Molecular Detection of Antibacterial Resistance Genes in Phenotypically Characterized Multidrug Resistance Pathogens Isolated from Surgical Wound Infections in Two Nigerian Teaching Hospitals

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ABSTRACT

Background of study: Postoperative surgical wound bacterial infection is a significant clinical challenge in hospitals in developing countries where proper healthcare delivery is hampered by limited resources. **Objectives**: This study was undertaken to search for and characterize chromosomally mediated multidrug resistant bacteria from surgical wounds and to detect the possible carriage of multidrug resistance genes from the isolates. **Methods:** A randomized sampling method was adopted. Three hundred and sixty two(362) swab specimens were collected from hospitalized surgical wound patients at University of Benin Teaching Hospital (UBTH), Edo State, Nigeria and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Anambra State, Nigeria.The specimens were bacteriologically investigated using standard methods. The antibiograms of the isolates were determined using Kirby Bauer disk diffusion technique. The antibiotic disks used were Augmentin (30µg), Oxacillin (4µg), Erythromycin (10µg), Gentamycin (30µg), Ceftazidime (30µg), Cefuroxime (30µg), Cefixime (5µg), Imipenem (30µg), Ofloxacin (5µg) and Ciprofloxacin (5µg). Plasmid DNA profiling was carried out on the multidrug resistant isolates and the chromosomally mediated multidrug resistant bacteria were phenotypically characterized and confirmed by molecular analysis. The chromosomally mediated multidrug resistant isolates were further tested for presence of TEM and gyrA resistance genes using polymerase chain reaction (PCR) technique. **Result:** Overall, 122 (33.7%) bacteria were isolated. These comprised 50(27.6%) from UBTH and 72 (39.8%) from NAUTH. The predominant pathogens from UBTH were *Ps. aeruginosa* (17=34.0%), *Esch.coli* (30=60.0%) and *Staph aureus* (3=6.0). Similarly,*Ps. aeruginosa* (26=36.1), *E.coli* (38=52.8%), *Proteus mirabilis* (2=2.8%), and *Staph aureus* (6=8.3) were the predominant isolates from NAUTH. There were no significant differences ($P > 0.05$) in the isolation rates of pathogens with respect to locations. The prevalence of multidrug resistant (MDR) isolates was higher in NAUTH 61(85%) when compared to UBTH 39(78%). The isolates from both locations exhibited high resistance to Augmentin, Cefuroxime,

cefixime, Ofloxacin and low resistance to Imipenem among the antibiotic discs tested against the Gram negative isolates. There was no significant difference in the prevalence of TEM resistance genes expressed by the MDR isolates in both locations (P value = 0.6). However, gyrA resistance genes expressed by the isolates were significantly different at the locations (OR = 16.10; P = 0.014). **Conclusion:** We conclude that bacteria isolated from surgical site infections harbor a large burden of multi-antibiotic resistance genes. Moreover, it became obvious that PCR technique is an, accurate and reliable method for determining the presence of antibiotic-resistant genes, which obviously constitute a major problem in the hospital setting due to inappropriate and sometimes reckless consumption of unprescribed drugs in developing countries.

Keywords: Surgical site infection**,** Antibacterial, Resistogram, Molecular characterization, Tem and GyrA resistance genes, NAUTH, UBTH.

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INTRODUCTION

Surgical site infection (SSI) is defined as an infection occurring within 30 days after a surgical operation or within 1 year if an implant is left in place after surgical procedure. These infections may be superficial, deep incision or infections involving organ/body space. The clinical signs of wound infection are pain, tenderness, localized swelling, redness or heat. Infection may be defined as invasion and multiplication of microorganisms in body tissues, which may be clinically apparent or result in local cellular injury because of competitive metabolite intracellular replication, or antigen-antibody response. This series of events leads to progressive tissue destruction and eventual death of the host if left unchecked (1). Historically, the analysis of nosocomial pathogens has relied on a comparison of phenotypic characteristics such as biotypes, serotypes and antimicrobial susceptibility profiles. This approach has been changing over the past 2 decades, with the improvement and deployment of new technologies based on DNA or molecular analysis (2). Studies of microbial pathogenicity at the molecular level have made substantial contributions to the understanding of the epidemiology, clinical manifestations, diagnosis, treatment, and immune prophylaxis of infectious diseases. One of the most exciting and profound technical advances in the past years has been the development of nucleic acid amplification techniques and their application to the study of microbial pathogenesis and the diagnosis of infectious diseases (2).

The developments of surgical wound infections are related to three factors which include; (a) the degree of bacterial contamination during the operation, (b) the duration of procedure, and (c) the underlying disease of the patients such as immune deficiency, diabetes, and malnutrition (3). Multidrug resistant bacteria have posed serious challenges to the treatment of surgical wound infections worldwide (4).The aim of this study is to employ phenotypic and molecular technologies for characterizing multidrug resistant bacteria isolates incriminated in surgical wound infection from hospitalized patients in UBTH (Edo state) and NAUTH (Anambra state) with emphasis on identifying associated drug- resistance genes from the isolates.

