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Research Article

Multidrug Resistance Tracing by Plasmid Profile Analysis and the Curing of Bacteria from Different Clinical Specimens

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Human-pathogenic bacteria resistant to one or multiple antibiotics have dramatically increased worldwide in the past decades. These bacteria possess great danger, have become a global issue, and it is now impossible to avoid developing strategies for the restoration of treatment options against infections caused by them. This research aims at profiling plasmids of multidrug-resistant bacteria from various clinical specimens such as ear exudate, sputum, urethral swab, wound swab, urine from the catheter, urine, nasal swab, high vaginal swab, stool, eye swab, and blood at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria. Our investigation used the agar diffusion method for susceptibility tests and identification of multidrug-resistant bacteria before plasmid extraction and gel electrophoresis. A plasmid curing test was performed with 10% sodium dodecyl sulphate. Of the 860 bacteria whose resistance profile was determined, only 42 were multidrug-resistant. These bacteria include *Pseudomonas aeruginosa* 16 (38.10%), *Staphylococcus aureus* 12 (28.57%), *Escherichia coli* 9 (21.43%), and *Klebsiella pneumoniae* 5(11.90%). The molecular weight of their plasmids ranges between 20.884 kbp and 187.50 kbp. As indicated by the plasmid bands, some bacteria had similar molecular weight while others had no plasmid. The bacterial pattern of the postcuring sensitivity test showed that the bacteria with plasmid bands were cured as they became susceptible to the drugs they were previously resistant to, while the bacteria without plasmid bands remained resistant to the antibiotics. This implies that the latter's multidrug resistance is nonplasmid mediated. Our analyses highlight the relationship between plasmids and multidrug resistance as well as the role of plasmids in the transmission of drug resistance across bacteria. Thus, in order to lessen the burden that multidrug-resistant bacteria cause and to improve bacterial infections treatments, there should be continued surveillance and periodic research on antibiotic resistance patterns of bacteria from various clinical settings.

1. Introduction

The multidrug-resistant (MDR) bacteria have continually posed severe global health challenges over the years [1]. When an organism simultaneously evades the effects of numerous antimicrobial medications from distinct chemical classes or subclasses through multiple methods, it is referred to as being multidrug-resistant [2]. Numerous bacterial species that were isolated from various clinical specimens displayed one or more antimicrobial agent resistance strategies [3–6].

Either or both mechanisms may cause multidrug resistance in bacteria. First, these bacteria may amass a number of genes, each of which codes for drug resistance within a cell. This buildup typically takes place on resistance (R) plasmids. Second, increased gene expression for multidrug efflux pumps, which ejects a wide range of medicines, may results in multidrug resistance. Antibiotic-resistant bacteria can transmit copies of the DNA that specify a defence mechanism to other bacteria, even closely related species. These other bacteria can then pass the resistant genes on, creating

new generations of antibiotic-resistant bacteria. Horizontal gene transfer is the name given to this phenomenon [7].

When certain bacteria are resistant to numerous antibiotics, there is limitation in therapy options. Therefore, the development of progressively resistant bacterial infections will continuously compromise the significant therapeutic advantages offered by the introduction of novel antimicrobial medicines [8]. To overcome these difficulties, there is need to monitor resistance profiles in health institutions.

Plasmids have been described as additional pieces of genetic material discovered in bacteria and a lot of cells that commonly bestow a unique attribute to the cell [9]. Drug resistance and toxin generation are among these characteristics. Plasmids play a crucial role in the emergence of antibiotic resistance, endangering human health by allowing pathogenic bacteria to pick up numerous resistance genes in a single transfer event [5, 10].

Microbiology relies heavily on the study of plasmids because, in addition to encoding genes for antibiotic resistance or virulence characteristics, plasmids can also act as identifiers for different bacterial strains when a system of typing known as plasmid profiling or plasmid fingerprint is applied [11]. This is performed following several procedures. First, deoxyribonucleic acid species that have been partially purified are separated by agarose gel electrophoresis based on molecular size. In the second method, restriction endonuclease-cleaved plasmid deoxyribonucleic acid can be separated by agarose gel electrophoresis, and the pattern of fragments that results can be used to confirm the identity of the bacterium. Plasmid profile typing has been used to track the inter- and intraspecies development of antibiotic resistance and look into outbreaks of numerous bacterial infections [10, 11].

The plasmids of multidrug resistant bacteria can be eliminated through curing, nevertheless. Plasmids are said to be "cured" when they undergo a mechanism that causes them to be lost during bacterial cell division [11–13]. Curing can also happen naturally through cell division or by administering physical and chemical agents like sodium dodecyl sulphate (SDS) to the cells. During the curing process, the plasmid that codes for antibiotics resistance is lost, thereby making the bacteria vulnerable to antibiotics attack. However, if the gene that codes for the antibiotic resistance is chromosomal, then the bacteria will retain its resistance after curing as curing does not remove the chromosomal gene [12].

The purpose of this study was to identify the plasmid profiles of multidrug-resistant bacteria from various clinical specimens in an effort to reduce the threat posed by the spread of pathogenic bacteria that are resistant to several medications and maintain a stronger public health system.

2. Materials and Methods

2.1. Source of Bacteria. The bacteria used for the study were sourced from the well-labelled stock cultures at the Microbiology Unit of the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka (formerly called Amaku General Hospital) in Anambra State, Nigeria. The stock bacteria were obtained from clinical specimens including ear discharge, sputum, wound swabs, urethral swabs, urine from catheters, urine, nose swabs, high vaginal swabs, stool, eye swabs, and blood that were taken from patients in various wards including the male medical ward (MMW), female medical ward (FMW), male surgical ward (MSW), female surgical ward (FSW), and outpatient.

