Original Research Article

Plasmid-Encoded Antibiotic Resistant Bacteria of Surgical Wound Isolates FromThree Hospitals in Akoko Land

ABSTRACT

The study evaluates a rapid and dependable method of identifying plasmid-encoded antibiotic resistance bacteria isolated from surgical wound samples of twenty-nine patients from three General hospitals in Akoko South West (Iwaro Oka), Akoko North East (Ikare), and Akoko North West (Irun),Ondo State, Nigeria, using standard microbiological techniques. Antibiotic sensitivity test (AST) was carried out, plasmid-encoded antibiotic resistance bacteria were determined and plasmids were cured. A total of seven bacteria were isolated, two were Gram-positive: *Staphylococcus aureus* and *Streptococcus viridians,*and five Gram-negative: *Enterobacter agglomerans, Klebsiella pneumoniae, Pseudomonas fluorescens, Morganella morganii biogrp 1* and *Serratia liquefaciens.* The total bacterial counts ranged from 4.3 x 10^4 to 9.9 x 10^4cfu/ml. The highest colony count (9.9 x 10^4 cfu/ml) was obtained from General Hospital, Iwaro-Oka, while the least (4.3 x 10^4 cfu/ml) was obtained from Irun General hospital. The AST result showed that most isolates were sensitive to some of the antibiotics within the range of 7 mm to 25 mm zones of inhibition, while only strains of *P. fluorescens* were resistant to ciprofloxacin, pefloxacin, streptomycin, chloramphenicol, amoxicillin, gentamicin, augmentin, sparfloxacin, tarivid, and septrin. Four out of the five resistant *P. fluorescens* strains had plasmid bands ranging from 2.27 kbp to 23.13 kbp molecular weight with thick bands. The plasmid-encoded antibiotic resistance bacteria were sensitive to the same antibiotics which were initially resistant. It was recommended that increased attention be paid to stricter infection control practices across the three local government areas. Health authorities should include profile epidemiology in infection control policies to detect resistance level of isolates and adopt effective methods of administration of antibiotics before widespread infection.

Keywords:Surgical wounds, resistance, plasmid-mediated, curing, multidrug

INTRODUCTION

Wound is any breach in the skin surface: trauma, accident, surgical operation or burns, which leads to bacterial infection following loss of skin integrity. It thus produces a moist, warm and nutritive environment that is conducive for colonization and proliferation of opportunistic and

pathogenic microorganisms (Bowler *et al*., 2001; Ohalete *et al.*, 2012). The common bacterial pathogens that are associated with wounds infections include both Gram positive and negative bacteria such as *Staphylococcus aureus, Escherichia coli, Streptococcus pyogens, Pseudomonas aeruginosa, Proteus* spp, *Streptococcus* sppand *Enterococcus* spp (Zhao *et al.*, 2010; Sani *et al.*, 2012).

Surgical wound infections are caused by microbial contamination of site or wound infection from surgical site within the convalescent period (Horan *et al*., 1992). All surgical wounds are contaminated by microbes, but in most cases, infection does not develop because innate host defenses are quite efficient in the elimination of contaminants. A complex interplay between host, microbial, and surgical factors ultimately determines the prevention or establishment of a wound infection, which subsequently results to prolong recovery, delay discharge and increase the cost of treatment for both the patients and health service (Quinn *et al*., 2009).This is a serious problem globally and particularly in Africa and other developing countries where inadequate resources and skilled personnel among others are contributing to the acquisition and spread of infections (Olowo-okere *et al.*, 2018).

Surgical wound infection (SWI) just as other healthcare-associated infections (HCAIs) is a major safety concern in hospitals. It tremendously impacts negatively on the patient's well-being as well as the health-care personnel and financial resources for managing the condition. Despite the numerous preventive measures recommended for its reduction, SWIs continue to occur among surgical patients with substantial increase in the cost of healthcare, prolonged hospitalisation and jeopardised health outcomes (Goyal *et al*., 2015). Prevention is indicative of the need to reduce the incident and severity of surgical wound infections in order to prevent the spread of wound infections to other patients, staff, visitors and the environment (Berrios –Torres *et al*., 2017). Antibiotics help both in the prevention and treatment of surgical wound infections such as use of prophylactic antibiotics. They are preferably narrow spectrum antimicrobials used to reduce the emergence of resistance and for covering the most likely wound contaminants microorganisms (Bratzler *et al*., 2013).

Plasmid-encoded antibiotic resistance encompasses most classes of antibiotics currently in clinical use therapy including cephalosporins, fluoroquinolones and aminoglycosides. Vancomycin resistance in Enterococci (VRE) is due to the presence of vanA gene cluster residing in Tn1546 transposon, which is carried on plasmids. Vancomycin resistant *S. aureus* (VRSA) resulted because of transfer of plasmids from VRE to MRSA. Tetracycline resistance in

Stapylococcus aureus, erythromycin resistance in *Enterococcus fecalis*, multidrug resistance in *S. aureus* are also plasmid-mediated. Resistance to penicillins and cephalosprorins in several members of enterobacteriaceae is by plasmid mediated extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases. Plasmid-encoded *Klebsiella pneumoniae* carbapenemases (KPCs) are now been found in a range of Enterobacteriaceae, including *Escherichia coli*, *Enterobacter* spp, *Citrobacter* spp, *Salmonella* spp, *Serratia marcescens*, *Proteus mirabilis,* and *Pseudomonas aeruginosa.* Beta-lactamase resistance to penicillin in *Haemophilus influenzae*, *Neisseria gonorrhoeae*, metallo-beta-lactamase (MBL) production in *Acinetobacter* spp and *Pseudomonas* spp, and tetracycline resistance in gonococci are other examples of plasmid-mediated antibiotic resistance (Ramirez *et al.*, 2014).

