PriMera Scientific
Medicine and Public Health
Volume 3 Issue 1 July 2023
DOI: 10.56831/PSMPH-03-074
ISSN: 2833-5627

Incidence, Antibiotic Susceptibility Pattern and Plasmid Profile of Bacterial Pathogens from Surgical site Infections in a Tertiary Hospital in Lagos, Nigeria

Type: Case Study
Received: June 23, 2023
Published: June 28, 2023

Citation:
Dauphin Dighitoghi Moro., et al. "Incidence, Antibiotic Susceptibility Pattern and Plasmid Profile of Bacterial Pathogens from Surgical site Infections in a Tertiary Hospital in Lagos, Nigeria". PriMera Scientific Medicine and Public Health 3.1 (2023): 23-34.

Dauphin Dighitoghi Moro[1]*, Hakeem Olanrewaju Bello[2], Taiwo Oluwabukola Bello[1], Ukhureigbe Miriam Oluchi[1], Hammed Kolawole Shittu[1], Zenas Chisom Agubata[1], Chinyere Theresa Nzomiwu[1], Collins Chidiebere Nwaji[1] and Jude Chidozie Nwaji[3]

[1]Department of Microbiology, Faculty of Science, Lagos State University, P. M. B. 0001, Ojo, Lagos, Nigeria

[2]Ogun State Institute of Technology, Department of Science Laboratory Technology, Igbesa, Ogun State

[3]Department of Microbiology, Faculty of Biological Science, Abia State University, P. M. B. 2000, Uturu Abia State, Nigeria

***Corresponding Author:** Dauphin Dghitoghi Moro, Professor of Microbiology, Department of Microbiology, Lagos State University, P. M. B. 0001, Ojo, Lagos, Nigeria.

Abstract

Surgical site infections [SSIs] remain a common postoperative complication despite the use of prophylactic antibiotics and other preventive measures, mainly due to increasing antimicrobial resistance. SSIs increase postoperative morbidity and mortality and may require hospital admission, intravenous antibiotics and even surgical re-intervention. A hospital based descriptive study was conducted on 100 consented postoperative patients with clinical SSIs. Data on patients was obtained using structured data collection form. Swab samples were collected aseptically from each patients. Bacteriological culture examination and identification was done following standard microbiological techniques. Antibiotics sensitivity test was done by Kirby-Bauer disc diffusion method. Ninety (90%) bacterial isolates were recovered from surgical site infection. Gram negative bacteria (GNB) were predominant (83.3%) with the dominant being *Escherichia coli* (27.78%) and *Staphylococus aureus* (16.67%). All the isolates were highly resistant to amoxicillin/clavunalate, ceftazidime, cefuroxime, levofloxacin and all the isolates were resistant to metronidazole but susceptible to imipenem, polymycin B and amikacin. The plasmid analysis in this study revealed that out of the 40 (44.4%) multi-drug resistance isolates, 35 (87.5%) of which were Gram-negative bacteria had 9 (22.5%) detectable plasmid pattern with the molecular weight of between 2027kbp to 23120kbp while the remaining 26 (74.6%) had no plasmid bands. The remaining 5 (12.3%) which was *Staphylococcus aureus* isolates had 2 (40%) detectable plasmid pattern with the molecular weight of between 23130kbp and 6557 while the remaining 3 (60%) had no plasmid bands. Imipenem is the drug of choice in the treat-

ment of surgical site infections in this study area These findings necessitates judicious antibiotic use and calls for surveillance of SSIs periodically as well as strict adherence to good sanitation practice to reduce spread of drug-resistant pathogens.

Keywords: Surgical Site Infections; Antimicrobial susceptibility; Gram negative; Bacteria; Gram positive; Plasmid Profile; Clinicians; Operating Room

Introduction

A wound is a break in the integrity of the skin or tissues, which may be associated with disruption of the structure and functions due to injury to the skin or underlying tissues or organs caused by surgery, a blow, a cut, chemicals, heat or cold, friction or shear force, pressure or as a consequence of disease, such as leg ulcers or carcinomas [1]. Wounds infection is the commonest and most troublesome disorder delaying wound's healing. When there is a breakdown of local and systemic host defenses, followed by an invasion of microorganisms through tissues, then the wound site is said to be infected [2]. Wound infections often result in sepsis, limb loss, long hospital stay, higher cost of treatment and account for significant human morbidity and mortality worldwide [3].

Wounds can be infected by a variety of microorganisms ranging from bacteria to fungi, and parasites as well as viruses [4]. Bacterial wound contamination are very common hospital acquired infections, causing more than 80% of mortality [5]. The most frequent post-surgical medical difficulties include wound infections by bacteria that are resistant to conventional antibiotics. Widespread use of vast groups of antibiotics together with the length of time causes a significant development of antibiotic resistance in wound infecting bacteria, which subsequently increase the complications of such infections and cost of treatment [6].

