Original Research Article
Antibiotics resistance and plasmid profile of clinical bacterial isolates obtained from Brait-Whyte Memorial Specialist Hospital, Port Harcourt, Rivers State, Nigeria

Abstract

Antibiotics resistance pattern of bacterial isolates have caused both economic and societal loses to mankind. In this study, ethical approval and samples were sought and obtained from the Brait-Whyte Memorial specialist hospital in Rivers State. Two hundred and seven samples (207) samples were obtained from both female (71%) and male (21%) sourced samples covering endocervical, throat, ear, wound, urethral, skin and high vaginal swabs. Biochemical and Molecular approaches were employed in identification of multidrug resistant isolates from the isolates obtained from the study. Kirby-Bauer method was employed in determination of antibiotics susceptibility profile. Female patients with the age 25-35 years and 35-44 years were observed to be most frequent for High vaginal and wound swab bacterial isolate colonization cases. Twenty-six isolates were observed to be resistant to Augmentin, Ceftazidine, Gentamicin, Ofloxcin, Cefuroxime .Over 90% of the bacterial isolates were resistant to Cloxacillin, 76% for Ceftriaxone. *Enterobacter ludwigii* was identified to be both multidrug resistant and plasmid-mediated form of resistance with multiple plasmids with Molecular weight of 13065, 9139, 2350 and 854bps whereas *Klebsiella aerogenes* was observed to have three distinct plasmid-bands with 10173, 2525 and 2118 bp. This study further supports the role of plasmids in the resistance profile and drug idiosyncrasies of nosocomial and pathogenic bacterial flora, reported in Port Harcourt metropolis. There is need to intensify public campaigns and awareness against unsafe drug administration practices and self-medication trends

Keywords: Plasmids, antibiotics susceptibility profile, Antibiotics Resistance Pattern, Kirby-Bauer method, multidrug resistant indices, drug idiosyncrasies

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Introduction

The increasing costs of health care delivery increased population and the increase in resistance of bacterial pathogens to conventional antibiotics have necessitated the need to underscore the battle between man and microbes in the administration of antimicrobial therapies. These have become imperative to ascertain the role of plasmids in the trends of microbial resistance.Antibiotics are microbial derived substances, can either be produced by microbial activities or they can be synthesized naturally or even both. They can be used to eliminate systemic or topical infections. Mostly they can inhibit the growth of pathogens or even kill them. Antimicrobial therapies can be categorized into two major groups, technically, the coverage or effectiveness of such antibiotics to both gram negative and gram negative is said to have a broad (wide) spectrum, but when it is effective against either the gram negatives or gram positive it regarded to have a narrow spectrum (Zhang, 2011). Bacterial resistance is a survival route in which bacterial groups react to either a strange toxicant, biological substances or even to a novel ecosystem (Davies and Davies, 2010).

Bacterial resistance to chemical substances has been linked to the mutational changes and uptake of novel plasmids by some form horizontal gene transfer. These resistance genes are often encoded on plasmids or on gene locus of the genome. Plasmids are extra-chromosomal genetic material, can be either linear or circular, with a self-replicating ability. They carry genes most essential for the initiation and control of replication while some others carry genes that ensure stable uptake of genes encoded in the locus of the genomes. These ones are often referred to as transposons also known as jumping genes (Carattoli *et al.,* 2001). This present study examined Occurrence, antibiotic susceptibility and plasmid profile of clinical isolates in Braith Waite Memorial Specialist hospital, Port Harcourt, Rivers State, Nigeria.

Rivers State, created on May 27, 1967 out of the former Eastern Region of Nigeria is located in the Niger Delta region. The state covers a land area of 11,077km, and its capital is Port Harcourt. Rivers state is amphibious, having both riverine and upland geographical areas (the State is about 45% riverine).Port Harcourt, the capital of Rivers State is one of the fastest growing urban centers in Nigeria. The progressive development of informal settlements along the waterfronts is as a result of this growth. These settlements that are inevitably densely populated, lack amenities such as toilets, waste collection points, roads, and water supply (Wokekoro and Inyang, 2014). The outcome of these settlements typically could aid the spread of infectious microorganisms with deadly mutational changes, thereby, contributing to frustrating the potentials of antibiotics and efforts made over the years in alleviating health standards in the medical history. The University of Port Harcourt Teaching Hospital and Braith Waite Memorial Specialist Hospital make up the two major hospitals in the state. Braith Waite Memorial Specialist Hospital being located in Port Harcourt metropolis is patronized majorly by the populace within the Obio/Akpo and Port Harcourt local government area (Wokekoro and Inyang, 2014).

METHODS

Ethical consideration

The clearance for this research was sought for and approved by the hospital management board.

Location of Study

This study was carried out in Brait-Whyte Memorial Specialist Hospital, Port Harcourt, Rivers State, Nigeria. Hence, For the purpose of advancement with this study in accordance with the ethical guidelines for Biomedical research involving human subjects, ethical approval was sought and obtained from the Rivers State Health Research Ethics, Rivers state Hospital Management Board, Port Harcourt, Rivers State.