MATERIALS AND METHODS

A total of 362 clinical wound swab samples were collected from post-operative hospitalized surgical patients. One hundred and eighty one of the samples were from UBTH in Edo state while another 181 was from Patients seen in NAUTH in Anambra state.

Sample collection:

Random samplings of patients with post-operative surgical wounds were used in this study as recently described (5).

Isolation and phenotypic characterization:

The wound swab specimens were inoculated on Blood agar and MacConkey agar (Oxoid Ltd UK) and were incubated aerobically at 37^0C for 24 hours. Duplicate blood agar plates were incubated anaerobically at 37^0C for 24 hours. All media used were prepared according to the manufacturers directives. Morphological characteristics and identification of isolates were carried out as earlier described by (6-7).

Antibiotic susceptibility testing:

This was done using the Kirby- Bauer Agar disc diffusion method described by Clinical Laboratory standard institute (8). The antibiotics discs were procured from Oxoid Ltd, UK. They include Ceftazidime (Caz 30µg), Gentamycin (GN 30µg), Ofloxacin (ofl 5µg), Ciprofloxacin (Cpr 5µg), Erythromycin (Ery 10ug), Imipenem (lmp 10ug), Oxacillin (oxa, 1ug,), Cefuroxime (Crx 30ug), Cefixime (CXM 5ug), and Augmentin (Aug, 30ug) (Oxoid). The disc was placed on the surface of the inoculated Muller Hinton agar and after incubation at 37^oC for 24 hours, the diameters of zones of inhibition were measured and compared with reference control strains held at Lahor Research Laboratories (*E.coli* ATCC 25922and *P.aeruginosa* ATCC 27853). They were then interpreted as susceptible or resistant (8).Isolates that were resistant to three or more antimicrobial agents were classified as multidrug resistant (MDR).

Molecular characterization:

Plasmid DNA analysis of the MDR) isolates was carried out to ascertain if the resistance exhibited by the isolate were due to plasmid or were chromosome mediated as recently described (5).

The chromosomally mediated multidrug resistant isolates (i.e. *Ps. aeruginosa* and *Esch. coli*) from both locations were further characterized and confirmed using PCR techniques. Two selected resistance gene primers (gyrA and TEM) were targeted against the multidrug resistant bacteria. They were all purchased from Inqaba Biotech industries, Harfield, South Africa.

Table1: Primers used for the study.

Gene Symbol	Primers 5^I-3^I (forward, reverse)	n moles (mg)	Molecular weight	Melting temperature (TM) (^{0}C)
Pa16S-F	GGGGGATCTTCGGACCTCA	28.19	5844.3	64.48
	TCCTTAGAGTGCCCACCCG	33.6	5724.3	64.48
URL – 301	TGTTACGTCCTGTAGAAAGCCC	49.8	6709.8	62.67
	AAAACTGCCTGGCACAGCAATT	30	6711.6	60.81
gyrA F	ATGACTGATATCACGCTGCCA	27.85	6389.6	60.61
	ATAACGCATCGCTGCCGGTGG	29.1	6446.5	66.47
TEMU1	ATGAGTATTCAACATTTCCG	38.98	6090.7	54.25
	CTGACAGTTACCAATGCT	32.02	5458.3	55.34

The specific *Ps.aeruginosa* primers (Pa16S), *Escherichia*coli primers (URL301) and resistance gene primers gyrA TEMU1 are detailed in Table 1.

DNA Extraction From isolates:

A pure colony of each isolate growing on Nutrient agar plate was inoculated into 3 ml of Luria broth and incubated at 37°C overnight with constant agitation at 120 rpm. One point five milliliters of the overnight culture was transferred into a micro centrifuge tube. The overnight culture was centrifuged at 13000rpm for 10 minutes and the supernatant was discarded. The pellet was resupended in 180µl digestion Solution. Two hundred micro litres of proteinase K solution was added and mixed thoroughly by vortexing to obtain a uniform suspension. The sample was incubated at 56°C in a thermomixer until the cells were completely lysed. Twenty microlitres of RNaseA solution was added to the mixture and incubated for 10 minute at room temperature. Two hundred micro litres of Lysis Solution was added to the sample and mixed thoroughly by vortexing for about 15 seconds to obtain a homogeneous mixture. Four hundred micro litres of 50% ethanol was added to the mixture and mixed by vortexing. The resultant lysate was transferred to a Gene JET Genomic DNA Purification Column inserted in a collection tube and centrifuged at 6000 x g for 1 minute. The resultant flow-through solution was discarded. The Gene JET Genomic DNA Purification Column was placed inside a new 2ml collection tube. Five hundred micro litres of Wash Buffer I was added to the column and centrifuged at 8000 x g for 1 minute. The flow-through was discarded. Five hundred micro litre of Wash Buffer II was added to the Gene JET Genomic DNA Purification Column and Centrifuged at 12000 x g for 3 minutes. Two hundred micro litres of the Elution Buffer was added to the center of the Gene JET Genomic DNA purification column membrane and incubated at 25^{0c} for 2 minutes. The column was centrifuged at 8000 x g for 1 minute to elute genomic DNA for PCR amplification.

Preparation of the 100µM stock solution of the primers.

