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Prevalence and Antibiotics resistance profile of *Staphylococcus aureus* isolated from post-operative wounds in secondary health facilities of Ilorin metropolis, Kwara State, Nigeria

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Abstract

Healthcare-associated infections are of different forms, with Surgical Site Infections (SSI) being the second most common type, they continue to be a relatively common postoperative complications and the most frequent reason for re-admission following surgery. Several data from around the world revealed Staphylococcus aureus to be the leading cause of surgical site infection. Therefore, this study aimed to determine the occurrence and drug resistance profile of Staphylococcus aureus and Methicilin Resistant Staphylococcus aureus isolated from post-operative wounds in secondary health facilities within the Ilorin metropolis. With the aid of sterile cotton swabs, a total of hundred and thirty-two (132) wound swab samples were obtained from patients who had been clinically diagnosed with surgical site infection. These samples were processed as per standard microbiological techniques. Staphylococcus aureus was isolated at an occurrence rate of 15.2%. Chi-square analysis showed that there was a significant difference in the number of isolates in relation to both hospitals (GHI and CHO) (p<0.05), the highest occurrence of Staphylococcus aureus (12.6%) was seen in GHI, while an occurrence rate of (24.1%) was recorded at CHO. The antimicrobial susceptibility profile revealed that 8(40%) of the S.aureus isolates were Methicilin Resistant Staphylococcus aureus (MRSA). S.aureus showed 100% susceptibility to Tigecycline and it was 100% resistant to Cefoxitin. Therefore, these findings affirmed that there is significant resistance in Staphylococcus aureus isolated from post-operative wounds in health facilities of the Ilorin metropolis.

Keywords: *(Surgical Site Infections, Post-operative wounds, Staphylococcus aureus, Prevalence).*

INTRODUCTION

Wounds or more specifically complex wounds are categorized as either chronic or acute (Guo and DiPietro., 2010). A wound may be referred to as chronic when its underlying etiology makes the healing process more challenging; postoperative wounds are an excellent example of chronic wounds. Hospital-acquired post-operative wound infections are known to increase hospital stays, cause pain and discomfort, and occasionally result in long-term or permanent disability. Exogenous and endogenous bacteria that penetrate the surgical site during the procedure are typically to blame for these infections (Nichols, 2004). The pathogenic bacteria that have been isolated from Surgical infected wounds include *Staphylococci, Enterococci, Streptococci, E. coli, Klebsiella, Pseudomonas, Acinetobacter, Enterobacter, Citrobacter, Proteus, etc.* As normal flora, these organisms coexist in various body parts (Oliveira *et al.*, 2018). Environmental transfer occurs as a result of their ease of transmission from one person to another, onto the hands and clothing of medical personnel, as well as onto objects and the air (Nichols, 2004; Shittu *et al.*, 2012). Conversely, some associated risk factors such as age (e.g. an elderly person), decreased immunity, Patients who have undergone transplants, underlying diseases, and therapeutic procedures could increase a patient's susceptibility to SSIs (Mangram *et al.*, 2011). Other key elements that impact whether infection occurs include the virulence and invasiveness of the implicated organism, the physiological state of the tissue surrounding the wound, and the host's immunological integrity. (Masaadeh and Jaran, 2009). With MRSA being a major problem, *Staphylococcus aureus* is one of the most often reported etiologic agents of Surgical site infections, accounting for up to 37% of infections in community hospitals (Anderson et al., 2007). *S. aureus* lives on skin

