International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijamr Volume 9, Issue 9 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2022.09.09.001

Prevalence of Methicillin resistant *Staphylococcus aureus* in patients with surgical wounds attending Esuth, Parklane

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Keywords

methicillin resistant, *Staphylococus aureus*, surgical wounds

Abstract

The spectrums of infections due to Methicillin-resistant *Staphylococcus aureus* are manifold and are associated with worse outcomes. A study on the prevalence of these pathogens and their sensitivity patterns will give updated information which is very helpful for health personnel responsible in the management of patients and timely monitoring of the emergence of resistant strains. This study aimed at assessing the prevalence of Methicillin-resistant *Staphylococcus aureus* and associated factors among patients with surgical wound infection at ESUTH, Parklane. A cross-sectional study was conducted among 120 patients attending ESUTH, Parklane from the period of June to October, 2021. Wound swab samples were collected aseptically and transported to Medical Microbiology laboratory of ESUTH, Parklane. Isolation of *Staphylococcus aureus* was done based on cultural and biochemical profiles. Drug susceptibility test was performed using the Kirby Bauer disc diffusion technique as per the standard and interpreted based on the Clinical and Laboratory Standards Institute guidelines. The data were entered and analyzed by using SPSS version 20. There was a statistical level of difference in isolates positive for *Staphylococcus aureus* bacteria isolated from wound swabs in ESUTH, Parklane ($p<0.05$). *Staphylococcus aureus* isolates from 120 processed surgical wound swabs were 84(86%). Of these, 79(94%) were identified as Methicillin-resistant *Staphylococcus aureus* and 5(6%) were Methicillin-sensitive *Staphylococcus aureus* using Cefoxitin and Oxacillin. The isolated Methicillin-resistant *Staphylococcus aureus* showed highest resistance to Ampiclox (87%) and

highest sensitivity to Erythromycin (25%). There was a statistical level of difference between the antimicrobial susceptibility pattern of the *Staphylococcus aureus* isolates ($p<0.05$). This research revealed that Methicillin Resistant *Staphylococcus aureus* had a high prevalence at ESUTH, Parklane with a prevalence of 94% of the total samples taken. It is important that adequate provisions are made to install fully equipped hand washing sinks at vantage points all around the hospital and especially at the entrance of the surgical wards in ESUTH, Parklane.

Introduction

Staphylococcus aureus infections continues to rise and are increasingly implicated in different infections states. Of particular interest is the Methicillin Resistant *Staphylococcus aureus* (MRSA) because of its increasing resistance. Most *Staphylococcus aureus* infection used to be in health settings, but they are now seen to cause serious infection even in the community (Knox *et al*., 2015). The major isolate from surgical site infections and other open wounds is *Staphylococcus aureus* (Bhattacharya, 2016). Awareness is necessary for Methicillin Resistant *Staphylococcus aureus* (MRSA), which is commonly resistant to B-lactam antibiotics drugs like penicillin, cephalosporin as well as carbapenems (Bhattacharya, 2016).

Staphylococcus is the main genus of the Staphylococcacceae family in the order of Bacillales. *Staphylococcus aureus* (*S. aureus*) is a non-spore-forming, non-motile, facultative anaerobic Gram-positive cocci-shaped bacterium, which is 0.5 to 1µm in diameter. It is both catalase and coagulase positive and appears as bunch of grapes when viewed under a microscope. When grown on mannitol salt agar and blood agar, it produces round, golden-yellow colored colonies with B- hemolysin on blood agarand ferments mannitol i.e turns media yellow (Ryan and Ray, 2014). When *Staphylococcus aureus* reproduces asexually by binary fission, the two daughter cells may not fully separate and may remain attached to one another, which explain why the cells are often observed in clusters. The bacterium is the most pathogenic of the staphylococci species; it has a generation time of 20-30 minutes and can grow in conditions of high salt concentration and at temperatures between 10 and 46°C. A study by Sotto *et al*. (2010), highlighted the virulence potential of the bacterium, and is considered one of the main cause of community acquired and nosocomial infections, leading to high morbidity and mortality (Bhateja *et al*., 2010). The organism forms part of the normal human microbial flora. It can be found on skin surfaces, intestines, upper respiratory tract and vagina. It can become pathogenic when temperature and pH conditions become favorable and nutrient is available to support overgrowth (Makgotlho, 2015). The pathogen is usually carried in the nasopharynx, on the skin, clothing and sometimes in the vagina, rectum or perianal areas. They can easily contaminate any other parts of the human body from these sites through direct contact with the hands or by aerosol transfer.