The lyophilized primers were spun down with the aid of the micro centrifuge before opening it to ensure that the primer pellets are at the bottom of the tubes. All primers were similarly prepared with slight modification:

Oligonucleotide forward (gyrA F): 278.47µl nuclease free water was added to the lyophilized primer as stock and further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (gyrA R): 291.02µl nuclease free water was added to the lyophilized primer as stock and further diluted 1:10 with nuclease water (10µM).

Oligonucleotide forward (TEM UI F): 389.8µl nuclease free water was added to the lyophilized as stock and further diluted 1:10 with nuclease free water (10µM).

Oligonucleotide reverse (TEM UI R): 320.22µl nuclease free water was added to the lyophilized primer as stock and further diluted 1:10 with nuclease free water (10µM).

Quick load One Taq One Step Polymerase Chain Reaction:

Quick load One Taq one step PCR master (2X) with catalog number NEB MO486S was purchased from lnqaba Biotech. Hartfield South Africa incorporated. See table 1 for details of the primers used. The system components were thaw and mixed by inverting ten times. The PCR was performed in 50µl reaction mixture containing 25µl Quick load One Taq one- step PCR master mix (2x), 1µL of each gene-specific forward primer (10µM), 1µL of each specific reverse primer10µM), 13ml of nuclease free water and 10µl of DNA template was added last.

The PCR was set as follows: Initial denaturation at 94^0C for 1 minute, denaturation at 94^0 for 30secs, annealing at Tm-5 for 30secs, extension at 72^0C for 1 minute, go to the denaturation step for 39 cycles, final extension at 72^0 C for 15mins and final holding at 4^0C.

Preparation of 1.5% agarose gel use for genomic DNA detection):

One point five percent agarose gel (1.5%) was prepared by dissolving 1.5g agarose powders in 100ml Tris EDTA Buffer. The mixture was heated in a microwave for 5 minutes to dissolve completely. It was allowed to cool to 56^0C and 6µl of ethidium bromide was added. The agarose gel was poured into the electrophoresis chambers with gel comb, and allowed to solidify.

Electrophoresis:

Five microlitres of the amplified PCR products was analyzed on 1.5% agarose gel containing ethidium bromide in Tris EDTA buffer. Electrophoresis was performed at 90v for 60 minutes. After electrophoresis the PCR products were visualized by Wealth Dolphin Doc UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard of the maker.

RESULTS

A total of 122 bacteria were isolated from both locations. Table 2 shows that out of 181 swab samples collected from each location, UBTH had 50(27.6) pathogens and NAUTH had 72(39.8). A statistically higher prevalence of wound infections was recorded among patients in NAUTH, when compared with study participants in UBTH.

Pseudomonas aeruginosa and Escherichia coli were the most frequently isolated pathogens associated with wound infections in UBTH and NAUTH. There was no statistically significant difference in the prevalence of *Esch. coli* isolated from UBTH (34%) and NAUTH (52.8%). However, *Ps. aeruginosa* showed significantly higher prevalence of (60.0%) in UBTHcompared to NAUTH (36.1%) from table 3.

Antibiotic susceptibility profile ofisolates from surgical wound infections in both locations shows that isolates from UBTH were more resistance to Cefixime (94%) while isolates from NAUTH exhibited more to Cefixime and Augmentin as shown in table 4.

As shown in Table 5, Ps.aeruginosa exhibited the highest resistance (90%) to antibiotics among isolates from UBTH while *Escherichia coli* displayed highest resistance (95%) among the isolates from NAUTH.

Table 2: Prevalence of surgical wound infection in UBTH and NAUTH

Locations	No. examined	No. infected (%)	OR	95%Cl
UBTH	181	50(27.6)	0.569	0.365,0.890
NAUTH	181	72(39.8)	1.756	1.120,2.798
T0TAL	362	122 (33.7)		

Keys: Cl=confidence Interval, OR=Odd ratio

Table 3: Distribution of etiologic agents of wound infection, according to location

Locations	No. infected	*Esch. coli* N (%)	*Ps.aeruginosa* N (%)	*Proteusmirabilis* N (%)	*S.aureus* N (%)
UBTH	50	17(34.0)	30 (60.0)	0(0.0)	3(6.0)
NAUTH	72	38(52.8)	26(36.1)	2(2.8)	6(8.3)

Total	**122**	**55(45.1)**	**56(45.9)**	**2(1.6)**	**9(7.4)**
p value		0.062	0.008	0.643	0.894

Key: P = P<0.05 were considered significant, Number of isolates in expressed (%).