Sample size

This study was investigating the proportion of swab samples in the total hospital attendees with clinical infection. Therefore, the sample size was determined using a qualitative variable (Charan and Biswas, 2013) employing the following equation:

$$Samplesize = \frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2} \quad Samplesize = \frac{1.96^2 \times 0.16(1-0.16)}{0.05^2} = 206.544$$

Sample size= ~207

Where: $Z_{1-\alpha/2}$= is the standard normal variate (at 5% type 1 error (P<0.05) it is 1.96 and at 1% type error (p<0.01) it is considered significant below 0.05 hence 1.96 is used in formula.
P= expected variation in population based on previous studies or pilot studies.
d= absolute error or precision (has to be decided by researcher)

Hence, the proportion of patients hypothetically with possible infections collected from clinical swabs specimens in hospital with bacterial origin among all age group according to laboratory statistics is estimated to be 16%. Using an absolute error of 5% therefore,

Population studies

During this study, isolates were collected from swab specimens of different patients. The populations of patients are made up of males and females across all ages that are clinically diagnosed for possibly infection that attended the hospital within the period of sample collection. Therefore, the specimen population is as follows (Table 1)

Isolation of pathogen from samples

The swabs were obtained from the patients by a physician, information on the form were obtained and document, with information on the age, sex, nature of sample and site of sample collection were reported. The swab stick was smeared on the solidified nutrient agar plates

Biochemical reaction

The method of Cheesebrough, (2006) was employed in the identification of the clinical pathogens. Gram reaction, spore staining, catalase, oxidase, sugar fermentation, Tripple sugar ion, Methyl red Voges-Proskauer and colonial morphology were used in the characterization of the isolates using dichotomous key response.

Standardization of inoculum

Approximately 85 ml of 1% sulfuric acid (H_2SO_4) was added to a 100ml volumetric flask. Using a volumetric pipette, 0.5ml of 1.175% anhydrous barium chloride ($BaCl_2$) was added drop-wise to the 1% sulfuric acid (H_2SO_4) while constantly swirling the flask. Then the solution was brought to a volume to 100ml with 1% H_2SO_4. Stir for 5 minutes while examining visually, until the solution appears homogeneous and free of clumps. The optical density (OD) of the McFarland standard was checked at a wavelength of 625nm

Determination of Antimicrobial Susceptibility

According to Clinical and Laboratory Standards Institute (CLSI, 2006) recommendations, the modified Kirby-Bauer disc diffusion method was used in determining the susceptibility pattern of the clinical isolates to antibiotics. Hence, a freshly prepared eighteen-hour culture of the bacterial isolates were inoculated into sterile distilled water, and compared with the equivalent of 0.5 Macfarland standard. About 0.1ml of the sample was spread on Mueller-Hinton agar and incubated at 37^{o}C for 2hrs. Hence, a multi-disc (the Gram negative and Gram-positive disc, containing 100 disc manufactured by Abtek Biological Ltd., UK) were used to determine the drug sensitivity and resistance pattern of the isolates) was aseptically placed on the Mueller-Hinton agar then incubated at 37^{o}C, after 24h the zones of inhibition were measured and compared with the zones of inhibition (breakpoints) as recommended by Clinical and Laboratory Standards Institute (CLSI, 2006).

Calculation of Multi-drug Antibiotics Resistance

The MAR (Multi-drug Antibiotics Resistance) index for Gram Positive and Gram Negative isolates was calculated using the method as described by Blasco et al., (2008) and Odjadjare et al., (2012) as follows:

MAR=a/b

a = No. of antibiotics to which the isolate was resistant; b = Total No. of antibiotics against

which individual isolate was tested.

Table1: Population study of samples collected

Specimens-type	Total
TS	5
SS	1
HVS	88
WS	80
ECS	5
US	12
ES	16
Total	207

Key: Wound Swabs-WS, High vaginal Swabs-HVS, Urethral Swabs-US, Endocervical Swabs-ECS, Ear Swabs-ES, Throat Swabs-TS, Skin Swabs-SS, M-Male, F-Female.

Abtek disc (manufatured by Abtek Biochemicals Ltd) were used for sensitivity.