While *Staphylococcus aureus* usually acts as a commensal bacterium, asymptomatically colonizing about 30% of the human population, it can sometimes cause diseases. In particular, *S. aureus* is one of the most common causes of bacteremia and infective endocarditis. Additionally, it can cause various skin and soft tissue infections, particularly when skin or mucosal barriers have been breached. The infections can spread through contact with pus from an infected wound, skin- to-skin contact with an infected person, and contact with objects used by an infected person such as towels, sheets, clothing or athletic equipment.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are the strains of the bacterium which have acquired the mecA gene that confers on them the ability to resist the effects of methicillin and several other beta-lactam based antibiotics. This resistant strain was first reported in 1961, after methicillin was approved for the treatment of penicillin-resistant staphylococci in humans

(Chambers and De Leo, 2015). MRSA was not that prevalent even in hospitals until the 1990s, when there was a surge in MRSA prevalence in hospitals and has since remained endemic (Johnson *et al*., 2011). Although MRSA causes similar infections as the methicillin-susceptible *Staphylococcus aureus* (MSSA), its resistance to most common antibiotics makes its treatment very challenging, and its management can result in considerable cost to a health facility (Guleri *et al*., 2011). Many studies have shown that MRSA-related infections are associated with higher risk of mortality and greater costs to the health-care system than infections caused by MSSA (Clements *et al*., 2010). Since MRSA was first discovered, its infection was only found in the hospitals through invasive procedures like urinary catheters, central venous lines, recent antibiotic use, and through contact with health care workers. It therefore became known as hospital-acquired MRSA (HA-MRSA). Recently, however MRSA infections have spread into the community and has become widespread (Bustamante, 2011).

Wound on the other hand is any interruption, by violence or by surgery, in the continuity of the external surfaces of the body or of the surface of any internal organ. According to him, legally, the whole thickness of the skin must be broken, and creating an internal injury wound. It is also a type of injury which happens relatively quickly in which the skin is torn, cut or punctured or where blunt force trauma causes a contusion. Wound can also be defined as injury to the skin or underlying tissues or organs by a blow or cut, missile or stab which includes injury to the skin caused by chemicals, cold, friction, heat, pressure and rays, and manifestation in the skin of internal conditions such as pressure sure and ulcers (Roper, 2014).

Patients with surgical wounds have been reported to be at high risk of MRSA infection (Quim, 2013).Wound sepsis is the infection of wound by pathogenic organisms. It is a life- threatening condition that arises when the body's response to infection causes injury to its own tissues and organs. Common signs and symptoms include fever, increased heart rate, increased breathing rate and confusion. (CDC, 2014).

Microorganism of clinical importance can be isolated from wounds and they include Actinomyces species, Bacteroides species, *Clostridium perfringes*, *Eschericha coli*, other gram negative enteric bacilli, like Mycobacterium species, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermis, Streptococcus faecalis, Clostridium tetani*.

The study was done to determine the prevalence of *Staphylococcus aureus* in patient with surgical wound attending ESUTH Parklane.

Materials and Methods

Study Area

Enugu state is one of the states in the Eastern part of Nigeria located at the foot of the Udi plateau. The state shares borders with Abia state and Imo state to the South, Ebonyi state to the East, Benue state to the Northeast, Kogi state to the Northwest and Anambra state to the West. The state has a good soil-land and climatic conditions all year round, sitting at about 223 meters (732ft) above sea level, and the soil is well drained during its rainy sessions.

