

Original Research Article

Characterization of Virulent Bacterial Isolates Associated with Multi-Drug Resistance in Patients with Surgical Site Infections.

Comment [VEUM1]: This title is similar to a review, I suggest you modify it and you can refer from here to the geographical site of the study

ABSTRACT

Aims: Increasing research findings have documented the continuous emergence and threats posed by drug resistant clinical isolates from post-operative wound infections to commonly used antibiotics globally. This hospital-based study investigated virulent bacterial pathogens implicated with post-operative wound infections among surgical site infection (SSI) patients in Calabar, Nigeria and determined their antibiotic resistance pattern.

Methodology: Within 12 months study period, a total of 127 bacterial isolates of different genus from 110 SSI patients, were isolated from pus and surgical wound exudates and fully characterized using standard bacteriological procedures. Antimicrobial susceptibility patterns of isolates were determined using Kirby- Bauer disk diffusion method, following the guidelines by Clinical Laboratory Standard Institute (CLSI).

Results: Multi-drug resistant bacteria isolated and their percentage frequency were Coagulase Negative Staphylococci (21.3%), *Staphylococcus aureus* (19.7%), *Pseudomonas aeruginosa* (14.2%), *Escherichia coli* (11.8%), *Klebsiella pneumoniae* (9.4%), *Enterococcus faecium* (6.3), *Enterobacter cloacae* (4.7%), *Proteus mirabilis* (4.7%), *Acinetobacter baumannii* (3.1%), *Pseudomonas putida* (3.1%) and *Aerococcus viridans* (1.6%). Among Gram-positive bacteria isolated, *S. aureus* showed highest resistance to several antimicrobials (100% to oxacillin, 96% to ciprofloxacin, 92% to Levofloxacin, and 76% resistance to Vancomycin). All recovered *S. aureus* isolates were Cefoxitin screen positive indicating possible MRSA isolates. Additionally, among Gram-negative isolates *K. pneumoniae* was found to possess higher resistance to several antibiotics (66.7% resistance to each of Ciprofloxacin, Levofloxacin, ceftazidime, Trimethoprim/Sulfamethoxazole, Cefazolin, Ampicillin, Tobramycin and 58.3% resistance to each of Ceftriaxone, Gentamicin, and Ampicillin/Sulbactam). Statistical analysis of categorical variables of study subjects revealed that length of hospital stay, type of surgery, previous

admission history, antibiotic use, and age were significant ($p < 0.05$) in SSI outcome of patients, while patients' gender was not significant ($p > 0.05$) in SSI outcome.

Conclusion: Adherence to measures of strict infection control, optimal preoperative, intraoperative, and postoperative patient care, including multifaceted approaches involving surveillance, and antimicrobial stewardship, are vital to SSI treatment outcomes.

Keywords: Bacteria, Antibiotics, Multi-Drug Resistance, Isolates, Surgical-Site, Infections

1.0 Introduction

Surgical site infection occurs due to contamination of surgical wound(s) by microorganisms.^[1] Its occurrence is within 30 days post-surgery or after a year (for surgical procedures that involve an implant). Surgical site infections are amongst the most common types of nosocomial infections, contributing about 13% of common hospital acquired infections.^[1,2] Pathogens that contaminate surgical wounds could arise from mainly exogenous sources which includes health workers, operating theatre environment, used materials and instruments. Such pathogens are mostly aerobic microbes, particularly Gram-positive organisms e.g. *Streptococci* and *Staphylococci*.^[2] A retrospective review did report that about 67% of implant infections were caused by Gram-positive organisms especially methicillin resistant *Staphylococcus aureus* (MRSA).^[2,3,4] The most common bacterial pathogens associated with SSIs include *Staphylococcus aureus*, coagulase negative *Staphylococci* (CoNS), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Enterococcus* sp, *Acinetobacter* sp.^[3,4]

SSI can be categorized into incisional or organ/space SSI, with incisional SSI further classified as either superficial (involving skin and subcutaneous tissues) or deep (involving fascia and muscles).^[5,6] For a surgical wound to qualify as a superficial SSI, the infection should have been known to occur within 30 days of the operative procedure and should involve only the skin and subcutaneous tissues.^[5,6] Conversely, deep incisional SSI occurs after 30 days of an operative procedure if an implant is not used, or one year of an operative procedure if an implant has been used. Additionally, an organ or space surgical site infection occurs in part(s) of the anatomy other than the incision which was manipulated during the surgery. Factors which could be patient specific or procedure specific may be responsible for surgical site infections, and it could be either modifiable or non-modifiable.^[5,6,7]

