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PREVALENCE OF NOSOCOMIAL INFECTIONS AND PLASMID PROFILE OF BACTERIA ISOLATES FROM HOSPITAL ENVIRONMENT

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ABSTRACT

Nosocomial infection is an infection that is acquired in a hospital or other health care facilities like nursing home, rehabilitation facility, outpatient clinic, or other clinical settings. The aim of the study was to access the Prevalence of Nosocomial Infections and Plasmid Profile of Bacteria Isolates from Hospital Environments. A total number of 300 patients were recruited for this study. Classification of infections into sites was based on the diagnosis in the folders and the type of specimen sent to the microbiology laboratory for investigations. Subsequent identification was done based on morphology and biochemical tests. Susceptibility pattern of the isolates using the disk diffusion method was performed. Presence of plasmids was determined using the agar gel electrophoresis technique. The prevalence of nosocomial infections by site showed that urinary tract infection had the highest prevalence rate of (14.7%) and the lowest prevalence was recorded in blood stream infection (4.9%). All the 15 multiple antibiotic resistant isolates subjected to agar gel electrophoresis analysis had plasmids. All plasmid borne multidrug resistant bacteria isolates in this study were cured of their plasmid upon treatment with 10% sodium dodecyl sulphate. This study justifies the need to strengthen infection control to prevent the spread of plasmid mediated multidrug resistant bacteria within Port-Harcourt metropolis of Rivers State.

KEYWORDS: Prevalence**;** Nosocomial infections**;** Plasmid Profile Bacteria; Hospital Environments; Port-Harcourt, Rivers State.

1. INTRODUCTION

Nosocomial infection, also known as a hospital-acquired infection (HAI), is an infection that is acquired in a hospital or other health care facilities like nursing home, rehabilitation facility, outpatient clinic, or other clinical settings. To emphasize both hospital and nonhospital settings, it is sometimes instead called a health care–associated infection (HAI or HCAI). Infection is spread to the susceptible patient in the clinical setting by various means (Akbari et al. 2015). Health care staff can spread infection, in addition to contaminated equipment, bed linens, or air droplets. The infection can originate from the outside environment, another infected patient, staff that may be infected, or in some cases, the source of the infection cannot be determined. In some cases the microorganism originates from the patient's own skin microbiota, becoming opportunistic after surgery or other procedures that compromise the protective skin barrier. Though the patient may have contracted the infection from their own skin, the infection is still considered nosocomial since it develops in the health care setting (Akbari et al. 2015).

The most common types are bloodstream infection (BSI), pneumonia (e.g, ventilator-associated pneumonia [VAP]), urinary tract infection (UTI), and surgical site infection (SSI). Many types are difficult to treat with antibiotics. In addition, antibiotic resistance can complicate treatment.

In the past most nosocomial infections were caused by gram positive microbes in which Staphylococcus aureus was the primary cause of nosocomial infection. Gram negative bacteria, such as E. coli and Pseudomonas aerugionsa that has the ability to cause opportunistic skin infections are also the major cause today (Qayyum et al. 2010). The prevalence rate of nosocomial infection as studied by WHO is averagely 11.85% which was reported by Samuel et al. (2010), and the risks are increasing in developing nations annually because it has been estimated that between 5 and 10% of patients

admitted to acute care hospitals in developing nations acquired one or more infections. This is by far more serious in low-resource countries that do not have the resources either to prevent and control or to manage such situations due to financial constraints (David and Famurewa 2010).

The economic cost are considerable, the increased length of hospital stay for infected patients is the greatest contributor to the cost (Saka et al. 2011). (Pittet et al. 1994) and (Saka et al. 2011) reported that the overall increase in the duration of hospitalization for patients with surgical wound infection was about 8 days, ranging 3 days for gynaecology to 9 days for general surgery and 19 days for orthopaedic surgery. Prolong stay not only increases direct cost to patient or payers but also indirect costs due to cost work. The increased use of drugs, the need for isolation and the use of additional laboratory and other diagnostic studies also contribute to the cost (Pittet et al. 1994; Samuel et al. 2010).

Infections cause by nosocomial pathogens are also one of the leading cause of deaths. More than 70% of these pathogens from the hospital environment are resistant to drugs or multi-drugs which are now the most leading cause of human death worldwide (Anton and David 2010). The occurrence of multi-drug resistance in hospital-associated pathogens has resulted in the emergence and re-emergence of difficult - to – treat nosocomial infections in patients. Therefore, hospital is not only a place where sick people recover from their sickness but also where the illnesses get complicated and healthy people get infected. Whenever clinical procedures are performed, clients are at risk of infection during and after the procedures (Kampf and Kramer 2004).

In Nigeria, public and hospital staff awareness of nosocomial infections either is very low or none with many healthcare institutions having no policy on disease management and guidelines on combating nosocomial infections. If the state of Nigerian hospitals and healthcare institutions is to be used as a yardstick to measure prevalence of nosocomial infection, then surely nosocomial infection rate in Nigeria could be relatively high. It would thus imply that a significant number of patients that go on admission in hospitals in Nigeria often have to spend much more money and time getting relief than would have been otherwise needed.