Table 4: Susceptibility profile of isolates to tested antibiotics

Class of Antibiotics	Type of antibiotics	UBTH (%) No. tested =50		NAUTH (%) No. tested = 72	
		R	S	R	S
Penicillin	Augmentin (30µg)	34(68)	16(32)	68 (94)	4 (6)
	Oxacillin (4µg)	0(0)	3(100)	1(17)	5(83)
Macrolides	Erythromycin (10µg)	0(0)	3(100)	1 (17)	5 (83)
Aminoglycoside	Gentamycin (30µg)	21 (42)	29(58)	38 (53)	34(47)
Cephalosporin	Ceftazidime (30µg)	24 (48)	26(52)	54 (75)	18(25)
	Cefuroxime (30µg)	34(68)	16(32)	57 (79)	15(21)
	Cefixime (5µg)	47(94)	3(6)	68 (94)	4 (6)
Carbapenem	Imipenem (30µg)	7(14)	43(86)	12 (17)	60(83)
Quinolones	Ofloxacin (5µg)	27(54)	23(46)	42(58)	30(42)
	Ciprofloxacin(5µg)	26 (52)	24(48)	50 (69)	22(31)

Multidrug resistant isolates were more prevalent at NAUTH (85%) when compared to UBTH (78%) as indicated in table 6.
Table 7 and figures 1 & 2 show that all 21 multidrug resistant isolates from UBTH (100.0%) were found to express TEM gene as against 24(82.8%) isolates from NAUTH. Indeed, Statistics however failed to show any significant difference in rate of expression of TEM gene (P = 0.06). Similarly all Multi-drug resistant bacteria isolates in UBTH resistant to flouroquinolones were observed to express gyrA gene, as against 18(69.2%) out of 26 of the isolates resistant to flouroquinolones in NAUTH (P = 0.014).

Table 5: Susceptibility rates of isolates from UBTH and NAUTH to antibiotics

	UBTH			NAUTH		
ISOLATES	**N**	**R (%)**	**S (%)**	**N**	**R (%)**	**S (%)**
Ps.aeruginosa	30	27 (90)	3(10)	26	24 (92)	2(8)
Esch.coli	17	12 (70.6)	5(29.4)	38	36(95)	2(6)
Proteus mirabilis	0	0 (0)	0 (0)	2	0 (0)	2(100)
Staph aureus	3	0 (0)	3 (100)	6	1(16.7)	5(83)
Total	50	39 (78)	11(22)	72	61(84.7)	11(18)

Keys: **N**=Number of isolates, **R**= Number of isolates resistant **(%)**, **S**= Number of isolates sensitive (**%).**

Table 6: Distribution of multidrug resistant isolates according to location.

Locations	**No. of isolates Tested**	**No. positive for MDR(%)**	**OR**	**95%CI**	**P Value**
UBTH	50	39(78.0)	0.982	0.492,2.106	1.000
NAUTH	72	61(85.0)	1.182	0.475,2.031	
Total	122	100(163.0)			

Key: CI= Confidence interval, OR= Odd ratio, P= P<0.05 were considered significant.

Table 7: Prevalence of expressed resistance genes among isolates according to location.

RESISTANCE GENES LOCATIONS	**N**	**NoExpressed (%)**	**OR**	**95% CI**	**P value**
TEM					
UBTH	21	21(100.0)	9.653	0.504,185.01	0.6
NAUTH	29	24(82.8)	0.104	0.005,1.986	
gyrA					
UBTH	17	17(100.0)	16.081	0.861,300.20	0.014
NAUTH	26	18(69.2)	0.0621	0.3425,1.567	

Keys: N=Number of isolates resistant to Augmentin (TEM) and Ofloxacin (gyrA).
CI=Confidence interval, **OR**=Odd ratio, **P** = P<0.05 were considered significant
No. = No. Positive expressed (%).

Table 8 and figures 4 & 6 show the prevalence of expressed resistance genes among *Esch.coli* isolates. All (100%) *Escherichia coli* isolates resistant to Augmentin in UBTH were found to express the TEM genes, in contrast to 9(69.0%) in NAUTH. However, with regards to expression of TEM gene, no statistically significant difference was observed between the two locations (P=0.249). Expression of gyrA gene by *E coli* was higher among MDR isolates in UBTH (100.0%) than value obtained in NAUTH (56.3%). The differences in expression of gyrA gene was found to be insignificant (P=0.046).

Table 9 and figure 3 & 5 show the prevalence of resistance genes among *Ps. aeruginosa* isolates from UBTH and NAUTH. Whereas all 14 isolates studied in UBTH (100%) expressed TEM gene among isolates from NAUTH. For the expression of gyrA gene, 100% and 90% of the studied isolates were respectively positive from UBTH and NAUTH.

Table 8: Prevalence of resistance genes among *Escherichia coli* isolates according to locations.

RESISTANCE GENES LOCATIONS	N	No. Pos expressed (%)	OR	95% CI	P value
TEM					
UBTH	7	7(100.0)	7.105	0.328,153.88	0.249
NAUTH	13	9(69.2)	0.142	0.006,3.048	
gyrA					
UBTH	6	6(100.0)	0.061	0.861,300.20	0.046
NAUTH	16	9(56.2)	16.467	0.793,341.61	

Keys
N=Number of *E.coli* isolates resistant to Augmentin (TEM) and Ofloxacin (gyrA).
CI=Confidence interval. **OR**=Odd ratio. **P** = P<0.05 were considered significant
No. = No. Positive expressed (%)

Table 9: Prevalence of resistance genes among *P.aeruginosa* isolates according to locations.