Enugu state is predominantly an area with residents comprising mainly civil servants and students; there are also traders and business men and women. The people of Enugu state are predominantly Igbo, although some other ethnic groups also reside in Enugu. There are numerous financial and banking institutions, medical facilities, telecommunications and postal services, radio and television houses in Enugu urban. This study was conducted in Enugu State University Teaching Hospital (ESUTH), Parklane. The University was established as a non-residential multi-campus institution.

Study Design

A cross sectional case control analytical study was conducted in the surgical ward of ESUTH, parklane. A total of one hundred and twenty (120) wound swab samples were collected and used for the study.

Sampling Technique/Sample size

A stratified sampling technique was used to select the study participants for the study.

The sample size was calculated based on the prevalence rate from former researchers. The prevalence rate of MRSA from surgical wounds according to Senati *et al*., 2016 at 95% confidence level and 5% precision is 8.6% (Senati *et al*., 2016).

$$N= \quad Z^2 PQ_{D2}$$

Where;
N= Minimum sample size required
Z= Normal standard deviation for a normal distribution taken as 95% confidence interval which corresponds to 1.96
P= Expected prevalence from literature available (8.6%) Q=1-P
D= Degree of activity set at 0.05 for 95% confidence interval

$$N=1.96^2 \text{ x } 0.086(1-0.086)$$

$$0.05^2$$
$$N=120.7$$

Thus, the sample size of 120 respondents was obtained using the above formula.

Study Population

One hundred and twenty (120) different clinical samples were gotten from the surgical ward in Enugu State University Teaching Hospital, Parklane which was diagnosed at Medical Microbiology laboratory, ESUTH. The Samples used are wound swabs and wound exudates or pus.

Inclusion Criteria

1. Patients with surgical wounds in the surgical ward seeking treatment in ESUTH
2. Patients who were willing to participate in the research

Exclusion Criteria

1. Patients with other forms of wounds as well as minor injuries
2. Outpatients seeking treatments in Enugu State University Teaching Hospital
3. Patients who were unwilling to participate in the research

Sample Collection

Wound swabs/exudates of patients attending ESUTH, Parklane were collected at the surgical wards and other wards where surgical wounds patients were moved to. The samples were collected with sterile cotton swabs. The cotton swabs were moistened with sterile normal saline before sampling the surgical area. Wound exudates on the other hand were collected with a sterile needle in a string and labelled. The sampled swabs were placed into sterile transport containers and taken to the microbiology laboratory, Enugu State University Teaching Hospital, Parklane for culture, Gram stain, biochemical testing and sensitivity testing. The sampled swabs were immediately inoculated into prepared medium.

Approval for the research was granted by the Chief Medical director of Enugu State University Teaching Hospital, Parklane. Additional information regarding the age, sex, and whether the participant was using any form of antibiotics were obtained from the participants and records. Those on any form of antibiotic treatment were not included. All the sites from where samples were obtained were grouped under two categories based on proximity and to aid efficient sampling and analyses.

Isolation of *Staphylococcus aureus*

Sterile swabs were partially moistened in a 2.5% saline solution and were used to take the samples by rotating it five times at the injury site. Sterile needles were also used to get exudates from wounds with much pus. The swabs were then pre-enriched in test tubes containing 3ml of Mueller–Hinton broth (MHB) supplemented with 2.5% sodium chloride (NaCl). The pre-enriched test tubes were then incubated at 37◦C for 24 hours and used to inoculate the mannitol salt agar (MSA) which was incubated at 37◦C for 24 hours. Suspected yellow *Staphylococcus aureus* colonies surrounded by yellow halo zones (Figure 3.1) were picked and further tests such as Gram staining, microscopy, catalase production, hemolysis on blood agar, and tube coagulase, were performed to confirm the bacteria (Benson, 2011).

Figure 1: Colonies observed on mannitol salt agar plates (colonies surrounded by yellow halo are suspected to be *Staphylococcus aureus* colonies; red colored backgrounds have no *Staphylococcus aureus* growth.

Colonial and Morphological Characteristics

The isolate was determined by studying the size, shape, elevation, edge, consistency, odor, fermentation, hemolysis and pigmentation. The results were recorded and later compared for presumptive identification.