According to Dohem (2008), surgical wound infection (SWI) among many others is said to be an important cause of postoperative mortality, despite the use of prophylactics, antibiotics and other preventive measures due to the antibiotics resistance developed and its increasing alarming rate. Such has been reported by Al-Qurayshi *et al.* (2019) that in the readmission of a significant mortality risk and high burden on the health system. Skin derived microorganism such as *Staphylococcus aureus* and coagulase negative *Staphylococcus* are mostly implicated in SWI, and the antimicrobial resistance among these and other clinically important pathogens is an increasing problem (Dohem *et al*., 2013). Thus, the emergence of antimicrobial resistance in the hospital pattern has also presented a challenge in the provision of good quality patient care as these microorganisms continue to exhibit high resistance to single and multi-antimicrobials (Mengesha *et al*., 2019).

The objective of this work is to study the plasmid-encoded antibiotic resistance of bacterial isolates from surgical wound from three hospitals in Akoko land, Ondo state, Nigeria, with the hope that the alarming trend of resistance microorganism will be stopped, and the ever-increasing number of infections caused by multiple drug resistant organisms will be dealt with.'

MATERIALS AND METHODS

Sample Collection

A total of twenty-nine wound swabs were aseptically collected from General hospitals located in three local government areas: Akoko South-west, Akoko North-east and Akoko North-west in Ondo State, Nigeria, using the standard microbiological technique (Cheesebrough, 1984). Sterile cotton wool swabs were used to collect samples from surgical wound patients with the evidence

of infection.The sample bottles were then transported to the Microbiology laboratory of Adekunle Ajasin University, Akungba Akoko, Ondo State.

Preparation and Inoculation of Samples

The pour plate method of Collins and Lyne (2004) was used. The pour plate method was used for culturing. The swab stick used in collecting the wound sample was aseptically transferred carefully into each of the test tubes containing 9.0 ml of cooled sterilized water, each wound sample in different test tubes was mixed thoroughly to ensure dislodgement and even distribution of microorganisms into the suspended sterile water. A ten-fold serial dilution of each 1.0 ml homogenate was prepared. Exactly 1.0 ml of dilution factor 10^{-4} and 10^{-6} were inoculated into the sterile Petri dishes for culturing. Incubation was carried out at 37^{o}C for 24 hours for bacterial growth. Colonies were counted in order to obtain the total viable count; discrete colonies were purified by sub-culturing and growth was observed under the microscope and then characterized.

Enumeration of Microbial Colonies

The samples were diluted and plating of several dilutions were made in order to ensure that it yielded CFU/ml (colony-forming units per milliliter) in a very good range. Colony counting was carried out visually by counting the number of visible colonies that appeared on the plates in order to obtain the total viable count. The calculation of colony forming unit (CFU) per ml for the bacteria was based on the formula:

$$\text{CFU/ML} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{ml of sample suspension}}$$

Isolation and Characterization of Pure Isolates

After 24 hours of incubation, the bacterial colonies that developed on nutrient agar plates and Mannitol salt agar plates were sub-cultured by streaking each colony on the surface of freshly prepared nutrient agar plates. The plates were then incubated at 37°C for 24 hours. Distinct colonies were picked into nutrient agar slant and stored in the refrigerator for further use (Fawole and Oso, 2001).

Colonial Morphology

The shape, size, pigmentation, elevation and marginal characteristics and Gram staing of the bacterial species were examined on the agar plates after appropriate incubation periods.

Biochemical Tests

This test is done to differentiate the spore-formers from the non-spore formers. On a clean grease free slide, a smear of the isolate was made. It was later stained with 5% malachite green for ten minutes. The stain was washed off with distilled water, 0.5% aqueous safranin was used to counter stain for 15 minutes. This was washed off with distilled water and allowed to dry. Spore stained green while the body of bacterial stain red when viewed under the microscope, The biochemical reagents used included Beta Galactosidase, Arginine Dihydrolase, Lysine Decarboxylase, Omithine Decarboxylase, Citrate Desimmons, Hydrogen Sulphide, Urease, Tryptophane De Amminase, Indole, Voges Proskauer, Oxidase and Nitrogen Dioxide.