Surgical wound infections also known as surgical site infections (SSIs) are of healthcare acquired infection (HAI) that occurs after a surgical intervention in an area of the body where the operation was carried out. SSI may involve the skin, tissues and organs or implanted materials, and they are revealed by a combination of signs and symptoms [7]. Increased morbidity and mortality associated with SSI range from wound discharge as a result of superficial skin infection to life-threatening conditions like severe sepsis [8]. Wound infections are still generally considered to be the most known nosocomial infections, especially in patients undergoing or who have undergone surgery, despite the technological breakthroughs that have been achieved over several years in surgical and wound management systems [9]. A surgical site infection typically occurs within 30 days after surgery. There are 3 types of SSIs which include superficial incisional SSI, which occurs just in the skin area where incision was made, deep SSI, which occurs beneath the incision area beneath the incision area in muscle and tissues surrounding the muscle and organ or space SSI, which occurs in any area of the body, excluding the skin, muscles, and surrounding tissues, that were engaged in the surgery [8, 9].

Infection of surgical sites can be induced by exogenous or endogenous microorganism. Most SSIs are caused by endogenous microbes present in the skin of the patients, especially if clean wounds are excluded [10]. Sources of contamination include the gastrointestinal, respiratory, genital and urinary tracts, the skin and anterior nares. A significant proportion of infections, particularly in clean wounds appear to be attributed to exogenous contamination, which may be responsible for many more infections during epidemics [11]. Exogenous contamination usually emerge from any individual or environmental sources, although most of it is likely to spread by the surgical team who come into direct contact with the wound.

The most widespread organism responsible for the occurrence and progression of SSI is *Staphylococcus aureus*, followed by *Escherichia coli*, coagulase-negative staphylococci (CONS), *Pseudomonas aeruginosa*, Enterococcus species, Enterobacter species, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Candida albicans* and *Streptococcus species*. A greater number of SSIs have been reported to be due to methicillin-resistant *Staphylococcus aureus* (MRSA) [12]. MRSA is the predominant isolate implicated in SSI and related to poor outcome, in clean surgeries which do not involve abdomen or genital tract such as neurosurgeries, cardio-thoracic, ophthalamic, orthopedic and breast surgeries [11, 12].

In order to effectively treat SSIs, antibiotics and anatomical sources may be necessary [13]. Uncomplicated superficial SSIs, such as cellulitis, may be effectively managed with oral antibiotics without surgical intervention and debridement [14]. Antimicrobial therapy is recommended for 5 days and extended if clinical signs of infection persists or the situation worsens. If physical examination (e.g. purulent drainage) and imaging suggests a deeper infection (I.e. deep or organ space), then suture removal, incision and drainage, and debridement of necrotic tissue should be performed [14, 15]. In addition, deep and organ space SSIs require surgical debridement and operative or interventions, such as drainage of the infected fluid collection [14, 16].

The risk of developing a surgical wound infection is largely determined by 3 factors: the amount and type of microbial contamination of the wound, the condition of the wound at the end of the operation (mainly determined by surgical technique and disease processes encountered during the operation), and the host susceptibility, which bothers on the patient's intrinsic ability to deal with microbial contamination [17]. Measures taken to prevent microbial wound infection begin before the operation and the treatment of acute wound infections is a vital preoperative measure. Even if the active bacterial infection is located far from the surgical wound, the risk of wound infection is much more higher for an infected patient than it is for an uninfected patient [16, 17].

This study was carried out to investigate the incidence, antibiotic susceptibility patterns and plasmid profile of pathogens responsible for surgical site infections.

Materials and Methods

Study Area

The cross-sectional study was conducted at the Surgical and Gynecology in Lagos State University Teaching Hospital (LASUTH), Ikeja, a medical organization and tertiary referral hospital with about 750-ed facility located 3 kilometers from Murtala Muhammed Airport. The coordinates of the sampling site were N 6.5895° E 3.3422° It is also a teaching hospital for the Lagos State University College of Medicine (LASUCOM) and it gives service to patients under different clinical disciplines which include surgery, obstetrics and gynecology, orthopedics, obstetrics, pediatrics and ear, nose and throat wards.

Period of Study

This was carried out on patients that underwent surgery from September, 2019 to September, 2020. During the collection of specimens for this study, hospital activities were disrupted at several points by industrial action undertaken by several staff unions within the hospital and the global pandemic, COVID-19, hence a smaller number of surgical operations than anticipated were carried out in the hospital.

Selection of Patients/Subjects

Purposive sampling, otherwise known as deliberate sampling, where participants are selected based on the purpose of the sample was used. All patients (male and females) aged 18 and above undergoing surgery cases, such as typhoid, abdominal injury, scrotal hernia, breast cancer including obstetrics and gynecology cases such as cesarean section and ruptured ectopic pregnancy, were included in the study.

The exclusion criteria include: patients under the age of 18 years, patients undergoing surgery involving permanent in-plants because such patients will require a follow-up and 1 year to effectively rule out surgical wound infection and this is beyond the sample collection time of this study.

Ethical consideration

The study was ethically reviewed and approved by the Health Research and Ethics Committee of the Lagos State University Teaching Hospital (LREC), Ikeja, with the reference number LREC/06/10/1239. The relevant ward nurses got written informed consent from operated patients, who became ill before being sampled. Information obtained at each course of the study was kept confidential.

