Bacterial Pathogens Isolated from Surgical Wound and Their Antimicrobial Resistance Pattern in A Government Hospital in Anambra, Nigeria

F.O. Nwajiobi*, C.O. Anyamene and C.U. Ezebialu

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

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Abstract: - Surgical wound infections are among the most common healthcare-associated infections as complications associated with them can have a significant long-term effect on the morbidity, mortality, and quality of life for patients. Knowledge on local pathogens and sensitivity to antimicrobial agents are crucial for successful treatment and management of surgical wound infection. This study evaluates the bacterial pathogens present in infected surgical wounds and their antimicrobial susceptibility profile. A total of 200 wound swabs from 112 males and 88 female patients of ages from 10 – 70 years with surgical wound infection were collected using clean, sterile swabs and analyzed using standard microbiological methods. Antibiotic disk diffusion method was used to determine the antibiotic resistance profile. Result showed that 142(71%) wound specimens were culture positive while 58(29%) showed no growth on culture media. Majority of the culture positive wounds (90.1%) showed single bacterial growth while the remaining (9.9%) revealed poly-microbial growth. The isolates were found to belong to both Gram-positive 53(37.3%) and Gram-negative 89(62.7%) bacteria. The most predominant isolate from the infected surgical wound was Gram-positive *Staphylococcus aureus* 53 (37.3%), followed by Gram-negative *Pseudomonas aeruginosa* 45(31.7%), *Escherichia coli* 32 (22.5%) and *Klebsiella pneumoniae* 12 (8.5%). The result of their antibiotic sensitivity test showed that majority of the wound isolates were highly resistant to ampicillin 126(88.7 %), followed by erythromycin 114(80.3%), gentamicin 109(76.7%) and trimetoprim-sulphametoxazole 103(72.5%). The overall findings on antimicrobial profile revealed high level of antimicrobial resistance from microorganisms isolated from surgical wound infections to commonly prescribed antibiotics. Therefore, there is a need for adequate intervention to control the spread of antimicrobial resistance.

Keywords: surgical wound infection, antimicrobial resistance, wound pathogenic bacteria, surgical wound

I. Introduction

Wound is a complex micro- environment where infections by bacterial pathogens represent major concerns in patient treatment. There are various types of wounds, including an incised wound, lacerated wound, surgical wound, ulcer and burn wound [1]. Surgical wounds are the most common wounds seen in hospitals and inappropriate management of the wound may delay wound healing making the wound prone to infection [2]. Surgical wound infections also known as surgical site infections are defined as infection occurring along the surgical wound within 30 days after surgery or within one year if an implant is used and left in place [3]. Surgical wound infections occur as a result of the presence of replicating microorganisms at or within a surgical incision [4]. Surgical wound infection continues to be a challenging healthcare problem. It increases overall hospital costs, the length of hospital stay and may have a significant effect on the morbidity and mortality for patients [5].

The causative agents of surgical wound infections may vary from hospital to hospital and from geographical location. Surgical wound contamination may occur at the time of surgery or at any time thereafter until healing is complete. The sources of bacteria in surgical wound can originate either from patient's own normal flora or from hospital environment [6]. Surgical wound can be infected with different groups of microorganisms including bacteria, fungi and viruses. The common surgical wound pathogens includes; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus species*, *Streptococcus species, Klebsiella species*, *Acinetobacter species* and *Enterobacter species* [7]. Many studies around the world have been conducted to identify the bacterial species isolated from surgical wound infections. A study carried out in Bangladesh, showed that the most common bacterial species isolated from different types of wounds including surgical wounds were *Staphylococcus aureus* (36.9%), followed by *Escherichia coli* (35.8%) *Pseudomonas spp.* (17.3%) and *Proteus spp*. (5.8%) [8].