Antibiotics have been utilized in clinical practice for decades and the use of antibiotics prophylaxis is justified at every surgical intervention because it reduces the rate of infection from about 5% to around 1%.⁽⁸⁾ Antibiotics can eliminate pathogens before they are intracellularly established in the macrophages or colonize implants^[8,9]. However, the challenges posed predominantly by resistant bacteria have increased remarkably, thereby threatening the efficacy of antibiotics in the treatment of SSIs.^[9] Generally, mMulti-drug resistance has been reported to be responsible worldwide for >700,000 deaths annually, which could rise to approximately 10 million by the year 2050. Infact, the World Health Organization (WHO) predicts that the world could be edging towards an era in which infections which were previously treatable becomes fatal.^[10,11,12]

The threat posed by antibiotic resistance in the treatment of SSIs remains a huge challenge to surgical patients as this contributes significantly to increased treatment cost, hospitalization time, as well as morbidity and mortality.^[13,14] Many studies have reported that microbial virulence as well as host factor are very crucial determinants of surgical site infections.^[15,16] Although several reports worldwide have documented the continuous emergence of drug resistance among clinical bacterial isolates associated with SSIs, relevant data regarding SSI isolates and their antibiotic resistance profile from major hospitals in Calabar remains inadequate. This study therefore investigated surgical site infections and the antibiotic resistance pattern of SSI isolates obtained from post-operative wound infection patients in major hospitals in Calabar, Nigeria. Result from this study will aid establish effective infection control strategies, and antibiotic therapeutic guidelines for the management and treatment of SSIs.

Comment [VEUM2]: You have to indicate the country

Comment [VEUM3]: Could you to provide more specific information

2.0 Materials and Methods

2.1.1 Study Settings and Inclusion Criteria

A hospital-based study was undertaken in selected hospitals in Calabar, Nigeria, and SSI samples were obtained within a period of 12 months. The hospitals selected for this study majorly provided services to patients under different clinical disciplines including surgery, orthopedics, obstetrics & gynecology, and Ear, Nose and Throat surgery (ENT). Study subjects were drawn from SSI patients admitted at the University of Calabar Teaching Hospital, General Hospital Calabar, Nigerian Navy Reference Hospital Calabar, Nigerian Airforce Clinic Calabar, and

Bakor Clinic Calabar. All patients in the present study were clinically diagnosed of SSI as contained in the Center for Disease Control (CDC) SSI classification system.^[17,18]

2.2 Exclusion Criteria

The exclusion criteria included patients presenting with open fractures (classified by the CDC as contaminated wounds with high risk of infection), patients with existing soft tissue or skin infection at operation site, patients with deep infections involving muscle layers, fascia, or an infected implant, and patients with osteomyelitis.^[17,18]

2.3 Sample Collection and Identification

A total of ~~One hundred and ten~~(110) samples were obtained from patients diagnosed clinically of having SSIs. These samples were collected using sterile cotton swabs from the respective infection sites. The samples obtained were processed using standard microbiological procedures as reported in similar studies.^[14,19,20,21] Samples were cultured on ~~n~~Nutrient agar, MacConkey agar, Eosin Methylene Blue agar, Blood agar and Mannitol salt agar. Gram staining was conducted on obtained isolates, as well as conventional biochemical tests (~~Indole production, methyl red test, Voges-Proskauer test, citrate utilization, coagulase test, arginine hydrolysis, urea hydrolysis, catalase test, oxidase test, motility test and H₂S production~~).^[21,22,23] All isolates were further confirmed using VITEK 2 microbial ID/AST system based on standard protocols as reported in a previous study^[24]. Methicillin resistance detection in obtained *S. aureus* isolates was performed using Cefoxitin disc diffusion test, Oxacillin screen agar test and VITEK 2 confirmation as reported in similar studies.^[6,25,26,27]

Comment [VEUM4]: In all the text, you have to standardize the use of upper and lower case

2.4 Antibiotic Resistance Assay

Antimicrobial resistance of SSI isolates from the study were determined according to the criteria provided by the Clinical Laboratory Standard Institute (CLSI) using Kirby Bauer method.⁽²⁸⁾ Antibiotic discs used include Ceftriaxone (CRO) 30µg, Cefepime (CEF) 30µg, Gentamicin (GM) 10µg, Ciprofloxacin (CIP) 5µg, Tobramycin (TOB) 10µg, Levofloxacin (LEV) 5µg, Ampicillin (AMP) 30µg, Tetracycline (TE) 30µg, Amikacin (AK) 30µg, Clindamycin (CLN) 2µg, Meropenem (MEM) 10µg, Ampicillin/Sulbactam (AMS) 20µg, Piperacillin (PIP) 30µg, Trimethoprim/Sulfamethoxazole (SXT) 25µg, Ceftazidime (CAZ) 30µg, Vancomycin (VA)

30µg, Oxacillin(OXA) 5µg, and Cefazolin(CZN)30µg. The plates were incubated for 18-24h at 37°C after which zones of inhibition was measured. Isolates were classified as either resistant or susceptible according to the CLSI guidelines.^[28]

2.5 Ethical Consideration

Ethical clearance was obtained from the Cross River State Health Research Ethics Committee with REC No: CRSMOH/RP/REC/2021/181. Written consent from the study participants was also obtained at each course of this study.