Collection of samples

All eligible patients that fulfilled the inclusion criteria were subjected to daily surveillance for the development of wound infection. In the same vein, all the factors related to SSIs present in the patient were noted down in the datasheet. Before the wounds were dressed, exudates were collected with a sterile cotton wool swab from the infected site. The swab was introduced gently into the wound sites and rotating the swab tip in the wound, taking care to avoid contamination of specimen with commensals from the skin, and then immersed immediately in a Stuart transport medium to avoid dessication, and to prevent the growth of some species at room temperature that may obliterate the true pathogens. Each sample was labeled carefully and transported to the microbiology laboratory immediately for microbiological investigation.

Isolation and identification of bacterial isolates

After the arrival of the samples at the microbiology laboratory, the swab swere inoculated onto MacConkey, chocolate agar, eosin methylene blue and blood agar plates by rolling the swab over the agar and streaking primarily using sterile bacteriological wire loop. The chocolate agar plate was placed in carbon dioxide enriched atmosphere in an anaerobic jar, macConkey and blood agar plates were placed in an ambient air incubator for some time, and they were incubated at 37°C for 24 - 48 hours.

The bacteria were identified using standard guidelines, by examining the colony, morphology, and using biochemical characteristics according to Cheesbrough, and CLSI [18, 19].

Antibiotic Susceptibility Testing

The susceptibility of the bacterial isolates was carried out using the Kirby-Bauer's disc diffusion technique and CLSI [19, 20]. From the pure culture of the isolates, a colony of each isolate was suspended in a sterile nutrient broth (Oxoid) diluted to match McFarland standards. Using aseptic techniques, the isolate was picked and streaked on Mueller-Hinton agar (Oxoid) plate to form a well spread colonies (lawn) and the disc containing antibiotics (Oxoid) with the recommended concentration was placed firmly on surface of the agar using sterile forceps. The plates were allowed to stand for 3-5 minutes to enable the antibiotics diffuse into the agar. The plates were incubated aerobically for 24 hours at 37°C. Diameters of growth inhibition around the discs were measured and interpreted as sensitive, intermediate or resistant in accordance to CLSI [19].

The following antimicrobial agents were used with their respective concentration in microliter (µ) : Ceftazidime (CAZ, 30), Cefuroxime (CXM, 30), Levofloxacin (LEV, 5), Imipenem (IPM,10), Metronidazole, (MTZ, 5), Amoxicillin-Clavulanate, (AMC, 30), Polymycin B (PB, 30), and Amikscin, (AK, 30) were used for Gram nedative bacteria, while Ceftazidime (CAZ, 30), Cefuroxime (CXM, 30), Levofloxacin (LEV, 5), Imipenem (IPM, 10), Metronidazole (MTZ, 5), Amoxicillin-Clavulanate (AMC, 30), Polymycin B (PB, 30), Amikacin (AK, 30) and Erythromycin (E, 10) was used for Gram-negative [19].

Plasmid DNA isolation and profiling

TENS protocol described by Liu et al [21], was employed in plasmid extraction. One and a half milliters (1.5 ml) overnight culture was poured into a centrifuge tube and spun at 10K for 1 min. To pellet the cells, followed by gentle decant of the supernatant keeping 150µl of media in the tube. The tube was inverted gently 3-4 times until the mixtures become sticky turning the liquid from turbid to clear. One and fifty microliters (150µl) of 3M NaOAc (pH 5.6) was then added to the preparation followed by vortex mixing. The tube was inverted gently 3-4 times where there was formation of a white precipitate. The preparation was spun at maximum speed for 5mins to pellet the white precipitate. The clear supernatant was pipette to a clean tube and mixed well with 900cl of 95 of ice absolute ethanol. It was spun at maximum speed of 2 min to pellet the DNA, the solution was poured off and 500µl of 70% was added to wash the pellet by vortexing. The solution was spun again for 1 min, and the 70% ethanol was poured off and the DNA pellet was dried. The DNA was dissolved in 50µl 10Mn Tris (pH 8).

Plasmid DNA Detection.

Ten Micoliters of the molecular markers was loaded into the first well. Two Microliters of the loading dye was mixed with 8 µl of the plasmid DNA extract and then loaded into the other wells. Electrophoresis was performed at 80V for 1 hour 30 min. after UV-illumination and photographed by polaroid camera. Molecular weights were estimated using Lambda DNA Hind III Marker (Jena Bioscience).

Results

The results of the 100 wound samples analyzed are reported below.

Distribution of subjects in the hospital wards

A total of 100 patients were under surveillance with signs of SSIs with the inclusion criteria, so were included in the study; 47 patients were from general surgical ward, 37 patients from obstetrics and gynecology ward, 13 from orthopedic and 3 patients from ophthamology ward. Overall, 90% of the patients developed SSIs, and fungal isolates were discarded because this study was focused only on bacterial pathogens. Escherichia coli was the leading cause of SSIs in this study constituting 27.7% of the total bacterial isolates (Fig. 1).