2.2. Identification of Multidrug Resistance (MDR) Bacteria and Determination of Multidrug Resistance Index (MARI). The MDR characteristics of the bacterial organisms were discovered by observing their resistance pattern to at least 5 out of the 12 antibiotics ((Oxoid, England). These antibiotics include piperacillin-tozabactam (P 110 μg), erythromycin (ERY 15 μg), cefotaxime (GX 30 μg), ciprofloxacin (CIP 5 μg), azithromycin (AZN 15 μg), cefixime (ZEM 5 μg), ceftriaxone (CRO 45 μg), ofloxacin (OFX 5 μg), levofloxacin (LBC 5 μg), Augmentin (AUG, 30 μg), clindamycin (CD 2 μg), and gentamicin (CN10μg)) used in this study. The bacteria must be resistant to at least two antibiotics from each of the following classes: quinolones, cephalosporins, macrolides, lincosamides, penicillin, and aminoglycosides [14]. Additionally, the number of antibiotics to which each of the chosen bacterial organisms was resistant was divided by the total number of drugs tested to establish the Multidrug Resistance Index (MDRI) for each of the microorganisms [15]. After that, plasmid profiling and curing were done using MDR bacteria.

2.3. Culture Purification for Plasmid DNA Extraction. The purity of the bacterial cultures used for the test was ensured by subculturing each bacterium on the nutrient agar and MacConkey agar [16].

2.4. Plasmid DNA Extraction. In a 5 ml double-strength Mueller Hinton broth, multidrug-resistant bacteria were cultured for 72 h at 37°C. The cultures were collected into clearly marked Eppendorf tubes and centrifuged (10,000 × g) for 10 minutes to produce pellets in a microcentrifuge (Biofuge, Biotra Bio-trade Hecrus Sepatech Co. Ltd. USA). After gently decanting the supernatant, the cell pellets underwent a 5-minute vortexing process. Then, to lyse the bacteria cell pellet, 300 μl of Tris EDTA (TE) buffer and 150 μl of 3.0 M aqueous sodium acetate were added at pH 5.2. After another 10-minute centrifugation of the samples, the supernatant was transferred to a new Eppendorf tube and thoroughly mixed with 0.9 ml of 70% ethanol that had been cooled to -20°C in order to precipitate plasmid DNA. The supernatant was discarded after it underwent a second centrifugation for 10 minutes. The pellet was twice washed with 1 ml of 70% ethanol, air-dried for 10 minutes, resuspended in 20–40 μl of TE buffer, and kept at 40°C pending use [17].

2.5. Preparation of Gel for Electrophoresis. The gel casting apparatus was set up as instructed in the product manual. The comb was straight, and there were few millimetres of clearance between the bottom of the comb and the bottom of the gel tray. A 1.0% gel was created by dissolving 0.5 g of agarose in 50 ml of Tris Borate EDTA buffer (TBE). After

the agarose was completely melted, the agarose solution was gently swirled, and any translucent "flecks" of agarose that had not completely melted were kept an eye out for. The heat was then kept on after that until all flecks were removed. Before pouring, the agarose solution was allowed to cool to a temperature of between 50 and 55°C. Then, gently adding 2 μl of a 10 mg/ml EtBr solution (per 50 ml gel), the liquid was swirled to mix, and then it was carefully placed into a gel tray. The comb was gently taken out of the opaque gel after it had solidified for 20 minutes. In the gel electrophoresis tank, the gel carrier was inserted after being withdrawn from the pouring tray. The electrophoresis tank was filled with 250 ml of 1X TBE until the gel was completely submerged. Air bubbles trapped in the sample well were carefully dislodged with a pipette as air bubbles will exclude the buffer and make the wells challenging to fill with the sample [18].

2.6. Plasmid DNA Sample Electrophoresis. A 50 μl sample of DNA and 3 μl of loading dye were combined using a micropipette, and the solutions were then gently mixed by pipetting the solutions up and down [19]. One sample per well was carefully inserted into the gel wells, which were then placed on the gel box at the electrophoresis machine's negative charge end. The agarose containing the sample DNA was sealed by the addition of buffered water, which also served as an electrolyte by directing the current and the sample plasmid DNA towards the positive end for 2 h at a voltage of 63 Volts. The standards and sample DNA were taken from the agarose and allowed to drain off. A photo displaying the size and movement of the sample plasmid DNA was captured using UV light, UV-certified safety glasses, and a camera.

2.7. Determination of Molecular Weight of the Bacterial Plasmids. This was done for all the extracted bacterial plasmids. The bacterial plasmid size was determined by using the distance of migration of the bands (plasmid unit) for each bacterium and matching it with the value of the marker in the standards. The standard plasmids' molecular weight, coupled with the distance travelled and the log of their molecular weight, respectively, has been represented (see Table 1). To do this, a plot of the log of known molecular weights of the standard plasmids (y) against the distance travelled (x) by the plasmids (mobility values) on the gel gave rise to a linear equation, $y = -0.0329x + 4.747$. This linear equation equivalent to $y = mx + b$, was used to determine the unknown molecular weights of the bacterial plasmids. As represented by the equation, $y = \log mw$, $m =$ slope, $x =$ mobility, and $b =$ intercept on y. Since y is equivalent to logmw, the antilog of y ($mw = 10^y$) gave the molecular weight of the bacterial plasmids.

2.8. Plasmid Curing Using Sodium Dodecyl Sulphate. Plasmid curing was performed on organisms resistant to a minimum of 5 antibiotics using a subinhibitory concentration of 10% sodium dodecyl sulphate (SDS) and according to a method by Sijhary et al. [20]. A 4.5 ml of nutrient broth was inoculated with overnight broth culture. A 0.5 ml SDS (10% concentration) was added, and the mixture was incubated at 37°C for 48 h. A freshly made 4.5 ml nutrient broth was supplemented with 0.5 ml of the broth and incubated at 37°C for an additional 24 h.

Table 1: The plasmid's molecular weight determination.

The molecular weight of standard (bp)	Distance moved by the standard (mm)	Log of molecular weight of standard
20000	15	4.30
15000	18	4.18
10000	20	4.00
8000	25	3.90
6000	30	3.80

2.9. Postcuring Susceptibility Test. This was done by following a standard method [19, 21]. An antibiotic susceptibility test was carried out on each bacterium. The suspension was uniformly spread onto Mueller-Hinton agar. The antimicrobial impregnated discs were placed using sterile forceps on the agar surface, and the plates were incubated at 37°C for 24 h.

3. Results and Discussion

3.1. Results

3.1.1. Distribution of Bacteria in Different Clinical Specimens. The distribution of bacterial isolates in various clinical specimens is displayed (see Table 2). Each organism's total number, together with the number and percentage of isolates from each type of specimen, are shown (see Table 2).