Gram Stain

A colony was picked from pure culture smeared on a clean grease free slide and allowed to air dry. It was then heat fixed by passing the slide over blue flame of a Bunsen burner for about three times. The smear was flooded with crystal violet stain for 60seconds and was washed off rapidly with clean water, it was then mordanted with lugol's iodine for 60seconds and was washed off rapidly again with clean water. It was also decolorized with acetone or alcohol for 2 seconds and washed off immediately. Finally, the counter stain safranin was added for 60seconds. It was then washed off with clean water and placed on a drinking rack to air dry. The smear was examined under high power oil immersion objective lens (x100) of light microscope. The Gram positive bacteria appeared purple while the gram negative organism appeared pink color.

Biochemical Tests. These tests were carried out for the complete identification of the organism isolated from the patients with significant growth.

Catalase Test

This test is carried out for the identification and differentiation of catalase positive organism (*Staphylococcus species*) from catalase negative organisms (*Streptococcus species*). A drop of 3% hydrogen peroxide was placed on a clean slide and a small portion of the solid culture to be identified was emulsified into it. A spontaneous effervescence reaction indicates a positive test.

Coagulase Test

This test was used to differentiate strains of staphylococcus by the production of coagulase. It differentiates between coagulase positive (*Staphylococcus aureus*) and coagulase negative (other *Staphylococcus species*). A colony from the test organism was emulsified in a drop of distilled water on a clean class slide. A loop of plasma was

added to the suspension and mixed gently. The presence of agglutination showed a positive test.

Antimicrobial Susceptibility Testing

The Kirby Bauer disc diffusion method was employed to test for antimicrobial susceptibility as recommended by Clinical Laboratory Standards Institute which indicated Cefoxitin disk diffusion or Cefoxitin MIC tests were preferred methods to determine resistance or susceptibility to cephalosporin and a wide array of other β-lactam antimicrobial agents. The test can thus be used to predict the presence of mecA-mediated methicillin resistance in *S. aureus*.

Test for Methicillin Resistant *Staphylococcus aureus*

All isolated *Staphylococcus aureus* strains were subjected to the antibiotics cefoxitin and oxacillin to differentiate Methicillin Resistant *Staphylococcus aureus* from Methicillin Sensitive *Staphylococcus aureus*. This was done by disc diffusion method. The strains were sub-cultured on Muller Hinton agar. Cefoxitin and oxacillin were placed on each ends of the plate and incubated for 24 hours at 37°C. Zone of inhibition and clearance were checked out for Methicillin Resistant *Staphylococcus aureus* from Methicillin Sensitive *Staphylococcus aureus.*

Statistical Analysis

The data generated from the study were analyzed using Analysis of Variance (ANOVA) to test for differences between treatment groups using statistical package for Social Sciences (SPSS) version 20. A value of ($p<0.05$) was accepted as significant.

Ethical Consideration

Ethical clearance was obtained from the ethical committee of the hospital. Informed consents of the patients were obtained and assurance were given to them that all information received would be handled confidentially. Participants were informed that participation is voluntary and safety was ensured. Also adequate client respect was given to the respondent. Also there were ethical clearance in the appendices.

Results

Table 4.1: Respondent's demographic/ personal information

Variables	Frequency (n=120)	Percentage (%)
Age (Years) (Mean±SD)	54.91±7.4	
18-27	26	22
28-37	19	16
38-47	26	22
48-57	22	18
58>	27	23
Gender		
Male	71	59
Female	49	41
Marital Status		
Married	83	69
Single	11	9
Divorced	3	3
Widowed	23	19

Occupation None Student Farmer Trader		
Civil servant	18	15
	29	24
Educational Status	11	9
Tertiary Secondary Primary None	27	23
	35	29
Religion Christianity Muslim		
Others	41	34
	29	24
	37	31
	13	11
	105	86
	10	8
	5	6

Table 4.2: Perception of knowledge of MRSA from patients attending ESUTH, Parklane