Treatment and management of surgical wound infection includes the use of antimicrobial drugs. The chosen antimicrobial agent should be able to kill or inhibit the growth of microorganism completely without causing harm to the host and at the same time, reduce the possibilities of the microorganism to have a tendency to develop resistance. However, antibiotic treatment is often ineffective due to resistance to antibiotics. With the rapid rise of antibiotic resistance by surgical wound pathogenic bacteria, management of surgical wound infection has become more challenging, and the problem is more pronounced in the developing countries where rational use of antibiotics is highly compromised [9].

In Nigeria, the cost of wound management is high and treatment of infected surgical wound is usually empirically, in which the causative agents is rarely identified [10]. Therefore, knowledge about the causative pathogens of surgical wound infection and their drug susceptibility profile would be valuable in reducing its economic and health consequences, especially in the context of growing resistance to antibiotics [8]. This study was carried out to identify the causative bacterial pathogens of surgical wound infections among patients attending a Government hospital in Anambra State and to determine the antibiotic susceptibility patterns of the isolated bacterial pathogens.

II. Materials and Methods

Data Collection and Study participants

The study participants were all patients with infected surgical wound in a Government Hospital in Anambra Nigeria, during the study period. A structured questionnaire including demographic data such as gender, age, sex, place of residence and wound location was used to obtain information on the health status of the patients.

Collection of wound specimens

Two hundred wound swabs were collected from several surgical wound infections site. Wound surface were cleaned with sterile normal saline, then specimens were collected using sterile cotton swabs. The inner surface of the infected area were swabbed gently, and swabs sticks were inserted immediately into a tube containing nutrient broth media and then transferred to the Microbiology Laboratory unit as soon as possible for further investigation

Isolation and identification of wound Isolates

Isolation and identification of the collected wound specimen was carried out as described by [11], [12]. Each wound specimen was inoculated onto nutrient agar MacConkey agar and then incubated at 37^{o}C for 24 hours. After the incubation, the different colonies were sub-cultured on nutrient agar and incubated for 24 hours at 37^{o}C to get a pure culture. The pure cultures were then identified based on their morphological and biochemical reactions. Morphological identification includes the colour and the size of the colonies.

Biochemical Characterization of Isolates from Infected Surgical Wound

Bacteria isolates were characterized based on colonial morphology, cultural characteristics and biochemical test as described by [12]. The biochemical tests that were carried out include; motility test, catalase test, indole test, coagulase test, mannitol salt agar test, Voges Proskauer test, citrate utilization test and cetrimide test.

Antimicrobial Susceptibility patterns of Bacterial Isolates from Wound Culture

Antibiotic susceptibility testing of the surgical wound pathogens was done on Mueller- Hinton agar (Oxoid, England) using Kirby Bauer disk diffusion technique [13] and the zones of inhibition produced after 24hrs incubation at 37^{o}C were measured according to Clinical and Laboratories Standards Institute (CLSI) [14]. The drugs tested were 9 widely used antibiotics for the treatment of wound infection in the study area namely; ceftriaxone (30µg), ciprofloxacin (5µg), gentamicin (10 µg), augmentin (30 µg), erythromycin (15 µg), Trimetoprim-sulphametoxazole (10 µg), doxycyline (30 µg), chloramphenicol (30 µg) and ampicillin (10µg) (Oxoid England). Diameters of zone of inhibition around the discs were measured to the nearest millimeter using a meter rule and classified as sensitive, intermediate and resistance according to Clinical and Laboratories Standards Institute (CLSI) guideline.

Ethical Consideration

Ethical approval was obtained from Hospital ethical committee for collection of surgical wound specimen with ethical approval Ref: IEH/REC/VOL.3/2022/009. All study participants were clearly informed of the objectives of the study and were provided with both verbal and written informed consent.

Data Analysis:

The collected data were analyzed using Microsoft Excel 2007 and SPSS version 23. Graph and tables were extracted from the data. Descriptive analysis such as frequencies and mean were used. Chi-square test was employed to compare the socio-demographic data, bacteria isolates and their antibiogram. P-value <0.05 was considered as significant.