3.1.2. Isolated Bacterial Distribution by Patients in Various Hospital Wards. The distribution of isolated bacteria is depicted in accordance with samples taken from patients in various hospital wards (see Table 3). The number and percentage of the eight different bacterial species from eleven different specimens collected from patients in nine wards are displayed (see Table 3).

3.1.3. Distribution of MDR Bacteria. Following the susceptibility testing, 42 (4.88%) out of the eight hundred and sixty (860) bacteria from different clinical specimens were multidrug-resistant (see Table 4).

3.1.4. The Multidrug Resistance Profile and Multidrug Resistance Index of the Bacteria. The bacterial multidrug resistance profile and multidrug resistance index are shown (see Table 5). The average MDRI was 0.6, while the lowest MDRI was 0.5. The peak MDRI (0.8) was exhibited by *S. aureus*.

3.1.5. Molecular Weights of the Extracted Bacterial Plasmids. The plasmid samples of the fifteen (15) selected multidrug-resistant organisms were subjected to electrophoresis. The plasmid samples on gel electrophoresis are shown (see Figure 1). Lane i is for the standards used, while the test bacterial plasmids occupy lanes ii-xvi. Bacterium on lane iii has

Table 2: Distribution of bacteria in different clinical specimens.

Specimen type	*S. aureus* 134	*CoNS* 71	*E. coli* 179	*P. aeruginosa* 197	*S. pyogenes* 53	*K. pneumoniae* 106	*S. typhi* 79	*Proteus spp* 41	Total 860 (%)
Urine	28 (3.26)	19 (2.21)	22 (2.56)	27 (3.14)	15 (1.74)	22 (2.56)	0 (0)	6 (0.70)	139 (16.16)
Nasal swab	10 (1.60)	10 (1.60)	12 (1.40)	2 (0.23)	1 (0.12)	3 (0.35)	0 (0)	0 (0)	38 (4.44)
U/S	4 (0.47)	1 (0.12)	16 (1.86)	5 (0.58)	2 (0.23)	11 (1.28)	0 (0)	4 (0.47)	43 (5.00)
HVS	19 (2.21)	7 (0.81)	17 (1.98)	17 (1.98)	6 (0.70)	17 (1.98)	0 (0)	6 (0.70)	89 (10.35)
Catheter (urine)	9 (1.04)	8 (0.93)	6 (0.70)	42 (4.88)	3 (0.35)	6 (0.70)	0 (0)	1 (0.12)	75 (8.72)
Blood	20 (2.33)	2 (0.23)	7 (0.81)	7 (0.81)	10 (1.60)	15 (1.74)	20 (2.33)	5 (0.58)	86 (10.00)
Eye swab	12 (1.40)	6 (0.70)	7 (0.81)	7 (0.81)	5 (0.58)	5 (0.58)	0 (0)	0 (0)	42 (4.88)
Stool	0 (0)	0 (0)	27 (3.14)	0 (0)	0 (0)	0 (0)	59 (6.86)	0 (0)	86 (10.00)
Sputum	14 (1.63)	3 (0.35)	19 (2.21)	10 (1.60)	3 (0.35)	10 (1.60)	0 (0)	10 (1.60)	69 (8.02)
Ear swab	6 (0.70)	3 (0.35)	5 (0.58)	29 (3.37)	4 (0.47)	15 (1.74)	0 (0)	8 (0.93)	70 (8.14)
Wound	12 (1.40)	12 (1.40)	14 (1.63)	51 (5.93)	4 (0.47)	2 (0.23)	0 (0)	1 (0.12)	123 (14.30
Total	134 (15.58)	71 (8.26)	179 (20.12)	197 (22.91)	53 (6.16)	106 (12.33)	79 (9.18)	41 (4.77)	860 (100)

Table 3: Isolated bacterial distribution by patients in several hospital wards.

Bacterial Isolate	Total No (%)	MMW No (%)	FMW No (%)	AW No (%)	FSW No (%)	MSW No (%)	OPD No (%)	CW No (%)	EW No (%)	PNW No (%)
S. aureus	134 (15.58)	31 (3.60)	28 (3.26)	7 (0.81)	4 (0.47)	11 (1.28)	16 (1.86)	19 (2.21)	13 (1.51)	5 (0.58)
CoNS	71 (8.26)	14 (1.62)	12 (1.40)	14 (1.62)	12 (1.40)	4 (0.47)	4 (0.47)	1 (0.12)	8 (0.93)	2 (0.23)
E. coli	179 (20.81)	29 (3.37)	28 (3.26)	11 (1.28)	19 (2.21)	18 (2.09)	19 (2.21)	27 (3.14)	20 (2.33)	8 (0.93)
P. aeruginosa	197 (20.91)	37 (4.30)	37 (4.30)	7 (0.81)	8 (0.93)	27 (3.14)	32 (3.72)	9 (1.05)	20 (2.33)	20 (2.33)
S. pyogenes	53 (6.16)	15 (1.74)	11 (1.28)	1 (0.12)	0 (0.00)	10 (1.16)	10 (1.16)	1 (0.12)	3 (0.35)	2 (0.23)
S. typhi	79 (9.19)	17 (1.98)	15 (1.74)	1 (0.12)	0 (0.00)	14 (1.63)	14 (1.63)	2 (0.23)	9 (1.05)	7 (0.81)
Proteus spp	41 (4.76)	7 (0.81)	8 (0.93)	2 (0.23)	2 (0.23)	4 (0.47)	7 (0.81)	0 (0.00)	4 (0.47)	7 (0.81)
K. pneumonia	106 (12.33)	30 (3.49)	23 (2.67)	0 (0.00)	0 (0.00)	17 (1.98)	22 (2.56)	1 (0.12)	11 (1.28)	2 (0.23)
Total	860 (100)	180 (20.99)	162 (18.84)	43 (5.00)	45 (5.23)	105 (12.21)	124 (14.42)	60 (6.98)	88 (10.22)	53 (6.16)

KEY: Male medical ward (MMW), female medical ward (FMW), male surgical ward (MSW), female surgical ward (FSW), antenatal ward (AW), postnatal ward (PNW), children's ward (CW), emergency ward (EW), outpatient department (OPD), cons = coagulase negative staphylococci, spp = species, no = number, % = percentage.

