eISSN 2255-0569

ORIGINAL

Sensitivity of bacteria from surgical wound Infection at Enugu State University Teaching Hospital

Sensibilidad de las bacterias procedentes de la atención de heridas quirúrgicas en el Enugu State University Teaching Hospital

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Received: 12 - IX - 2023
Accepted: 11 - X - 2023

doi: 10.3306/AJHS.2024.39.01.130

Abstract

Introduction: 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.
Methods: 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. Purposive sampling was employed and a total of 118 samples were collected from patients who gave their consent. The samples were processed through Gram stain, Culture, and an array of Biochemical tests. Subsequently, antibiotic susceptibility tests by Kirby Bauer disc diffusion technique were performed on the isolated bacteria. Data collection was analyzed using Statistical Package for the Social sciences (SPSS) version 25 and Microsoft Excel.
Results: Based on the Sensitivity report, most of the SSI bacteria isolates were sensitive to Ciprofloxacin (67.3%) while most of the isolates were resistant to Chloramphenicol (55.1%). Staphylococcus aureus (16.1%) was the most prevalent organism while the highest bacteria isolates were seen among patients who had laparotomy (61.9%).
Conclusions: Routine Culture should be performed whenever SSI is suspected and choice of Antibiotics for treatment of SSIs should be guided by routine antimicrobial sensitivity testing. Ciprofloxacin should replace first line antibiotics for empirical treatment of SSIs; and strict guidelines for antibiotics prescriptions in treatment of SSIs should be established.

Key words: Sensitivity, bacteria, surgical wound, infections.

Resumen

Introducción: Las infecciones quirúrgicas (ISQ) son un problema mundial en el campo de la cirugía que contribuye a aumentar la mortalidad y la morbilidad. Sin embargo, a pesar de los avances en el control de las ISQ el riesgo de adquirir estas infecciones no se ha eliminado por completo debido a la aparición y propagación de bacterias resistentes.
Metodología: Se realiza un estudio transversal de pacientes con sospecha de infección quirúrgica en las salas del hospital. Se utilizaron cuestionarios estructurados para recoger los datos de los pacientes. Se empleó un muestreo intencional y se recogieron 118 muestras de pacientes que dieron su consentimiento. Las muestras se procesaron mediante tinción de Gram, cultivo y una serie de pruebas bioquímicas. Posteriormente, se realizaron pruebas de susceptibilidad antibiótica a las bacterias aisladas mediante la técnica de difusión en disco de Kirby Bauer. Los datos recogidos se analizaron con el paquete estadístico para las ciencias sociales (SPSS) versión 25 y Microsoft Excel.
Resultados: Según el informe de sensibilidad, la mayoría de las bacterias de ISQ aisladas eran sensibles a la ciprofloxacina (67,3%), mientras que la mayoría eran resistentes al cloranfenicol (55,1%). Staphylococcus aureus (16,1%) fue el organismo más prevalente, mientras que el mayor número de bacterias aisladas se observó entre los pacientes sometidos a laparotomía (61,9%).
Conclusiones: Deben realizarse cultivos sistemáticos siempre que se sospeche una ISQ y la elección de los antibióticos para el tratamiento de las ISQ debe guiarse por las pruebas sistemáticas de sensibilidad a los antimicrobianos. La ciprofloxacina debería sustituir a los antibióticos de primera línea en el tratamiento empírico de las ISQ, y deberían establecerse directrices estrictas para la prescripción de antibióticos en el tratamiento de las ISQ.

Palabras clave: Sensibilidad, bacterias, herida quirúrgica, infecciones.

Cite as: Maureen ChCh, Obu JO, Obeagu EI. Sensitivity of bacteria from surgical wound Infection at Enugu State University Teaching Hospital. *Academic Journal of Health Sciences 2024*; 39 (1):130-134 doi: 10.3306/AJHS.2024.39.01.130

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[1-3]. 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[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].

Deposition and multiplication of microorganisms create wound infections in surgical site of a susceptible host. Most infections of post-operative wounds are hospital acquired and vary from one hospital to another[8].

Lack of standardized criteria for diagnosis of SSIs present a challenge to monitor the global epidemiology of surgical site infection5. 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]. Surgical antimicrobial prophylaxis can help prevent SSIs. In the hospital setting, 30-50% of antibiotics are prescribed for surgical prophylaxis, and 30-90% of this prophylaxis is unnecessary. This incorrect use raises selection pressure, favoring the formation of pathogenic drug-resistant bacteria[14], complicating the selection of empirical antimicrobial drugs and thereby raising the risk of post-operative wound infections.

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, $n = (1.96^{2*} 0.145* 0.855) / 0.052 = 190$

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. 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 60 seconds 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 60 seconds. 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 100 mcg, GN-Gentamicin 10 mcg, CIP-Ciprofloxacin 10 mcg, C-Chloramphenicol 10 mcg, OF-Ofloxacin 10 mcg, MP-Meropenem 10 mcg, PF-Pefloxacin 10 mcg,CT-Cetriaxone 30 mcg, AX-Amoxicillin 30 mcg, ST-Streptomycin 30 mcg. For Gram positive organisms the following antibiotics were used AM-Ampicillin 30 mcg, CL-Cloxacillin 10 mcg, LV-Levofloxacin 10 mcg, CX-Cephalexin 30 mcg, CIP-Ciprofloxacin 5 mcg, GN-Gentamicin 10 mcg, OF-Ofloxacin 10 mcg, CD-Clindamycin 10 mcg, E-Erythromycin 10mcg, CT-Cetriaxone 30 mcg 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.