Cloxacillin	CXC	30 µg
Ceftriaxone	CTR	30 µg
Cefuroxime	CRX	30 µg
Cefixim	CXM	5 µg
Augmentin	AUG	30 µg
Erythromycin	ERY	5 µg
Gentamicin	GEN	10 µg
Ciprofloxacin	CPR	5 µg
Oflaxacin	OFL	5 µg
Nitrofurantoin	NIT	300 µg

Results

Table 2 describes the summarized antibiogram of the clinical isolates to conventional isolates, eight (8) isolates did not reveal antibiotics resistance pattern , hence had 0-MAR indices. Table 3 revealed that a total of 26 isolates were resistant to Augmentin, Cefazidime, Gentamicin, Ofloxacin and Cefuroxime. Twenty (20) isolates were observed to be resistant to augmentin, alone, whereas 18 had no resistance. Figure 9 reveals the plasmid profiling of multidrug resistant clinical isolates gel bands for the bacterial isolates. Figure 10 describes the molecular weight determination protocol

Table2 Number of Antibiotics showing resistance pattern of clinical isolates

Number of antibiotics resistant to	Number of isolates showing pattern	MAR index
None	8	0
One	5	0.1
Two	9	0.3
Three	14	0.4
Four	24	0.5
Five	14	0.6
Six	13	0.8
Seven	7	0.9
Eight	8	1.0

Key: Multi-Drug Resistant index (MAR index)

Table 3: Antimicrobial profiles of isolates resistant to broad-spectrum antibiotics

Antimicrobial resistance profile	Number of isolates showing profile
No Resistance	18
Augmentin	20
Ceftadizine	14
Gentamicin	12
Ofloxacin	3

Cefuroxime	1
Augmentin, Ceftadizine	9
Augmentin, Ofloxacin	2
Augmentin, Cefuroxime	17
Ceftazidine, Gentamicin	8
Ceftazidine, Ofloxacin	8
Ceftazidine, Cefuroxime	6
Gentamicin, Ofloxacin	2
Gentamicin, Cefuroxime	1
Augmentin, Ceftazidine, Gentamicin	1
Augmentin, Ceftazidine, Ofloxacin	6
Augmentin, Ceftazidine, Cefuroxime	5
Augmentin, Gentamicin, Ofloxacin	1
Augmentin, Ofloxacin, Cefuroxime	7
Ceftazidine, Gentamicin, Ofloxacin	3
Ceftazidine, Gentamicin, Cefuroxime	2
Ceftazidine, Ofloxacin, Cefuroxime	1
Gentamicin, Ofloxacin, Cefuroxime	1
Augmentin, Ceftazidine, Gentamicin, Ofloxacin	9
Augmentin, Ceftazidine, Gentamicin, Cefuroxime	12
Augmentin, Ceftazidine, Cefuroxime, Cefuroxime	4
Augmentin, Gentamicin, Cefuroxime, Ofloxacin	3
Ceftazidine, Gentamicin, Ofloxacin, Cefuroxime	5
Augmentin, Ceftazidine, Gentamicin, Ofloxacin, Cefuroxime	26

Zone of inhibition: Ab*≤0*Antibiotics

Table 4.0: Percentage of isolates showing resistance pattern to broad-spectrum antibiotics

Number of antibiotics resistant to	Number of isolates showing pattern
Zero	18(8.7%)
One	50(24.2%)
Two	53(25.6%)
Three	27(13.0%)
Four	33(15.9%)
Five	26(12.6%)

Table5.0 Percentage occurrence of broad-spectrum antibiotics resistant isolates

	Total number of isolates-n	Total number of isolates resistant to broad spectrum antibiotics-n'	Percentage when ∑n'=26	Percentage when ∑n=207
HVS	88	7	27	3.4
WS	80	13	50	6.3
ES	16	3	12	1.4
US	12	2	8	1.0
TS	5	1	4	0.5
Total	207	26		12.6

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES) and Wound Swab (WS).

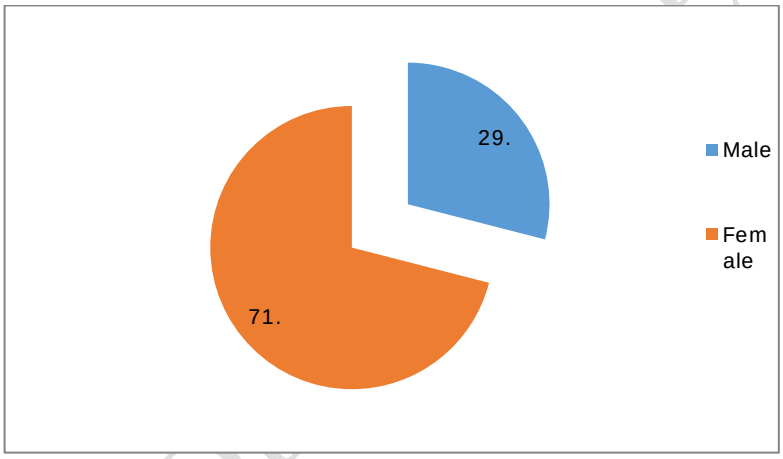

Figure 1.0: population study by gender in percentages. Female were 71% and males 29%.