2.6 Quality Assurance

The reliability of the study findings was guaranteed by implementing quality control measures throughout the laboratory process.

3.0 Data and Statistical Analysis

A structured questionnaire was used to obtain data from study subjects. Data were entered and properly analyzed using the statistical package for social science (SPSS) software version 20. Descriptive analyses including frequencies, percentage, mean and standard deviation were utilised as performed in a similar study.^[29] Chi-square test was employed in comparing the association of socio-demographic data, categorical variables, and SSI predisposing factors with surgical wound infection status or outcome. *P*-value of <0.05 was considered statistically significant.

4.0 RESULTS AND DISCUSSION

4.1 Patients' Characteristics and SSI Prevalence

In the current study, a total of 1,202 surgeries were carried out within the study period in the five hospitals surveyed, from which 110(9.2%) cases developed surgical site infections. Among those who developed SSI, the study participants were majorly females 57(51.8%), compared to males 53(48.2%). Male study participants with SSI had a mean age of 40.73±19.87 (SEM= 2.73) while among female study subjects who developed SSI, the mean age was 46.01±20.74 (SEM=2.74). Conversely, among Non-SSI cases, male patients had a mean age of 34.96±19.25 (SEM= 0.84) compared to a mean age of 34.86±18.88 (SEM=0.79) for Non-SSI cases among female surgery

patients. Majority of study participants who developed SSIs were admitted to General Surgery (35.5%), closely followed by Obstetrics/Gynecology surgery (34.5%), Orthopedic Surgery (10.9%), Cardiothoracic Surgery (9.1%), Ophthalmology Surgery (7.3%), ENT Surgery (2.7%). There was previous history of admission for 83.6% of the study subjects with SSI cases, and an aggregate of 62.7% of SSI patients on admission had a length of stay greater than 5 days (Table.1). Statistical significance in each group of categorical variables of study subjects when evaluated using Chi-square test revealed that factors such as length of hospital stay, type of surgery, previous admission history, antibiotic use and age were significant (at p-value <0.05) in SSI outcome of patients, while patients' gender was not significant in patients' SSI outcome (Table 1). This correlates with findings from similar studies that factors such as type of surgery, infrequent usage of drugs, antibiotic/prophylaxis use, age, poor nourishment, previous admission history, lower immune status, and length of hospital stay amongst others contributes to SSI outcomes. [6,20]

Table.1: Categorical Variables of Subjects and SSI Outcome.

Characteristics	Non-SSI Cases n=1092	SSI Cases n=110	P- value
Gender	Total (%)	Total (%)	
Male	524(48)	53(48.2)	0.969
Female	568(52)	57(51.8)	
Age Category (Years)	Total (%)	Total (%)	
≤10	177(16.2)	5(4.5)	
11-20	132(12)	11(10)	
21-30	228(20.9)	18(16.4)	
31-40	119(10.9)	15(13.6)	0.001*
41-50	231(21.2)	24(21.8)	
≥51	205(18.8)	37(33.6)	
Length of Hospital Stay	Total (%)	Total (%)	
<5 Days	631(57.8)	41(37.3)	
5-7 Days	276(25.3)	37(33.6)	0.0001*
> 7 Days	185(16.9)	32(29.1)	
Previous Admission History	Total (%)	Total (%)	
Yes	229(21)	92(83.6)	0.0001*
No	863(79)	18(16.4)	

Antibiotic Use	Total (%)	Total (%)	
Pre-operative and Intraoperative	935(85.6)	69(62.7)	0.0001*
Post-operative	157(14.4)	41(37.3)	
Type of Surgery	Total (%)	Total (%)	
General Surgery	302(27.7)	39(35.5)	0.028*
Cardiothoracic Surgery	106(9.7)	10(9.1%)	
Obstetric and Gynecologic Surgery	321(29.4)	38(34.5)	
ENT Surgery	137(12.5)	3(2.7%)	
Orthopedic Surgery	109(10)	12(10.9)	
Ophthalmology Surgery	117(10.7)	8(7.3)	

*Statistically significant at $P < 0.05$

127 bacterial isolates were identified by Gram staining; 62 were Gram positive (48.8%) and 65 were Gram negative (51.2%). (Fig. 1). Among bacterial pathogens isolated were *Staphylococcus aureus* (19.7%), *Pseudomonas aeruginosa* (14.2%), *Escherichia coli* (11.8%), and *Klebsiella pneumoniae* (9.4%). (Table. 2). The findings from the present study align with several reports previously published on post-operative wound infections from different regions of the world that *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the frequently isolated bacteria among SSI patients.^[30] The presence of *S. aureus* as observed in this study is justified by the fact that *S. aureus* is an endogenous bacterial contaminant, and the disruption of the natural skin barrier promotes *S. aureus* access into surgical wounds. According to similar studies, about 85% of SSIs caused by *S. aureus* could be traced to patients' endogenous colonization. Similarly, it's been reported that surgery patients who are *S. aureus* carriers are 2 to 9 times more likely to develop SSI, and that *S. aureus* may also result from the contamination of surgical instruments as well as contaminated hospital environment.^[31]