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III. Result

Data Collection and Study Participant

Out of 200 participants, 112 were males (56.0%) and 88 were females (44.0%). The participants were aged between 10 – 70 years for both male and female and majorities (32.5%) were in the age group of 31 – 40 years. Majority of the study participants were from rural area 123(61.5%) compared to those from urban area 77(38.5%). Of the 200 participants, 131(65.5 %) had wound on the leg, 62(31%) had wound on the abdomen and 7(3.5%) had wound on the hand. Rate of isolation was higher in males 112(56.0%) compared to females 88(44.0%). The socio-demographic characteristics of the participants are shown in Table 1.

Table 1: Socio-demographic Characteristics of study participants with Surgical Wound Infections (n=200)

Characteristics	Number tested	Number of culture positive
Sex		
Male	112 (56.0)	90 (63.4)
Female	88 (44.0)	52 (36.6)
Total	**200 (100)**	**142 (100)**
Age		
≤ 20	18 (9.0)	18 (12.6)
21 – 30	38 (19.0)	25 (17.8)
31 – 40	65 (32.5)	50 (35.2)
41 – 50	52 (26.0)	39 (27.4)
≥ 50	27 (13.5)	10 (7.0)
Total	**200 (100)**	**142(100)**
Residence		
Urban	77 (38.5)	51 (35.9)
Rural	123 (61.5)	91 (64.1)
Total	**200 (100)**	**142(100)**
Location of wound		
Leg	131(65.5)	101(71.1)
Abdomen	62 (31.0)	38 (26.8)
Hand	7 (3.5)	3 (2.1)
Total	**200 (100)**	**142 (100)**

Distribution of Pathogens in Infected Surgical Wound Swabs n=200

The overall result of bacteriological investigation revealed that out of the 200 surgical wound specimens analyzed, 142 (71%) were found culture positive while 58 (29%) showed no growth on culture media (Figure 1)

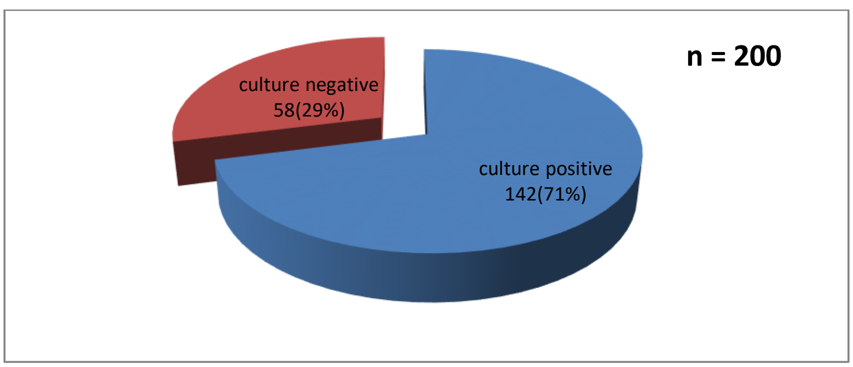

Figure 1: Distribution percentage of surgical wound swabs on growth character

Prevalence of Pathogens in Infected Surgical Wound Isolated

The most predominant isolates from the infected wound was Gram-positive *Staphylococcus aureus* 53 (37.3%), followed by Gram-negative *Pseudomonas aeruginosa* 45(31.7%), *Escherichia coli* 32(22.5%) and *Klebsiella pneumoniae* 12(8.5%). Table 2 shows the morphological and biochemical characteristics of the wound isolates while Table 3 shows the prevalence of Pathogens in infected surgical wound

Table 2: Biochemical Characteristics of Isolate from Infected Surgical Wound

Isolates	Characteristics										
	Colony Morphology	GM	MT	CST	INT	CGT	MAT	VPT	CT	CMT	Presumtive Organisms
A	Circular, yellow colonies on Nutrient agar	+ cocci	–	+	–	+	+	+	–	–	*S. aureus*
B	Flat, green Pigment on macconkey agar	– rods	+	–	–	–	–	+	–	+	*P. aeruginosa*
C	Circular colonies MacconKey agar	– rods	+	–	+	–	–	–	–	–	*E. coli*
D	Mucoid, pink Colony on Nutrient agar	– rods	–	+	–	–	–	+	+	–	*K. pneumoniae*

+ = Positive, – = Negative

GM = Gram staining, MT=Motility test, CST=Catalase Test, INT=Indole test, CGT=Coagulase test, MAT=Manitol agar test, VPT= Voges proskauer test, CT=Citrate test,, CMT= Cetrimide Test.