In spite of the seriousness of nosocomial infections and the problems it poses to successful management of health in health institutions as well as the attendant effects on patients, little or no previous attempts have been made to investigate the problems of nosocomial infections in Port-Harcourt. Therefore this study was carried out to access the Prevalence of Nosocomial Infections and Plasmid Profile of Bacteria Isolates from Hospital Environments in Port Harcourt, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Braithwaite memorial specialist hospital, a government owned hospital, named after Eldred Curwen Braithwaite, a British doctor and pioneer of surgery. It is located inside Portharcourt City Local Government Area. This study was carried out between June 2016 and May 2017.

2.2 ETHICAL PERMISSION

Ethical permission was obtained from the hospital authorities and the consent of patients was sort before the study was carried out.

2.3 RECRUITMENT OF PATIENTS FOR THE STUDY

Patients that were newly admitted in the hospital were recruited for the study. A total number of 300 patients were recruited from (6) major wards which includes the male medical ward (MMW), female medical ward(FMW), female surgical ward(FSW), male surgical ward (MSW), maternity ward (MW) and pediatric ward (PW). They were monitored from the day of admission to the time they were discharged from the hospital. The following parameters were taken from the patients and recorded using a predesigned questionnaire.

a. The time spent in the hospital before operation.
b. Underlining diseases.
c. Age of the patient
d. Antibiotics usage before admission/operation.

During the period of admission/operation, the bacteria load of the air and floor of the hospital wards were studied.

2.3. STUDY OF NOSOCOMIAL INFECTIONS

Data's were collected on all inpatients hospitalized before 8 am on the first day of the survey. The sampling units were wards. Trained medical doctors collected data from clinical records, temperature charts, laboratory reports, and information provided by physicians and nurses in each ward (i.e data like patients hospital numbers, names, age, sex, date of admission, diagnosis on admission, specimen sent to the microbiology laboratory and results, type of organism(s) isolated, susceptibility of the isolates to the antibiotics tested, second diagnosis and date and interval between the first and second diagnosis).

Infections of more than one site in the same patient were counted as separate infections. Antibiotics prescribed at the time of the survey were recorded. Preoperative and perioperative doses of antibiotics were registered separately as prophylactic. Records were computer analyzed and data were entered in duplicate to minimize transcription errors.

To classify an infection to be nosocomial, the following criteria, as specified by WHO/CDC (2002) were used:

a. Time interval between the first and second diagnosis is not less than 48 hours
b. Positive culture results in the first and second diagnosis
c. Isolated pathogen in second diagnosis is different from the first isolated pathogen

2.4 CLASSIFICATION OF INFECTIONS INTO SITES

Classification of infections into sites was based on the diagnosis in the folders and the type of specimen sent to the microbiology laboratory for investigations. When record indicated surgical operation and wound swab was sent to microbiology laboratory for culture and the culture was positive, the infection acquired is considered as surgical site. Where the sample involved was urine, with significant bacteria level, the infection is considered urinary. Whereas, when the specimens cultured are sputum and blood and the culture produced positive result, the patients were deemed to have developed respiratory and blood stream infections respectively.

2.5 DETERMINATION OF RATES OF INFECTIONS

Rate of infection was determined by dividing total number of patients infected by total number of patients discharged multiply by 100.
Infection rate = Total no of patients infected / Total no of patients discharged × 100

2.6 BACTERIOLOGICAL STUDY OF THEATRE AIR

The bacterial load of the air in the 6 hospital wards was studied using settling plate method. Plates containing Nutrient agar (NA), MacConkey agar, and Chocolate agar were exposed and placed in different areas of the hospital wards and left exposed for 1hr. Thereafter, they were covered and taken for incubation. The plates were incubated at 37^{o}C in an ambient incubator. Colonies that developed after 24hr of incubation in the plates were enumerated and recorded. Based on the number of colonies per plate, the number of exposed plates and the duration of the surgical operation, the number of colonies in the air per hour was determined and recorded number of colonies forming unit per cubic meter of air (cfu/m^{3}). Colonies showing different morphological characteristics were isolated, purified into pure cultures, coded, sub-cultured onto nutrient agar slant and kept in refrigerator for subsequent characterization.

2.7 BACTERIOLOGICAL STUDY OF THEATRE FLOOR

The bacteria load of the theater floor was studied using swabbing method. The floor of theater which has already been demarcated (one meter square) was sub divided into 16 sub-squares, and one of these squares was randomly chosen and swabbed using sterile cotton swab moistened with sterile normal saline. The swab was then put into test tube containing 9ml sterile normal saline and mixed properly as to discharge it contents. One milliliter (1ml) each of this mixture was then used to flood plates of Nutrient agar, MacConkey agar and Chocolate agar. Plates of nutrient agar and MacConkey agar were incubated at 37^{o}C for 24 hours (Favero et al. 1984). The colonies that developed after 24 hours of incubation, were counted, streaked onto nutrient agar slants and stored at 7^{o}C in refrigerator for further processing.