RESISTANCE GENES LOCATIONS	N	No Pos (%)	OR	95% CI	P. value
TEM					
UBTH	14	14(100.0)	2.806	0.106,74.624	1.000
NAUTH	16	15(93.8)	0.003	0.001,0.082	
gyrA	11	11(100)	3.632	0.132,99,927	
NAUTH	10	9(90.0)	0.275	0.01,7.577	0.476

Keys: N = Number of *P. aeruginosa* isolates resistant to Augmentin (TEM) and ofloxacin (gyrA), **CI** = confidence interval, **OR** = Odd ratio

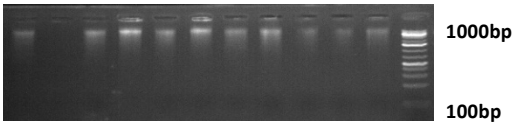

Figure 1: Shows agarose gel electrophoresis for ***Pseudomonas aeruginosa***, L is 100bp-1kb DNA ladder (molecular marker). Lanes 1N, 2N, 4N, 5N and 6N (NAUTH)while lanes 7U, 8U, 9U and 10U (UBTH)are all positive isolates with bands at **1000bp.** NC is a no DNA template control.

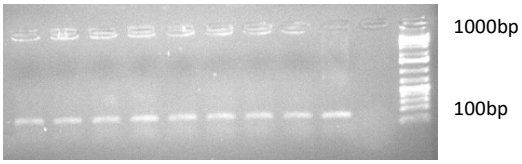

Figure 2: Shows Polymerase chain reaction results for *Escherichia coli*. L is 100bp-1kb DNA ladder (molecular marker). Lanes 20N,21N, 22N and 23N (NAUTH); 24U, 25U, 26U, 27U and 28U (UBTH), were all positive isolates with bands at 160bp.NC is a no DNA template control.

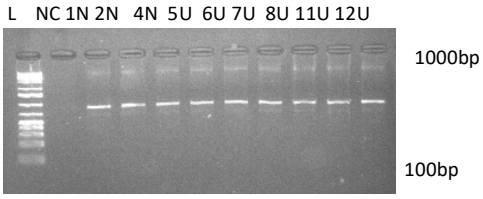

Figure 3: Shows TEM resistant genes detected in *Pseudomonas aeruginosa*. L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Lanes 1N, 2N, 4N and 5N (NAUTH); 5U, 6U, 7U, 8U, 11U and 12U (UBTH) are all positive for resistant genes with bands at 650bp.

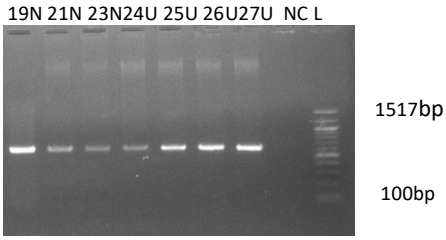

Figure 4: Shows Polymerase chain reaction results of TEM resistant genes detected in *Escherichia coli*. L is 100bp-1517bp DNA ladder (molecular marker). NC is a no DNA template control.Lanes 19N, 21N and 23N (NAUTH); 24U, 25U, 26U and 27U (UBTH) are all positive for Augmentin (TEM) resistant genes with bands at 700bp.

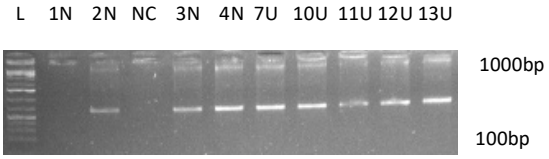

Figure 5: Shows polymerase chain reaction results of gyrA resistant genes detected in *Pseudomonas aeruginosa.* L is 100bp-1kb DNA ladder (molecular marker). NC is a no DNA template control. Lane 1N is negative for gyrA resistant from NAUTH, Lanes 2N, 3N and 4N(NAUTH); 7U, 10U, 11U, 12U and 13U (UBTH)are all positive for Ofloxacin (gyrA) resistant gene with bands at 400bp.

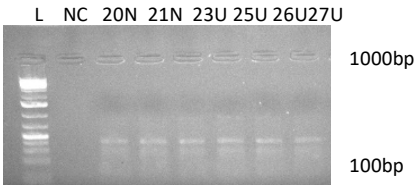

Figure 6: Shows polymerase chain reaction results of gyrA resistant gene detected in *Escherichia coli* isolated. L is 100bp-1kb DNA ladder (molecular marker). NC is a known DNA template control. Lanes 20N and 21N (NAUTH) together with lanes 23U, 25U, 26U and 27U (UBTH) areall positive for Ofloxacin (gyrA) resistant gene with bands at 400bp.

DISCUSSION

Of the 362 patients investigated at UBTH and NAUTH, 122 (33.7%) of them were infected with at least a bacterial pathogen in their surgical wounds. The most predominant pathogens were *Ps. aeruginosa* (45.9%) *Escherichia coli* (45.1%), *Staph. aureus*(7.4%) and *Proteus mirabilis* (1.6%). Patients from NAUTH recorded a higher infection rate of 39.8% than those from UBTH (27.6%)

Isolation rates bacterial pathogens from surgical wound infections had been reported by various workers as 39.9% (9); 9.6% (10); 5-34% (11); (75% (12) and 11% (13). Our overall infection rate of 33.7%) and 39.% or 27.6% for NAUTH and UBTH respectively are statistically similar to findings from Kano by Mohammed and his Colleagues (9) and the range of 5-3% reported by WHO (11). Compared to our present findings, the report by Reiye *et al.*, (12) of 75% was much higher while the 11% reported by Sale and his associates (13) was much lower.