Microbact (24E) Kit Identification

The Microbat (24E) used consists of a microplate format of twenty-four substrates in a combination of both 12A and 12B strips. Preparation of 18 to 24 hours old culture of the organism to be identified, oxidase test was performed which must be negative or positive for 24E kit. The selected isolate colony was emulsified in saline, test strips were placed in a holding tray and the back seal was peeled and addition of four drops of bacterial suspension to each well, the addition of two drops of mineral oil to the wells, the seal was replaced and incubated at 37°C for 18 to 24 hours, the tray was removed from the incubator and appropriate reagents were added. The result was then recorded and interpreted using the MicrobactTM identification software package (Balows *et al*., 1991). A parity check was performed by inoculating a purity plate with one drop of bacteria suspension and incubating at 37°C for 24 hours. Well 13 were read at 24 to 48 hours for Enterobacteriaceae and at 48 hours for miscellaneous Gram-negative bacilli (MGNB). Well, 24 was interpreted differently at 24 and 48 hours. A nitrate reduction test was done on well 7 after reading the O-nitrophenyl-beta-D-galactose ranoside (ONPG) reaction. Performance was monitored by testing appropriate control strains (Oxoid Limited)

Antibiotics Susceptibility Testing

The antibiotics used and their corresponding concentrations are as follows: ciprofloxacin-30µg, amoxicillin-5µg, septrin-30µg, rocephin-30µg, gentamicin-30µg, zinnzce-30µg, streptomycin-30µg, erythromycine-5µg, ampiclox-30µg, cephalexin-10µg and pefloxacin-10µg for Gram-positive organism and ciprofloxacin-30µg, septrin-30µg, amoxicillin-5µg, augmentin-10µg, streptomycin-30µg, chloraphenicol-30µg, gentamicin-30µg tarivid-10µg, pefloxacin-10µg, sparyloxacin-5µg, cephalexin-10µg were used on Gram-negative organisms.The plate diffusion technique of Williamson *et al*. (2017) was used. Overnight cultures of the organisms were

swabbed on sterile Muller Hilton solidified Agar plates using sterile swab sticks. The multiple antibiotic discs were placed on agar surface and pressed using sterile forceps to ensure complete contact with the agar. All the plates were incubated at 37^0C for 24 hours. The zones of inhibition were measured at the point at which an obvious demarcation between growth and no growth could be seen using a meter rule. The zones of inhibition were measured and interpreted according to (NCCLS, 2000).

Plasmid Extraction and Profiling

The previously selected five *Pseudomonas fluorescens* isolates recovered from surgical wounds that showed resistance to multidrug (pefloxacin, tarivid, streptomycin, septrin, chlorophenicol, sparyloxacin, amoxicillin, ciprofloxacin) were used. The method of Mbim *et al*. (2016) and Munita and Arias (2016) were used, and the cultures were inoculated on TBS and incubated at 37^oC for 24 hrs. Half of one milliliter (0.5ml) of the culture were transferred into a microfuge tube and same volume of phenol: chloroform: isoamyl-alcohol (25:24:1) was added. The phenol was saturated with Tris EDTA buffer (10Mm Tris, 1mM EDTA with final pH 7.5) before mixing with chloroform and isoamylalcohol. The microfuge tube containing mixtures was vortexed speed and tubes were centrifuged at 12,000 rpm for 5 minutes, then upper aqueous phase (0.45ml) leaving the interphase intact was collected in another microfuge tube containing 0.5ml isopropanol. The microfuge tubes were mixed well and centrifuged immediately at 12,000 rpm for five minutes. The supernatant was discarded, 70% ethanol (0.5ml) was added to the side of the tube carefully and centrifuged at 14,000 rpm for 10 minutes. The supernatant was discarded and the pellet air dried. Then 25µl of deionized water was added to the dried pellet and stored at -20^oC till further use. The resultant plasmid of multidrug-resistant *Pseudomonas fluorescens* was separated using gel electrophoresis Agarose gel (0.8%) containing 1X TBE buffer. 90V for 1hr. The resulting bands were visualized under the UV trans-illuminator and compared with 100bp and 1kb ladder (Mbim *et al.,* 2016; Munita and Arias, 2016).

Plasmid Curing

The methods of Spengler *et al*. (2006) and Mengesha *et al.* (2019) were used. Ten milliliters of each of the five selected multidrug-resistant *Pseudomonas fluorescens* isolates were inoculated into peptone water and incubated for 24 hours then introduced into test tubes containing 50 mg/ml of ethidium bromide and incubated for 24 hrs at 37^oC. Later, one milliliter of the selected culture was exposed to 50 mg/ml of ethidium bromide for plasmid curing and was inoculated onto nutrient agar plates and then incubated at 37^oC for 24 hrs. From the growth observed after 24 hrs,

the curing of the plasmid in the isolate was confirmed by testing with the same antibiotics they were resistant to previously, to know if they have been partially or fully cured.

RESULTS

Morphological Characteristics of Bacterial Isolates

The colonial and morphological characteristics of Seven (7) isolates: *Enterobacter agglomerans, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus, Morganella morganii biogrp1, Serratia liquefaciens* and *Streptococcus viridians*, Table 1 showed different colours such as slow brownish, slimy white, green-brown, orange, grayish white, white red and grey beaded respectively. Out of the seven isolates, *E.agglomerans, P.fluorescens, S. aureus, M. morganii biogrp1,S.liquefaciens,* and *S.viridans* had circular shapes, while *K. pneumoniae* had irregular shape.