2.8 DETECTION OF SURGICAL SITE NOSOCOMIAL INFECTIONS (SSI)

The operated patients in the ward were visited on daily bases, and the sites of operations were inspected for signs of infection. Patient's folders were also checked on daily bases for information that might indicate signs of infection in the surgical sites.

The classification of an operations site as positive for surgical site infection (SSI) is based on the definition of WHO/CDC (2002), which states that "SSI is an infection that occurred within 30 days after operative procedure". The patient is deemed to have developed SSI when at least one of the followings is noticed in the patient.

a. Purulent drainage from the superficial incision.
b. Organism is isolated from an aseptically obtained culture of fluid or tissue from superficial incision.
c. At least one of the following signs or symptoms of infection occurs: pain, tenderness, or heat and superficial incision deliberately opened by surgeon unless incision is culture negative. Where there was occurrence of any of the above signs or symptoms on a patient, the consultant attention was drawn and decision on the status of the sign made. Such sites were then swabbed using sterile cotton swab stick, inoculated in 9 ml sterile peptone water broths, inoculated on Nutrient agar, MacConkey and Chocolate agar and appropriately incubated.

2.9 CHARACTERIZATION OF BACTERIA ISOLATES

All the isolated bacteria already sub-cultured onto Nutrient agar slants were characterized based on their cultural, morphological and biochemical reactions as described by Cowan and Steel (1974), and Monica (1991).

2.1.0 CULTURAL CHARACTERIZATION

Cultural characterization of the isolates was based on their morphological and growth characteristics on media (Cowan and Steel 1974). Odor and pigmentation were used for the preliminary identification of isolated bacterial species.

2.1.1 GRAM STAINING REACTION

Gram staining was performed on the isolates as described by Monica (1991). Following examination under a binocular microscope, isolates were classified as Gram positive or negative cocci, cocco-bacilli or rods.

2.1.2 MOTILITY TEST (MOTILITY STAB METHOD)

Semi solid culture media were prepared in test tubes. This was done by using half of the weight of the required medium in the preparation. After sterilization and cooling to room temperature, the organisms were inoculated by stabbing. Using sterile inoculation needle, the tubes were properly stoppered and incubated at 37^0C for 24-48hours. Zigzag and diffuse growth into the agar away from the stab line indicated motility while growth only along the stab line meant no motility.

2.1.3 BIOCHEMICAL TEST

The tests distinguished biochemical tests between bacteria of different genera and species using their biochemical characteristics. The methods according to Singleton (1999), were used. These tests includes catalase test (slide method), oxidase test (dry filter paper method), coagulase test, indole test, methyl red (MR) test, vogues proskauer test, citrate utilization test, nitrate reduction test and hydrogen sulphide test.

2.1.4 HAEMOLYTIC PROPERTIES OF THE ISOLATES

All the bacterial isolates were cultured on Blood Agar to determine their hemolytic properties. This was done by inoculating them into blood agar plates. Plates were incubated at 37^oC for 24hours. A clearing zone surround the bacterial colony was observed and recorded (Baron et al. 2013). The presence of hemolysis Alpha or incomplete haemolysis and Beta or complete haemolysis indicated ability to lyse Red blood cell (RBC) which could be used to indicate pathogenicity. Alpha haemolysis was indicated by the presence of greenish discoloration that surrounds a bacterial colony growing on the agar. Alpha haemolysis can sometimes be called incomplete or partial or greenish haemolysis. Beta haemolysis represents a complete breakdown of the haemoglobin of the red blood cells in the vicinity of the bacterial colony. The area appears lightened (yellow) and transparent.

2.1.5 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed for all bacterial isolates using disk diffusion method on Mueller-Hinton agar (Oxoid Basingstoke, Uk) according to the direction of the Clinical and Laboratory Standards Institute (CLSI, 2014). Briefly, 3–5 colonies of the test organism was emulsified in 5 ml of nutrient broth and mixed gently. The suspension was incubated at 37 °C and the turbidity of the suspension becomes adjusted to 0.5 McFarland standards. The suspension was uniformly rapped onto Mueller-Hinton agar. The antimicrobial impregnated disks were placed using sterile forceps on the agar surface and the plates were incubated at 37 °C for 24 hours and the zone of inhibition was determined. The antimicrobials agents on the disks and their concentrations are as follows: gentamicin (GEN, 10 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (CHL,10 µg), tetracycline (TET, 10 µg) and ceftriaxone (CTR, 30 µg). The rest are amoxicillin-clavulanic acid (AMC, 30 µg), co-trimazole (COL, 25 µg), imipenem (IMP, 10µg) and amikacin (AMK, 30µg). The zones of inhibition were measured to the nearest millimeter using a transparent foot ruler. The results obtained were interpreted as sensitive or resistant according to the direction of the Clinical and Laboratory Standards Institute (2014).

2.1.6 IDENTIFICATION OF MULTI-DRUG RESISTANCE (MDR) ISOLATES

The multi-Drug Resistance (MDR) characters of the isolates in this study were identified by observing the resistance pattern of the isolates to at least 3 of the antibiotics used in this study.