Age and sex distribution of subjects

In this study, samples were collected from age groups ranging from 18 to 77 years, which were categorized into 6 main groups: 18-27, 28-37, 38-47, 48-57, 58-67 and 68-77 (Table 1). The highest incidence of SSIs (41%) was recorded in the 28-37 age group.

Fifty eight percent of the samples were collected from males and 42% from females. In contrast however, 53 (58.8) of the bacterial isolates were recovered from females and 37 (41.2%) from males, as growth from females was higher [Table 1].

Distribution of bacterial isolates among subjects

Using standard microbiological techniques on the bases of cultural, morphological and biochemical characteristics, 90 bacterial isolates were recovered, which comprised of Staphylococcus aureus 15 (16.7%), as the only Gram positive bacterium and Gram negative bacteria were: *E. coli*, 25 (27.7%), *Pseudomonas aeruginosa*, 11 (12.22%), *Enterobacter aerogenes*, 9 (10%), *Acinetobacter baumanii*, 8 (8.89%) and *Klebsiella oxytoca*, 1(1.11%) (Table 2).

Occurrence of bacterial isolates in hospital wards

Out of the 90 bacterial isolates from the hospital, the highest number of Gram negative bacteria was *E.coli*, 25 (27.7%), 14 (56%) , and 10 (11.11%) from general surgery obstetrics and gynecology wards respectively. The least bacterium isolated was *Klebsiella oxytoca*, 1 (2.4%), from the general surgery ward. Forty one (45.56%) were recovered from the general surgery ward, followed by 26 (28.89%) from the obstetrics and gynecology ward, 6(6.67%) from orthopedic and 2(2.22%) from ophthalmic ward. Eleven (73.3%) *S. aureus* were recovered from obstetrics and gynecology while only 4 (26.7%) *S. aureus* were recovered from general surgery as the only Gram positive bacterium (Table 3).

Antibiotics sensitivity testing

Antibiotics resistance demonstrated by both Gram negative and Gram positive bacteria is shown table 5. All the 75 Gram negative bacteria showed high resistance to Amoxicillin/clavulanate, Ceftazidime, Cefuroxime, Levofloxacin, and a 100% resistance to Metronidazole, but relatively susceptible to Imipenem, PolymicinB and Amikacin respectively (Table 4).

Staphylococcus aureus which was the only Gram positive bacterium demonstrated high level of resistance to Ceftazidime, Levofloxacin, Amoxicillin/Clavulanate, Polymycin B, Erythromycin, Metronidazole and Cefuroxime but showed a high susceptibiliy to Imipenem and Amikacin in-vitro as shown in table 4.

Plasmid profile of bacterial isolates

Plasmid profiling was carried out on 40 (44.4%) multidrug resistant bacterial isolates. The fragment bands observed were directly compared with the molecular weight marker bands Lambda DNA Hind III Marker (Jena Bioscience) which has seven distinct visually observed bands with 5molecular weight 23130Kbp, 9416Kbp, 6557Kbp, 4361Kbp, 2322Kbp, 2027Kbp, and 564Kbp. The representation of the various plasmids are as shown in figures 2, 3 and 4.

Figures 2-3, show the plasmid profile of Gram negative bacteria isolates: S. typhi, Serratia marcescens, Acinetobacter baumanii, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *E. coli* ranging from 2027Kbp to 23830Kbp, being the most common plasmid detected. Eight of the Gram negative isolates (*S. typhi, Serratia mascescens, Acinetobacter baumanii, Enterobacter aerogene, Klebsiella pneumoniae and Pseudomonas aeruginosa*) carried only one plasmid each with molecular weights ranging from 2027Kbp to 231320Kbp, while K. neumoniae in lane 20 had four plasmids with sizes 23130Kbp, 23830Kbp, 3482Kbp and 2027Kbp [Table 5].

Figure 4 shows that the Gram positive bacterium, Staphylococcus aureus in lanes 15 and 17 had 2 genes and 1 plasmid gene respectively, with molecular weight of 23130Kbp and 23130Kbp as shown in table 5.

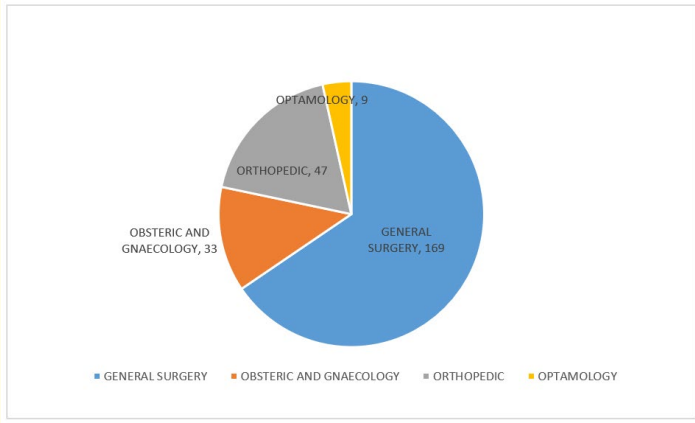

Figure 1: Distribution of samples in hospital.