Table 1: Colonial and Morphological Characteristics of Bacteria Isolated From Surgical Wound Samples

S/	Colour	Shape	Edge	Elevation	Organism
1	Slow brownish	Circular	Lobate	Convex	*Enterobacter agglomerans*
2	Slimy white	Irregular	Lobate	Flat	*Klebsiella pneumoniae*
3	Green to brown	Circular	Entire	Convex	*Pseudomonas fluorescens*
4	Orange	Circular	Entire	Convex	*Staphylococcus aureus*
5	Grayish white	Circular	Entire	Convex	*Morganella morganii bio grp*
6	White to red	Circular	Smooth	Raised	*Serratia liquefaciens*
7	Grey beaded	Circular	Entire	Flat	*Streptococcus viridans*

Bacterial Count of Surgical Wound Samples

The average total bacteria count in colony forming unit per ml (cfu/ml) of each surgical wound sample showed the range, Irun: from 4.3×10^4 to 7.0×10^4 cfu/ml, Ikare: 4.8×10^4 to 7.4×10^4 cfu/ml, and Iwaro: 5.6×10^4 to 9.9×10^4 cfu/ml. The highest bacterial count 9.9×10^4 cfu/ml obtained for surgical samples from Iwaro Oka General hospital while the least count of 4.3×10^4 cfu/ml counts was in the surgical samples from Irun General hospital. The *Staphylococcus* count for the sample sites ranged from Irun – 1.5×10^4 to 4.8×10^4 cfu/ml, Ikare – 2.5×10^4 to 6.2×10^4 cfu/ml, and Iwaro – 3.1×10^4 to 7.2×10^4 cfu/ml. The highest count

7.2 x 10^4 cfu/ml was for the surgical samples from Iwaro Oka General hospital, while the least count 1.5 x 10^4 cfu/ml was for the surgical samples from Irun General hospital. The average bacterial count for the samples are: Irun – 5.6 x 10^4 cfu/ml, Ikare – 5.8 x 10^4 cfu/ml, and Iwaro – 8.0 x 10^4 cfu/ml. The count was significantly different at 95% confidence limit. The female surgical samples had a higher count than the male which are 21 and 8 respectively.

Biochemical Characteristics of Wound Isolates Using Microbact Kit

Biochemical characteristics of wound isolates using Microbat Kit confirmed the isolated organisms: *Enterobacter agglomerans, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus, Morganella morganii biogrp1, Serratia liquefaciens and Streptococcus viridans* based on the differences in the biochemical activities (Table 2). The result of sugar fermentation on wound isolates using Microbact Kit. to determine the ability of the organisms to ferment sugar (Table 2). *Enterobacter agglomerans, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus, Morganella morganii biogrp1, Serratia liquefaciens, and Streptococcus viridans* to ferment sugar glucose.

Table 2. Biochemical characteristics and Sugar fermentation of wound isolates using microbact kit

ORGANISM	ON	AD	LD	OD	CIT	H_2S	UR	TD	IND	VP	OX	NO_2	GEL	GLU	MA	INO	SOR	RHA	SAC	MEL	AM	ARA
Enterobacter agglomerans	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+v	+ve	+ve	+v	+v	+v	+v	+ve	+ve	-ve	-ve	+ve	+ve	+ve
Klebsiella eumoniae	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+v	-ve	-ve	-ve	+v	+v	+v	+ve	-ve	-ve	-ve	-ve	+ve	+ve
Pseudomonas fluorescens	-ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+v	+v	+v	+v	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Staphylococcus aureus	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+v	+ve	-ve	+v	+v	+v	+v	+ve	+ve	-ve	+v	+ve	+ve	+ve
Morganella morganii biogrp 1	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+v	+v	+v	+v	-ve	+ve	+ve	+v	+ve	+ve	+ve
Serratia liquefaciens	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+v	+v	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Streptococcus viridans	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+v	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve

KEYS **:** +ve = sugar fermented -ve = sugar not fermented

Names of biochemical reagents used:

ONPG = Beta Galactosidase **ADH** = Arginine Dihydrolase **LDC** = Lysine Decarbonxylase **ODC** = Omithine Decarbonxylase

CIT = Citrate Desimmons **H_2S** = Hydrogen Sulphide **URE** = Urease, **TDA** = Tryptophane De Amminase,

IND = Indole **VP** = Voges Proskauer **OX =** Oxidase **NO_2** = Nitrogen Dioxide

KEY: +ve = sugar fermented -ve = sugar not fermented

Names of biochemical reagents used:

GEL - Gelatin Hydrolyse **GLU**- Glucose **MAN-** Mannitol, **INO**- Inositol

SOR - Sorbitol **RHA** –Rhaminose **SAC** –Saccharide **MEL** -Melibiose, **AMY** – Amygdalin **ARA** -Arabinose

Table 3: Identity of the isolated bacteria using microbact identification kit

S/N	NUMBER	ORGANISM
1	47734160	*Enterobacter agglomerans*
2	147774560	*Enterobacter agglomerans*
3	547126664	*Enterobacter agglomerans*
4	45276164	*Enterobacter agglomerans*
5	144752300	*Morganella morganiibiogrp1*
6	46630144	*Proridencia stuartti*
7	540146522	*Pseudomonas fluorescens*
8	452072600	*Pseudomonas fluorescens*
9	541432000	*Pseudomonas fluorescens*
10	47274556	*Serratia liquefaciens*

Antibiotic Sensitivity Tests on Surgical wound Isolates

Gram positive Isolates

The antibiotic sensitivity test on eight Gram-positive isolates: three strains of *Staphylococcus aureus* and five strains of *Streptococcus viridan*s was presented in Table 4. Out of three the strains of *S.aureus*, two strains were susceptible to streptomycin, and resistant to ciprofloxacin, septrin, rocephin, gentamicin, zinacef, amoxicillin, erythromicin, ampiclox, pefloxacin, and cephalexin respectively while one strain was resistant to all the antibiotics used. Among the five strains of S.*viridans,* one strain was susceptible to rocephin and cephalexin but resistant to others. while four strains were resistant to all the antibiotics used. Differences on the inhibition zones across the test organisms at P = 0.05.