2.1.7 DETERMINATION OF MULTIPLE ANTIBIOTICS RESISTANCE (MAR) INDEX

The Multiple Antibiotic Resistance (MAR) index was determined for each of the selected bacterial isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman 1983; Paul et al. 1997).

2.1.8 PLASMID PROFILE STUDIES USING AGAROSE GEL ELECTROPHORESIS

a. Culture purification

Purity of bacterial cultures to be used for the test was ascertained by sub-culturing the isolates on Nutrient and MacConkey agars.

b. Extraction of Plasmid DNA.

Selected resistant isolates were grown in a 5 ml double strength Mueller Hinton broth for 72 hours at 37^0C. The 72 hours grown cultures were harvested into Eppendorf tubes which has already been labeled with the name of each isolates and centrifuged in a micro centrifuge for 10 min at 10,000 rpm to obtain pellets. The supernatant was gently decanted and the cell pellets were vortexed for 5 min. Thereafter, 300µl of Tris EDTA (TE) buffer and 150 µL of 3.0 M sodium aqueous acetate was added at pH 5.2 and was vortexed for 3mins to lyse the bacteria cell pellet. The samples were centrifuged again for 10 min in a microcentrifuge (Biofuge, Biotra Bio-trade Hecrus Sepatech Co. Ltd USA) and the supernatant was transferred to a fresh eppendorf tube, mixed well with 0.9 ml of 70 % ethanol which had been precooled to -20^0C (in a refrigerator) to precipitate the plasmid DNA. It was centrifuged again for 10 min and the supernatant was discarded. The pellet was rinsed twice with 1 ml of 70 % ethanol and was air dried for 10 min(excess alcohol was wiped away from the sides of the tube surrounding the pellet very carefully using an absorbant paper), after which it was resuspended in 20 - 40 µL of TE buffer for further use. It was stored at 4^0C or frozen until needed (Lech and Brent 1987).

c. Preparation of Gel

The gel casting apparatus was set up as instructed in the product manual. The comb was straight, and there were few millimeters of clearance between the bottom of the comb and the bottom of the gel tray. A 0.5g of agarose was dissolved in 50 ml of Tris Borate EDTA buffer (TBE), thus forming 1.0% gel. The agarose was completely melted and the agarose solution was gently swirled while looking out for translucent "flecks" of non-melted agarose. Heat was later applied until all flecks are gone. The agarose solution was allowed to cool to a temperature of about 50-55^{0}C before pouring. Thereafter ethidium bromide [2µl of a 10 mg/ml EtBr solution (per 50ml gel)] was added, swirled to mix and the mixture poured into a gel tray. Hand glove was worn when handling Ethidium bromide as Ethidium bromide is a well-known mutagen. This was allowed for 20 min to solidify (the solution turned from translucent to opaque when solidified) and the comb was carefully removed from the gel. The gel carrier was removed from the pouring tray and was placed in the gel electrophoresis tank. A 250 ml of 1X TBE was used to fill the electrophoresis tank until the gel was submerged. Air bubbles left in the sample well were carefully dislodged with a pipette because presence of air bubbles will exclude the buffer and make the wells difficult to fill with the sample.

d. Electrophoresis of the DNA Samples

Using micropipette, a 50 µL sample of DNA and 3 µL of loading dye were added together and carefully mixed by pipetting the solutions up and down (Kraft et al. 1988). Each sample was loaded carefully into the gel wells, one sample per well and this was placed on the gel box at the negative charge end of the electrophoresis machine. Buffered water was added which sealed the agarose containing the sample DNA and acts as electrolyte by moving the current as well as the sample DNA towards the positive end for 2 h with a voltage of 63 V. The agarose containing the sample DNA was removed and allowed to drain off. With the aid of UV light, UV certified safety glasses and camera, a picture showing size and movement of the sample DNA was taken to determine the mobility in millimeter using a known sample standard (Maniatis et al. 1982).

e. Determination of Molecular Weight of Plasmids of the Isolates

Using the distance of migration of the bands (plasmid unit) in each isolate and matching the value of the marker in the standard with it, the plasmid sizes of the isolates were determined.

f. Plasmid DNA curing Using Use of sodium dodecyl sulphate (SDS)

Plasmid curing was carried out on organisms using sub-inhibitory concentration of 10% sodium dodecyl sulphate (SDS) as described by Sijhary et al. (1984). Overnight broth culture was inoculated into 4.5 ml nutrient broth. About 0.5 ml of Sodium deodecyl sulphate (5% concentration) was added and incubated for 48 hours at 37°C. 0.5 ml of the broth was added into a freshly prepared 4.5 ml nutrient broth, it was incubated for another 24 hours at 37°C, after which another plasmid antibiotic susceptibility test was carried out on each of the isolates. This was repeated at 10% and 20% SDS concentration. The suspension was uniformly spread onto Mueller-Hinton agar. The antimicrobial impregnated disks were placed using sterile forceps on the agar surface and the plates were incubated at 37 °C for 24 hours.

