INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 10, Issue 8 - 2023

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2023.10.08.005

Isolation and identification of bacteria from surgical wound attention at Enugu State University Teaching Hospital, Enugu

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Abstract

Surgical site infections are worldwide problems in the field of surgery contributing to increased mortality and morbidity. However, despite advances in the control of surgical site infections, the risk of acquiring these infections had not fully been eliminated due to the emergence and spread of resistant bacteria pathogens. The aim of this study was to isolate and identify bacteria from surgical wounds patient. This was a cross sectional study of patients with suspected surgical site infections in the hospital wards. Structured questionnaires were used to collect patient's data. A total of 49 (41.5%) Bacteria were isolated from the Culture positive swabs. The pathogens Isolated include; *Staphylococcus aureus (16.1%), Escherichia coli (15.3%), Proteus mirabilis (3.4%), Klebsiella pneumoniae (3.4%)* and *Pseudomonas aeruginosa (3.4%).* Surgical site infections can be quite challenging to detect and then treat. Prevention necessitates meticulous patient care and all required interventions. This study identified *Staphylococcus aureus* as the leading causative organism of SSIs among surgical patients at Enugu State University Teaching Hospital, Enugu.

Keywords: Isolation, identification, bacteria, surgical wound, infection

Introduction

The incidence of surgical site infection is a major concern in many hospitals. It affects the patient's wellbeing as well as the healthcare personnel [1-2]. Therefore, surgical site infection (SSI) could be defined as an infection that occurs within 30 days of a surgical procedure or one year if an implant is left in place after the surgery and affects either the incision or the deep tissues at the surgical site. Infections involving organs or bodily space might be superficial or deep incisional infections [3-4].

Surgical site infections are a worldwide problem in the area of surgery; linked to longer hospital stays, higher treatment costs, and increased rates of morbidity and mortality [5]. SSIs are the second most common kind of nosocomial infection in hospitals in the United States, SSIs are linked to a 3.0% mortality rate, according to the Center for Disease Control and Prevention (CDC) [6]. Pre-existing medical disorders, the amount and type of resistant skin bacteria, and preoperative, intraoperative, and post-operative care are all factors that influence the risk of surgical site infection [7].

Lack of standardized criteria for diagnosis of SSIs present a challenge to monitor the global epidemiology of surgical site infection [5]. In addition to this, emergence of high antimicrobial resistance among bacterial pathogens has made the management and treatment of post-operative wound infection difficult [8].

Moreover, rapidly emerging nosocomial pathogens and the problem of multidrug resistance necessitates periodic review of isolation pattern and their sensitivity[9]. Many studies in different part of the world found that the most frequently isolated bacteria from surgical wound infections were *Staphylococcus aureus,* coagulase negative *Staphylococcus* (CoNS), *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus* species [10].

Infected wounds are wounds that are colonized with bacteria or other microorganisms that cause its deterioration and a delay in wound healing. In other words, infected wounds result when immune defenses of the body are stunned or cannot withstand common bacterial growth. Wound infection caused by surgery is a severe health challenge and surgical wounds are mostly contaminated by bacteria, previous studies have revealed that about 70 percent of the deaths of patients who have undertaken surgical operations are triggered by surgical site infections [11-13].

Methodology

Study Area

The study was conducted at ESUT teaching hospital G.R.A Enugu Urban, the capital city of Enugu state, Nigeria**.**

Study design

A cross sectional research technique was used whereby the samples were collected from surgical wounds patients currently admitted at ESUTH.

Study population

The population is a mix of both urban and rural dwellers. Enugu State University Teaching Hospital, Parklane Enugu.

Sample size

Sample size was calculated using Cochran's formula

$n = Z^2pq / d^2$

Where:
n (minimum sample size/desired sample size) = ?
p (the percentage of target population estimated to have a particular characteristic) = 14.5% (0.145) [14].
Z (standard normal deviation) = 1.96 (corresponding to 95% confidence interval)
d (margin of error) = 5% (0.05)
q = 1 – p =1 – 0.145 = 0.855
Therefore, $n = (1.96^2 * 0.145 * 0.855) / 0.05^2 = 190$

Sampling technique

The study adopted purposive sampling, The patients with suspected surgical site infection were identified by surgeons during the routine daily ward rounds. The surgeons would then document the clinical signs of infection in the patient file. The patients were briefed about the research and informed consents obtained prior to their inclusion as study participants. This included patients with SSI infection in the hospital wards.