Findings from this study also revealed that Coagulase Negative Staphylococci (CoNS) did account for 21.3% of isolated bacteria (Table. 2). This is generally not unexpected because CoNS are frequently isolated from surgical wound infections.^[32,33] Infact, the prevalence of methicillin-resistant CoNS has been reported to account for about 13.4% of bacterial isolates recovered from

SSI patients in a recent study in Egypt, and culture-confirmed SSI incidence rate of CoNS was reported to be 24.7% in a similar study in Ethiopia. [32,33]

Other bacteria isolated and their percentage occurrence include *Enterococcus faecium* 8(6.3), *Enterobacter cloacae* 6(4.7%), *Proteus mirabilis* 6(4.7%), *Acinetobacter baumannii* 4(3.1%), *Pseudomonas putida* 4(3.1%), *Aerococcus viridans* 2(1.6%)(Table 2).

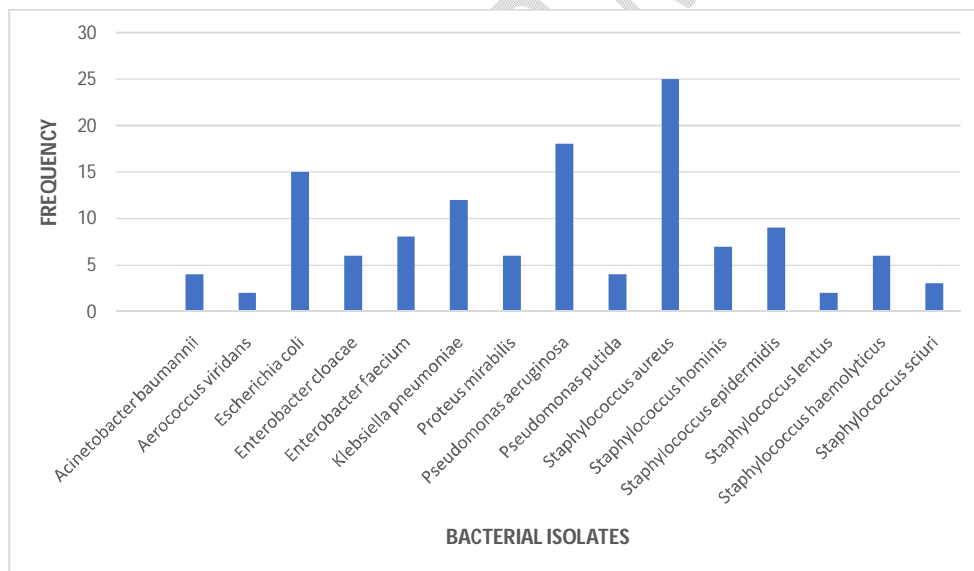


Fig.1: Number of corresponding bacterial isolates recovered from SSI patients.

Table. 2: Bacterial isolates from study subjects and their percentage occurrence.

Bacterial isolates	Number of Isolates (%)
<i>Acinetobacter baumannii</i>	4(3.1)
<i>Aerococcus viridans</i>	2(1.6)
<i>Escherichia coli</i>	15(11.8)
<i>Enterobacter cloacae</i>	6(4.7)
<i>Enterococcus faecium</i>	8(6.3)
<i>Klebsiella pneumoniae</i>	12(9.4)
<i>Proteus mirabilis</i>	6(4.7)
<i>Pseudomonas aeruginosa</i>	18(14.2)
<i>Pseudomonas putida</i>	4(3.1)
<i>Staphylococcus aureus</i>	25(19.7)
<i>Staphylococcus hominis</i>	7(5.5)
<i>Staphylococcus epidermidis</i>	9(7.1)
<i>Staphylococcus lentus</i>	2(1.6)
<i>Staphylococcus haemolyticus</i>	6(4.7)
<i>Staphylococcus sciuri</i>	3(2.4)
TOTAL	127

4.2. Antimicrobial Susceptibility Pattern of Bacterial Isolates.

In the present study, resistance to selected antibiotics by bacterial isolates was observed to be very high. On average, the percentage resistance of SSI isolates was 82.7% (Table 3). This corroborates findings from previous studies conducted in other world regions on the increased resistance of SSI bacterial pathogens to antibiotics, and affirms that the high resistance of SSI isolates to antibiotics, is likely as a result of several factors including self-medication practices, poor antibiotic stewardship and inappropriate use of antibiotics. ^[34,35,36]