2.1.9 REFERENCE STRAINS

Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATTC 27853) were used as reference strains for culture and sensitivity testing.

2.2.0 STATISTICAL ANALYSIS

Statistical analysis was carried out using the SPSS 21.0 window based program. The proportion of isolated bacteria with patient demographic information, and susceptibility to commonly used antibiotics was compared using the chi-square test. A value of $P < 0.05$ was considered to be statistically significant**.**

3. RESULTS

The results of prevalence of nosocomial infections by site showed that urinary tract infection had the highest prevalence rate of (14.7%) and the lowest prevalence was recorded in blood stream infection (4.9%) (Table 1).

Table I: Prevalence of Nosocomial Infections by Sites.

Sites of infection	No of patients discharged	No of patients infected	Infection rate(%)
Surgical wound	54	4	7.4
Blood Stream	41	2	4.9
Urinary Tract	109	16	14.7
Respiratory Tract (Pneumoniae)	96	9	9.4
Total	300	31	

Table 2 showed the rate of nosocomial infections in the various wards. As indicated in this table, male surgical ward had the highest nosocomial infection rate with 45% followed by the female surgical ward with 25%. The least infection rate was in Paediatrics ward with 4.7%.

Table II: Nosocomial Infection Rates in the Various Wards.

Wards	No of Patients discharged	No of Patients infected	Infection Rates (%)
Male medical ward (MMW)	80	4	5
Female medical ward (FMW)	68	2	2.9
Male Surgical ward (MSW)	20	9	45
Female Surgical Ward (FSW)	32	8	25
Maternity ward (MW)	57	6	10.5
Pediatric ward (PW)	43	2	4.7
	300	31	

Table 3 shows the distribution of the causative agents (bacteria) of nosocomial infections among the hospital wards. A total 81 bacterial isolates were isolated from the hospital wards. Bacteria that were frequently isolated were S. aureus 26(32.1%), S. epidermidis 10(12.3%), E. coli 14(17.3), P. aeruginosa 19(23.5), K. pneumonia 8(9.9), S. Pyogenes 4(4.9).

Table III: Distribution of Isolates among Hospital wards.

Isolated	No (%)	MMW No(%)	FMW No(%)	MSW No (%)	FSW No (%)	PW No (%)	MW No (%)
S. aureus	26(32.1)	4(15.4)	2(7.7)	8(30.8)	6(23.1)	1(3.8)	5(19.2)
S. epidermidis	10(12.3)	1(10.0)	1(10.0)	4(40.0)	2(20.0)	0	2(20.0)
E. coli	14(17.3)	2(14.3)	1(7.1)	5(35.7)	3(21.4)	1(7.1)	2(14.3)
P. aeruginosa	19(23.5)	3(15.8)	2(10.5)	6(31.6)	4(21.1)	1(5.3)	3(15.8)
K. pneumoniae	8(9.9)	1(12.5)	1(12.5)	2(25.0)	2(25.0)	0	2(25.0)
S. pyogenes	4(4.9)	1(25.0)	1(25.0)	1(25.0)	1(25.0)	0	0
Total	**81(27.0)**	**12(14.8)**	**8(9.9)**	**26(32.1)**	**18(22.2)**	0	**14(17.3)**

KEY: MMW = Male medical ward, FMW = Female medical ward, MSW – Male surgical ward, FSW = Female surgical ward, PW = Pediatric ward, MW = Maternity ward.

Tables 4 present the bacterial level of the environmental air and floors of the 6 hospital wards. The bacteria levels of the floors were higher than those of the corresponding airs. The bacterial counts of the Pediatric ward and Female medical ward were considerably less than those of the Male surgical ward, Female surgical ward and maternity ward.

Table IV: Bacteriological Studies of Hospital Wards (Air and Floor).

Hospital wards	Bacteria level (cfu/m^3)	Floor (cfu/m^3)
Male medical ward (MMW)	2.2×10^2	2.4×10^2
Female medical ward (FMW)	1.8×10^2	1.5×10^1
Male surgical ward (FSW)	4.1×10^3	3.8×10^4
Female surgical ward (FSW)	3.2×10^3	3.7×10^3
Maternity ward (MW)	3.5×10^2	2.1×10^3
Pediatric ward (PW)	1.0×10^1	0.9×10^2

3.1 HAEMOLYTIC PROPERTIES OF THE BACTERIAL ISOLATES

S. aureus, S. pyogenes and Pseudomonas aeruginosa showed complete haemolysis on blood agar (Beta haemolysis). There was no haemolysis in E coli and K. pneumoniae (Gamma haemolysis).

3.2 ANTIMICROBIAL SUSCEPTIBILITY TEST

The susceptibility of bacteria to different antibiotics is shown in table 5. Among gram positive bacteria, S. aureus was 88.5% sensitive to gentamicin, 96.2% sensitive to ciprofloxacin, 46.2% sensitive to chloramphenicol, 92.3% sensitive to amoxicillin clavulanic acid and 100% sensitive to amikacin. Among the gram negative bacteria, P. aeruginosa was 52.6% sensitive to gentamicin, 94.3% sensitive to ciprofloxacin, 68.4% sensitive to chloramphenicol, 47.4% sensitive to tetracycline, 89.5% sensitive to amoxicillin clavulanic acid and 100% sensitive to amikacin. Generally all the bacteria were 14.8% resistant to ciprofloxacin, 47.7% resistant to ceftriaxone and 48.1% to Imipenem.

