patterns of Methicillin Resistant S. aureus in Patients Attending the Laquintinie Hospital Douala, Cameroon. *European Journal of Clinical and Biomedical Sciences.* 2016;2(6):92-6.

33. Buzaid N, Elzouki AN, Taher I, Ghenghesh KS. Methicillin-Resistant S. aureus (MRSA) in a Tertiary Surgical and Trauma Hospital in Benghazi, Libya. *The Journal of Infection in Developing Countries.* 2011;5(10):723-6.

34. Udobi CE, Obajuluwa AF, Onaolapo JA. Prevalence and Antibiotic Resistance Pattern of MRSA from an Orthopedic Hospital in Nigeria. *Biomed Research International* .2013;860467.

35. Ismail H, Bello HS, Isa MA. Multidrug Resistance Pattern of S. aureus Isolates in Maiduguri Metropolis. *Research gate.* 2015:16-20.

36. Ibanga IA, Akan OD, Uyanga FZ, Mantu EC, Asuquo M. Multidrug Resistant S. aureus from Pediatric Ward, General Hospital, ikot-Ekpaw, Mkpat Enin LGA, Akwa Ibom State, Nigeria. *Archives of Clinical Microbiology.* 2020;11(1):105.