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 I 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 II** shows sensitivity and resistance for *Pseudomonas aeruginosa*, **table III** shows sensitivity and resistance for *Proteus Mirabilis*, **table IV** shows sensitivity and resistance for Staphylococcus aureus and **table V** shows sensitivity and resistance for *Klebsiella pneumoniae*.

Table I: Sensitivity and Resistance for *E. Coli*.

Antibiotics	Sensitive	Resistant
N	3	15
GN	7	11
CIP	8	10
C	2	16
OF	6	12
MP	2	16
PF	5	13
CT	4	14
AX	7	11
ST	5	13

N-Nitrofurantion, GN-Gentamicin, CIP-Ciprofloxacin, C-Chloramphenicol, OF-Ofloxacin, MP-Meropenem, PF-Pefloxacin, CT-Cetriaxone, AX-Amoxicillin, ST-Streptomycin.

Table II: Sensitivity and Resistance for *Pseudomonas aeruginosa.*

	Sensitive	Resistant
N	0	4
GN	0	4
CIP	4	0
C	0	4
OF	1	3
MP	0	4
PF	0	4
CT	1	3
AX	1	3
ST	0	4

N-Nitrofurantion, GN-Gentamicin, CIP-Ciprofloxacin, C-Chloramphenicol, OF-Ofloxacin, MP-Meropenem, PF-Pefloxacin, CT-Cetriaxone, AX-Amoxicillin, ST-Streptomycin.

Table III: Sensitivity and Resistance for *Proteus Mirabilis.*

	Sensitive	Resistant
N	0	4
GN	0	4
CIP	2	2
C	0	4
OF	1	3
MP	0	4
PF	0	4
CT	1	3
AX	0	4
ST	0	4

N-Nitrofurantion, GN-Gentamicin, CIP-Ciprofloxacin, C-Chloramphenicol, OF-Ofloxacin, MP-Meropenem, PF-Pefloxacin, CT-Cetriaxone, AX-Amoxicillin, ST-Streptomycin.

Discussion

The majority of the SSI bacteria isolates were Gram-negative rods 61.2% (30) with Gram-positive bacteria accounting for 38.8% (19) of the total isolates. These results were attributed to antibiotic resistance, because majority of the Gram-negative bacteria isolates were more resistant compared to Gram-positive isolates. These findings were in harmony with observations made by other research works which observed the predominance of Gram-negative bacteria over Gram-positive bacteria 56.5% versus 43.5% and 85 versus 27. However, the results were contrary to the work of two researchers who observed that Gram-positives were more compared to Gram-negatives[15].

Gram-negative isolates were mostly resistant to Chloramphenicol, Amoxicillin, Meropenem and Streptomycin. Nonetheless, relative success was observed with Ciprofloxacin, Ofloxacin and Nitrofurantion. While the gram-positive isolates were mostly sensitive to Ciprofloxacin, Cetriaxone, Levofloxacin and Ofloxacin and showed great resistance to Ampicillin, Cloxacillin and Cephalexin.

This study discovered that a majority of the SSI isolates were highly resistant to Chloramphenicol and Cloxacillin proving that the two drugs were no longer very effective in the treatment of surgical site infection at the hospital16. The sensitivity of SSI bacteria isolates to these drugs in this present study was actually low, this may be due to bacteria developing a very high resistance against the two drugs, it may also be attributed to the indiscriminate use of the drugs in the study area.

Table IV: Sensitivity and Resistance for *Staphylococcus aureus.*

	Sensitive	Resistant
- AM	3	16
- CL	1	18
- LV	9	10
- CX	2	17
- CIP	15	4
- GN	4	15
- OF	5	14
- CD	1	18
- E	2	17
- CT	14	5

AM-Ampicillin, CL-Cloxacillin, LV-Levofloxacin, CX-Cephalexin, CIP-Ciprofloxacin, GN-Gentamicin, OF-Ofloxacin, CD-Clindamycin, E-Erythromycin, CT-Cetriaxone.

Table V: Sensitivity and Resistance for *Klebsiella pneumoniae*

	Sensitive	Resistance
N	3	1
GN	2	2
CIP	4	0
C	1	3
OF	3	1
MP	0	4
PF	2	2
CT	2	2
AX	1	3
ST	3	1

N-Nitrofurantion, GN-Gentamicin, CIP-Ciprofloxacin, C-Chloramphenicol, OF-Ofloxacin, MP-Meropenem, PF-Pefloxacin, CT-Cetriaxone, AX-Amoxicillin, ST-Streptomycin.

Conclusion

This study identified *Staphylococcus aureus* as the leading causative organism of SSIs among surgical patients at Enugu State University Teaching Hospital, Enugu with Ciprofloxacin as the most sensitive antibiotic and Chloramphenicol, Cloxacillin and Erythromycin respectively as least sensitive, pointing to the necessity of clinicians and microbiologists working hand in hand for the timely diagnosis and treatment of such infections.

Conflict of interest

No

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