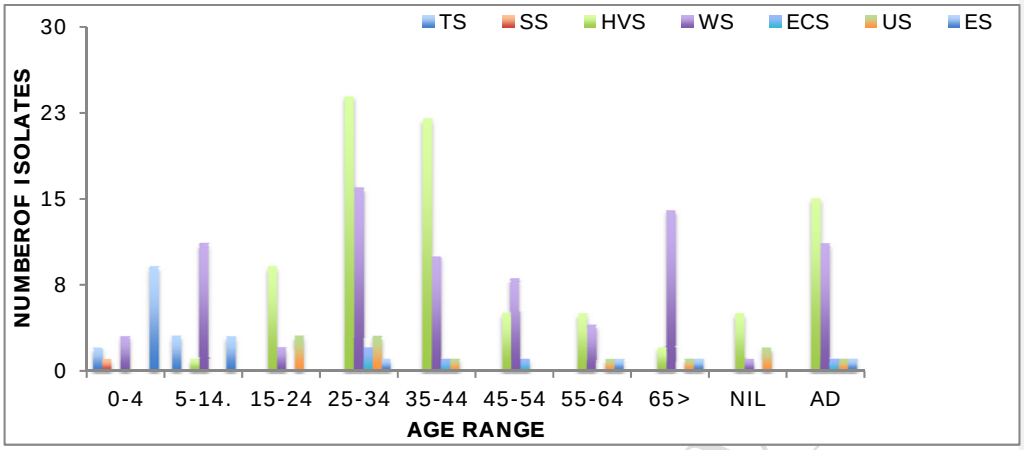

Figure 2: A clustered column chart showing population study by Age distribution.

Key: Wound Swabs-WS, High vaginal Swabs-HVS, Urethral Swabs-US, Endocervical Swabs-ECS, Ear Swabs-ES, Throat Swabs-TS, Skin Swabs-SS, AD-patients with unknown age, NIL-patients without age record.

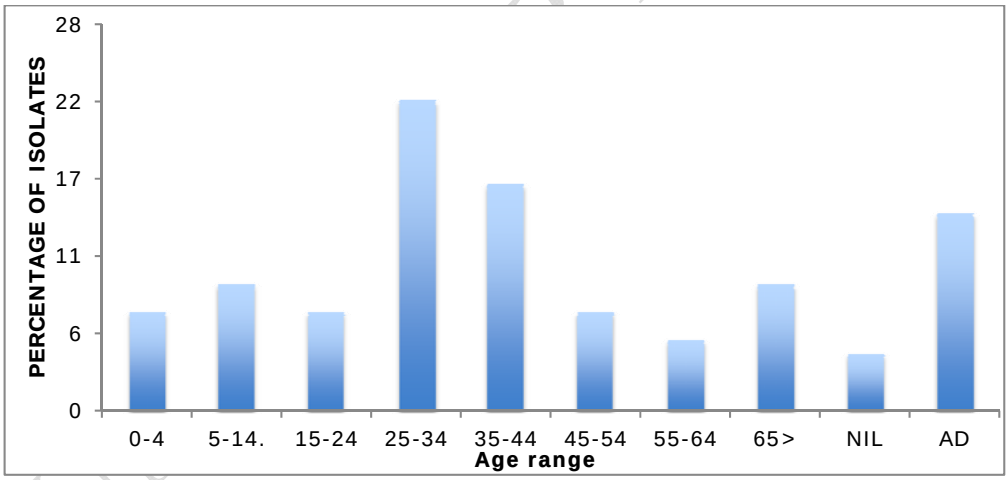

Figure 3: A column chart showing summary of population study in percentage of Age distribution.

Key: AD-patients with unknown age, NIL-patients without age record.

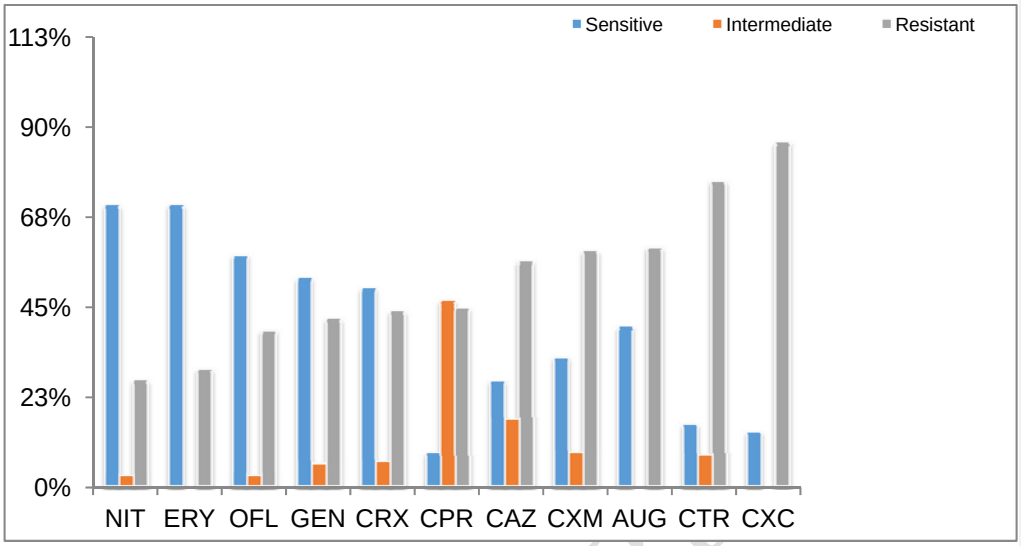

Figure 4: A clustered column chart showing Antibiotics susceptibility profile.