The present study shows that *Ps. aeruginosa* and *Esch coli* were the most frequently incriminated pathogens in the surgical wound infectious. The finding is in agreement with the similar reports by Ezebialu *et al.*, (14) and Sulochana and Diswajeet (15) who Found *Ps. aeruginosa* and *Esch. coli* to be more prevalent in surgical site infections. However, *Staph. aureus* predominated in other studies (16-17). *Esch. coli* is reported to be extremely predominant in hospital acquired infections worldwide making it the number one nosocomial pathogen (18). In a study of health care associated infection in Nigeria, (19) *Esch. coli* (34.4%) was the predominant

isolate from surgical wounds infections. However, in other studies (16; 20), *Ps. aeruginosa* was mostly recovered from postoperative surgical wounds. This organism is known to rank second among nosocomial pathogens isolated from hospitals, often contaminating hospital equipment such as wound dressing sinks and other surgical apparatus (21). Antibiotic resistant strains may 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.

Isolates incriminated with surgical wound infectious in this study were generally resistant to various antibiotics tested. Previous studies had reported that Ceftazidime and Augmentin were mostly resisted by bacterial isolates from surgical wound (22 -23). This is most likely due to the presence of Cephalosporinase and Penicillinase enzymes produced by such organisms as *E. coli* and *Ps. aeruginosa,* which prevent the action of the Beta-lactam ring structure of the antibiotics (24).
In the present study, *Ps. aeruginosa* (90%) ranked highest in resistance to antibiotics among UBTH isolates followed by *Esch. coli* (70.6%) and *Staph. aureus,* (0%) while in NAUTH, *Esch. coli* (95%) displayed highest resistance capacity followed by *Ps. aeruginosa* (92%) and *Staph. aureus* (16.7%). It has been suggested that bacterial infections may be 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 (25). Prevalence of multidrug resistant isolates was found to be very high in both locations (UBTH= 78%, NAUTH= 85%; OR= 1.182; P value= 1.000). This high resistant pattern is an indication of the evolving survival strategies by the clinical bacteria isolates. Studies have shown that emerging high prevalence of multidrug resistant bacteria maybe due to the high selective pressure exerted on bacteria due to numerous factors such as poor adherence to hospital antibiotic policy and excessive and indiscriminate use of broad-spectrum antibiotics (25-26). The odd ratio indicates that patients from both locations have equal chances of being infected with multidrug resistant strains in this study.

Bacteria resistance can be expressed through their ability to colonize new hospital environments where selective pressure prevails (25). This selective pressure is usually imposed by widespread use or misuse of Antimicrobials (AMs), which is the primary reason for emergence of antimicrobial resistance (AMR). When microbes are exposed to AMs, susceptible organism are eradicated, whilst resistant once persist, passing on their resistant genes to off-springs by replication or to other species through horizontal gene transfer. It is generally believed that the higher the use of AMs, the greater is the resistance of microbes to them. Similarly, facilities with higher AM use are most likely to be associated with high incidence of microbial resistance. Our finding of higher prevalence of multidrug-resistance isolates in NAUTH, therefore suggests a more irresponsible use of antimicrobials in this location. Overuse and misuse resulting from poor prescribing and dispensing behaviour, uninformed patient demand and lack of adherence to prescribed treatment regimen, use of low-quality drug formulations, inadequate dosage regimens and insufficient duration of therapy are important contributions to the development of AMR.

Some opportunistic pathogens such as *Esch. coli* and *Ps. aeruginosa* are able to adapt to new environments through the acquisition or development of mechanisms of resistance and persistence (27). The high rates of

multidrug resistance reported in this study is worrisome especially as the two most incriminated isolates from both locations possessed very high percentage of multidrug resistant genes. Isibor *et al*., (28) reported a high percentage of multidrug resistance in *Ps. aeruginosa* isolates associated with diabetic wounds. Similarly, Gima *et al,* (29) incriminated *Esch. Coli* as their third ranking multidrug resistant Gram negative isolates.

According to Lambert (30), *Ps.aeruginosa,* a Gram negative opportunistic nosocomial pathogen is responsible for a wide range of infections associated with antimicrobial resistance. The genome of *Ps.aeruginosa* is reported to be among the largest in the bacterial world, allowing for great genetic capacity and high adaptability to environmental change via horizontal gene transfer (31). This suggests that multidrug resistance pattern associated with Gram negative pathogens could result in increased morbidity and mortality. It has been opined that acquired resistant patterns of Gram negative pathogens was a consequence of mutational events associated with drug selective pressure (32). This study also showed that all sixty eight (68) phenotypically characterized MDR bacterial isolates from UBTH and NAUTH were confirmed by molecular technique. With PCR technique, we were able to target specific resistance genes responsible for MDR in the Isolates. 1t has also beenreported that molecular techniques undoubtedly had the potential to play an essential part in the laboratory for the screening, tracking and monitoring of spread of infective agents in the community and hospital setting (33).