Table V: Antibiotic Sensitivity pattern of bacteria Isolates.

Bacteria Isolates	No	GEN	CIP	CHL	TET	CTR	AMC	COL	IMP	AMK
S. aureus	26	23(88.5)	25(96.2)	12(46.2)	19(73.1)	17(65.4)	24(92.3)	20(76.9)	18(69.2)	26(100.0)
S. epidermidis	10	8(80.0)	9(90.0)	7(70.0)	8(80.0)	9(90.0)	9(90.0)	4(40.0)	5(50.0)	9(90.0)
Total Gram + ve	36	31(91.4)	34(94.4)	19(52.8)	27(75.0)	26(72.2)	33(91.7)	24(66.7)	23(63.9)	35(97.2)
E.coli	14	8(57.1)	13(92.9)	7(50.0)	4(28.6)	8(57.1)	12(85.7)	7(50.0)	6(42.9)	13(92.9)
P.aeruginosa	19	10(52.6)	18(94.3)	13(68.4)	9(47.4)	11(57.9)	17(89.5)	14(73.7)	10(52.6)	19(100.0)
K. pneumoniae	8	8(100.0)	4(50.0)	3(37.5)	3(37.5)	3(37.5)	7(87.5)	4(50.0)	2(25.0)	8(100.0)
S. pyogenes	4	3(75.0)	0	1(25.0)	1(25.0)	0	4(100.0)	1(25.0)	1(25.0)	3(75.0)
Total Gram - ve	45	29(64.4)	35(77.8)	24(53.3)	17(37.8)	22(48.9)	40(88.9)	26(57.8)	19(42.2)	43(95.6)
Grand Total	81	**60(74.1)**	**69(85.2)**	**43(53.1)**	**44(54.3)**	**48(52.3)**	**73(90.1)**	**50(61.7)**	**42(51.9)**	**78(96.3)**

KEY: GEN = Gentamicin, CIP = Ciprofloxacin, CHL = Chloramphenicol, TET = Tetracycline, CTR = Ceftriaxone, AMC = Amoxicillin-clavulanic acid, COL = Co-trimazole, IMP = Imipenem, AMK = Amikacin.

3.3. MULTIPLE ANTIBIOTICS RESISTANCE BACTERIA.

The multiple antibiotic resistance (MAR) of the bacteria isolates is found on table 6. The percentage MAR of S. aureus, E coli, P. aeruginosa and K. pneumoniae to 2 to 5 drugs was 40.0%, 20.0%, 33.3% and 5.9% respectively.

Table VI: Multiple Antibiotic Resistance (MAR) Bacteria.

Name of Bacteria	No of MAR Isolates	Percentage of bacteria with MAR (%)	No of Antibiotics resistant to isolates
S. aureus	6	40.0	2 - 5
E coli	3	20.0	2 - 4
P. aeruginosa	5	33.3	3 - 5
K. pneumoniae	1	5.9	2
Total	15	100	

3.4 MULTIPLE ANTIBIOTICS RESISTANCE INDICES OF BACTERIAL ISOLATES

The Multiple Antibiotics Resistant Profile (MAR) and Indices (MARI) of Bacterial Isolates are found in table 7. The MARI range was between 0.3 to 0.4.

Table VII: Multiple Antibiotics Resistant Profile and Indices of Bacterial Isolates

Isolates	Multi-Antibiotic Resistant Profile	Multi-Antibiotic Resistant Indices
S. aureus1	IMP-CHL-TET-COL	0.4
S. aureus2	CHL-CTR- IMP -AMC	0.4
S. aureus3	CIP- CHL – TET	0.3
S. aureus4	CHL- IMP - TET	0.3
S. aureus5	GEN – COL – IMP	0.3
S. aureus6	TET-COL-IMP	0.3
E coli1	CHL – TET – CTR	0.3
E coli2	GEN – IMP- TET	0.3
E coli3	GEN – CIP – CTR	0.3
P. aeruginosa1	COL- CHL – CTR – IMP – TET	0.6
P. aeruginosa2	CHL – COL – TET – IMP	0.4
P. aeruginosa3	GEN – TET – CHL	0.3
P. aeruginosa4	COL – CTR – AMC	0.4
P. aeruginosa5	CTR – AMC – COL	0.3
K. pneumoniae1	CHL- AMC – GEN	0.3

KEY: TET=Tetracycline, AMC=Amoxicillin clavulanic acid, COL=Cotrimoxazole, CHL=Chloramphenicol, CIP=Ciprofloxacin, GEN= Gentamicin, IMP=Imipenem, CTR=Ceftriaxone, AMK=Amikacin

3.5 PRESENCE OF PLAMIDS, PLAMID SIZES AND MOLECULAR WEIGHT

All the 15 multiple antibiotic resistant isolates subjected to agar gel electrophoresis analysis had plasmids, with number of band ranging from 1 to 2. All the isolates had one plasmid common to them in size (figure 1). The plasmid bands had a molecular weight ranging from 9.82kbp to 18.15 kbp.