Key: CXC-Cloxacillin; CAZ-Ceftazidine, CTR-Ceftriaxone, CRX-Cefuroxime; CXM-Cefixim; AUG-Augmentin; ERY-Erythromycin; GEN-Gentamicin, CPR-Ciprofloxacin, OFL-Oflaxacin; NIT-Nitrofurantoin; S-Susceptible; I-Intermediate and R-Resistant.

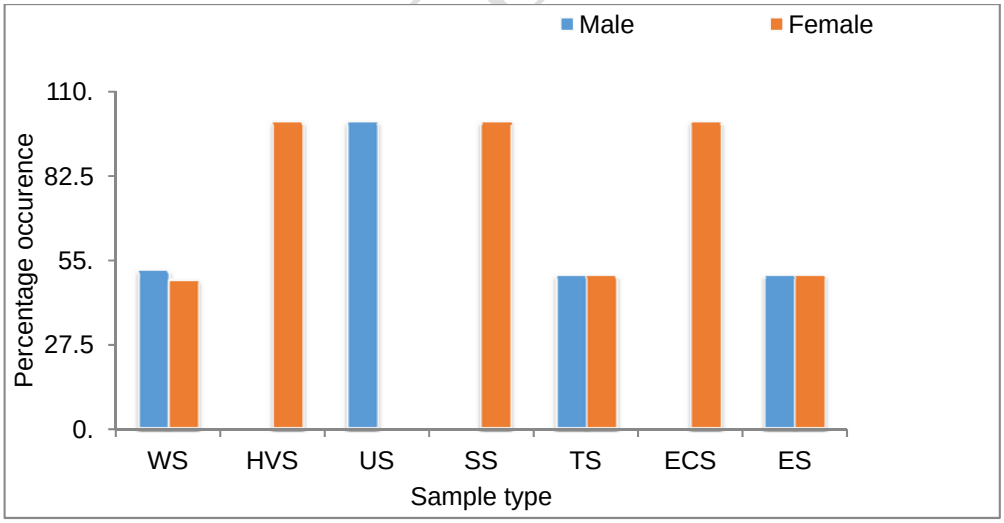

Figure 5: A clustered column chart showing Sample-type and percentage distribution for Gram positive isolates by gender from Brait wait memorial hospital, Rivers State

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS)

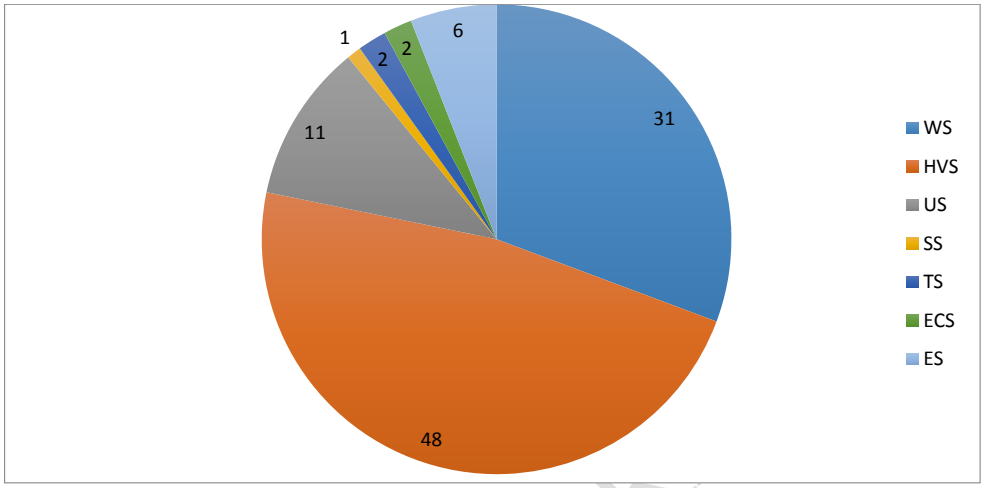

Figure 6: Percentage occurrence of Gram positive pathogens in clinical isolates from Brait Waite memorial hospital, Rivers State

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS).