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surfaces, and it is estimated that 31% (with a range of 6-56%) of the general population has *S. aureus* colonizing their anterior nares at any given moment. Deep-seated infections as well as cutaneous lesions on the surface of the skin can both be caused by *S. aureus*. Over the past few decades, there has been an increase in the prevalence of SSI caused by S. aureus, mostly due to the ongoing emergence of isolates resistant to certain drugs. The center for disease control and Prevention (CDC) reports that between 1992 and 2002, the percentage of SSIs caused by S. aureus rose from 16.6% to 30.9%, and the number of isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA) similarly climbed from 9.2% to 49.3% (Jernigan., 2004). Patients who are carriers of *S. aureus* are typically two to nine times more likely to experience an SSI, and research has indicated that endogenous patient colonization accounts for 85% of SSIs. (Skramm *et al.*, 2014).
Studies in Nigeria have indicated that SSIs in post-operative wounds are endemic in some parts of the country (Shittu *et al.*, 2012; Kolmos *et al.*, 2013) ranging between a prevalence rate of 5.1% to 60.7% (Olowo-Okere *et al.*, 2019). However, compared to Nigerian studies, the infection rate recorded in many overseas studies is fairly low; for instance, it is 2.8% in the USA and 0.6-9.5% in European nations. (CDC, 2016; Satyanarayana *et al.*, 2011). The main contributing factors for greater infection rates in Nigerian hospitals may include improper hand hygiene techniques, a lack of focus on infection control measures, and overcrowding in the hospitals.
For efficient antibiotic use and infection management, it is important to understand the occurrence, and patterns of antibiotic resistance of organisms associated with SSI. Accordingly, this study aimed to give an overview of the prevalence rate of SSI caused by *Staphylococcus aureus* in secondary health facilities within the Ilorin metropolis.

MATERIAL AND METHODS

Study Population

Patients clinically diagnosed with Surgical Site Infection were enrolled for this
Cross-sectional study. A total of one hundred and thirty-two (132) wound swab samples were collected from two health facilities namely General Hospital Ilorin and Cottage Hospital Ogidi, both of which are government-run hospitals located in Ilorin, Kwara State. Approval was obtained from the Ethical Committee of Kwara state ministry of Health for all samples used for this study. Informed consent was obtained from patients before enrollment.

Collection of wound swab Samples

The wound swab samples were collected from post-operative patients with the aid of two sterile swabs under aseptic conditions, of which one was meant for smear preparation and the other for culture preparation. The samples were labeled and immediately conveyed to the Department of Microbiology for processing according to Standard microbiological procedures in Cheesbrough (2012).

Cultivation, Isolation, and Identification

The wound swabs were inoculated on Chocolate agar and incubated at 37ºC for 24 hours according to standard procedures (Cheesbrough., 2012). Colonies that were suggestive of *S. aureus* were further identified by Gram staining, catalase test, tube coagulase test, and mannitol fermentation tests.

Antimicrobial Susceptibility testing

The Inoculum of each of the confirmed *Staphylococcus aureus* isolates was standardized by matching its turbidity with that of 0.5 McFarland standards. Antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method. The test was performed by inoculating the standardized bacterial inoculum onto the surface of the Mueller-Hinton Agar plate. The antibiotics used were cefoxitin, clindamycin, erythromycin, gentamycin, tigecycline, linezolid, and ciprofloxacin. Methicillin-resistant *Staphylococcus aureus* was detected by using cefoxitin (30ug) as a surrogate marker for Methicillin. The discs were placed on the agar plate within 15 minutes of inoculation using sterile forceps and pressed firmly against the plate. The plates were inverted and incubated for 18-24 hours at 37˚C. The diameters of the zone were related to the susceptibility of the isolate and the diffusion rate of the drug through the agar medium. The diameter of the inhibitory zones of both the isolate and control was measured and interpreted as sensitive, intermediate, or resistant, according to the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2021).

Quality Control: ATCC 25922 was used as a control strain to ensure the quality of the antimicrobial susceptibility procedure.

Data Analysis

Descriptive statistics such as percentages were used to determine the prevalence of *Staphylococcus aureus* and also the percentage of the antimicrobial susceptibility pattern. The chi-square test was used to determine the association between the occurrence of *staphylococcus aureus* and sociodemographic factors, as well as patient medical information.

RESULTS

Out of the 132 wound swab samples screened, a total of 20 *Staphylococcus aureus* were isolated, giving an occurrence rate of 15.2% as presented in Figure 1.