Inclusion and exclusion criteria

Inclusion criteria:

i. Patients of all age groups except neonates
ii. Presence of suspected post-operative SSIs
iii. Giving informed consent to participate.

Exclusion criteria:

i. Neonates
ii. Infection occurring 30 days after operation if no implant is in place
iii. Burn injuries and donor sites of split skin grafts
iv. Refusal to give consent for participating in the study

Ethical issues

Approval for study was given by the Ethical Review committee of Enugu state university teaching hospital. Consent was obtained from the patients of the various post-surgical wards of Enugu state university teaching hospital. The study was carried out with the highest level of transparency and professionalism.

Patient data collection

Structured questionnaires were used to extract data from the patients case notes; the information included were; demographic data, existing chronic disease (such as diabetes mellitus), past medical history, current drug use such as steroid, smoking, length of preoperative hospital stay, duration of operation and physical examination was done to determine location of the wounds.

Specimen collection

The specimens were collected aseptically from patients presented with clinical evidence of infection (purulent drainage from incision or drain) before the wound was cleaned with antiseptic. Collection of pus and serous fluid from the deep viable tissues of the wound was done using moistened sterile swab sticks by Levine method (rotating the swab over 1 cm^2 area of viable tissues for 5 seconds).

Laboratory procedure

Swab specimens were processed and tested in the microbiology laboratory. Specimens were immediately cultured upon arrival in the laboratory. Culturing for colony characteristics followed by Gram stain and biochemical tests were used to identify pathogenic bacteria. Culture media used were Blood agar, Nutrient agar and MacConkey agar. Culture media were made by reconstituting the commercial powder in distilled water and sterilizing at 121°C for 15 minutes in an autoclave as per manufacturer's instructions.

Culture procedure

Culture of pus and surface swabs was carried out according to the set standards and procedures in bacteriology (Cheesbrough, 2006). A small amount of the specimen was applied on the agar surface of both MacConkey and Blood agar. Then using a sterile wire loop, the specimen was spread on the agar surface using the streaking method. Each swab was inoculated on a separate plate and after labeling them, the plates were incubated aerobically at 35-37°C for 18-24 hours. After incubation, Individual bacteria isolates were identified from their respective plates by observation of the growth pattern which included checking the form (circular), elevation (raised, flat or convex), margin (undulate or entire), opacity (translucent, opaque or transparent), hemolysis (beta, alpha or gamma), surface (smooth, dry or mucoid) and pigmentation (pink,

golden yellow or white) of the colonies. Swarming characteristics of bacteria on blood agar surface was also used in the identification process. Plates with no growth after 18 hours of incubation were re-incubated while those with mixed growth were sub-cultured on separate plates until pure growth of discrete colonies was observed. Reporting of no growth was only done after the plates were incubated for 48 hours.

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.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of all identified isolates from the surface swab samples was done according to the criteria of the Clinical and Laboratory Standards Institute method {CLSI). Briefly, from a pure culture a loopful of bacterial colony was taken and transferred to a tube containing 5 ml of normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 (Mary-l'Etoil, France) in order to standardize the inoculum size.
A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Nutrient agar. The inoculated plates were left at room temperature to dry for 3-5 minutes.

With the aid of sterile forceps, the appropriate antibiotic sensitivity discs (gram positive or gram negative) were placed on the surface of the nutrient agar. For Gram negative the following antibiotics were used N-Nitrofurantion 100mcg, GN-Gentamicin 10mcg, CIP-Ciprofloxacin 10mcg,C-Chloramphenicol 10mcg,OF-Ofloxacin10mcg,MP-Meropenem10mcg, PF-Pefloxacin 10mcg,CT-Cetriaxone30mcg,AX-Amoxicillin 30mcg,ST-Streptomycin 30mcg. For Gram positive organisms the following antibiotics were used AM-Ampicillin 30mcg,CL-Cloxacillin 10mcg,LV-Levofloxacin 10mcg,CX-Cephalexin 30mcg,CIP-Ciprofloxacin 5mcg,GN-Gentamicin 10mcg,OF-Ofloxacin10mcg,CD-Clindamycin 10mcg,E-Erythromycin 10mcg,CT-Cetriaxone 30mcg The plates were then incubated at 37ºC for 24 hours. Diameters of the zone of inhibition around the discs were measured using a digital caliper, and the isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI (2014).