Table 4: Distribution of multidrug-resistant bacteria.

Name of bacteria	No. of multidrug resistant bacteria	% of organism with MDR bacteria	No. of antibiotics resistant to bacteria
P. aeruginosa	14	33.33	6-7
S. aureus	14	33.33	6-7
E. coli	9	21.43	6-8
K. pneumonia	5	11.91	6-7
Total	42	100%	

a plasmid weight of 22.490 kbp, while bacteria on lanes vi and vii have the same plasmid weight of 20.844 kbp. The bacteria on lanes xii and xiv have a common plasmid weight of 161.435 kbp, while the bacteria on lanes xiv, xv, and xvi have a similar plasmid weight of 187.499 kbp. Bacteria on lanes ii, iv, v, viii, ix, x, and xi showed no plasmid band.

The molecular weight of plasmids, the bacteria, the specimen were sourced from, and the wards where the patients who gave the samples were admitted to the hospital are shown (see Table 6).

3.1.6. The Drug-Resistant Pattern of the Selected MDR Bacteria before and after Plasmid Curing. The drug-resistant pattern of the selected MDR bacteria before and after plasmid curing is shown (see Table 7). The plasmids on lanes iii, vi, vii, xii, xiii, xiv, xv, and xvi that conferred the bacteria resistant to the listed antibiotics were cured of the plasmid after treating them with 10% SDS, thereby making them susceptible to those antibiotics. However, the bacteria with plasmids on lanes ii, iv, v, viii, ix, x, and xi were resistant to the above-listed antibiotics and maintained their resistance totally or partially to the same drugs after curing.

4. Discussion

Infectious pathogens' multidrug resistance is a serious risk and a barrier to finding the appropriate chemotherapy. Understanding the resistance pattern is therefore essential

Table 5: MDR profiles of bacteria and their MDRI.

S/no	Bacteria	MDR profile	MDRI
1	*S. aureus*	LBC-P-ERY-AUG-CIP-CD-CRO	0.6
2	*S. aureus*	GX-CRO-ZEM-LBC–CIP–AZN–P	0.6
3	*S. aureus*	ZEM–CIP-CN-CD-P- OFX AUG–CN	0.6
4	*S. aureus*	CIP–AZN–CD-CN–P-LBC CRO–ERY	0.7
5	*S. aureus*	ZEM–CD-CIP–OFX–P–CN–LBC–CRO	0.7
6	*S. aureus*	ZEM-ERY–AZN-P–CIP–OFX	0.5
7	*S. aureus*	CN-CD-OFX–P-LBC–ERY-GX	0.6
8	*S. aureus*	LBC–CN–AZN–ERY CIP–P–OFX	0.6
9	*E. coli*	LBC–AZN–ERY-CD–CIP–P–OFX	0.6
10	*E. coli*	CIP–LBC–ERY–P–CD–OFX	0.5
11	*E. coli*	LBC–P–GX–CN-OFX-AZN ERY–CRO	0.7
12	*E. coli*	CRO–ERY–P-LBC–OFX–AZN–AUG-ZEM	0.7
13	*E. coli*	ZEM–LBC–CIP–CRO–P–CN	0.5
14	*E. coli*	AZN–LBC–ERY–P–CIP–CRO	0.5
15	*E. coli*	CD–CN–GX–P–CIP–CRO	0.5
16	*E. coli*	OFX–CD–CN–CRO–AZN AUG–LBC	0.6
17	*E. coli*	OFX–CD–CN–AZN–AUG–LBC	0.5
18	*S. aureus*	P–GX–CD–CN–AZN–OFX–AUG–CIP	0.7
19	*S. aureus*	CD–AZN–AUG–CRO–CIP–GX–LBC–ERY–P	0.8
20	*S. aureus*	GX–CD–CN–OFX–CIP–CRO–LBC-ERY	0.8
21	*S. aureus*	AZN–GX–CD–CN–CRO–CIP	0.5
22	*K. pneumoniae*	ZEM-LBC-CIP-ERY-CN-CD-GX-CRO	0.7
23	*K. pneumonia*	ERY-CRO-AZN-P-CIP-LBC-CD	0.6
24	*K. pneumoniae*	LBC-CN-P-CIP-OFX-CRO	0.5
25	*K. pneumonia*	CRO-CN-GX-CIP-OFX-P	0.5
26	*K. pneumoniae*	AZN-P-CIP-CN-GX-CRO	0.5
27	*P. aeruginosa*	AZN-P-CIP-CN-GX-CRO	0.5
28	*P. aeruginosa*	AZN–CRO–OFX–P–LBC–CIP	0.5
29	*P. aeruginosa*	ZEM–P–CIP–OFX–AZN–AUG	0.5
30	*P. aeruginosa*	P–CIP–OFX–CRO–LBC–CN–GX	0.6
31	*P. aeruginosa*	GX–ZEM–P–CIP–CN–CRO LBC	0.6
32	*P. aeruginosa*	ZEM–CRO–LBC–P–CIP–ERY	0.5
33	*P. aeruginosa*	CN–P–CIP–OFX–LBC–ERY	0.5
34	*P. aeruginosa*	CN–P–CIP–OFX–LBC	0.5
35	*P. aeruginosa*	AZN–CRO–GX–ZEM–CD–AUG	0.5
36	*P. aeruginosa*	P–CIP–AZN–ERY–AUG–OFX	0.5
37	*P. aeruginosa*	P–CIP–AZN–ERY–LBC–AUG	0.5
38	*P. aeruginosa*	LBC–AUG–CN–OFX–CD–AZN	0.5
39	*P. aeruginosa*	ZEM–LBC–CD–AUG–OFX–CN	0.5
40	*P. aeruginosa*	GX–CRO–CIP–LBC–ERY–CN-OFX	0.6
41	*S. aureus*	CRO–CIP–LBC–GX–P–AZN–CD	0.6
42	*S. aureus*	CN–CRO CIP-LBC-GX–P–CD	0.6

Key: MDR = multidrug resistant, MDRI = multidrug resistant index, piperacillin-tazobactam = P, cefixime = ZEM, erythromycin = ERY, Augmentin = AUG, cefotaxime = GX, levofloxacin = LBC, azithromycin = AZN, gentamicin = CN, ceftriaxone = CRO, cipfloxacin = CIP, clindamycin = CD, ofloxacin = OFX.

to fighting the battle against bacterial drug resistance. This research on plasmid profiling of multidrug-resistant bacteria from different clinical specimens was conducted at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka. Different isolated bacteria from various clinical samples were selected, and the multidrug-resistant ones were confirmed before bacterial plasmid extraction, analysis, and curing using SDS.