3.6 PLASMID DNA CURING USING 10% SODIUM DODECYL SULPHATE (SDS)

All plasmid borne multidrug resistant bacteria isolates in this study were cured of their plasmid upon treatment with 10% sodium dodecyl sulphate (Table 8).

Table VIII. Resistant Pattern of Multi Antibiotic Resistant Bacteria from hospital wards before and after Plasmid curing at 5% SDS.

Isolates	Hospital Ward	Resistance Profile before Curing at 10% SDS	Resistance Profile after Curing at 10% SDS
S. aureus1	MSW	IMP-CHL-TET-COL	NIL
S. aureus2	MSW	CHL-CTR- IMP –AMC	NIL
S. aureus3	FSW	CIP- CHL – TET	NIL
S. aureus4	PW	CHL- IMP – TET	IMP
S. aureus5	MW	GEN – COL – IMP	NIL
S. aureus6	MMW	TET-COL-IMP	NIL
E coli1	MMW	CHL – TET – CTR	NIL
E coli2	FSW	GEN – IMP- TET	NIL
E coli3	MSW	GEN – CIP – CTR	NIL
P. aeruginosa1	FMW	COL-CHL–CTR–IMP–TET	NIL
P. aeruginosa2	MSW	CHL – COL – TET – IMP	NIL
P. aeruginosa3	FSW	GEN – TET – CHL	NIL
P. aeruginosa4	MMW	COL – CTR – AMC	NIL
P. aeruginosa5	MSW	CTR – AMC – COL	NIL
K. pneumoniae1	FSW	CHL- AMC – GEN	NIL

KEY: TET=Tetracycline, CTR=Ceftriaxone, CHL=Chloramphenicol, GEN=Gentamicin, AMC = Amoxicillin Clavulanic acid, COL = Clotrimazole, IMP = Imipenem, AMK = Amikacin, CIP=Ciprofloxacin, NIL = No Isolate Seen

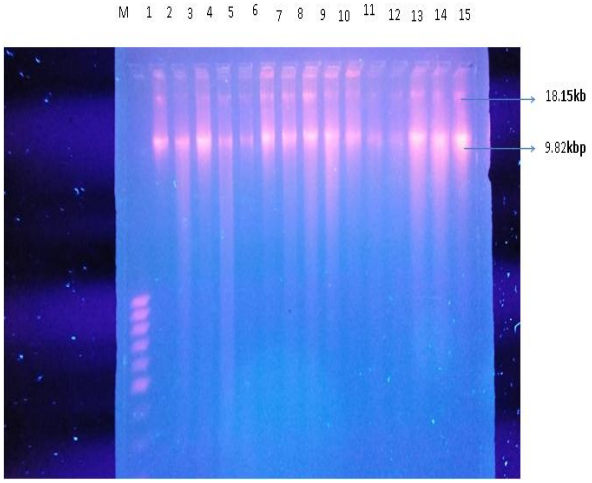

Figure 1: Plasmid DNA Profile of Multi-drug Resistant Bacterial Isolates.

Key: lane L =S. aureus, Lane 2 = S. aureus, Lane 3 = S. aureus, Lane 4 = S. aureus, Lane 5 = S. aureus, Lane 6 = S. aureus, Lane 7 = E coli, Lane 8 = E coli, Lane 9 = E coli, Lane 10 = P. aeruginosa, Lane 11 = P. aeruginosa, Lane 12 = P. aeruginosa, Lane 13 = P. aeruginosa, Lane 14 = P. aeruginosa, Lane 15 = K. pneumoniae

4. DISCUSSION

Classification of infections by sites showed that urinary tract site had the highest prevalence rate, followed by respiratory tract site. This order is similar with the finding of Theodore and Eichhoff (1998), who reported that Urinary tract infection site, is the most prevalent infection site in University of Lagos Teaching hospital (LUTH). But it is in contrast with the work of Dumps et al. (2003), who posited that surgical site is the leading nosocomial infection.

The reason for the higher infection rate in urinary tract sites in the hospital as reported in this work might not be unconnected to the unsatisfactory hygienic condition in the bathroom and toilet (rest room). Patients can contact bacteria from the surfaces of the toilet seat or toilet wall/bathroom wall. Due to shortage of water in the hospital, patients sometimes fail or forget to wash their hands after visiting the rest room, and thereby transfer these bacteria to the mouth during meals.

Comparison of the infection rate among the hospital wards showed that, there is no significant difference in the infection rates. Pediatrics ward which has been widely reported as epidemic jungle in teaching hospitals (Kappstein et al. 1999; Lizioli et al. 2003) had comparatively lower nosocomial infection rate (4.7%) in this hospital.