Table 1 reveals that the occurrence of *Staphylococcus aureus* was higher in the Caesarean section having a rate of 6.8%, followed by Appendectomy at 3.0% and Open reduction with external fixation at 3.0%, the occurrence of 1.5% was seen in Skin grafting and occurrence rate of 0.8% was seen in Wound closure. The table also reveals that S. aureus was not isolated from surgical sites such as Hip replacement, Knee replacement, Amputation, Tooth extraction, Internal knee closure, and Arthroscopy of the knee. It is statistically significant at p-value <0.05 (p=0.001).

Table 2 shows the occurrence of *Staphylococcus aureus* in wounds according to various health facilities. Among the health facilities investigated, Staphylococcus aureus occurrence rates were observed at 12.6% at General Hospital Ilorin and 24.1% at Cottage Hospital It is statistically significant at p-value <0.05 (p=0.000).

Table 3 shows the occurrence of *Staphylococcus aureus* in wounds according to gender. Among female patients, Staphylococcus aureus was isolated at a rate of 13.4%, while among male patients, it was isolated at a rate of 20.0%. With a p-value of 0.05, it is not statistically significant (p=0.289).

Table 4 represents the occurrence of *Staphylococcus aureus* isolated from wounds in different age groups with *Staphylococcus aureus* showing a higher occurrence of infection in age groups 41-50 and 21-30 at rates of 35% and 16.3% respectively and the least occurrence in age groups 11-20 and 61-70 recorded at 11% and 8.3% respectively. It is statistically significant at P value <0.05 (p=0.039).

Table 5 reveals the susceptibility profile of *Staphylococcus aureus* isolates from post-operative wounds in Ilorin Metropolis. Of all 20 *Staphylococcus aureus* isolates, 8 (40%) were c MRSA as shown by resistance to cefoxitin. 10% of all *Staphylococcus aureus* isolates showed resistance to linezolid and no resistance was recorded for tigecycline. There was 40% resistance to ciprofloxacin, 10% to clindamycin, 35% to erythromycin, and 20% were resistant to gentamycin.

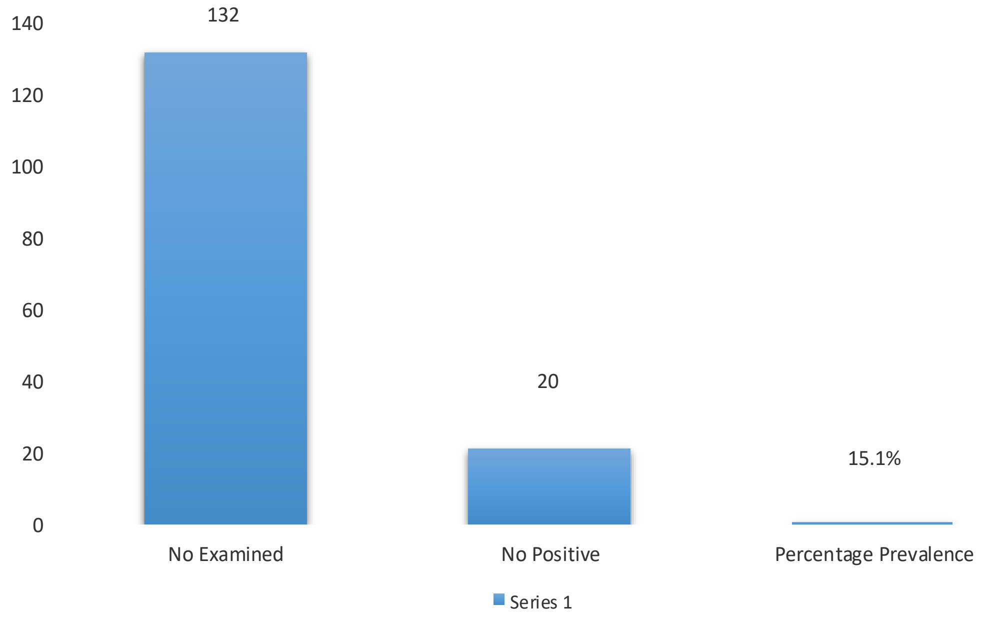

Figure 1: Occurrence of *Staphylococcus aureus* in post-operative wound sites investigated in the Ilorin metropolis.