In this investigation, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Coagulase Negative*

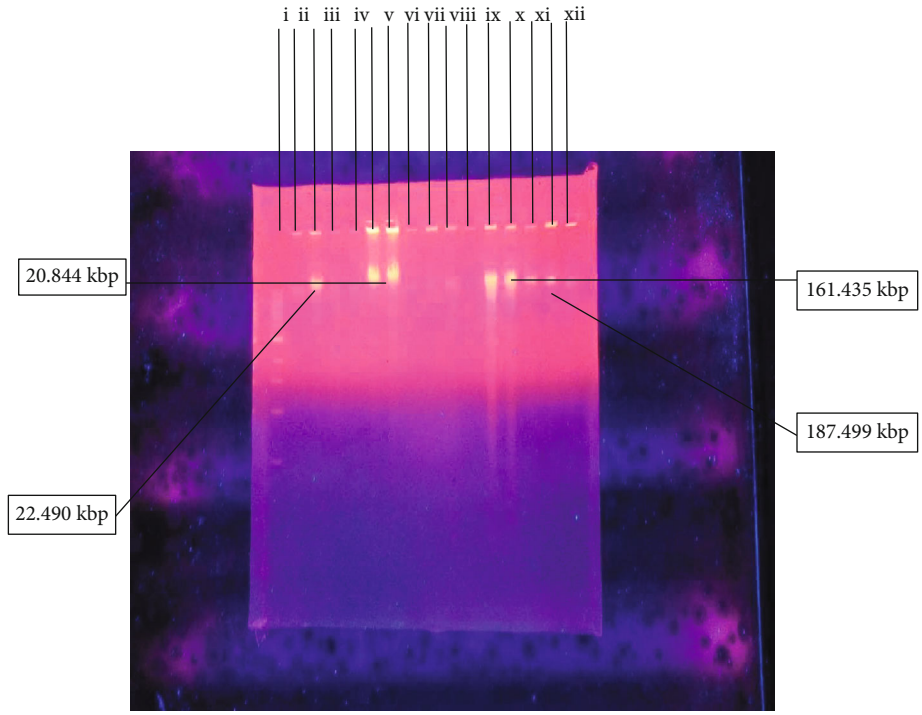

Figure 1: Plasmid lanes on gel. Gel showing the distance travelled by the loaded plasmids following electrophoresis.

Staphyloccocus (CoNS), *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Proteus spp* were among the numerous bacteria recovered from the varied clinical samples (see Table 2). *P. aeruginosa* had the highest prevalence rate in the examined clinical samples (22.91%), followed by *E. coli* (20.81%), *S. aureus* (15.58%), *K. pneumonia* (12.33%), *S. typhi* (9.18%), CoNS (8.26%), and *Proteus* spp. (4.77%) (see Table 2). Some previous studies, [22–24], which isolated *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *Proteus* spp, had reported a similar trend. A study by Huai et al. [25] almost followed a similar pattern except for *S. aureus* which had higher occurrence than *P. aeruginosa* and *E. coli*. Also, a report showed that the most prevailing bacterial isolates from their study were *E. coli* and *S. aureus* [26]. It is common knowledge that *S. aureus* and *E. coli* can coexist as healthy skin flora, but they can also become contagious when there is a break in the skin due to a wound or surgery, or if the immune system is weak or suppressed [27, 28].

According to the distribution of bacteria isolates among the various specimens, the urine sample had the most isolates, totaling 139 (16.16%), followed by wounds (123 (14.30%)), HVS (89 (10.35%)), stool (86 (10.00%)), blood (86 (10.00%)), catheter (urine) (75 (8.72%)), ear swab (70 (8.14%)), sputum (69 (8.02%)), U/S (43 (5.00%)), eye swab (42 (4.88%)), and nasal swab (38 (4.44%)) [see Table 2]. This is in line with the findings of Amsalu et al. [29], who found that the frequency of bacteria in urine was 44.3%. According to Fahim's [30] analysis of data from another study conducted in Egypt, the majority of pathogens were discovered in blood cultures (44.84%), followed by urine (41.41%), and wound swabs (13.75%). The bacterial frequency seen in different samples in our study could be attributed to the relative sample sizes in response to the demands for clinical studies. The surroundings, patient conditions, personal hygiene, the number of patients involved in each study, the source of the sample, and laboratory techniques, among others, can all have an effect.

Further look into the result [see Table 2] reveals the frequency of specific isolates from the various samples. *S. aureus* had the highest number of 28 (3.26%) in the urine sample. This was closely followed by *P. aeruginosa* (27 (3.14%)). Both *K. pneumoniae* and *E. coli* contained 22 (2.56%) isolates. This is consistent with the findings of Ekwealor et al. [31] who showed that *S. aureus* was the most common urine pathogen to be isolated in Awka, followed by *E. coli*. They continued by pointing out that earlier research had connected the rise in *Staphylococcus* urinary tract infections (UTIs) to an increase in the use of instruments like bladder catheterization. The reported high percentage of *Staphylococcus* differed from other earlier studies that were published and identified *E. coli* to be the main pathogen of the urinary tract [31]. This is in contrast to the findings of Amsalu et al. [29], who said that between January 2012 and December 2014 in Southern Ethiopia, the most common isolate from urine was *E. coli* (42.9%), followed by *K. pneumoniae* (12.7%), and *S. aureus* (12.7%). However, the difference in geographic location and period of investigation may be the reason for this departure from the findings of our study.