Variables	**Frequency (120)**	**Percentage (100%)**
	YES	**NO**
a. Heard of *Staphylococcus aureus*	101(84%)	19(16%)
b. Encountered bacterial infection caused by *Staphylococcus aureus*	96(80%)	24(20%)
Heard of Methicillin Resistant Staphylococcus *aureus*	31(26%)	89(74%)
Diagnosed of an infection due to Methicillin Resistant Staphylococcus *aureus*	23(19%)	97(81%)
e. Have sought advice because of a problem with wound other than the routine lanned follow up treatment at ESUTH, Parklane.	72(60%)	48(40%)

Table 4.3: Factors that may lead to antibiotics resistance amongst patients attending ESUTH, Parklane

Variables	**Frequency (120)**	**Percentage (100%)**
	YES	**NO**
a. Heard of Antibiotics	109(91%)	11(9%)
Consults physician before taking antibiotics	53(44%)	67(56%)
c. Always finish an entire antibiotics course	89(74%)	31(26%)

d.	Currently on any antibiotics	120(100%)	0(0)
e.	Any history of hospitalization before now	51(43%)	69(57%)
f.	Run microbiological culture before taking antibiotics	83(55%)	67(45%)
g.	Presence of any in dwelling medical device	0(0)	120(100)
h.	Did you take antibiotics that was prescribed for a different person?	83(69%)	37(31%)

		Viral infection	**Bacterial infection**	**Other Reasons**
i.	What are antibiotics used for?	31(26%)	53(44%)	36(30%)
		Antibiotics	**Anti-inflammatory**	**Analgesics**
j.	Medication used more frequently	109(91%)	9(8%)	2(2%)
		Once a Month	**Twice a Month**	**> Thrice a Month**
k.	How many times do you take antibiotics?	31(26%)	46(38%)	43(36%)

Table 4.4: Occurrence of positive and negative cases from wound swab cultures of patients attending ESUTH, Parklane according to age group.

Age group	No of samples	No of positive (%)	No bacteria growth (%)	p-value
18-28	26	24(92)	2(8)	0.000*
28-39	19	16(84)	3(16)	0.096
40-50	26	21(81)	5(19)	0.026*
51-61	22	17(77)	2(23)	0.000*
>62	27	20(74)	7(26)	0.000*
Total	**120**	**98(82)**	**22(18)**	

P>0.05 (Not significant)

Table 4.5: Occurrence of *Staphylococcus aureus* from wound swab cultures of patients attending ESUTH, Parklane according to age group.

Age group	No of samples	*S. aureus* positive (%)	*S. aureus* negative (%)
18-28	24	22(92)	2(8)
28-39	16	13(81)	3(19)
40-50	21	19(90)	2(10)
51-61	17	11(65)	6(35)
>62	20	19(95)	1(5)
Total	**98**	**84(86)**	**14(14)**

Table 4.6: Methicillin sensitive *Staphylococcus aureus* (MSSA) prevalence and methicillin resistant *Staphylococcus aureus* (MRSA) according to gender and age of patients with surgical wounds attending ESUTH, Parklane.

Parameters	Total n=84 Overall =86%	MRSA prevalence (%)	MSSA prevalence (%)
Gender Male Female			
	59	56(95)	3(5)
	25	23(92)	2(8)
Age Group			
18-28	22	22(100)	0(0)
29-39	13	12(92)	1(8)
40-50	19	17(89)	2(11)
51-61	11	11(100)	0(0)
>62	19	17(89)	2(11)
Total	**84**	**79(94)**	**5(6)**

Table 4.7: Methicillin sensitive *Staphylococcus aureus* (MSSA) prevalence and methicillin resistant *Staphylococcus aureus* (MRSA) using antibiotics cefoxitin and oxacillin

Parameters	Frequency	Percentage
MRSA prevalence (%) MSSA prevalence (%)	79	94
Total	5	6
	84	**100**

Table 4.8: Antibiotic susceptibility pattern of Methicillin resistant *Staphylococcus aureus* isolated from surgical wounds of patients attending ESUTH, Parklane.
P>0.05 (Not significant)