Gram negative Isolates

A total of fourteen Gram–negative isolates: two strains of *Enterobacter agglomerans*, one strain of *Klebsiella pneumoniae*, seven strains of *Pseudomonas fluorescens,* three strains of *Serratia liquefaciens*, and one strain of *Morganella morganii biogrp 1* were used for antibiotic sensitivity test against eleven antibiotics: ciprofloxacin, septrin, chloramphenicol, amoxicillin, streptomycin, tarivid, sparyloxacin, cephalexin, pefloxacin, augmentin and gentamicin (Table 5). Out of the two strains of *Enterobacter agglomerans*, one strain was resistant to tarivid and amoxicillin, and the other strain was resistant to ciprofloxacin and cephalexin, but the two strains were sensitive to other antibiotics. *K.pneumoniae* was resistant to streptomycin and cephalexin but susceptible to ciprofloxacin, septrin, chloramphenicol, amoxicillin, tarivid, sparyloxacin, pefloxacin, augmentin and gentamicin respectively. *Morganella morganii biogrp1* was resistant to septrin, chloramphenicol, sparyloxacin, and amoxicillin but sensitive to ciprofloxacin, tarivid, cephalexin, augmentin, gentamicin, pefloxacin and streptomycin respectively. Three strains of *S.liquefaciens* were susceptible to all antibiotics. Seven strains of *P. luorescens* were resistant to all the antibiotics.

Antibiotic Sensitivity Test of Five Multidrug Resistant Pseudomonas Fluorescens Curred Plasmids

Multidrug resistance was common for Gram–negative isolates. Out of the fourteen isolates used for Gram–negative Antibiotic Sensitivity Test, seven strains of *Pseudomonas fluorescens* were found susceptible to augmentin and gentamicin, but resistant to pefloxacin, tarivid, streptomycin, chloramphenicol, septrin, sparyloxacin, amoxicillin, ciprofloxacin, and cephalexin used for the test. Out of seven strains of *Pseudomonas fluorescens*, five strains were analyzed(Table 6),

Table 4: Antibiotic sensitivity test of the Gram positive surgical wound isolates

ORGANISM	CPX	ZIN	STR	ERY	PEF	AMX	SXT	ROC	APX	GEN	CNX
Staphylococcus aureus	11.0±1.	13.6±0.3^{e}	0.0	13.0±0.5^{c}	13.0±0.0^{e}	13.0±0.5^{b}	10.0±1.0^{a}	20.0±0.5^{f}	13.0±0.1^{f}	20.0±0.1^{f}	20.0±1.0^{g}
Staphylococcus aureus	10.0±0.	12.0±0.3^{c}	0.0	14.0±0.8^{d}	12.0±0.6^{b}	11.0±0.2^{a}	13.0±0.3^{d}	16.0±0.1^{c}	11.0±0.5^{c}	10.0±0.2^{b}	15.0±0.4^{e}
Staphylococcus aureus	11.0±0.	8.0±0.1^{a}	7.0±0.0^{b}	12.0±0.5^{b}	11.0±0.2^{a}	13.0±0.3^{b}	12.0±0.3^{c}	17.0±0.2^{d}	11.0±0.1^{d}	15.0±0.7^{e}	14.0±0.0^{d}
Streptococcus varidians	13.0±0.	9.0±0.0^{b}	13.0±0.2^{e}	12.0±0.0^{b}	25.0±0.5^{h}	21.0±0.4^{f}	14.0±0.5^{e}	13.0±0.5^{b}	13.0±0.5^{f}	11.0±0.0^{c}	12.0±0.3^{c}
Streptococcus varidians	11.0±0.	13.0±0.3^{c}	10.0±0.6^{c}	14.0±0.5^{d}	17.0±0.5^{f}	18.0±0.0^{d}	13.0±0.0^{d}	13.0±0.2^{b}	10.0±0.3^{a}	20.0±0.4^{g}	14.0±0.4^{d}
Streptococcus varidians	13.0±0.	12.0±0.0^{c}	13.0±0.5^{e}	11.0±0.6^{a}	16.0±0.5^{e}	14.0±0.4^{c}	11.0±0.2^{b}	0.0	11.0±0.1^{e}	12.0±0.1^{d}	0.0
Streptococcus varidians	11.0±0.	14.0±0.3^{c}	10.0±0.0^{c}	17.0±0.3^{f}	21.0±0.0^{g}	19.0±0.0^{e}	13.0±0.6^{d}	23.0±0.1^{g}	24.0±0.2^{g}	25.0±0.4^{h}	18.0±0.0^{f}
Streptococcus varidians	12.0±0.	13.0±0.2^{d}	12.0±0.3^{d}	15.0±0.3^{d}	14.0±0.2^{c}	14.0±0.2^{c}	12.0±0.0^{c}	18.0±0.0^{e}	10.0±0.4^{b}	8.0±00^{a}	11.0±0.7^{b}

Keys: - Different alphabets on the same column indicate significant difference of the zones of inhibition at P > or = 0.05.