Age Range (Years)	*Male*		*Female*		*Total*	
	No of Samples	*No of Isolates (%)*	*No of Samples*	*No of Isolates (%)*	*No of Samples*	*No of Isolates (%)*
18-27	6	4 (67)	7	7 (100)	13	11 (83)
28-37	16	16 (100)	25	24 (96)	41	40 (98)
38-47	15	11(73)	11	10 (91)	26	21 (81)
48-57	0	0 (0)	12	10 (83)	12	10 (81)
67	4	4 (100)	3	3 (100)	7	7 (100)
68-77	1	1 (100)	0	0 (0)	1	1 (100)
	42	36	58	54	100	90

Table 1: Demographic Factors of Participants (Age and Sex).

Bacteria	***No (%)***
Escherichia coli	25 (27.78)
Staphylococcus aureus	15 (16.67)
Pseudomonas aeruginosa	11 (12.22)
Enterobacter aerogenes	9 (10)
Acinetobacteria baumanni	8 (8.89)
Serratia marcescens	5 (5.56)
Proteus mirabilis	4 (4.44)
Citrobactor freundii	4 (4.44)
Salmonella typhi	3 (3.33)
Klebsiella pneumonia	3 (3.33)
Citrobacter diversus	2 (2.22)
Klebsiella oxytoca	1 (1.11)
	90 (100)

Table 2: Frequency Distribution of Bacteria Isolates.

	Ward	***Obs & Gyn No. %***	***Ortho No, %***	***Ophth No. %***	***Gen. Surgery No. %***	***Total***
Gram Negative Bacteria	*Escherichia coli*	10 (38.4)	1 (16.7)	0	14 (34.1)	25
	Pseudomonas aeruginosa	1 (3.8)	0	1 (16.7)	9 922)	11
	Enterobacter aerogenes	3 (11.5)	1 (16.7)	1 (50)	4 (39)	10
	Acinetobacteria baumanni	3 (11.5)	0	1 (16.7)	4 (39)	8
	Serratia marcescens	0	1 (16.7)	1 (50)	3 (7.3)	5
	Proteus mirabilis	3 (11.5)	0	0	1 (2.4)	4
	Citrobactor freundii	2 (5.4)	0	0	2 (4.9)	4
	Salmonella tyhpi	0	0	0	3 (7.3)	3
	Klebsiella pneumoniae	3 (11.5)	0	0	0	3
	Citrobacter diversus	1 (3.8)	1 (16.7)	0	0	2
	Klebsiella oxytoca	0	0	0	1 (2.4)	1
Total		26 (100)	6 (100)	2 (100)	41 (100)	

Table 3A: Distribution of Gram-negative bacteria isolated by wards.

	Ward	***Obs & Gyn No. %***	***Ortho No.%***	***Ophth No. %***	***Gen. Surgery No. %***
Gram positive bacteria	*Staphylococus aureus*	11 (100)			4 (100)
Total		11 (100)			4 (100)

Table 3B: Distribution of Gram-positive bacteria isolated by wards.

Isolated organisms	***CAZ***		***LEV***		***IPM***		***MTZ***		***AMX***		***PB***		***CXM***		***AK***	
	%		***%***		***%***		***%***		***%***		***%***		***%***		***%***	
	R	***S***	***R***	***S***	***R***	***S***	***R***	***S***	***R***	***S***	***R***	***S***	***R***	***S***	***R***	***S***
Escherichia coli (25)	88	12	84	16	89	1	100	0	96	4	20	80	96	4	24	76
Pseudomonas aeruginosa (11)	73	27	64	36	9	91	100	0	73	27	36	64	100	0	36	64
Enterobacter aerogenes (9)	100	0	56	44	22	78	100	0	100	0	11	89	100	0	22	78
Acinetobacteria baumanni (8)	100	0	100	0	50	50	100	0	100	0	13	87	100	0	25	75
Serratia marcescens (5)	60	40	60	40	0	100	100	0	100	0	40	60	100	0	40	60
Enterobacter freundii (4)	100	0	100	0	25	75	100	0	100	0	25	75	100	0	25	75
Proteus mirabilis (4)	100	0	100	0	25	75	100	0	100	0	50	50	100	0	50	50
Klebsiella pneumoniae (3)	100	0	67	33	100	0	100	0	100	0	33	67	100	0	33	67
Salmonella tyhpi (3)	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0
Enterobacter diversus (2)	100	0	100	0	100	0	100	0	100	0	50	50	100	0	100	0
Klebsiella oxytoca (1)	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0
Staphylococcus aureus (15)	87	13	67	33	27	73	100	0	87	13	80	20	60	40	40	60

Table 4: Antibiotics resistance pattern of bacteria isolates from subjects.