Data analysis

Statistical analysis Package for Social Science (SPSS) version 25 and Microsoft excel were used for statistical analysis of the data generated. Chi square was used to compare between two or more variables. Statistical significance was considered at *p*-value <0.05 and confidence level of 95%.

Results

Table 1 presents the background age and sex of the patients. Their age ranged from 18-87 years with mean and standard deviation, 44.70±16.69 and modal age group, 31-40 years (25.4%). Females (61.9%) were more than males (38.1%). Previous social history of alcohol consumption and smoking were 28.8% and 4.2% respectively.

Table 1: Demographic Characteristics of the Patients

		Frequency	Percent (%)	Range	M±SD
Age				18-87	44.70±16.69
-	< 30	29	24.6		
-	31-40	30	25.4		
-	41-50	17	14.4		
-	51-60	14	11.9		
-	61-70	17	14.4		
-	71 +	11	9.3		
Sex					
-	Male	45	38.1		
-	Female	73	61.9		
Previous social history					
-	None	83	70.3		
-	Alcohol	34	28.8		
-	Smoking	5	4.2		

From Table 2, the pre-operative diagnoses of the patients were majorly diabetic foot (27%)

Table 2: Showing the Pre-Operative diagnosis of the Patients

		Frequency	Percent (%)
Pre-operative diagnosis			
-	Fractured tibia and hypertensive	1	0.8
-	Diabetic foot	33	27.0
-	Peritonitis	9	7.6
-	Multiple gestation	1	0.8
-	Hypertensive and diabetic	7	5.9
-	Chronic leg ulcer	3	2.5
-	Gestational hypertension	8	6.8
-	Diaphragmatic hernia	1	0.8
-	Multiple fracture of tibia and femur	2	1.7
-	Intestinal perforation/hernia	7	5.9
-	Multiple births	3	2.5
-	Broken tibia and fibula/femur	5	4.2
-	Intrauterine fetal death	1	0.8
-	Broken humerus/ankle	6	5.1
-	Previous CS.	8	6.8
-	Fetal macrosomia	7	5.9
-	Appendicitis	5	4.2
-	Open fracture	1	0.8
-	Obstructed labour	4	3.4
-	Advanced Patient's age and hypertensive	1	0.8

From Table 3, 44.9% had 1-day pre-operative stay in the hospital while 14.4% had 4 days or more. Majority had metronidazole for antibiotic prophylaxis 82(69.4%)

Table 3: Showing the Pre-operative hospital stay and Antibiotics prophylaxis given before the surgery

Pre-operative hospital stay		
- 1 day	53	44.9
- 2 days	24	20.3
- 3 days	23	19.5
- 4 days and above	17	14.4
- No response	1	0.8
Antibiotic prophylaxis		
- Metronidazole	82	69.4
- Augmentin	1	1.6
- Ciprofloxacin	11	9.3
- Ceftriaxone	22	18.6
- Gentamycin	1	0.8
- No response	1	0.8

From Table 4, Amputation (33.1%) and Caesarean section (32.2%) were the main surgery types performed by the patients. Most of them had the complaints of feeling pains at the surgical site (84.7%).

Table 4: Surgery Related Characteristics

	Frequency	Percent
Type of surgery		
- Surgical wound debridement and external fixation	13	11.0
- Amputation	39	33.1
- Laparotomy	21	17.8
- Caesarian section	38	32.2
- Open reduction and internal fixation	3	2.5
- Others	4	3.3
Present complain		
- No pain	17	14.4
- Pain	100	84.7
- Insomnia and pain	1	0.8
- Pus	8	6.8
- Swelling	2	1.7
- Gaping at site	1	0.8

Table 5 presents the prevalence of the isolated organisms. The prevalence of bacterial growth was there was a total of 49(41.59%) isolates from culture positive swabs specifically, *E.coli* was(15.3%),*Pseudomonas aeruginosa* (3.4%),*Proteus mirabilis* (3.4%), *Staphylococcus aureus* was (16.1%) and *Klebsiella pneumoniae (* 8.2%).