Other urinary tract samples had the followings [see Table 2]. HVS had *S. aureus* (19 (2.21%)) and 17 (1.98%) isolates of *P. aeruginosa* and *E. coli* each. Urethral swab had *E. coli* (16 (1.86%)), *K. pneumoniae* (11(1.28%)), and *S. aureus* (4 (0.47%)). *P. aeruginosa* (42, 4.88%),

Table 6: Molecular weight of the bacterial plasmids.

Lane in gel	Molecular weight (kbp) band 1	Bacteria	Sample	Ward
ii	55.847	*S. aureus*	U/S	OPD
iii	22.490	*S. aureus*	Urine (catheter)	MMW
iv	55.847	*S. aureus*	Urine	MMW
v	55.847	*P. aeruginosa*	Wound	FSW
vi	20.844	*P. aeruginosa*	Wound	MSW
vii	20.844	*P. aeruginosa*	Wound	MSW
viii	55.847	*P. aeruginosa*	Wound	FSW
ix	55.847	*P. aeruginosa*	Urine (catheter)	MSW
x	55.847	*E. coli*	Wound	MSW
xi	55.847	*E. coli*	Urine	OPD
xii	161.435	*E. coli*	Urine	MMW
xiii	161.435	*K. pneumonia*	Urine	MMW
xiv	187.499	*K. pneumonia*	HVS	FSW
xv	187.499	*K. pneumonia*	HVS	FSW
xvi	187.499	*K. pneumonia*	HVS	FSW

Key: Male medical ward (MMW), female medical ward (FMW), male surgical ward (MSW), female surgical ward (FSW), and outpatient department (OPD).

Staphylococcus aureus (9, 1.04%), and *Escherichia coli* (6, 0.70%) were all found in the urine collected via the catheter. These findings are consistent with those of Kolawole et al. [32], who said that *E. coli*, *S. aureus*, *Proteus species*, *K. pneumonia*, and *P. aeruginosa* are the most prevalent pathogenic organisms of urinary tract infections (UTI).

P. aeruginosa was the most common isolate among bacteria isolated from urinary tract samples. The least number of isolates were *S. aureus*, *E. coli*, *K. pneumoniae*, *CoNS*, and *Proteus* spp. [see Table 2]. This is consistent with the findings of Mohammed et al. [33], who indicated that the predominant bacteria they isolated for their study were *E. coli* and *S. aureus*. *S. aureus* and *E. coli* were the two bacteria that were most commonly isolated, according to Amin et al. [34]; however, *E. coli* has consistently been the isolate that causes urinary tract infections in similar research. Also, this is in accordance with the findings of Demilie et al. [26], who observed that *E. coli*, which accounted for 90% of the cases in their study, is the main etiologic agent causing urinary tract infections. According to Rachid et al. [35], the urinary tract (42.9%) was the site of infection most frequently impacted by *Staphylococcus*, followed by *E. coli*, and *K. pneumoiae*. The highest frequency of all isolates in this investigation, however, was found in the urinary tract samples (Urine 16.16%, HVS 10.35%, U/S 5.00%, and urine from catheter 8.72%). The report of Unegbu et al. [36] stating that urinary tract infection has the greatest prevalence of 36.33% is directly related to this. The high occurrence of urinary tract infections may be caused by unsanitary hospital and restroom conditions.

P. aeruginosa (51 (5.93%)) was the most prevalent isolate in the wound sample out of a total of 123 (14.30%) isolates [see Table 2]. Then came *CoNS* (12 (1.40%), *S. aureus* (12 (1.40%), and *E. coli* (14 [4.7%]). This is consistent with the findings of Motayo et al. [37], who suggested that an endogenous infection source may be to blame for the high prevalence of *P. aeruginosa* and *S. aureus* infections. Environmental contamination may also be the cause of these organisms' infections. *S. aureus* may easily enter wounds due to the breakdown of the natural skin barrier. According to Basu et al. [38], the relatively high number of *P. aeruginosa* isolates is indicative of a high incidence of nosocomial infections, particularly in hospitalised patients. This raises the issue of the necessity of strict infection control procedures and good hygiene, such as frequent hand washing and the sterilization of wound care providers' tools. According to the research by Ehiaghe et al. [39], surgical wounds exposed to numerous pervasive environmental infections, such as unsterile surfaces, water, and dirt, had the largest number of clinical isolates of *P. aeruginosa* in their wound swabs. In contrast, Shittu et al. [40] observed that *S. aureus* was the most common etiologic agent of wound infection in numerous healthcare institutions in their research area after microbiological examination of wound sample.

From blood samples, 86 bacteria in all (10.00%) were recovered. There are several of them, including *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *CoNS*, *S. pyogenes*, and *K. pneunomiae*. As a consequence, *S. aureus* and *S. typhi* had the highest number of isolates (20 each, or 2.33%), followed by *K. pneumoniae* (15, or 1.74%), and *S. pyogenes* (10, or 1.60%), according to the results [see Table 2]. This is closely similar to the study by Mehta et al. [41] which revealed that the most frequent organisms linked to septicemia include *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *CoNS*, and *K. pneunomiae*. On the other hand, Alam et al. [42] revealed that the main septicemia-causing organisms were gram-positive pathogens, coagulase-negative staphylococci, *S. aureus*, and gram-negative *S. typhi*. This is supported by a research by Falagas et al. [43], which found that coagulase-negative staphylococci are the most common cause of septicemia. However, *S. typhi* was identified as the main pathogen by Onile et al. [44]. According to Taiwo et al. [45], this variation may be due to the selection of drug-resistant bacteria isolates that were better adapted for survival, which has changed the etiology of blood stream infection.

Our research showed that only two bacteria were recovered from 86 (10.00%) isolates found in stool samples (see Table 2). These are *E. coli* (27) and *S. typhi* (59, 6.86%). This was somewhat supported by the findings by Amsalu et al. [29], who isolated only *S. typhi* (41.70%) and *Shigella* spp. (58.39%).These bacteria are primary intestinal commensals present in endothermic animals [46].

Nasal swab samples produced 38 (4.44%) bacterial isolates [see Table 2]. These comprised *P. aeruginosa* (2

Table 7: Drug-resistant pattern of some multidrug-resistant bacteria before and after plasmid curing.