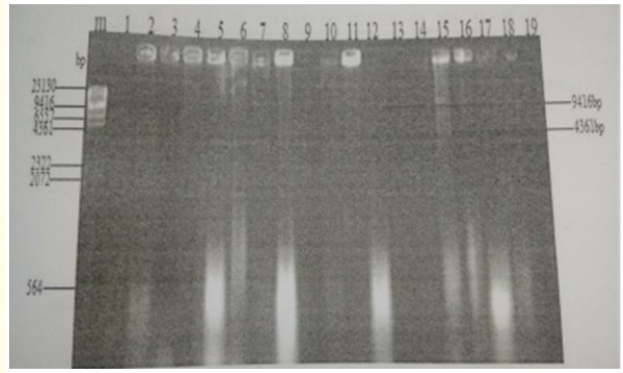

Figure 2: Plasmid profile of isolate in lane 1-19 (Gram-negative}.

M-Maker

Lane:

1. Salmonella typhi
2. Salmonella typhi
3. Serratia marcescens
4. Serratia marcescens
5. Escherichia coli
6. Pseudomonas aeruginosa
7. Salmonella typhi
8. Escherichia coli
9. Citrobactor freundii
10. Klebsiella pneumoniae
11. Serratia marcescens
12. Acinetobactor baumanni
13. Enterobacter aerogenes
14. Escherichia coli
15. Enterobacter aerogenes
16. Klebsiella oxytoca
17. Acinetobacter baumanni
18. Escherichia coli
19. Salmonella typhi

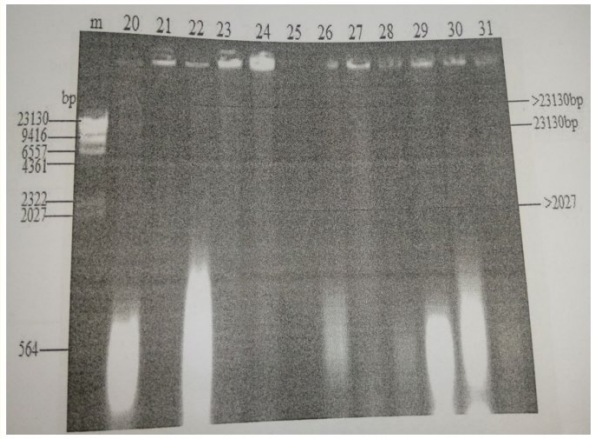

Figure 3: Plasmid profile of isolate in Lane 20-31 (Gram-negative).

M-Maker

Lane:

20. Klebsiella pneumoniae
21. Proteus mirabilis
22. Pseudomonas aeruginosa
23. Enterobacter aerogenes
24. Pseudomonas aeruginosa
25. Escherichia coli
26. Citrobacter freundii
27. Escherichia coli
28. Enterobacter aerogenes
29. Escherichia coli
30. Acinetobacter baumanni
31. Citrobacter freundii

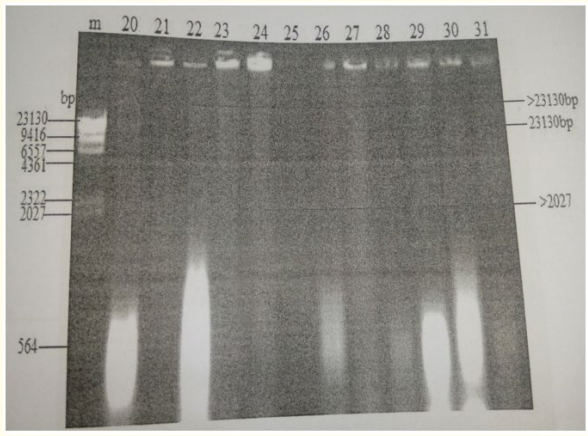

Figure 4: Plasmid profile for Gram-positive isolates.

M: Maker

Lane:

15. Staphylococus aureus
17. Staphylococus aureus
20. Staphylococus aureus
34. Staphylococus aureus
49. Staphylococus aureus
53. Staphylococus aureus
73. Staphylococcus aureus
76. Staphylococus aureus

Organism code	*Gram reaction*	*Resistant pattern*	*Number of plasmid isolated*	*Plasmid band size (pb)*
Salmonella typhi	-ve	0%	1	4561
Klebsiella pneumoniae	-ve	37%	1	4561
Serratia marcescens	-ve	37%	1	4561
Acinetobacter baumanni	-ve	37%	1	9418
Enterobacter aerogenes	-ve	37%	1	4561
Klebsiella pneumoniae	-ve	25%	4	23830, 23130, 3482; 2027
Pseudomonas aeruginosa	-ve	37%	1	23130
Pseudomonas aeruginosa	-ve	12%	1	2027
Acinetobacter baumanni	-ve	50%	1	23130
Staphylococcus aureus	+ve	11%	2	23130, 6557
Staphylococcus aureus	+ve	15%	1	23130

Table 5: Number of Plasmids and their corresponding sizes of multidrug resistant bacteria isolated.