Table 3: Prevalence of Pathogens Isolated from Infected Surgical Wound Swabs (n=142)

Organisms	Number	Percentage (%)	M±SD
Gram positive			
S. aureus	53	(37.3)	13.940 ± 2.613^b
Gram negative			
P. aeruginosa	45	(31.7)	8.712 ± 2.135^b
E. coli	32	(22.5)	9.350 ± 2.375^b
K. Pneumoniae.	12	(8.5)	2.930 ± 2.313^b
Total	**142**	**(100)**	

$P\geq 0.05$ Mean with subscript ‘ b’ are not significant

Antimicrobial Susceptibility Patterns of Surgical Wound Pathogens.

Table 4, shows the antimicrobial susceptibility patterns of the wound pathogens. A total of 9 widely used antibiotics for treatment of wound infection in the study area were tested against the bacterial species (surgical wound isolates) for antibacterial susceptibility pattern. The predominant isolate, *Staphylococcus aureus*, revealed high level of multidrug resistance pattern as it was resistant to all tested antibiotics except chloramphenicol 45(84.9), ciprofloxacin 43(81.1%), ceftriaxone 41(77.3%) and doxycyline 36(67.7%).

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The second predominant isolate, Gram-negative *Pseudomonas aeruginosa,* showed also high level of resistance to erythromycin 35(77.8%), ampicillin 38(84.4%), gentamicin 36(80.0%), ciprofloxacin 29(64.4%) and trimetoprim-sulphametoxazole 29(64.4%), however, they were moderately susceptible to other antibiotics tested. All isolates of *Escherichia coli* were (100%) resistant to ampicillin. It was also found to be resistance to trimetoprim-sulphametoxazole 22(68.7%) and erythromycin 21(65.6%). However, they were susceptible to doxycyline 25(78.2%), ciprofloxacin 22(68.7%) and ceftriaxone 21(65.6%) and moderately susceptible to augmentin. *Klebsiella pneumoniae* was only sensitive to ceftriaxone 8(66.7%) but showed extensively multidrug resistance to other antibiotics tested.

Table 4: Antibiotic Susceptibility patterns of Bacteria isolated from infected wound Swab Cultures (n=142)

Bacterial Isolates	**Number of resistance pathogens to antimicrobial agents (%)**									
	Pattern	**CIP**	**CL**	**ER**	**RX**	**DO**	**GM**	**SXT**	**AP**	**C**
S. aureus	S	43(81.1)	41(77.3)	9(16.9)	26(49.1)	36(67.7)	7(13.3)	10(18.9)	9(16.9)	45(84.9)
(n= 53)	R	10(18.9)	12(22.6)	44(83.1)	27(50.9)	17(33.3)	46(86.7)	43(81.1)	44(83.1)	8(15.1)
P. aeruginosa	S	16(35.6)	27(60.0)	6(13.3)	35(77.8)	27(60.0)	9(20.0)	16(35.6)	7(15.6)	36(80.0)
(n = 45)	R	29(64.4)	18(40.0)	39(86.7)	10(22.2)	18(40.0)	36(80.0)	29(64.4)	38(84.4)	9(20.0)
E. coli	S	22(68.7)	21(65.6)	11(34.4)	18(56.3)	25(78.2)	16(50.0)	10(31.3)	0(0)	20(62.5)
(n = 32)	R	10(31.3)	11(34.4)	21(65.6)	14(43.7)	7(21.8)	16(50.0)	22(68.7)	32(100)	12(37.5)
K. pneumonia	**S**	3(25.0)	8(66.7)	2(16.7)	5(41.7)	5(41.7)	1(8.3)	3(16.7)	0(0)	0(0)
(n= 12)	R	9(75.0)	4(33.3)	10(83.3)	7(58.3)	7(58.3)	11(91.7)	9(75.0)	12(100)	12(100)
Total	**S**	**84(59.2)**	**97(68.3)**	**28(19.7)**	**84(59.2)**	**93(65.5)**	**33(23.3)**	**39(27.5)**	**14(11.3)**	**101(71.1)**
(n= 156)	**R**	**58(40.8)**	**45(31.7)**	**114(80.3)**	**58(40.8)**	**49(34.5)**	**109(76.7)**	**103(72.5)**	**126(88.7)**	**41(28.9)**