Bacteria lane	Resistant profile before curing	Resistant profile after curing
S. aureus ii	GX-CRO-ZEM-LBC-CIP-AZN-P	GX-ZEM-LBC-P
S. aureus iii	ZEM-CIP-CN-CD-P-OFX-AUG-GX	NIL
S. aureus iv	CIP-AZN-CD-CN-P-LBC-CRO-ERY	CIP-AZN-CD-CN-P-LBC-CRO-ERY
P. aeruginosa v	AZN-P-CIP-CN-GX-CRO	AZN-P-CIP-CN-GX-CRO
P. aeruginosa vi	AZN-CRO-OFX-P-LBC-CIP	NIL
P. aeruginosa vii	ZEM-P-CIP-OFX-AZN-AUG	NIL
P. aeruginosa viii	P-CIP-OFX-CRO-LBC-CN-GX	P-CIP-OFX-CRO-LBC-CN-GX
P. aeruginosa ix	GX-ZEM-P-CIP-CN-CRO-LBC	GX-ZEM-P-CIP-CN-CRO-LBC
E. coli x	LBC-AZN-ERY-CD-CIP-P-OFX	LBC-AZN-ERY-CD-CIP-P-OFX
E. coli xi	CIP-LBC-ERY-P-CD-OFX	ERY-P-CD-OFX
E. coli xii	LBC-P-GX-CN-OFX-AZN-ERY-CRO	NIL
K. pneumoniae xiii	REM-LBC-CIP-ERY-CN-CD-GX-CRO	NIL
K. pneumoniae xiv	ERY-CRO-AZN-P-CIP-EBC-CD	NIL
K. pneumoniae xv	LBC-CN-P-CIP-OFX-CRO	NIL
K. pneumoniae xvi	CRO-CN-GX-CIP-OFX-P	NIL

KEY: SDS = Sodium dodecyl sulphate, NIL = No resistance drugs, piperacillin-tazobactam = P, cefixime = ZEM, erythromycin = ERY, Augmentin = AUG, cefotaxine = GX, levofloxacin = LBC, azithromycin = AZN, gentamicin = CN, ceftriaxone = CRO, cipfloxacin = CIP, clindamycin = CD, ofloxacin = OFX.

(0.23%), *S. aureus* (10 (1.60%), *K. pneumoniae* (3 (0.35%), and *E. coli* (12 (1.40%). This is consistent with the findings of the article by Amsalu et al. [29], which demonstrated the isolation of *Proteus* spp. (8.3%), *K. pneumonia* (58.3%), *P. aeruginosa* (8.3%), and *E. coli* (16.7%).

Our findings on the relationship between the different hospital wards and the bacterial is shown [see Table 3]. According to the results, the male medical ward (MMW) had the most isolates, 180 (20.99%), followed by the female medical ward (FMW) and the outpatients department (OPD), which had 162 (18.84%) and 124 (14.42%), respectively, isolates. The antenatal ward (AW) has the fewest isolats, with 43 (5.00%). In general, the surgical wards (FSW and MSW) had a total of 150 (17.44%) isolates, which was followed by the medical wards (MMW and FMW), which had the highest number of isolates (342 (39.77%)). These results are consistent with those of Rachid et al. [35], who said that the medical and surgical wards, with 392 (32.8%) and 379 (31.7%) infections, respectively, had the largest number of infections. But according to Lizioli et al. [47], the rate of surgical site infections appears to be higher than what is generally reported around the world.

All the isolated bacteria were subjected to the effects of different antibiotics. A total of 42 (4.88%) out of the 860 bacteria from different clinical specimens were multidrug (6-8 antibiotics) resistant (see Table 4). *P. aeruginosa* and *S. aureus,* with fourteen (14 (33.33%)) each, had the highest number of drug resistance, to 6-7 of the twelve (12) antibiotics used. Also, nine (9 (21.43%)) *Escherichia coli* were resistant to 6-8 of all the drugs used, while five (5 (11.90%)) *K. pneumoniae* were resistant to 6-7 antibiotics used. In comparison to the results of this investigation, a prior study on the bacterial profile and multidrug (>4 drugs) resistance pattern of bacterial isolates among probable cases of septicemia in Ethiopia showed a multidrug resistance rate of 74.2% [48]. Similarly, in another study in Ghana and Nigeria [49, 50], *P. aeruginosa, E. coli*, and *K. pneumonia* were reported to be among the Gram-negative multidrug-resistant bacterial pathogens. Different studies have also reported *P. aeruginosa*, S. *aureus*, *E. coli*, and *K. pneumonia* are the most frequently reported multidrug resistant isolates [51, 52]. The environment's inappropriate and unregulated use of antibiotics as well as inadequate infection control practices may be to blame for the multidrug resistant bacteria. It has been stated that in order to stop the establishment of new resistant strains, it is imperative to analyse resistant isolates using susceptibility testing given the overuse of antibiotics in hospitals and the continued growth in antibiotic resistance [53]. It is crucial to remember that resistance patterns may differ from region to region due to genetic mutation brought on by inappropriately given antibiotics.

The results of multidrug resistance profile and multidrug resistance index of the bacteria have been shown (see Table 5). The average MDRI was 0.6, while the lowest MDRI was 0.5. The peak MDRI (0.8) was exhibited by *S. aureus.* The prevalence of bacterial resistance in a particular community has reportedly been revealed by MDRI [50]. A study recorded *Streptococcus* spp. as the only isolate that gave MDRI below 0.20 while other isolates were above 0.20 [31]. However, our findings revealed that all 42 bacteria had a high MDRI index value above 0.2. When the MDRI index is greater than 0.2, it indicates that the organisms were isolated in an environment where antibiotics are misused. Additionally, a significant portion of the bacterial isolates have been exposed to multiple antibiotics and have subsequently developed resistance to these antibiotics [31, 54]. The results indicate that this is the case in this study.

The results of the plasmids analyses are shown (see Figure 1). As observed, some of the multidrug-resistant bacteria harbour varied molecular weights plasmids while others harbour no plasmid, indicating that the latter's resistance was chromosomally borne. This agrees with the report

that some of the multidrug-resistant isolates had plasmid band during electrophoresis while others did not, despite that they were all multidrug-resistant [22]. Plasmid mediated bacterial resistance has been widely reported [55–58]. Our study indicates that some of the multidrug resistant isolates harbored resistance plasmids which probably must have been acquired.