Among Gram-positive bacteria isolated in the present study, *Staphylococcus aureus* isolates showed highest resistance to several antimicrobials (100% to oxacillin, 96% resistance to ciprofloxacin, 92% resistance to Levofloxacin, and 76% resistance to vancomycin amongst others). (Table. 4). All multi-drug resistant *S. aureus* isolates recovered in the present study were Cefoxitin screen positive when analysed using Vitek 2 system which indicates possible detection of MRSA isolates. This result is in line with previous studies in India and a referral hospital in Ghana on the high occurrence of MRSA strains amongst patients. ^[34,38] Also, result from a similar study in Iran, posit that 83.33% of *S. aureus* isolates recovered from patients with SSI were MRSA strains. ^[34] The increased prevalence of resistant strains including MRSA strains narrows treatment options for SSI patients because frequently, this results in cross-resistance to majority of other antibiotic drugs. ^[37,39]

Additionally, among Gram-negative isolates, *K. pneumoniae* was found to be highly resistant to several antibiotics tested (66.7% resistance to each of Ciprofloxacin, Levofloxacin, ceftazidime, Trimethoprim/Sulfamethoxazole, Cefazolin, Ampicillin, Tobramycin and 58.3% resistant to each of Ceftriaxone, Gentamicin, and Ampicillin/Sulbactam) (Table. 4). In the current study, reduced resistance to ciprofloxacin by *Pseudomonas aeruginosa* was observed. It has been reported that Ciprofloxacin is highly potent for the treatment of infections caused by *P. aeruginosa*. This is consistent with result from this study as Ciprofloxacin recorded the least resistance (27.8%) to *P. aeruginosa* isolates. Similarly, *P. aeruginosa* reduced resistance to ciprofloxacin has been reported in Ilorin Nigeria (24.7%), Latin America (28.6%), in India (26.22%), Jamaica (19.6%), Kuala Lumpur, and Malaysia (11.3%). ^[20,30]

It is worthy of note that presently ciprofloxacin is one of the most effective antibiotics against *Pseudomonas aeruginosa* in wound infections treatment, compared to most used antibiotics. Also, a similar study conducted in Nigeria posited that ciprofloxacin was one of the most effective antibiotics in treating SSI when compared to other antibacterial agents.^[20]

Resistance to meropenem, a carbapenem and third generation cephalosporins by *P. aeruginosa* (38.9%) and *K. pneumoniae* (41.7%) was observed in the present study, and this is a serious threat.^[30] This is consistent with a report in a previous study in Alexandria that in a sub-set of 65 isolates from SSI, half of the Gram-negative bacteria especially *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, were resistant to carbapenem antibiotics.^[13] The result from this study supports the trends reported on antibiotic resistance and indicates reduction efficacy of several classes of antibiotics including carbapenems, second and third generation cephalosporins, fluoroquinolones against gram negative bacilli, and extended-spectrum beta-lactamase inhibitor resistance amongst others.^[13]

Table. 3: Percentage resistance of Bacterial Isolates Recovered from Patients with SSI

Comment [VEUM5]: In all the text, you have to standardize the use of upper and lower case

Bacterial isolates	Total No of Isolates involved	Percentage resistance (%)
<i>Acinetobacter baumannii</i>	4	2(50)
<i>Aerococcus viridans</i>	2	2(100)
<i>Escherichia coli</i>	15	10(66.7)
<i>Enterobacter cloacae</i>	6	5(83)
<i>Enterococcus faecium</i>	8	7(87.5)
<i>Klebsiella pneumoniae</i>	12	9(75)
<i>Proteus mirabilis</i>	6	4(66.7)
<i>Pseudomonas aeruginosa</i>	18	16(88.9)
<i>Pseudomonas putida</i>	4	2(50)
<i>Staphylococcus aureus</i>	25	25(100)
<i>Staphylococcus hominis</i>	7	6(85.7)
<i>Staphylococcus epidermidis</i>	9	8(88.9)

<i>Staphylococcus lentus</i>	2	2(100)
<i>Staphylococcus haemolyticus</i>	6	4(66.7)
<i>Staphylococcus sciuri</i>	3	3(100)
TOTAL	127	105(82.7)