**Numbers in parentheses indicate percentage*

S= Susceptible, R= Resistance, CIP= Ciprofloxacin, CL= ceftriaxone , ER= Erythromycin, RX= Augmentin, DO= Doxycyline, GM= Gentamicin, SXT= Trimetoprim-sulphametoxazole, AP= Ampicillin, C= Chloramphenicol.

IV. Discussion

Surgical wound infections are among the most common complications following surgical procedures. It represents a significant healthcare burden on the patients as treatment cost and general wound management practices becomes more resources demanding. Therefore, knowledge about the causative microorganisms as well as their drug susceptibility profile is important for proper management and reducing its economic and health consequences.

This study has shown that the incidence of surgical wound infection were more common in male gender (56%) as compared to female gender (44%). This finding is in agreement with study done by [15] who found male gender to be a significant risk factor for surgical site infection in patients undergoing elective pancreatectomy. Another study conducted previously by Warren *et al.*, also reported similar results showing that male sex is an independent risk factor for surgical wound infection [16]. In contrast to our finding, a retrospective case control study published by Pedroso-Fernandez *et al.*, demonstrated a significant high risk of surgical wound infection to female patients than male after colorectal surgery [17] suggesting that sex does not contribute to the pattern or rate at which infection occur in surgical wounds. The discrepancy in sex variation in surgical wound infection may be attributed to the type of surgery that were performed, procedure specific, differences in microbiome composition and body immune constitution.

In the present study, out of the 200 surgical wound specimens analyzed, bacterial pathogens were isolated from 142 (71%) surgical wound specimens while 58(29%) showed no growth on culture media. This finding was similar with studies conducted in different parts of Nigeria [19], [20] and in Bangladesh [9] with an isolation rate of 70.1%, 71% and 72.4% respectively. However, it was higher than report from Nepal [21] with isolation rate of 64.5%, but lower than the report from Nigeria [22] and Ethiopia [23] with an isolation rate of 92% and 100% respectively. The differences in rate of bacterial isolation from the surgical wound infection may be due to the differences in the management approaches among health facilities to prevent distribution of nosocomial pathogens and infection control.

The predominant isolate in the present study was found to be Gram positive *S. aureus* accounting for 57(36.5%). This finding was similar to number of studies previously done on surgical wound infection in different part of the country [24], [25]. The high prevalence of *S. aureus* in wound infections maybe because it's a microbial flora of the skin and mucous membrane, with the breakage or damage of skin barrier, *S. aureus* can migrate to the wound and contaminate wound leading to infections. Our observation of Gram positive *S. aureus* as the most predominant pathogen in surgical infected wounds differs from previous other study by Okoro *et al.*, [26] which reported Gram-negative *E. coli* as the most common organism from surgical wound infection.