Antibiotics	Number of *S. aureus* Sensitivity and Percentage	Number of *S. aureus* Resistant and Percentage	p-value
	N=79		
Pefloxacin (PEP)	15(19)	64(81)	0.000*
Gentamycin (CN)	13(16)	66(84)	0.002*
Ampiclox (APX)	10(13)	69(87)	0.000*
Zinacef (Z)	18(23)	61(77)	0.000*
Amoxacillin (AM)	13(16)	66(84)	0.000*
Rocephin (R)	11(14)	68(86)	0.000*
Ciprofloxacin (CPX)	18(23)	51(77)	0.000*
Streptomycin (S)	12(15)	67(85)	0.000*
Septrin (SXT)	13(16)	66(84)	0.000*
Erythromycin (E)	20(25)	59(75)	0.000*

Discussion

This present study was carried out to determine the prevalence of Methicillin Resistant *Staphylococcus aureus* in patients with surgical wounds attending ESUTH, Parklane. A total number of one hundred and twenty (120) wound swab samples were collected from patients with surgical wounds attending ESUTH, Parklane. From the total samples, 98(82%) showed positive pathogens isolate while 22(18%) had no case of bacterial growth and were referred to as negative cases. This result showed a case of high prevalence of bacterial contamination with wound swabs in ESUTH, Parklane. There was a statistical level of difference between the occurrence of positive and negative cases bacteria isolated from wound swabs in ESUTH, Parklane ($p<0.05$).

In this present study, the overall prevalence of Methicillin Resistant *Staphylococcus aureus* was 94%. The observed high prevalence of Methicillin Resistant *Staphylococcus aureus* 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, Methicillin Resistant *Staphylococcus aureus* wound infections were significantly associated with occupation (farmers), and (Traders). This might be because farmers may not have knowledge of utilizing healthcare services; in addition, their occupation may expose them to wound infection and make them use antibiotics without prescription. High prevalence of Methicillin Resistant *Staphylococcus aureus* in admitted patients may be attributed by resistant strain bacterial cross-contamination in health institutions. Based on this current study, the null hypothesis (H_0: Methicillin-resistant *taphylococcus aureus* (MRSA) is highly associated with wound sepsis) was accepted and the alternative hypothesis (H_A: Methicillin-resistant *Staphylococcus aureus* (MRSA) is not associated with wound sepsis) was rejected.

Highest prevalence (100%) of Methicillin-resistant *Staphylococcus aureus* was found amongst the age 18-28. This is contrary to a similar study carried out by Monnet, 2014 who reported a low prevalence rate of 7.14% between the same age group. This could be as a result of lower socio- economic status associated with poor personal hygiene methods.

Concerning the antimicrobial susceptibility pattern of the isolates as shown in table 4.7 in the present study, *Staphylococcus aureus* isolates showed highest resistance to Ampiclox (87%) and highest sensitivity to Erythromycin (25%). There was a statistical level of difference between the antimicrobial susceptibility patterns of the *Staphylococcus aureus* isolates.

Conclusion

This research revealed that Methicillin Resistant *Staphylococcus aureus*had a high prevalence at ESUTH, parklane with a prevalence of 94% of the total samples taken. It is important that adequate provisions are made to install fully equipped hand washing sinks at vantage points all around the hospital and especially at the entrance of the surgical wards in ESUTH, parklane. The study also revealed that both males and females stand an equal chance of being infected with Methicillin Resistant *Staphylococcus aureus* and also becoming agents of its transmission to others. Children and adults also stand an equal chance of being infected or becoming agents of transmitting the bacteria to others. However, prevalence of Methicillin Resistant *Staphylococcus aureus* was also found to be significantly high among the age group of 18-28.

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Website: www.ijarm.com

Subject: Microbiology

Quick Response Code

DOI:10.22192/ijamr.2022.09.09.001

How to cite this article:
Chidimma Maureen Chukwueze, Ogochukwu Vivian Okpala and Emmanuel Ifeanyi Obeagu. (2022). Prevalence of Methicillin resistant *Staphylococcus aureus* in patients with surgical wounds attending Esuth, Parklane. Int. J. Adv. Multidiscip. Res. 9(9): 1-12.
DOI: http://dx.doi.org/10.22192/ijamr.2022.09.09.001