All 21(100%) multi-drug resistant bacteria isolates from UBTH that were resistant to Augmentin were found to express TEM gene (at 700bp) as against 24 (82.8%) from NAUTH(at 650bp). Similarly, all 17(100%) multi-drug resistant bacteria isolates from UBTH that were resistant to Ofloxacin (flouroquinolones) were found to express gyrA genes at 500bp as against 18 (69.2%) of the bacteria isolates from NAUTH. Interestingly, multi-drug resistant bacteria isolates in UBTH were found to be more likely to express gyrA gene when compared with those from NAUTH (P = 0.014). This, however could be because UBTH isolates were more resistant to broad spectrum antibiotics probably due to constant exposure to the same. This relatively high level of resistance of the MDR to Augmentin and Ofloxacin clearly indicated a general misuse or abuse of these antibiotics in both locations. This often happens especially when antibiotic prescriptions in hospital are given without clear evidence of infection. When broad spectrum antibiotics are given in place of narrow spectrum antibiotics as substitute for culture and susceptibility testing the consequent risk is that of dangerous side effects (34). Our findings are in agreement with previous report that the continuous overuse and misuse of antibiotics including Augmentin and Ofloxacin in human medicine had contributed directly to the incidence of multi-drug resistant bacteria isolates (35). It has been further reported that frequent exposure of bacteria strains to a multitude of β- lactam antibiotics including Augmentin has induced dynamic, continuous production and mutation of β-lactamase enzymes in bacterial isolates, thereby expanding their activity even against newly developed β-lactam antibiotics (36-37).

CONCLUSION

This study confirmed that with molecular techniques, definitive resistance genes coding for MDR in clinical isolates of bacteria could readily be identified. We conclude that though phenotypic characterization of bacterial isolates will continue to remain useful and relatively reliable in low-resource laboratories,

molecular diagnostics has rapidly become the "gold standard" because of its accuracy and conclusiveness.

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REFERENCES

1. ManGram, AJ, Horan, TC, Pearson, ML, Silver, LC, Jarvis, WR (1999). Hospital infection Control Practice Advisory Committee. Guideline for prevention of surgical site infection. Infection *Control and Hospital Epidemiology* 20:247 - 278.

2. Snyder, L Champness, W (2007). Molecular Biology Manipulations with DNA, Molecular Genetics of Bacteria, 3rd edition, ISBN-13: 978-1-55581-399-4Staphylococcal endocarditis. *Infectious Disease Clinic of North America* **16**: 297–318.

3. Dunn, DL, Beilman, GJ (2005). surgical infections. Schwartz's Principles of Surgery. 8th ed. McGraw-Hill Companies, New York.

4. Mofikoya, B Neimogha, M Ogunsola, F Atoyebi, O (2009). Bacterial agents of abdominal surgical site infections in Lagos Nigeria. *European Journal of Science Research***38(3):** 509-513.

5. Ehiaghe, JI, Nwobu, RAU, Ehiaghe, FA, Agbakoba, NR, Agbonlahor, DE, Uwabor, CI (**2016**). Plasmid profiling of Multidrug Resistant Bacteria Isolates from Surgical Site Infection in Nigeria.*American Journal of Biotechnology and Molecular Sciences.* **5(1): 23-32.**

6. Cheesbrough, M (2000). District Laboratory Practice Manual in Tropical Countries, part 2 Cambridge University Press, New York pp. 178-179.

7. Cowan, ST, Steel, KS (1993). Manual for the identification of Medical Bacteria. Cambridge University Press, London pp. 986.

8. Clinical and Laboratory Standards Institute (CLSI) (2009). Performance Standards for Antimicrobial Susceptibility Testing,' 18th informational supplement.

9. Mohammed, A Adeshina, GO, Ibrahim, YKE (2013). Retrospective incidence of wound infections and antibiotic sensitivity pattern: A study conducted at the Aminu Kano Teaching Hospital, Kano, Nigeria. *International journal of medicine and medical sciences* (55):60-66.

10. Ezechi, OC, Edet, A Akinlade, H Gab-Okafor, CV, Herbertson, E (2009). Incidence and risk factors for caesarean wound infection in Lagos Nigeria. *BMCResearch Notes* **2:**186-204.

11. WHO, Bulletin of the World Health Organization (2011). 89:757 – 765.

Medical Laboratory Department, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.

12. Reiye, E M, Berhe, GK, Muthupandian, S Derbew, FB, Araya, GW (2014). Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia 7: 575 - 580.

13. Sule, AM, Thanni, L Sule Odu, AO, Olusanya, O (2002). Bacterial pathogens associated with infected wounds in Ogun State University Teaching Hospital, Sagamu, Nigeria. *African Journal of Clinical and Experimental Microbiology* 3:13-16.

14. Ezebialu, CU, Chukwura, EI, and Ezebialu IU (2010). Bacterial pathogens associated with wound infections at national orthopaedic hospital. Enugu. *Nigerian Journal of Microbiology* 24 (1):1987 – 1992.