The second predominant organism from the present study was Gram negative *P. aeruginosa* 45(31.7%) followed by *E. coli* 32(22.5) and *K. pneumoniae* 12(8.5). Similar result was reported in Bayelsa Nigeria by [27], who revealed that predominant isolate among the Gram negative bacteria from surgical wounds were *P. aeruginosa* (17.07%), followed by *E. coli* (11.58%) and finally *K. pneumoniae* accounted for (10.37%). This finding of *P. aeruginosa* as the predominant gram-negative organisms from surgical wound may be due to the fact that *P. aeruginosa* are among major bacteria species incriminated in nosocomial wound infection and prolonged hospital stay. However, the present result differs from the previous report from Okoro *et al.*, with *E. coli* accounting the predominant gram negative bacteria isolated from surgical wound infection [26]. These variations in prevalence of bacteria in surgical wound infections could be attributed to several factors such as the wound type, site and location of the wounds, nature of the surgery itself and the level of care and precaution taken to prevent nosocomial wound infection.

In the study, the antimicrobial susceptibility test revealed that ampicillin was the antimicrobial drug towards which the isolated surgical wound pathogens showed the highest percentage of resistance (88.7%), followed by erythromycin (80.3%), gentamicin (76.7%) and trimetoprim-sulphametoxazole (72.5%). On the contrary, the most active antibiotics towards isolated bacteria were found to be chloramphenicol, ceftriaxone, doxycyline and ciprofloxacin. The predominant isolate *Staphylococcus aureus* tends to be resistant to a wider spectrum of antibiotics. More than 80% of isolated *S. aureus* was resistance to gentamicin (86.7%), ampicillin (83.1%), erythromycin (83.1%) and trimetoprim-sulphametoxazole (81.1%). This was comparable with study done by [28], [29] who reported more than 80% resistance of *Staphylococcus aureus* to ampicillin, gentamicin and erythromycin. This high resistance pattern of isolated *S. aureus* to antibiotics may be due to overuse of these drugs as empiric option for most of the patients. The second predominant isolate *P. aeruginosa* showed high resistance ≥80% to commonly prescribed antibiotics like; erythromycin (86.7%), ampicillin (84.4%) and gentamicin (80.0%). This report was similar with result of other study in which *P. aeruginosa* showed high resistance to ampicillin (100%) and gentamicin (82%) [30]. However, this is in conformity with the result of other study in which gentamicin recorded the least resistance 6.2% [31]. The inappropriate use of antibiotics is responsible for the development of resistance of *P. aeruginosa* to antibiotic drugs. The results obtained showed a high resistance rates of *E. coli* to ampicillin (100%), erythromycin (71%) and trimetoprim-sulphametoxazole (68.4%). However, it is moderately susceptible to other antibiotics. Similar to most of the studies previously done elsewhere, ciprofloxacin, augmentin and ceftriaxone was detected among the most effective antibiotics against wound isolates [32], [33]. *K. pneumoniae* was only sensitive to ceftriaxone but showed extensively multidrug resistance to other antibiotics tested. A high rate of resistance 100% to chloramphenicol and ampicillin by *K. pneumoniae* was observed from the present study. The result also showed over 70% resistant to ciprofloxacin, erythromycin, gentamicin and trimetoprim-sulphametoxazole. These antibiotics are usually used at random to cure a generalized wound infection. Our finding was in agreement with report done in Nigeria and other part of the world where *K. pneumoniae* showed to be resistant to most commonly used antibiotics [34-35]. High resistance to antibiotics by surgical wound bacteria from our study may be due to variations in prescription process and self-medication because of affordability and availability [35]. The overall findings on antibiotic profile revealed a high level of antimicrobial resistance from microorganisms isolated from surgical wound infections and this high level of resistance is a cause for concern.

V. Conclusion

In conclusion, the predominant isolate from surgical wound infection in the study was Gram- positive *S. aureus*, followed by Gram-negative *P. aeruginosa*, *E. coli* and *K. pneumoniae*. There is a high level of resistance to ampicillin, erythromycin, gentamicin and trimetoprim-sulphametoxazole among the isolates, Therefore, routine microbial analysis of wound samples and their antibiogram is necessary if treatment of surgical wound infection is to be successful**.** Proper surgical wound infection control measures and actions to reduce antimicrobial resistance should be strengthened. Rational use of antimicrobials should be encouraged to prevent the emergence of resistant pathogens in the study area.

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Competing Interest

The authors declare that they have no competing interest.

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