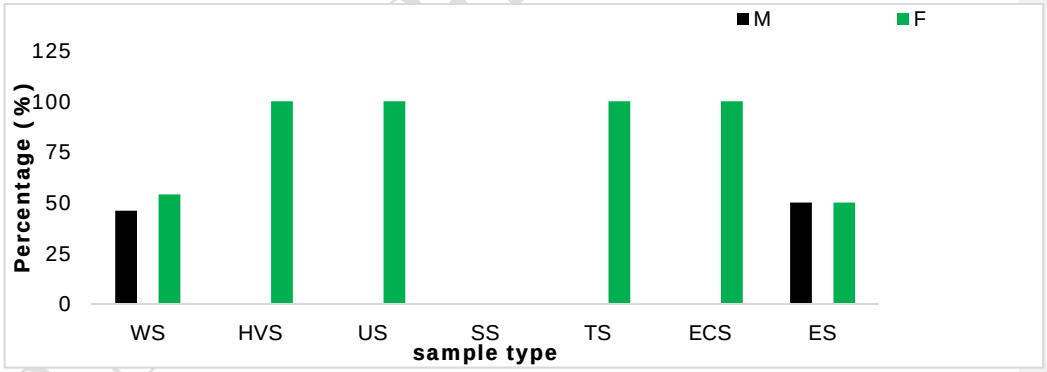

Figure 7: A column chart showing percentages of Gram negative isolates according to gender

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES), Wound Swab (WS).

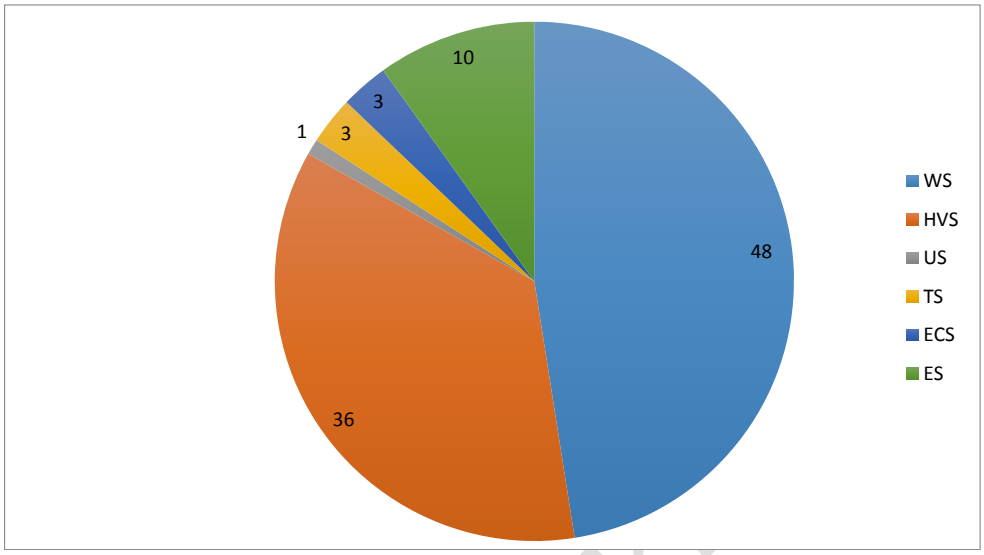

Figure 8: A pie chart showing Percentage occurrence of Gram negative pathogens in clinical isolates from Brait waite memorial hospital, Rivers State

Key: Skin Swab (SS), Throat Swab (TS), High Vaginal Swab (HVS), Urethral Swab (US) Endocervical Swab (ECS), Ear Swab (ES) and Wound Swab (WS)

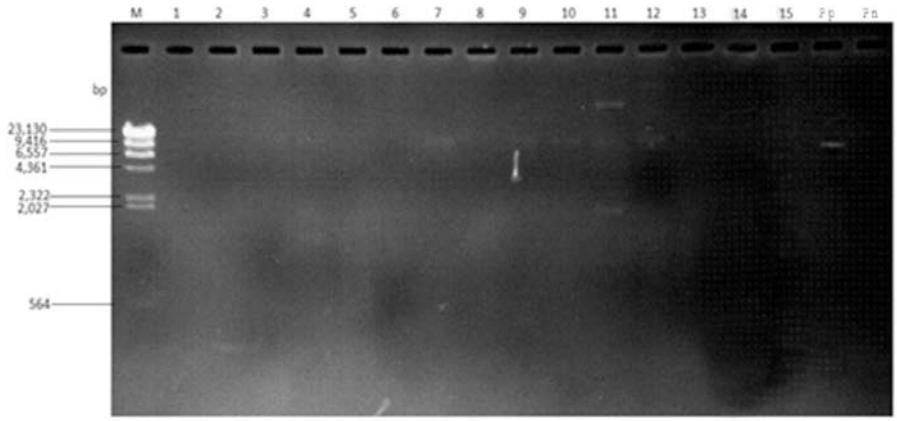

Figure 9: Plasmid profile of Gram-negative broad spectrum resistant isolates.

Lane one to lane fifteen are the isolates screened for plasmid. Pp positive control (plasmid of known molecular weight of 9416bp) and Pn negative control (double distilled water-ddH_2O), M ladder.