UNDER PEER REVIEW

Table 4: Antibiotic Resistance Profile of Recovered SSI Bacterial Isolates

Bacterial Isolates	Antibiotic Resistance n (%) [*]																	
	CIP	CEF	AK	GM	LEV	CRO	CAZ	MEM	TE	VA	SXT	OXA	CZN	AMS	CLN	AMP	PIP	TOB
<i>K. pneumoniae</i>	8 (66.7)	5 (41.7)	6 (50)	7 (58.3)	8 (66.7)	7 (58.3)	8 (66.7)	5 (41.7)	-	-	8 (66.7)	-	8 (66.7)	7 (58.3)	-	8 (66.7)	6 (50)	8 (66.7)
<i>E. coli</i>	3 (20)	6 (40)	0 (0)	2 (13.3)	2 (13.3)	7 (46.7)	9 (60)	0 (0)	-	-	9 (60)	-	8 (53.3)	6 (40)	-	8 (53.3)	9 (60)	2 (13.3)
<i>A. baumannii</i>	2 (50)	2 (50)	-	1 (25)	1 (25)	1 (25)	2 (50)	0 (0)	-	-	2 (50)	-	2 (50)	0 (0)	-	-	2 (50)	1 (25)
<i>P. mirabilis</i>	3 (50)	1 (16.7)	1 (16.7)	3 (50)	3 (50)	0 (0)	0 (0)	1 (16.7)	-	-	3 (50)	-	0 (0)	2 (33.3)	-	3 (50)	1 (16.7)	1 (16.7)
<i>E. faecium</i>	6 (75)	-	-	-	7 (87.5)	-	-	-	6 (75)	7 (87.5)	-	-	-	5 (62.5)	-	6 (75)	-	-
<i>E. cloacae</i>	0 (0)	3 (50)	0 (0)	2 (33.3)	0 (0)	3 (50)	4 (66.7)	0 (0)	-	-	3 (50)	-	3 (50)	-	-	-	3 (50)	3 (50)
<i>P. aeruginosa</i>	5 (27.8)	8 (44.4)	6 (33.3)	7 (38.9)	7 (38.9)	-	6 (33.3)	7 (38.9)	-	-	-	-	14 (77.8)	-	-	-	12 (66.7)	10 (55.6)
<i>P. putida</i>	2 (50)	0 (0)	2 (50)	2 (50)	2 (50)	1 (25)	0 (0)	0 (0)	-	-	2 (50)	-	2 (50)	-	-	-	0 (0)	2 (50)
<i>A. viridans</i>	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	-	-	-	0 (0)	2 (100)	0 (0)	0 (0)	-	-	2 (100)	-	-	-
<i>S. aureus</i>	24 (96)	-	-	7 (28)	23 (92)	-	-	-	13 (52)	19 (76)	12 (48)	25 (100)	-	-	0 (0)	-	-	-
<i>S. epidermidis</i>	7 (77.8)	-	-	3 (33.3)	7 (77.8)	-	-	-	8 (88.9)	1 (11.1)	7 (77.8)	8 (88.9)	-	-	7 (77.8)	-	-	-
<i>S. haemolyticus</i>	3 (50)	-	-	4 (66.7)	3 (50)	-	-	-	3 (50)	0 (0)	3 (50)	4 (66.7)	-	-	3 (50)	-	-	-
<i>S. hominis</i>	5 (71.4)	-	-	6 (85.7)	6 (85.7)	-	-	-	1 (14.3)	1 (14.3)	1 (14.3)	5 (71.4)	-	-	5 (71.4)	-	-	-
<i>S. lentus</i>	2 (100)	-	-	1 (50)	2 (100)	-	-	-	0 (0)	2 (100)	2 (100)	2 (100)	-	-	2 (100)	-	-	-
<i>S. sciuri</i>	1 (33.3)	-	-	0 (0)	1 (33.3)	-	-	-	0 (0)	0 (0)	1 (33.3)	3 (100)	-	-	3 (100)	-	-	-

Keys: Ciprofloxacin, CIP; Cefepime, CEF; Amikacin, AK; Gentamicin, GM; Levofloxacin, LEV; Ceftriaxone, CRO; Ceftazidime, CAZ; Meropenem, MEM; Tetracycline, TE; Vancomycin, VA; Trimethoprim/Sulfamethoxazole, SXT; Oxacillin, OXA; Cefazolin, CZN; Ampicillin/Sulbactam, AMS; Clindamycin, CLN; Ampicillin, AMP; Piperacillin, PIP; Tobramycin, TOB.

The relatively high antibiotic resistance by gram-positive and gram-negative bacterial isolates as reported in this study is worrisome because most of these antibiotics are administered as first line drugs. ^[14,39] SSI isolates and their antibiotic susceptibility pattern may vary from patient to patient, time to time, and place to place. The ease of access to antibiotics without medical prescription, which is commonly practiced in most developing and under-developed countries, is an important factor that should be addressed to curb antibiotic resistance. Also, routine antibiotics sensitivity screening prior to prescription is encouraged. Furthermore, there is an urgent need for developing nation's health systems to facilitate policies for antibiotic use or administration. ^[20,34] A functional surveillance network for Surgical Site Infections with relevant feedback data to hospital authorities and surgeons is greatly recommended.

5.0 CONCLUSION

Surgical site infections represent the second most frequent type of nosocomial infections and remains relatively common as a postoperative complication. It is the most common cause for post-surgery patients' readmission. SSIs have serious implications for patients, surgeons, and institutions which includes prolonged treatment, double-risk of patient mortality, and economic burden. Although SSIs are preventable, it is a significant contributor to healthcare associated infections globally. Despite advances in modern surgical techniques and better understanding of post-operative wound infections pathogenesis, surgical site infections management is a significant concern for physicians and surgeons in healthcare facilities especially in underdeveloped and developing countries. Moreover, patients with SSIs are further exposed to rapidly spreading and unrestrained resistance to the array of antibiotics which further exacerbates the existing challenge.