Names of antibiotics used:

CPX = Ciprofloxacin -30µg AMX = Amoxicillin -5µg GEN = Gentamicin -30µg APX = Ampiclox -30µg

SXT = Septrin -30µg STR = Streptomycin -30µg ZIN =Zinacef -30µg PEF = Pefloxacin -10µg

ROC = Rocephin -30µg ERY= Erythromycin -5µg CNX = Cephalexin -10µg

Table 5: Antibiotic sensitivity test of Gram negative surgical wound isolates

ISOLATES	AU	GE	PEF	OFX	STR	SXT	CHL	SPX	AMX	CPX	CNX
Enterobacter agglomerans	14.0	22.	14.0.	0.0	13.0	18.0	15.0	14.0	0.0	13.0	12.0
Enterobacter agglomerans	21.0	17.	13.0	15.0	17.0	21.0	15.0	19.0	23.0	0.0	0.0
Klebsiella pneumoniae	14.0	8.0	18.0	20.0	0.0	13.0	13.0	7.0	21.0	8.0	0.0
Morganella morganii biogrp 1	17.0	12.	10.0	13.0	10.0	0.0	0.0	0.0	0.0	10.0	12.0
Pseudomonas fluorescens	10.0	21.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	13.0	10.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	7.0	10.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	13.0	12.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	10.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	9.0	10.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas fluorescens	12.0	21.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Serratia liquefaciens	15.0	21.	20.0	21.0	17.0	17.0	23.0	25.0	15.0	13.0	17.0
Serratia liquefaciens	11.0	23.	21.0	17.0	14.0	15.0	19.0	21.0	20.0	12.0	16.0
Serratia liquefaciens	7.0	8.0	13.0	20.0	21.0	18.0	17.0	15.0	14.0	15.0	17.0

Keys: - Names of antibiotics used:

CPX	=	Ciprofloxacin -30µg	AMX	=	Amoxicillin -5µg
SXT	=	Septrin -30µg	STR	=	Streptomycin -30µg
AUG	=	Augmentin -10µg	CHL	=	Chloramphenicol -30µg
GEN	=	Gentamicin -30µg	OFX	=	Tarivid -10µg
PEF	=	Pefloxacin -10µg	SPX	=	Sparyloxacin -5µg
CNX	=	Cephalexin -10µg			

Table 6: Antibiotic sensitivity test of five multidrug resistant *Pseudomona fluorescens* whose plasmids have been cured with 50mg/ml ethidium bromide isolates (mm)

ISOLATES	AUG	GEN	PEF	OFX	STR	SXT	CHL	SPX	AMX	CPX	CNX
Pseudomonas fluorescens	17.4±0.5[a]	14.0±1.0	15.0±8.2[b]	18.0±0.4	23.0±0	21.0±1.0[a]	26.0±0.3[d]	27.0±0.0[a]	32.0±0.7[a]	27.0v0.4[c]	17.0±0.5[a]
Pseudomonas fluorescens	21.0±0.7[c]	16.0±0.3	17.0±0.4[c]	16.0±0.5	21.0±0	23.0±0.4[b]	24.0±0.1[b]	32.0±0.6[c]	34.0±0.3[b]	24.1±0.2[b]	28.0±±0.0[e]
Pseudomonas fluorescens	27.0±0.6[e]	18.0±0.0	15.0±0.2[a]	26.0±0.0	25.0±0	23.1±0.2[b]	25.0±0.0[c]	31.0±0.7[c]	32.0±0.1[a]	29.0±0.1[d]	19.0±0.5[b]
Pseudomonas fluorescens	19.0±0.2[b]	31.0±0.6	17.0±0.5[d]	24.0±.0.	27.0±0	26.0±0.1[c]	25.0±0.0[c]	34.0±0.1[d]	31.0±0.1[a]	23.0±0.2[a]	27.0±0.1[d]
Pseudomonas fluorescens	25.0±0.5[d]	27.0±0.3	17.0±0.5[e]	23.0±0.6	26.0±0	23.0±0.6[b]	21.0±0.6[a]	29.0±0.0[b]	36.0±0.5[c]	30.0±0.5[e]	26.0±0.1[c]

Keys: - Different alphabets on the same column indicates significant difference at P = 0.05.

Names of antibiotics used:

AUG = Augmentin 30µg GEN = Gentamicin 30µg

PEF = Pefloxacin 10µg OFX = Tarivid 5µg

STR = Streptomycin 5µg SXT = Septrin 30µg

CHL = Chloramphenicol 30µg SPX = Sparyloxacin 5µg

AMX = Amoxicillin 30µg CPX = Ciprofloxacin 5µg CNX = Cephalexin 10µg

Plasmid Profile of *Pseudomonas Fluorescens* Isolated From Wound Samples

The plasmid profiling of *P.fluorescens* showed resistance to cephalexin, pefloxacin, tarivid, streptomycin, septrin, chloramphenicol, sparyloxacin, amoxicillin, and ciprofloxacin(Table 6). The plasmid mediated analysis of the five selected multidrug-resistant *Pseudomonas fluorescens* isolates from surgical wound as observed by agarose gel electrophoresis showed plasmid bands in different combinations ranging from 2.27 kbp to 23.13 kbp molecular weight and thick band(Figure 1) showing the bacterium resistance as plasmid mediated, and not chromosomal mediated. The molecular markers showed the molecular weight and thickness of each band. The plasmid profile before curing showed two thick bands, while the bands were reduced to a band not as thick as the cured bands.