Table 6.0 : Plasmid profile standard curve

Distance of ladda	Base pairs
44.1	25130
50.1	9416
56	6557
63	4361
79	2322
84	2027
135.3	564

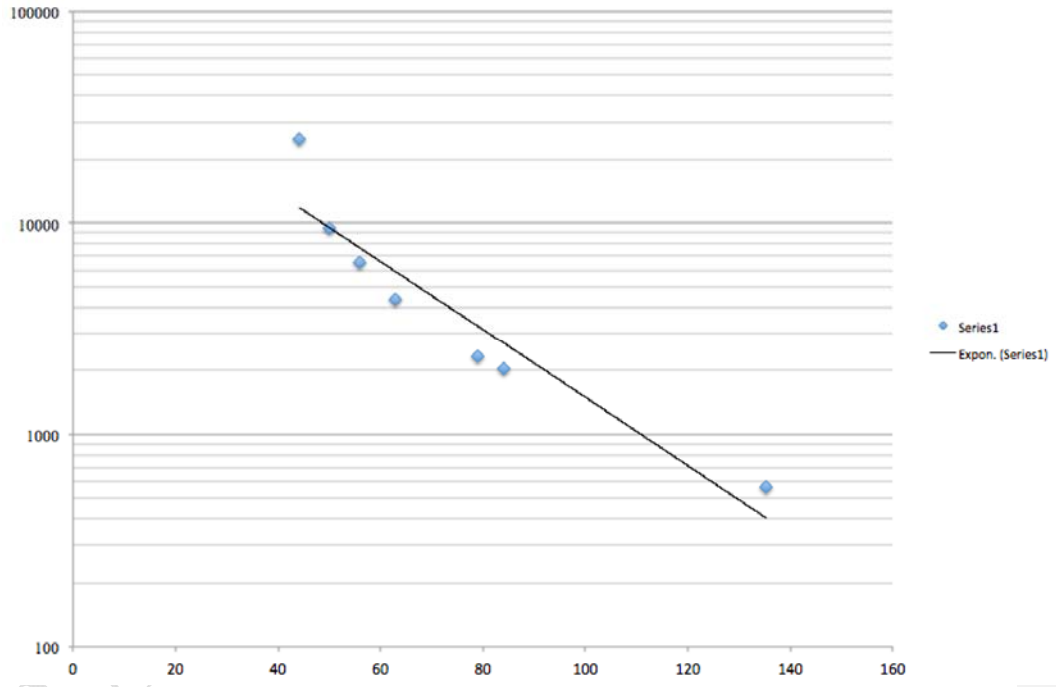

Figure 10: Plasmid profile standard curve

Table 4.24: Correlation Of Plasmid And Gene-Typing

PMC	Isolate Identity	Isolate Code	Molecular weight of plasmid (bp)	Age	Sex
E1	*Enterobacter ludwigii*	E3	13065, 9139, 2350, 864	68	F
E2	*Bacillus sp. B-26*	H6	0	2Mt	M
E3	*Pseudomonas fluorescens ex-17*	W5	0	2Mt	F
H1	*Staphylococcus epidermis ECwu-Ha2*	W8	0	27	F
H2	*Pseudomonas sp. DJ5*	W11	0	28	F
H4	*Staphylococcus epidermis LCR40*	H2	0	34	F
H5	*Staphylococcus epidermis HXV-L23cs*	W9	48	34	F
H6	*Pseudomonas marginalis*	W10	0	37	F
H7	*Burkholderia cenocepacia H111*	H7	10173	40	F
H8	*Enterobacter sp.Vm-12*	W2	10173	41	F
T1	*Enterobacter cloacae ST23*	E1	0	14	F
U1	*Brevibacillus sp. SSB1*	W6	0	61	M
U2	*Streptococcus pyogenes. E-231*	H8	0	65	M
W1	*Streptococcus sp. ZT16*	W4	0	12	M
W10	*Providencia vermicola PDMZnCd1502*	T1	10173	77	F
W11	*Pseudomonas aeruginosa strain R4*	W3	0	72	F
W12	*Bacillus valezensis P13*	U1	0	75	M
W13	*Bacillus subtilis*	E2	0	71	M
W2	*Providencia vermicola CGS9*	W13	10173	25	F
W3	*NO AMPLIFICATION*	W1	0	25	F
W4	*Proteus mirabilis*	H5	0	29	M
W5	*Citrobacter sp. XT-7*	H4	0	35	F
W6	*Klebsiella sp.VDS.42_A*	W12	10173	53	M
W7	*Staphylococcus aureus*	W7	0	55	F
W8	*Klebsiella aerogenes*	U2	10173, 2525, 2118	62	F
W9	*Enterobacter sp. XBBSY5*	H1	10173	70	F

Key: Throat Swab (T), High Viginal Swab(H), Urethral Swab(U), Ear Swab(E), Wound Swab(W), Male (M), Female (F), Month(Mt), PMC-Plasmid and Molecular study Code, Gram-positive(+), Gram-negative(-)