A total of 81 bacterial isolates were isolated from the wards, giving overall prevalence of 27.0%. Although this is lower than an earlier report of 39.9% Oni et al. (2006), it is still on the high side when compared to the result of Dellinger et al. (2005), who reported a prevalence rate of 9.6%. However, World Health Organization in 2011

gave a prevalence of 5 – 34% of nosocomial infection and this is in line with the result of this study.

All the wards sampled and analysed had S. aureus, P. aeruginosa, E coli, S. epidermidis, K. pneumoniae and S. pyogenes in decreasing order as nosocomial bacteria. These predominant bacteria species are normal body microbial flora most likely originating from the hospital personnel, patients and visitors. S. aureus, S. epidermidis, P. aeruginosa, and E. coli have been reported notorious causative agents of surgical wounds, Urinary tract and blood stream infections (Abdallah et al. 1998; Liziol, et al. 2003).

In the work of Nejad et al. (2011), E. coli (34.4%) was mostly isolated from surgical wounds infections. However, in this study P. aeruginosa have been mostly recovered from post-operative surgical wounds despite the site of infection and location of specimens due to its high survival characteristics in hospital environment (Christopher et al. 2011; Dalhatu et al. 2014). It is known to rank second among nosocomial pathogens isolated from hospitals, often contaminating hospital equipments such as wound dressing sinks and other surgical apparatus; and even antibiotic resistant strains can survive in supposedly sterile equipment used in the hospitals, making it a dangerous nosocomial pathogen widely distributed in the hospital environments where they are particularly difficult to eradicate (Masaadeh and Jaran 2009).

The high prevalence of isolates from the male surgical ward, MSW (32.1%) is probably due to poor hygienic practice in the ward which may have been the cause of high rate of multidrug bacterial surgical wound infections among patients there. Bacterial infections of surgical wounds are attributed to overcrowding of hospital wards and lack of basic facilities for standard hygiene condition which is common in sub-Saharan African countries including Nigeria (Fontana et al. 2000).

Antibiotic resistant pattern of the isolated bacterial species showed that most of the isolates were multiple antibiotics resistant. S. aureus and P. aeruginosa was the most resistant of all the isolates. The implication of this is that most of the patients may not respond positively if they are infected with any of the isolated organisms and treated with any of the antibiotics tested in this study. This high resistant pattern is an indication of the multidrug resistant strategies possessed by the clinical bacteria isolates. Many studies have been carried out on multidrug resistant bacteria and such resistance may be due to the high selective pressure exerted on bacteria due to numerous reasons like poor adherence to hospital antibiotic policy and excessive and indiscriminate use of broad-spectrum antibiotics (Akoachere et al. 2014; Garba et al. 2012). Cephalosporins and Penicillins have been found to be highly resisted by surgical wound pathogens. In the works of Eduardo et al., 2008, Ceftazidime and Augmentin were mostly resisted by surgical wound etiologic bacterial. This is mostly likely due to the presence of Cephalosporinase and Penicillinase enzymes which prevent the action of the Beta-lactam ring structure of the antibiotics (Fontana et al, 2000; Livermore 1995).

All the 15 multi-drug resistant isolates had MAR indices greater than > 0.2. This was similar to the work of Razaq (2008), who recorded a high level of MAR index of between 0.6 – 0.8 within Ilorin metropolis, Kwara State. When the MAR index is greater than 0.2, it shows that the organisms were isolated in an environment where antibiotics are abused widely (Razaq 2008), which was the case in this study.

All the 15 multidrug resistant isolates were subjected to plasmid profile studies and the result showed that some all the isolates have plasmid. Some have plasmid band of the same number and sizes which indicates that they are likely of same origin, most probably the same community or close to the hospitals were this study was carried out.

The presence of these plasmids found in the isolates can be attributed to poor hygienic practices by people living in these areas giving room to frequent acquisition of resistant plasmid among bacterial isolates from nosocomial infections. Bacteria resistance can be expressed through their ability to colonize new hospital environments where selective pressure prevails, some opportunistic pathogens such as E. coli and P. aeruginosa are able to adapt to this new environment through the acquisition or development of mechanisms of resistance and persistence (Alejandro et al., 2013; Prescott et al. 2008).

All plasmid borne multidrug resistant bacteria isolates in this study were cured of their plasmid upon treatment with 10% sodium dodecyl sulphate. This made them susceptible to the drugs to which they were once resistant as they have now lost their resistant markers. The loss of all the plasmid DNA in the 15 isolates after curing with 10% sodium dodecyl sulphate suggests that their antimicrobial resistance is plasmid-mediated.

5. CONCLUSION

Findings in this study show that Urinary tract infection was the most prominent nosocomial infection followed by respiratory tract infection. The least was blood stream infection. S. aureus and P. aeruginosa were the predominant bacteria isolates causing nosocomial infection. The resistant found among the multidrug resistant isolates were plasmid mediated. This justifies the need to strengthen infection control to prevent the spread of plasmid mediated multidrug resistant bacteria. Therefore, efforts to promote the appropriate use of antimicrobials are paramount to avoid therapeutic failure in Port-Harcourt metropolis of Rivers State.

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