Table 5: Prevalence of Organisms isolated from surgical sites

	Frequency	Percent (%)
- No bacterial growth	69	58.5
- Bacterial growth	49	41.5
- *E. Coli*	18	15.3
- *Pseudomonas aeruginosa*	4	3.4
- *Proteus mirabilis*	4	3.4
- *Staphylococcus aureus*	19	16.1
- *Klebsiella pneumonia*	4	3.4

Table 6: Association of Bacterial Growth with Patients' Characteristics

	Organism Isolated				
	Yes	No	Total	Chi-square	p-value
Age				2.004	.849
- < 30	13(44.8)	16(55.2)	29		
- 31-40	10(33.3)	20(66.7)	30		
- 41-50	7(41.2)	10(58.8)	17		
- 51-60	6(42.9)	8(57.1)	14		
- 61-70	9(52.9)	8(47.1)	17		
- 71 +	4(36.4)	7(63.6)	11		
Sex				2.753	.097
- Male	23(51.1)	22(48.9)	45		
- Female	26(35.6)	47(64.4)	73		
Alcohol				.213	.644
- Yes	13(38.2)	21(61.8)	34		
- No	36(42.9)	48(57.1)	84		
Smoking				.996	.401
- Yes	1(20.0)	4(80.0)	5		
- No	48(42.5)	65(57.5)	113		
Pre-operative hospital stay				2.018	.569
- 1 day	18(34.0)	35(66.0)	53		
- 2 days	11(45.8)	13(54.2)	24		
- 3 days	11(47.8)	12(52.2)	23		
- 4 days and above	8(47.1)	9(52.9)	17		
Antibiotic prophylaxis				1.069	.586
- Metronidazole	31(38.3)	50(61.7)	81		
- Ciprofloxacin	6(54.5)	5(45.5)	11		
- Ceftriaxone	9(40.9)	13(59.1)	22		
Type of surgery				12.226	.007
- Surgical wound debridement	7(53.8)	6(46.2)	13		
- Amputation	20(51.3)	19(48.7)	39		
- Laparotomy	13(61.9)	8(38.1)	21		
- CS	8(21.1)	30(78.9)	38		

Pain					.628	.428
-	Yes	40(40.0)	60(60.0)	100		
-	No	9(50.0)	9(50.0)	18		

Discussion

In this study, a total of 118 swab samples from surgical sites were investigated to determine and isolate the bacteria present in post-surgical sites their types and antimicrobial susceptibility pattern. The findings demonstrated the predominance of *Staphylococcus aureus* 19(16.1%) being the commonest isolated organism followed by *E,coli 18*(15.3), These two bacteria (that is S*taphylococcus aureus* and *E.coli)*are mostly skin and gastro-intestinal tract flora respectively. Therefore, their high isolation rate from surgical wound site in the present study was ascribed to endogenous contamination of the exposed tissues with skin and gastro-intestinal tract flora during surgery. This pattern of organisms causing SSIs in the current study is in concurrence with previous studies from the same study setting and elsewhere within the region which reported *S.aureus*as the most common SSI bacterial pathogen [14-]. The possible reason for uniformity in these studies could be attributed to similarities in the populations investigated; homogeneousness of surgical procedures performed on the study participants, as well as timing of specimen collections.

In the present study, the majority of the isolates were obtained from patients who were already on antimicrobial treatment. This could have led to the high number of no bacteria growth observed.

Regarding the frequency of isolation of organisms in different surgery types, the present study showed the highest number of bacteria isolates were seen in patients who had laparotomy (61.9%) and the lowest in patients who had caesarian section (21.1%) agreeing with a number of works which saw laparotomy as the surgery with the highest number of isolates example; Alkaaki*et al* in a 2019 study showed that of the isolates seen in SSIs 35% were from sites of laparotomy [15]. These findings suggest that the aetiologic agents of SSIs depend on where the procedures are performed and whether skin was incised or gastrointestinal tract was opened [16]. When gastrointestinal tract is opened, organisms usually include aerobic Gram negative rods such as *E. coli*, *P.aeruginosa.*

Conclusion

Surgical site infections can be quite challenging to detect and then treat. Prevention necessitates meticulous patient care and all required interventions. This study identified *Staphylococcus aureus* as the leading causative organism of SSIs among surgical patients at Enugu State University Teaching Hospital, Enugu.

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Subject: Medical Sciences

DOI: 10.22192/ijcrcps.2023.10.08.005

How to cite this article:
Chidimma Maureen Chukwueze, Judith Onyedikachi Obu and Emmanuel Ifeanyi Obeagu. (2023). Isolation and identification of bacteria from surgical wound attention at Enugu State University Teaching Hospital, Enugu. Int. J. Curr. Res. Chem. Pharm. Sci. 10(8): 35-43.
DOI: http://dx.doi.org/10.22192/ijcrcps.2023.10.08.005