Discussion

This study revealed that Gram negative bacteria constituted 83.3% of the bscterial agents recovered from the surgical site infections and the remaining 16.7% was constituted by Gram positive bacteria, with *E. coli* having the highest prevalence of 27.7%. A similar finding was reported by Olufunmilola [22] in a study carried out at the Federal Medical Center, Idi-Aba, Abeokuta, Nigeria, where out of 160 organisms, 31% of the pathogens were Gram positive and 69% were Gram negative, with *E. coli* having the highest prevalence of 32.5%. The fact that majority of the infected patients in our study had undergone abdominal surgery and that Gram negative bacteria are typically reported to be engaged in intra-abdominal procedures may be the cause of the high prevalence of Gram negative bacterial pathogens as earlier reported [23]. In this study, there was no mixed growth as all samples has single bacterial agent, a finding which is similar Olufumilola [22], where there was no mixed infections as all samples had a single causative agent. This finding is however in contrast to that of Giacometti et al [24], who reported mixed organisms in over a half of their study population. This may be due to the different methods of isolation and variation in the subjects and the areas where the studies were carried out.

The predominant organism among the Gram negative bacteria was *E. coli* as it had the highest prevalence of 27.78% and *S. aureus* (16.7%) was the only Gram positive bacterial isolate. A similar finding was reported by Pradeep and Rao [25] in which, *E.coli* was the predominant Gram negative bacterial isolate (42.1%) and *S.aureus* (44.8%) was the predominant Gram positive bacterial pathogen in SSIs. A contrary report by Bisi-Johnson and Olowe [26], reported that *S.aureus* was the second most predominant bacteria in their study. Despite the notable shift in etiology of SSIs, *S.aureus* has remained an important nosocomial pathogen accounting for a remarkable proportion of hospital acquired infections. Even in this study, *S. aureus* was the predominant bacterium isolated from the obstetrics and gynecology ward. *E. coli* was more prevalent in obstetrics and gynecology ward as earlier reported by Olufunmiola [22]. Several other enteric bacterial pathogens with varying prevalence were recovered from SSIs in this study which included *Pseudomonas aeruginosa*, 11 (12.2%), *Enterobacter aerogenes*, 9 (10%), *Acinetobacter baumanii*, 8 (8.89%), *Serratia marcescens*, 5 (5.56%), *Citro-*

bacter freundii, 4 (4.44%), *Proteus mirailis*, 4 (4.44%), *Klebsiella pneumoniae*, 3 (3.33%), *S. typhi*, 3(3.33%), *Citrobacter diversus* and *Klebsiella oxytoca* with 2 (2.22%) and 1(1.11%) respectively.

A study of the frequency distribution of SSIs on age showed that 41% was recorded in the age group of 28-37 years, followed by 26% in the 38-47 years age group, and 13% in 18-27 years age group. The lowest incidence of was seen in the older age group of 68-77 years (1%), followed by 58-67 years age group (3%) and age group 48-57 (12%). The low incidence of SSIs among the elderly may be due to their very careful approach to life, being very conscious of their life style, with many living quite sedentary live and precautionary in whatever they do.

Antimicrobial susceptibility testing is very critical for accurately prescribing antibiotics for patients' treatment. Antibiotics susceptibility testing in-vitro estimates the activity of drugs which assists the clinicians in selecting an antibiotic effective in inhibiting or out rightly killing an infecting microorganisms in-vivo. Most of the antibiotics tested which are commonly used in our clime, appear to be ineffective against the isolated bacterial pathogens. All the bacteria tested were absolutely resistant (100%) to Metronidazole which is one of the most commonly used over-the-counter drug, and more than a half of the bacterial isolates tested were also absolutely resistant to Cefuroxime and Amoxicillin/Clavulanate. Similar findings were reported by Kemebradikumo [27], where absolute resistance (100%) to Amoxicillin/Clavulanate was found. Majority of the bacterial isolates tested were found to be resistant to 6 of the antimicrobial agents with nine different resistant patterns of antibiotics resistance. This means that most of the treatment using this drugs may not be effective, thereby necessitating the need to search for new potent antibiotics and attempt to treat such common and unavoidable infections like SSIs with combination of different antibiotics, which may yield positive treatment and successfully control same.

Conclusion

E. coli and *S. aureus* among several enteric bacteria constitute common bacterial pathogens among SSIs in the tertiary hospital where the study was carried, which origin may be the patients, the surgeons or the operating theater. Imipenem, Polymycin B and Amikacin are very potent antibiotics against bacterial pathogens that cause SSIs among postoperative patients. The recovery of multidrug resistant bacterial pathogens causing SSIs requires a good hospital infection prevention in the form of adequate surveillance, ensuring standard aseptic techniques as well as proper preparation and maintenance of operating room.

Acknowledgements

The authors sincerely appreciate the contributions of the following to the successful execution of this research project. Mr. Fukpene Baitei for his secretariat assistance and the statistical presentation of the results, as well as the assistance of the Laboratory staff of Department of Microbiology, Lagos State University and staff of Department of Medical Microbiology, Lagos State University College of Medicine (LASUCOM), Ikeja, Nigeria for their technical assistance.