REFERENCES

1. Ako-Nai, A.K., Abumere, G., Akinyoola, A.L., Ebhodaghe., B.I., Attah, O.T., and Kassim, O.O. (2013). Characterization of bacterial isolates from patients' wounds and environmental factors predictive of post-surgical infections at the orthopaedic ward in Ile-Ife, Nigeria. *East African Medical Journal*, 90(12), 380-385.
2. Sartelli, M., Pagani, L., Iannazzo, S., Moro, M.L., Viale, P., Pan, A... Catena, F. (2020). A proposal for a comprehensive approach to infections across the surgical pathway. *World Journal of Emergency Surgery*, 15, 13.
3. Negi, V., Pal, S., Juyal, D., Sharma, M.K., and Sharma, N. (2015). Bacteriological profile of surgical site infections and their antibiogram: A study from resource constrained rural setting of Uttarakhand State, India. *Journal of Clinical and Diagnostic Research*, 9(10), 17-20.
4. Feldman, E.M., Kontoyiannis, D.P., Sharabi, S.E., Lee, E., Kaufman, Y., and Heller, L. (2010). Breast implant infections: is cefazolin enough? *Plastic and Reconstructive Surgery*, 126, 779-85.
5. Lubega, A., Joel, B., and Lucy, N.J. (2017). Incidence and etiology of surgical site infections among emergency postoperative patients in Mbarara regional referral hospital, south western Uganda. *Surgery Research and Practice*, doi.org/10.1155/2017/6365172.
6. Priya, K., Somasundar.V.M., Rajesh, J.G., Priya, P., Kamal R.M. (2016). Surgical Site Infection and Incidence of MRSA Using Phenotypic and Genotypic Methods from Tertiary Care Hospital. *IOSR Journal of Dental and Medical Sciences*, 15(7), 111-115.
7. Kalayul, A.A., Diriba, K., Girma, C., and Abdella, E. (2019). Incidence and bacterial etiologies of surgical site infections in a public hospital, Addis Ababa, Ethiopia. *The Open Microbiology Journal*, 13, 301-307.
8. Aiken, S.S., Cooper, J.J., Florance, H., Robinson, M.T., and Michell, S. (2015). Local release of antibiotics for surgical site infection management using high-purity calcium sulfate: An in- vitro elution study. *Surgical Infections*, 16(1), 54-59.
9. Hassan, M.M., Gaber1, A., Attia, A.O., and Baiuomy, A.R. (2014). Molecular Characterization of Antibiotic Resistance Genes in Pathogenic Bacteria Isolated from Patients in Taif Hospitals, KSA. *American Journal of Phytomedicine and Clinical Therapeutics*, 2(2), 2321-2748.

Comment [VEUM6]: In this reference, you don't include the volume and pages. Also, if you will include the DOI in one reference, you have to include the DOI for all references.

Formatted: Space After: 0 pt, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

10. World Health Organization. (2021). Antimicrobial resistance. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>
11. World Health Organization. (2020). Antimicrobial resistance. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance>
12. World Health Organization. (2019). Antimicrobial resistance: Global report on surveillance. Retrieved from <https://www.who.int/publications/i/item/9789241564748>
13. Yehouenou, C.L., Kpangon. A.A, Afolabi, D., Rodriguez-Villalobos, H., Van Bambeke, F., Dalleur, O., and Simon, A. (2020). Antimicrobial resistance in hospitalized surgical patients: a silently emerging public health concern in Benin. *Annals of Clinical Microbiology and Antimicrobials*, 19, 54.
14. Adegoke, A. A., Mvuyo, T., Okoh, A. I., and Steve, J. (2010). Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. *Scientific Research and Essays*, 5(24), 3876-3881.
15. Pietsch, F. (2015). Evolution of Antibiotic Resistance. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine*, 1150, 67.
16. Calina, D., Docea, A.O., Rosu, L., Zlatian, O, Rosu, A.F., Anghelina, F....and Gofita, E. (2016). Antimicrobial resistance development following surgical site infections. *Molecular Medicine Reports*, 15, 681-688.
17. National Healthcare Safety Network. (2022). *Procedure associated module SSI events*. Retrieved from <https://www.cdc.gov/nhsn/pdfs/pscmanual/9pscscsscurrent.pdf>
18. National Healthcare Safety Network. (2021). *Procedure-associated module surgical site infection SSI events*. Retrieved May 8, 2021 from <https://www.cdc.gov/nhsn/pdfs/pscmanual/9scscsscurrent.pdf>
19. Adwan, G., Hasan, N.A., Sabra, I., Sabra, D., Al-butmah, S., Odeh, S...and Badran, H. (2016). Detection of bacterial pathogens in surgical site infections and their antibiotic sensitivity profile. (2016). *International Journal of Medical Research & Health Sciences*, 5(5),75-82.
20. Akinkunmi, E.O., Adesunkanmi, A., and Lamikanra, A. Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles (2014). *African Health Sciences*, 14(4), 802-808.

21. Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z.A., Yaseen, I. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. *International Journal of Research Publication*, 54(1), Retrieved from <https://www.ijrp.org/paper-detail/1225>
22. Upula, S.A., Bassey, E.E., & Ije, U.E. (2021). Antiseptic soaps and body cleansing agents and its effects on the normal flora of the human skin. *World Journal of Pharmaceutical and Medical Research*, 7(4), 28-34.
23. Upula, S.A., Ikeh, K.E., & Ije, U.E. (2019). Characterization of a clinical isolate of *Staphylococcus aureus* and the action of linezolid on growth properties and toxins production. *European Journal of Pharmaceutical and Medical Research*, 6(1), 642-653.
24. Bazzi, A.M., Rabaan, A.A., Fawarah, M.M., & Al-Tawfiq, J.A. (2017). Direct identification and susceptibility testing of positive blood cultures using high speed cold centrifugation and Vitek II system. *Journal of Infection and Public Health*, 10, 299-307.
25. Cartwright, E.J., Paterson, G.K., Raven, K.E., Harrison, E.M., Gouliouris, T., Kearns, A., and Török, M.E. (2013). Use of Vitek 2 Antimicrobial Susceptibility Profile To Identify mecC in Methicillin-Resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 51(8), 2732–2734.
26. Broekema, N.M., Van, T.T., Monson, T.A, Marshall, S.A, and Warshauer, D.M. (2009). Comparison of Cefoxitin and Oxacillin Disk Diffusion Methods for Detection of mecA-Mediated Resistance in *Staphylococcus aureus* in a Large-Scale Study. *Journal of Clinical Microbiology*, 47(1), 217–219.
27. Alipoura, F., Ahmadi, M., & Javadi, S. (2014). Evaluation of different methods to detect methicillin resistance in *Staphylococcus aureus* (MRSA). *Journal of Infection and Public Health*, 7, 186-191.
28. Clinical Laboratory Standards Institute. (2012). Performance standards for antimicrobial susceptibility testing. Wayne, PA, USA: CLSI: M100-S22.
29. Tolera, M., Abate, D., Dheresa, M., Marami, D. (2018). Bacterial Nosocomial Infections and Antimicrobial Susceptibility Pattern among Patients Admitted at

HiwotFanaSpecialized University Hospital, Eastern Ethiopia. *Advances in Medicine*, Retrieved from <https://www.doi.org/10.1155/2018/2127814>

30. Goswami, N.N., Trivedi, H.R., Goswami, A.P., Patel, T.K., and Tripathi, C.B. (2011). Antibiotic sensitivity of bacterial pathogens. *Journal of Pharmacology and Pharmacotherapeutics*, 2(3), 158-164.
31. Pal, S., Sayana, A., Joshi, A., and Juyal, D. (2019). Staphylococcus aureus: A predominant cause of surgical site infections in a rural healthcare setup of Uttarakhand. *Journal of Family Medicine and Primary Care*, 8, 3600-3606.
32. Ahmed, E.F., Gad, G.F., Soliman, W.E., El-Asady, R.S., Hasaneen, A.M., and Abdelwahab, S.F. (2021). Prevalence of methicillin-resistant coagulase-negative staphylococci among Egyptian patients after surgical interventions., *Tropical Doctor*, 51(1): 40–44.
33. Kalayu, A.A., Diriba, K., Girma, C., & Abdella, E. (2019). Incidence and bacterial etiologies of surgical site infections in a public hospital, Addis Ababa, Ethiopia. *The Open Microbiology Journal*, 13, 301-307.
34. Shamsundar, B.V. (2015). Multidrug resistance wound pathogens - A serious challenge ahead. *International Journal of Health Sciences & Research*, 5(2): 173-181.
35. Bayram, Y., Parlak, M., Aypak, C., and Bayram, I. (2013). Three-year review of bacteriological profile and anti-biogram of burn wound isolates in Van, Turkey. *International Journal of Medical Sciences*, 10(1):19–23.
36. Biadlegne, F., Abera, B., Alem, A., and Anagaw, B. (2009). Bacterial isolates from wound infection and their antimicrobial susceptibility pattern in a referral hospital, North West Ethiopia. *Ethiopian Journal of Health Sciences*, 19(3):173–178.
37. Li, B., and Webster, T.J. (2018). Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopaedic Infections. *Journal of Orthopedic Research*, 36(1): 22–32.
38. Asante, J., Govinden, U., Owusu-Ofori, A., Bester, L.A., & Essack, S.Y. (2019). Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates from a

Comment [VEUM7]: Homogenize in all references

hospital in Ghana. *African Journal of Clinical and Experimental Microbiology*, 20(3), 164-174.

39. Akhia, M.T., Ghotasloua, R., Alizadeha, N., Pirzadehb, T., Beheshtirouyd, S., & Memar, M.Y. (2017). High frequency of MRSA in surgical site infections and elevated vancomycin MIC. *Wound Medicine*, 17, 7–10.

UNDER PEER REVIEW