Table 1: Prevalence of *Staphylococcus aureus* According to Types of Surgical Operation (N=132)

Types of Surgical Operation	Number examined	Number (%) S. aureus	X^2	P value
Caesarean section	57	9 (6.8)		
Wound closure	9	1 (0.8))		
Hip replacement	9	0(0.0)		
Appendectomy	22	4 (3.0)		
Knee replacement	1	0(0.0)	169.88	0.001
Tooth extraction	6	0(0.0)		
Open reduction with external fixation	9	4 (3.0)		
Internal knee closure	1	0(0.0)		
Arthroscopy of the knee	7	0(0.0)		
Amputation	5	0(0.0)		
Skin Grafting	6	2 (1.5)		
Total	132	20(15.2)		

Statistically significant at p value <0.05 (p=0.001).

Table 2: Prevalence Of *Staphylococcus aureus* According To Various Health Facilities (N=132)

Health facilities	**Number examined**	**Number (%) S. aureus**	**X^2**	**P value**
General Hospital Ilorin	103	13(12.6)		
Cottage Hospital	29	7(24.1)	47.86	0.000
Total	132	20(15.2)		

Statistically significant at p value <0.05 (p=0.000).

Table 3: Prevalence of *Staphylococcus aureus* According to Gender of the Patients (N=132)

Gender	Number examined	Number (%) S aureus	X^2	P value
Male	35	7(20.0)		
Female	97	13(13.4)	13.07	0.289
Total	132	20(15.2)		

Not statistically significant at p value<0.05 (p=0.289).

Table 4: Prevalence of *Staphylococcus aureus* According to Age Group (N=132)

Age	Number examined	Number (%) S. aureus	X^2	P-value
0-10 years	3	0(0.0)		
11-20 years	9	1(11.1)		
21-30 years	43	7(16.3)		
31-40 year	29	4(13.8)		
41-50 years	20	7(35.0)	100.26	0.039
51-60 years	12	1(8.3)		
61-70 years	10	0(0.0)		
71-80 years	6	0(0.0)		
Total	132	20(15.2)		

Statistically significant at P value <0.05 (p=0.039).

Table 5: Antibiotic Susceptibility Profile of *Staphylococcus aureus* From Post-Operative Wounds in Ilorin Metropolis (N=20)

Antibiotic agent	Disc content(ug)	Susceptibility profile		
		Sensitive (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	5	11(55.0)	1(5.0)	8(40.0)
Tigecycline	15	20(100)	0(0.0)	0(0.0)
Cefoxitin(MRSA)	30	12(60.0)	0(0.0)	8(40.0)
Clindamycin	2	12(60.0)	6(30.0)	2(10.0)
Erythromycin	15	3(15.0)	10(50.0)	7(35.0)
Gentamycin	10	16(80.0)	0(0.0)	4(20.0)
Linezolid	30	18(90.0)	0(0.0)	2(10.0)

DISCUSSION

The prevalence of *Staphylococcus aureus* of 15.2% from surgical site infections obtained in our study is comparable with previous studies in Nigeria by Iduh *et al.* (2014) who reported a 13% occurrence rate; Anjum *et al.* (2010) reported 19.7% and Akinjogunla *et al.* (2010) who reported 14.3% occurrence rate. However, the rate of surgical site infections reported from other part of the world is low compared to reports from Nigeria. , For example, SSI was 2.8% in the United States and about 2-5% in the European countries (Satyanarayana *et al.,* 2011).in this study, the occurrence of *Staphylococcus aureus* in SSI was highest in Caesarean section (6.8%), followed by Appendectomy (3.0%), skin grafting (1.5%), and a lower prevalence was seen in wound closure (0.8%). Inappropriate preoperative or post-operative prophylaxis and multiple risk factors such as age, and medical complications during pregnancy are reasons for the increased occurrence of Surgical site infection in C-section surgeries. Other possible risk factors are obesity and prolonged duration of rupture of the membrane for longer than 18 hours.