References

1. Soresen LT, Karlsmark T and Gottrup F. "Abstinence from smoking reduces incisional wound infection: a randomized controlled trial". Annals of surgery 238.1 (2003):1-5.
2. Farhan S, Zeeshan S, Moshin Z and Shahzad A. "Frequency of Post-operative Surgical Site Infections in a Tertiary Care Hospital". Journal of Dental and Medical Sciences 7.11 (2019): 42-43.
3. Shrestha A and Sharma VK. "Bacteriological study of wound infection and antibiotic susceptibility pattern of the isolates". Nepal Journal of Science and Technological 14.2 (2013): 143-150.
4. Church D. "Burn Wound Infections". Clinical Microbiology Review 19.2 (2006): 403-434.
5. Manikandan C and Amsath A. "Antibiotic susceptible of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnadu, India". International Journal Current Microbiology Applied Science 2 (2013): 195-203.
6. Anguzu JR and Olila D. "Drug Sensitivity Patterns of Bacterial Isolates from Septic Post-operative Wounds in a Regional Referral Hospital in Uganda". Africa Health Sciences 7 (2007): 148-154.

7. CDC Guideline for the Prevention of Surgical Site Infection 152.8 (2017): 784-791.
8. Owens CD and Stoessel K. Surgical site infections: epidemiology, microbiology and prevention". J Hosp Infect 2 (2008): 3-10.
9. Horan TC, Gaynes RP, Martone WJ, Jarvis WR and Emori TG. "CDC definitions of nosocomial surgical site Infections: A modification of CDC Definitions of nosocomial Surgical Site Infections: A modification of CDC definitions of Surgical Wound Infections". Infection Control Hospital Epidermiology 13.10 (1992): 606-608.
10. Altemeier WA. "Sepsis in Surgery. Presidential address". Arch Surg 117.2 (1982): 107-112.
11. Garner JS, Javis WR, Emori TG, Horan TC and Hughes JM. "CDC definitions for nosocomial infection". American Journal of Infection Control 16 (1988): 128-40.
12. Cantlon CA, Stemper ME and Schwan WR. "Significant Pathogens isolated from surgical site infections at a community hospital in the Midwest". America Journal of Infection Control 34.25 (2006): 526-529.
13. Barie PS and Wilson SE. "Impact of evolving epidemiology on treatments for complicated skin and skin structure infections: The surgical perspective". Journal of America College Surgery 220.1 (2015): 105-106.
14. Anderson DJ. "Surgical site infections". Infectious diseases and Clinic of North America 25.1 (2011): 135-153.
15. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL and Wade JC. "Practice guidelines for the diagnosis and management of skin and soft tissue infections: Update by the infectious Diseases Society of America". Clinical Infectious Disease 2 (2014): 10-52.
16. Lawson EH, Hall BL and Ko CY. "Risk factors for superficial vs deep/organ-space surgical site infections: implications for quality improvement initiatives". Jamaica Surgery 148.9 (2013): 849-858.
17. Malik A, Mohammad Z and Ahmad J. "The diabetic foot infections: biofilms and antimicrobial resistance. Diabetes and Metabolic Syndrome". Clinical Research and Reviews 7.2 (2013): 101-107.
18. Cheesbrough M. "Medical Laboratory Manual for Tropical Countries". Microbiology. Butterworth and Co. Ltd 3 (2007): 258-270.
19. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. Wayne, PA, USA: CLSI (2012): M100-S22.
20. Kirby QMM. and Bauer AW. "Antibiotic susceptibility testing by a standardized single disc method". Am. J. Clin. Path 45 (1966): 493-6.
21. PY Liu, YJ Lau, BS Hu, JM Shyr, ZY Shi, WS Tsai, YH Lin and CY Tseng. "Analysis of clonal relationships among isolates of Shigella sonnei by different molecular typing methods". Journal of Clinical Microbiology 33 (1995): 1779-1783.
22. Olufunmilola BM, Olugbenga AO and Folasoge AA. "Bacterial Agents of Surgical Site Infections in South-Western Nigeria". American. Journal of Biomedical Sciences 5 (2010): 217-225.
23. Bibi SGA, Channa TR, Siddiqui and Ahmed W. "Pattern of bacteria pathogens in postoperative wounds and their sensitivity patterns in Karachi, Pakistan". Journal of Surgery Pakistan 17.4 (2012): 164-167.
24. Bisi-Johnson MA and Olowe OA. "Studies on bacteria agents of surgical site infection in Osogbo, South Western Nigeria". International Journal of Infectious Diseases 1.21 (2014): 346.
25. Giacometti A, Cirioni O, Schimizzi AM, Del Prete MS, Barchiesi F and D'errico MM. "Epidemiology and microbiology of surgical wound infections". Journal of Clinical Microbiology 38.2 (2000): 918-922.
26. Kemebradikumo P, Fente BG and Oladapo O. "Current microbial isolates from wound swabs. Their culture and sensitivity pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria". Tropical Medical Health. 41.2 (2013): 49-53.
27. Pradeep M and Rao KVV. "A study on surgical site infections, their bacteriological profile and antimicrobial susceptibility pattern". International Journal of Medical Microbiology Tropical Disease 5.1 (2019): 9-13.