A comparison of plasmid sizes and numbers (see Table 6) shows that some of the bacteria have plasmid bands of the same number and sizes, suggesting that they are likely of the same origin, most probably in the same community or close to the hospital where this study was carried out. As they can be produced and selected by horizontal transfer, it has been proposed that multidrug-resistant plasmids could be acquired by susceptible bacteria during antibiotic treatment [4, 59]. The existence of drug resistance plasmids may also result from selective pressure brought on by the increased use and abuse of antimicrobial drugs, according to studies [2, 60]. However, other investigations suggested that, in addition to environmental factors, the development of resistance can be caused by chromosomal mutations or plasmids that can be transferred from one strain of an organism to another across species [61, 62]. As a result, the gene responsible for antibiotic resistance may be found on both chromosomal and plasmid DNA, or both. This has been supported by a report that some multidrug-resistant isolates without plasmid bands, following gel electrophoresis, indicated that their resistance might be chromosomal mediated since some remained resistant after curing using 10% sodium dodecyl sulphate [63]. Additionally, it has been noted that in some bacteria, the absence of a plasmid may not be sufficient proof to demonstrate that the trait is plasmid-encoded [64, 65] due to the fact that numerous plasmids are capable of integrating into the bacterial host genome.

A crucial technique for investigating the numerous properties of plasmids is plasmid curing. By acting on the bacterial membrane and creating pores there, SDS is mostly used to cure plasmids. From our findings, some bacteria were cured totally by the treatment with 10% SDS, while others were either partially cured or unaffected by the curing agent (see Table 7). Eight bacteria on lanes iii, vi, vii, xii to xvi were cured totally, two bacteria on lanes ii and xi were partially cured, while five others on lanes iv, v, viii, ix, and x were not affected by the curing agent as they remained resistant to those drugs after treatment. Given the high frequency of SDS in curing resistant bacteria, our study is in accordance to previous studies [66, 67]. The efficacy of curative agents against multidrug resistant bacterial isolates cannot be predicted because there are no standard methods that are relevant to all plasmids [64]. While some curing agents damage and disrupt the cells in a nonspecific manner, others act specifically [68]. It is understood that no plasmid curing agent is capable of removing all plasmids from various bacteria [65]. Given that SDS is the only curing agent employed in our investigation, this may help to explain the incomplete or no curing reported in some bacteria. In the future, it will be required to test a wide range of curing techniques before finding one that works well for each bacterium [64]. SDS has been identified as the most effective curing agent for *P. aeruginosa*'s resistant plasmid [66]. Resistant plasmid-containing cells may be more sensitive to SDS because of the plasmid-specific pili on the cell surface [64]. Through the pili necessary for aggregation, SDS can interfere with bacterial cell-to-cell transmission [69, 70]. By preventing the plasmid's conjugational transfer, this may limit the spread of antibiotic-resistant plasmid in the environment.

Furthermore, it should be highlighted that even while many plasmids are refractory or difficult to cure, this does not always suggest that the resistant trait is not encoded by the plasmid [64]. However, investigations have indicated that plasmid curing can improve antibiotic efficacy because of the greater susceptibility with post-plasmid curing compared to pre-plasmid curing [71, 72]. Given that plasmid-mediated resistance to many antimicrobial drugs has been shown [see Table 7], this claim is consistent with the findings of our study.

5. Conclusions

In order to establish the most appropriate empirical and definitive therapies, it is now important to update knowledge of the antibiotic susceptibility pattern of bacteria. This work on the plasmid profiling and the cure of multidrug-resistant bacteria from different clinical specimens revealed this. This study revealed that multidrug-resistant bacteria are widespread, and the majority of them had plasmids, which made it easier for the resistant genes to spread. To stop the spread of multidrug-resistant bacteria caused by plasmids, infection control must be strengthened. As the resistance seen in some bacteria was not just plasmid-mediated but chromosomally transmitted, there is also a requirement for stringent infection control procedures and adequate cleanliness. Due to the low frequency of resistant bacteria seen after curing, the study also showed that SDS is a suitable agent for curing a resistant plasmid. Being rapid, affordable, adequate in providing insights, and able to influence antibiotic management policies, this curative agent is particularly promising in the fight against antibiotic resistance. This study revealed that successfully treating a resistance plasmid can change the bacterial phenotype back to one that is susceptible. Therefore, greater research into this process and continuous monitoring are needed to drastically reduce bacterial resistance to routinely prescribed antibiotics.

Abbreviations

SDS: Sodium dodecyl sulphate
NIL: No resistance drugs
P: Piperacillin-tazobactam
ZEM: Cefixime
ERY: Erythromycin
AUG: Augmentin
GX: Cefotaxime
LBC: Levofloxacin
AZN: Azithromycin
CN: Gentamicin
CRO: Ceftriaxone

CIP: Ciprofloxacin
CD: Clindamycin
OFX: Ofloxacin
MMW: Male medical ward
FMW: Female medical ward
MSW: Male surgical ward
FSW: Female surgical ward
AW: Antenatal ward
PNW: Postnatal ward
CW: Children's ward
EW: Emergency ward
OPD: Outpatient department
TE: Tris EDTA
MDR: Multidrug resistance
MARI: Multidrug resistance index
COOUTH: Chukwuemeka Odumegwu Ojukwu University teaching hospital.

Data Availability

All the data relevant to this research are available in the body of the manuscript as supporting figure and tables. We do not have any ethical or legal consideration for not to make our data publicly available.

Ethical Approval

The ethical approval obtained from the Ethical Committee of COOUTH, Amaku, Awka, Anambra State, Nigeria. (Approval reference number: COOUTH/AA/VOL.067, Date: 10-11-2017)

Consent

Consent is not applicable.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

This work was carried out in collaboration among the authors. All authors contributed to the study conception and design. Material preparation and data collection were performed by CK, ELO, ETU, and IO. All authors analysed the data. ETU and ELO wrote the first draft of the manuscript. All authors discussed results of the experiments, edited, and approved the final version of the manuscript. All authors read and approved the final manuscript.

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