During the study, we discovered that female patients typically had a higher rate of surgical site infection than male patients, which resulted in a higher positive rate for *Staphylococcus aureus* in the female patients. This finding is consistent with previous studies from Kolmos *et al* (2013), and contrasts with studies from Naik *et al.,* (2011) and Iduh *et al.,* (2014) where male patients were more infected with *Staphylococcus aureus* than females. The increasing rate of C-section surgeries in the modern era and the increased mobility of the female population may both contribute to this.

In the study population, a higher infection rate was observed in the age group of 21-30 years and 41-50 years where both have an occurrence rate of 35% and 16.3% respectively and a lower rate of infection was seen in the age group 11-20 and 51-60 with an occurrence rate of 11.1% and 8.3% respectively. This finding is consistent with a study by Kaye et al (2005), which was conducted in 11 hospitals, there was an increased incidence of SSI up until age 65. The occurrence of SSI reduced after the age of 65. SSI in the young and elderly, however, raise interesting questions such as, do younger and older patients who have a higher risk of SSI have surgery less frequently than their healthy peers, likely because of bad clinical outcomes. Does the "hardy survivor" effect hold true, which suggests that older and younger patients may have genetic factors which help them resist infections. However, Further research is necessary to determine whether a combination of these factors results in an elderly surgical population that is generally in good health.

Although, Linezolid and Tigecycline were the most sensitive antibiotics for *S. aureus*. Antibiotic susceptibility results revealed that a degree of resistance was seen for many bacterial isolates, and the commonly used drugs were found to be more intermediate and resistant. 40% of the Isolates were resistant to cefoxitin and ciprofloxacin and exactly half of the isolates showed intermediate susceptibility to erythromycin. The incidence of Methicillin-resistant *S. aureus* in our study was 8 (40%) as shown by resistance to cefoxitin. This finding agrees with the reported incidence of MRSA in a study conducted by Eagye et al. (2009) at 45% and Keith *et al.* (2009) at 58.2%. But, observations by Iduh *et al* (2015) have shown a higher incidence of 84.4%. Staphylococcal resistance to methicillin occurs when an isolate carries an altered Penicillin-binding protein, PBP2a, which is encoded by the mecA gene (Soleto *et al.,*2003) (Lilani *et al.,*2005). This penicillin-binding protein binds beta-lactams with lower avidity, which results in resistance to this class of antimicrobial agents (Soleto *et* *al.*,2003) (Kownhar *et al.*,2008). A study by Harbarth et al. (2008) observed that methicillin-resistant S. aureus (MRSA) alone It is an undisputed fact that these Surgical site infections may originate from the patient's normal flora or that they may be derived from the hospital environment. Multidrug-resistant S. aureus is commonly associated with the hospital environment, this fact needs to be kept in mind by surgeons and healthcare practitioners.

Finally, these findings indicate that to deliver high-quality surgical services and reduce the spread of these resistant organisms, Kwara State Hospital management must provide a consistent, tight clinical interaction between the surgical team, the microbiologist, and the infection control staff. In addition, the study revealed that the majority of the *Staphylococcus aureus* isolates were sensitive to tigecycline and linezolid which are antibiotics that are used as a last resort to treat infections. However, the medical microbiologist and in rare or emergency cases, the clinician, should always have access to recent data on the occurrence and antibiotic susceptibility pattern of the commonly encountered organisms.

constituted 5.1% of surgical site infections (Harbath *et al.,* 2008).

CONCLUSION

The study revealed that Staphylococcus aureus (15.2%) remains one of the leading causes of surgical site infection. Likewise, the study reported a high prevalence (40%) of Methicillin-resistant Staphylococcus aureus (MRSA) among the patients infected with S. aureus. We credit this high rate of MRSA to the high degree of transmission in the hospital environment, over antibiotic-prescribing practices, and new more, virulent strains; another important risk factor is the carrier state of MRSA. However, we observed that irrespective of Methicilin resistance, the majority of the S. aureus isolates were sensitive to Tigecycline and Linezolid, most likely because they're a last resort to treat infections. In summary, the findings therefore, demonstrate a significant increase in S. aureus isolated from post-operative wounds and a high degree of MRSA in the selected Kwara State health facilities. Early identification of MRSA-positive patients undergoing a surgical procedure through pre-operative screening may decrease the incidence of MRSA.

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