Discussion

Epidemiological survey of clinical isolates for both resistance and multidrug resistance is critical for a robust curative and preventive measure for limiting the spread of these microorganisms (Umolu *et al*., 2006). The emergence of infections caused by antibiotic-resistant pathogens is a growing problem and has now become a major health issue (Yoshikawa, 2002). Pathogenicity of clinical isolates has the ability to increase in resistance profile (Karlosky *et al*., 2004). The study conducted by Umolu *et al*., (2006) observed in a total of eighty-six clinical samples collected

high vaginal swab (HVS) accounted for samples reported for enteric pathogen accounted for 4 while wound samples were 14 for similar cases. In this study over 207 samples were collected wound swab (80), 88 (HVS), Ear Swab (16), Urethra Swab (12). The occurrence of enteric pathogen in females was significantly higher in the samples collected for females than for males as seen in urethra swab samples. This line of argument negates or disagrees with the position of Jombo et al., (2011). Although the loss in medical history, presentation of disease cases might have been identified as crucial.

The impact of age of patients presented with obvious of microbial surface colonization. When age ranges of 25-34 and 65≥ displayed the highest cases resistance. Hassan *et al*., (2012) in a separate but related study reported similar findings but had a far different report where. The HVS had highest cases still within the ages of 25-34 while wound swab also had a high occurrence. The female patients had the highest cases of these multidrug resistance compared to the male. Surprisingly, the children within the ages 0-4 were also observed to have these multidrug resistances as well.

The most resisted were the beta-lactam antibiotic, in this case was Cloxacillin (86%) this findings agree with report of Aibinu *et al*., (2004); Stelling *et al*., (2005) and who reported a 100% amoxicillin. Also resisted Ceftrioxin (76%), Augumentin (60%), Cefixime (59%) while the most susceptible antibiotics was Erythromycin (71%), Nitrofurantoin (70%), Ofloxacin (58%), Gentamicin (52%) and Cefuroxime (50%) whereas. The use of Nitrofurantoin is currently discouraged due to its toxicity and side effects, but in extreme cases of urinary tract infections it is limited for use for children, Which Jumbo *et al*., (2012) reported that children have uncontrolled ability to produce the enzyme beta-lactamases which neutralizes most penicillin group, further suggesting use of Ofloxacin with a remarkable sensitivity of 58%. This agrees with the findings of this current study cephalosporins like Erythromycin and Ciprofloxacin was slightly resistant. The weakest or poorly resistant Nitrofurantoin (27%) while the weakly sensitive Ciprofloxacin (9%) whereas Idu and Odjimogho (2003) showed that ciprofloxacin is the most effective quinolone, In a study conducted among fresh students in Ahmadu Bello University for Pseudomonas showed a uniform susceptibility to ciprofloxacin (Olayinka *et al*., 2009) while contrary to this, the result suggest that the cephalosporin and beta-lactam were most resisted by the pathogens while Aminoglycoside, Azolidines and quinolones were susceptible although no certain group of antibiotics dominated the susceptibility profile.

Since the widespread use of antibiotics in animal husbandry and agricultural activities is propelled by economic motives, especially higher yield from the veterinary and agricultural world, and from food producers and pharmaceutical companies, to combat the spread of multi-drug resistant bacteria effectively (Lutter *et al*., 2005). Strong adherence of appropriate chemotherapy to disease treatment and control has its foundations surveillance, control of resistance, administration of the proper antibiotics and adjustment of doses. Health facilities must be encouraged to adhere to these recommendations (Cheng et al., 2009; Chakupurakal *et al*., 2010). Also, the high rate of resistance (100%) of ESBL producing strains of *E. coli* against cephalosporins in Turkey (Akyar, 2008); the high rate of resistance of Enterobacteriaceae against

quinolones in Sweden (Ostholm-Balkhed *et al*., 2010); and the high resistance of *Enterobacter* against ceftazidime in Brazil (Sader *et al*., 2011) clearly shows the global variations in antimicrobial susceptibility patterns. Laboratory physicians and scientists should always develop local antimicrobial susceptibility profiles (antibiograms) of local bacterial isolates, and the patterns regularly updated for ready consultations (Morosini *et al*., 2006; Bouchillon *et al.,* 2005).

The number of samples reported for Gram negative were 105 samples while Gram positive had 102 two samples. Among these samples enteric pathogens were most frequent (Hassan *et al*., 2012) agrees with this study result when they reported that the *Enterobacteriacea* groups suggesting that *Escherichia coli* as the dominant isolate especially in High vaginal swab than in wound swab. The susceptibility. Isolates like the *Enterobacer ludwigii*, *Pseudomonas fluorescens* ex-17, *Pseudomonas* sp. DJ5, *Pseudomonas marginalis*, *Enterobacter cloacae* ST23, *Providencia vermicola* CGS9, and *Enterobacter* sp. XBBSY5 and *Burkholderia Cenocepacia*. The isolates obtained in this study agrees with the findings of Hassan *et al*., (2012) whom in his study was able to identify *Enterobacter cloacae, Citrobacter freundii*, Sadly the preponderance of *Pseudomonas* sp. were hardly seen in previous studies *Proteus mirabilis.*

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