15. Sulochana, D Mamta, D Biswajeet, S (2014). Susceptibility Pattern of Aerobic Bacterial Isolates from Wound swab. *Indian Medical Gazette* PP. 355 – 359.

16. Aisha, M Gbonjubola, OA, Yakubu, KI (2013). Incidence and Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound Infections in a Tertiary Hospital in Nigeria. *Tropical Journal of Pharmaceutical Research* 12 (4): 617-621.

17. Ojo, SKS, Sargin, BO, Esumeh, FI (2014). Plasmid curing Analysis of Antibiotic Resistance Beta – Lactamase Producing *Staphylococci* from Wounds and Burns Patients. *Pakistan Journal of Biological Sciences* 17 (1): 130 – 133.

18. Teresa, C Horan, MPH, John, W White William, R Jarvis, MDT, Grace, ERN, David, MSH, James, M Hughes, MD (1986). Nosocomial Infection Surveillance: Hospital Infections ProGram Center for Infectious Diseases. Morbidity and Mortality Weekly Reports 35(1):17-29.

19. Nejad, SB, Allegranzi, B, Syed, SB, Ellis, B Pettet, D (2011). Health care associated infection in Africa: a systematic review. *Bulletin of the World Health Organization* 89:757-765.

20. 31. Dalhatu, A Yunusa, U Ahmad, S Timothy, G Jari, S Musa, M (2014). Bacterial Agents of Abdominal Surgical Site Infections in General Hospital Funtua, Katsina State, North-Western Nigeria. *Journal of Dental and Medical Science* pp. 48 – 52.

21. Masaadeh HA, Jaran AS. Incident of *pseudomonas aeruginosa* in postoperative wound infection. *American Journal of Infectious Diseases.* 2009, 5(1):1-6.

22. Eduardo, L Airton, DF, Jonas, P Pedro, A (2008). Prevalence of bacterial resistance in surgical wound infections in peripheral arterial surgery. *Journal Vascular Brasileiro* 7(3): 1677 – 5449.

23. Yah, SC, Eghafona, NO, Oranusi, S Abouo, A (2011). Widespread plasmid resistance genes among *Proteus species* in diabetic wounds of patients in the Ahmadu Bello

University Teaching Hospital (ABUTH) Zaria. *African Journal of Biotechnology* 6: 1757 – 1762.

24. Livermore, DM (1995). Beta-lactamases in laboratory and clinical resistance. *Clinical Microbiology Reviews* 8(4): 557-584.

25. Akoachere, JF, Tatah, K Palle, J Ngunde, M Soupsop, E Nkwelang, G Roland, N (2014). Risk factors for wound infection in health care facilities in Buea, Cameroon: aerobic bacterial pathogens and antibiogram of isolates. *Pan African Medical Journal* 18: 6 – 8.

26. Garba, I Lusa, YH, Bawa, E Tijani, MB, Aliyu, MS, Zango, UU, Raji, MIO (2012). Antibiotics susceptibility pattern of *Pseudomonas aeruginosa* isolated from wounds in patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Nigerian Journal of Basic and Applied Sciences* 20 (1): 32 – 34.

27. Alejandro, B Maria, T German, B (2013). Successful or Deleterious Association in the Bacterial World. *Clinical Biology Review* 26: 185-230.

28. Isibor, JO, Samuel, SO, Eyaufe, A Edomwonyi, EO, Odiase, V Ehiaghe, JI, Eidangbe, A2013. Aerobic Bacteria Associated With Diabetic Wounds In Patients Attending Clinic In A Rural Community In Nigeria. Global Research Journal of Microbiology.2013, 3(1): 8 –12.

29. Girma, G Gebre, K Himanot, T (2013). Multidrug resistant bacteria isolates in infected wounds at Jimma University Specialized Hospitals, Ethiopia. *Annals of Clinical Microbiology and Antimicrobials* 12:1-17.

30. Lambert, PA (2002). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. *Journal Soc. Medical* 95:22-26.

31. Goergios, M Maria, B (2013). *Pseudomonas aeruginosa*: Multi-Drug Resistance Development and Treatment. *Options***10**:34 – 56.

32. Sibhghatulla, S Jamole, F Shazi, S Syed, M Danish, R Mohammed, AK. (2014). Antibiotic resistance and extended spectrum beta lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences* 22: 90 – 101.

33. Prescott LM, Harley LP, Klein DA. Microbiology. 4th Edition, New York. McGraw Hill. 1999, Pp. 678 – 697.

34. Alekshun, MN, Levy, SB (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell.***128**: 1037 – 1050.

35. Paterson, DL, Bonomo, RA (2005). Extended-spectrum p-lactamases. A clinical update. *Clinical Microbiology Review***18:** 657-686.

36. Pitout, JD, Lavpland, KB (2008). Extended-spectrum Beta-lactamase producing Enterobacteriaceae: an emerging public health concern. *The Lan of infectious Diseases*. 8:159 – 166.

37. Yimaz, A Cobian, AY, Yelz, Y Tamverdi, C Yidrim, TT, Erturan, Z.Z, Dunipnar, BB, Bozdogan, BB (2011). Investigation of Plasmid-Mediated quinolone Resistance in *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients. *Microbiological Bulletin***45:** 602-608