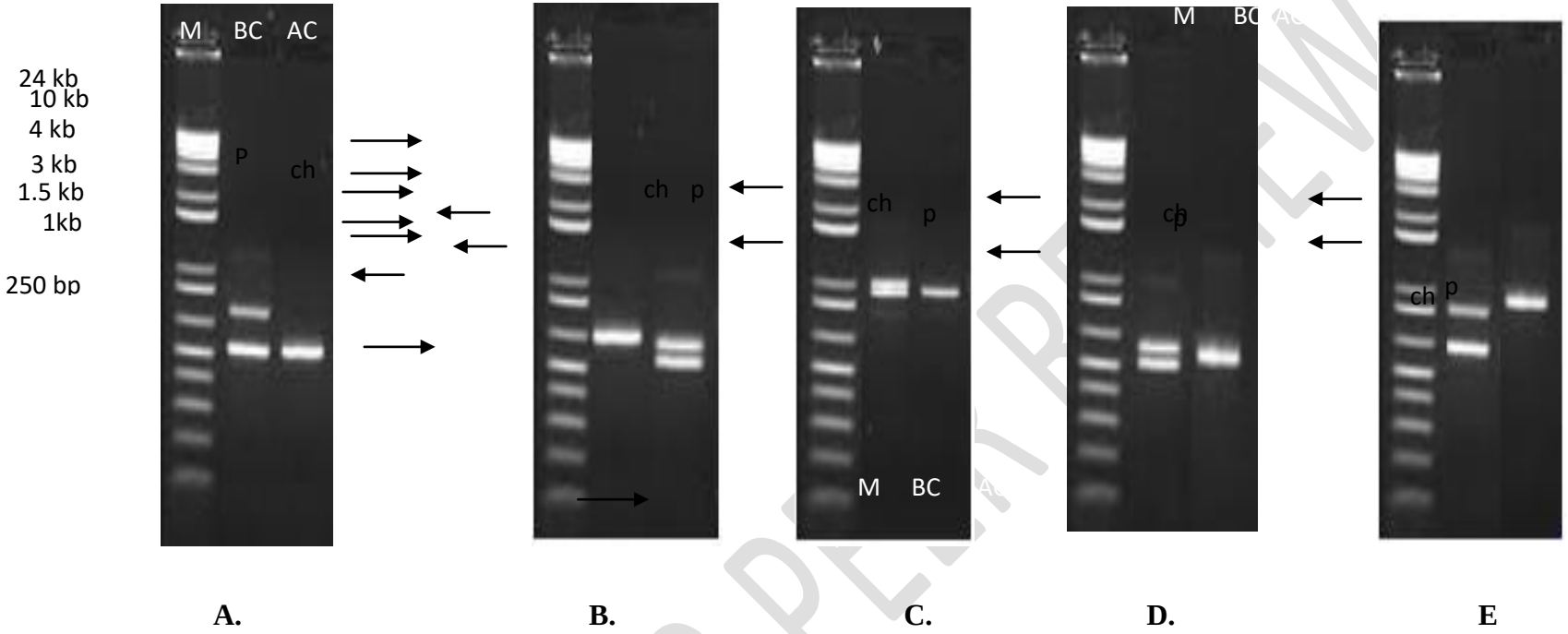

Figure 1: (A-E): Gel profile of plasmid DNA of *Pseudomonas fluorescens*isolated from surgical wound sample before and after curing.

Note: M-molecular marker, ch-chromosomal DNA, p-plasmid DNA, BC-plasmid profile before curing, AC-plasmid profile after curing. The same molecular marker was used throughout the experiment.

DISCUSSION

One of the most significant problems which are to be raised in connection with Pseudomonas is their antibiotic resistance, particularly in the wound management care unit of hospitals. High consumption of antibiotics has led to an increase in vulnerability of hospitalized patients to opportunistic infections (David, 2002). As a result of antibiotic resistance especially in form of multidrug resistance, *P. florescens* has created a lot of problems. The resistance of this bacterium to various antibiotics as observed in Table 5 increased the significance of controlling this bacterium in hospital surgical wards. From the seven strains of *Pseudomonas* isolated, all were resistant towards pefloxacin, tarivid, streptomycin, septrin, chlorophenicol, sparyloxacin, amoxicillin and ciprofloxacin.

The information obtained from this study and other research showed that β-Lactam drugs, although are being introduced as one of the anti-*Pseudomonas* drugs, today they have become ineffectual as a result of the organisms developing resistance to the drugs (Mbim *et al*., 2016). The rate of resistance of *Pseudomonas* towards antibiotics is different in various parts of the world due to diverse strains. Plasmids have been described as extrachromosomal elements capable of independent replications, they are different from chromosomal DNA present in bacteria (Mbim *et al*., 2016). As observed by Raghada *et al.* (2013) and Pattnaik *et al.* (2013), plasmid plays a very significant role in bacteria species developing multidrug resistance.

Plasmid profile analysis reveals the presence, type, size, and number of plasmids in bacterial isolates, which explains to a large extent the drug resistance among the isolates. The agarose gel electrophoresis of plasmid DNA showed that of the five strains of *Pseudomonas fluorescens* subjected to analysis. All of them (1, 2, 3, 4, and 5) possessed *SHV* and *CTX-M* genes, *CTX-M* genes are an extended spectrum of ß-lactamases (*ESBL*) with molecular weight ranging between 250bp to 24kb. Plasmid with a size range of 2.9 - 66kb had been reported by Al-Qurayshi *et al (*2019). Another study conducted in Bangladesh reported plasmid from *Escherichia coli* isolates, with sizes ranging from 0.5 kb to 40kb (Alam *et al.*, 2010). The relatively large plasmid sizes can be directly attributable to multiple genes coding for various resistant phenotypes which may be why the organisms showed resistance to two or more classes of antibiotics (Farshad *et al.*, 2012).

A plasmid analyses study conducted on uropathogenic *E. coli* isolated from children had plasmid sizes from 1 to 33kb, while some isolates harbor only 1 plasmid of size ranging from 5 to 9kb (Farshad *et*

al., 2012). Similarly, in another studyplasmid copy number was observed ranging from 0 to 3kb (Hussein *et al.*, 2018). The slight variation in results might be due to differences in the origin of the isolated organisms, the geographical distribution of the bacteria, and exposure to different antimicrobials, which explains to a large extent the type of drug resistance among the isolates.

These findings are in agreement with the observations of Oteo *et al*. (2006). Picao *et al*. (2009 and Amaya *et al*. (2012) which revealed that *P*. *fluorescens* strains possessed blaCTX-M enzymes which hydrolyze cefotaxime, but are weakly active against ceftazidime. David (2002) also stated that *P*. *fluorescens* species possess unique characteristics such as the ability to acquire resistance either via mutation or extra-chromosomal elements, as well as efflux pump mechanisms; these findings are also consistent with reports of Du *et al*. (2010) and Mbim *et al*. (2016). It is noted that plasmids encoding *SHV* and *CTX-M* enzymes influence the resistance of these species of organisms to antibiotics globally (David-Regli, 2015). These combined features enhance the resistance of these organisms to almost all the commonly used antibiotics (Maina *et al*., 2012). In this study, it probably presumed that presence of plasmids encoding *SHV* and *CTX-M* enzymes influenced their resistance to pefloxacin, tarivid, streptomycin, septrin, chlorophenicol, sparyloxacin, amoxicillin, and ciprofloxacin. Spengler *et al*. (2006) reported that the curing agent Ethidium bromide acts on the plasmid either through inhibition of plasmid efflux pumps on the plasmid membrane or inhibition of DNA gyrase responsible for plasmid DNA replication.

The susceptibility of the isolates to antimicrobial agents previously resistant confirms the fact that the resistant genes were harbored on the plasmids. This agrees with Raghada *et al*. (2013 that Ethidium bromide that achieved cured cells showed enabling susceptibility to all antibiotics initially resistant, confirming that most of the β-lactamases produced by some of the isolates were plasmid-encoded, and most likely responsible for the resistance of the isolates to antibiotics.

CONCLUSION

This study has revealed some identified bacteria associated with surgical wound infections in three local government areas: Akoko South-west (Iwaro Oka), Akoko North-east (Ikare), and Akoko North-west (Irun) in Ondo State, Nigeria. Seven bacteria were identified, out of which two were

Gram-positive: *Staphylococcus aureus* and *Streptococcus viridans* while five were Gram-negative: *Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Morganella morganii biogrp 1* and *Serratia liquefaciens.* Their response to antibiotic sensitivity, *SHV* plasmid, and *CTX-M* enzymes probably influenced the resistance. This will help epidemiologists in Ondo State to arrest the spread of resistant isolates.

RECOMMENDATIONS

Most of the surgical wound samples analyzed in this study were found to be normal floral or opportunistic pathogens that possess resistance mechanisms due to the high occurrence of resistant *Pseudomonas* spp in hospitals and its environment. Patients and people working in these 3 Local Government areas must pay close attention to their personal hygiene in order to avoid bacterial infections such as *Pseudomonas fluorescens*. Furthermore, due to high occurrence and frequency of penicillin, cephalexin, and vancomycin-resistant *Pseudomonas* spp., it is suggested that these antibiotics should not be considered as drugs of choice for treatment of surgical infections. In contrast, ciprofloxacin, gentamicin, and ticarcillin could be used as drugs of choice, as well as piperacillin, ceftriaxone, imipenem, and amikacin as alternatives for the treatment of nosocomial infections due to *Pseudomonas* spp. in this geographical area. It is unfortunate that there has been no strict application of legal standards relating to the hygienic conditions in our healthcare facilities in Akoko North-west, Akoko South-west, and Akoko northeast local government areas in Ondo State, Nigeria. The microbial contamination may also come from the environment, individuals, healthcare facilities, hospital equipment, or staff. The result suggests that medical practices should ensure the safety of patients now and in the future. The public health implication of the continuous dissemination of multi-resistant bacteria with high-frequency transmissible DNA cannot be overlooked in healthcare facilities. The efforts to develop antimicrobials that can withstand and compete with this trend should continue, and be greatly aided by the rapidly accumulating bank of microbial genome sequences. This will permit the use of bioinformatics and genomic techniques to identify and study new targets. It is hoped that further work will develop alternatives to antibiotics such as bacteriophage derived therapy or chemical agents that can block or reverse resistance pathways. It is also possible that new agents may be found in natural products, an age-old source of antimicrobials (Munita and Arias, 2016).

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