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Plasmid Profile Analysis of *Pseudomonas aeruginosa* Isolated from Wound Infections in a General Hospital in Southern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FZU designed the study and wrote the protocol. Author FZU wrote the first draft of the manuscript and proof reading. Authors FZU and IAI managed the analyses of the study and performed the statistical analysis. Author IAI managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background/Purpose: Open wound infection is a serious problem especially with extended-spectrum beta lactamase (ESBL) producing Gram negative bacteria such as *P. aeruginosa.* The purpose of this research was to identify open wound infections due to *Pseudomonas aeruginosa* and also determine their plasmid profile.
Methods: A total of 50 *Pseudomonas aeruginosa isolates* were obtained from clinical wound swabs in a secondary health care facility. *Pseudomonas aeruginosa* isolates were identified using Microbact 24E system kit. *Pseudomonas aeruginosa* were isolated and subjected to antibiotic susceptibility testing by disc diffusion method. ESBL production was detected using Double Disk Synergy Test (DDST) and CHROMagar ESBL (France). Six different antibiotic discs were used to determine the susceptibility pattern of the isolates. Plasmids were extracted using ZR plasmid Miniprep classic extraction Kit. Electrophoresis of the DNA was carried out on 0.8% w/v agarose gel.
Results: The prevalence of *Pseudomonas aeruginosa* was 63%. The antibiotic resistance pattern showed that *Pseudomonas aeruginosa was* highly resistant against cefotaxime (90%), ofloxacin

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(80%), ceftazidime (55%), azetronam (60%), imipenem (25%), amoxicillin clavulanic acid (35%) and amikacin (30%). Plasmid profile was carried out on 20 selected multidrug resistant isolates; those resistant to three or more classes of antibiotics. Plasmid content of the isolates were found to be 90%. All isolates that had plasmid were resistant to cefotaxim, ceftazidime, azetronam, ofloxacin and amikacin.

Conclusion: Antibiotic resistance by *P. aeruginosa* is increasingly high in wound infections and appears to be linked to the presence of plasmid and ESBL enzymes.

Keywords: P. aeruginosa; plasmid profile multidrug resistance.

1. INTRODUCTION

Pseudomonas aeruginosa, is the major pathogenic species that belongs to the family *Pseudomonadaceae* and is easily identified as gram-negative straight or slightly curved rods. The organism is primarily encountered as an opportunistic hospital pathogens which reflects its propensity to grow in a variety of environments with minimal nutritional components such as water, soil and on plants. *Pseudomonas aeruginosa* is resistant to several antibiotics because it carries multiple genetically based resistant determinants, which may act independently or in concert with others [1] and therefore become dominant when more susceptible bacteria of the normal flora are suppressed [2-3]. It is the fifth common pathogen among hospital microorganisms and causes 10% of all hospital acquired infections [4,5]. *P. aeruginosa* are naturally resistant to large number of antibiotics that are used during treatment of its infection [6,7] and this result in treatment failure [8,9]. Consequently, the effect of this high resistant pattern is responsible for high mortality rate associated with pseudomonas infections [9].

Wound is an injury caused by a cut in which the skin is broken leading to the exposure of the subcutaneous tissue, thereby creating an avenue and environment for the proliferation and entry of pathogenic organisms [10,11]. An open wound is an injury that involves either an external or internal break in the body tissue. Examples of open wound include incision, laceration, gunshot and abrasion [11,12]. Wound infection is a serious concern among health care practitioners because some wound takes a long time to heal and need special care. Thus, leading to increase in financial burden for the patient as well as increase in the budget and economic management within the health care system. The high intrinsic antibiotic resistance of this organism is attributed to factors such as active drug efflux and β-lactamase production [3]. This study examined the antibiotic susceptibility pattern and plasmid profile of *P. aeruginosa* isolates from open wound swabs from patients attending a general hospital in the southern part of Nigeria.

2. MATERIALS AND METHODS

A total of 100 wound swabs were collected from July 2018 to December, 2018. All wound specimens were obtained from patients attending a public health care facility. Routine clinical wound was swabbed with a sterile swab stick before dressing and care was taken not to swab the surrounding skin around the open wound. The wound swabs were transported immediately to the laboratory for processing. The samples were cultured on MacConkay, incubated aerobically at 37℃ for 24h. Samples were also inoculated on CHROMagar ESBL and incubated at 37ºC for 24 hours. Samples were Gram stained and also subjected to Microbact 24E identification.

2.1 Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar (Oxoid, UK) according to Clinical and Laboratory Standard Institute (CLSI) guidelines. ESBL-producing isolates were screened using double-disk synergy test in accordance with CLSI guidelines [13]. According to CLSIs guidelines isolates showing inhibition zone size of $\leq$22 mm with Ceftazidime (30µg), $\leq$25mm with Cefotaxime (30 µg), $\leq$27 mm with Azetronam (30 µg) and $\leq$22 mm with Cefodoxime (10 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production [14]. *P. aeruginosa* ATCC 49189 were used as quality control strains.

2.2 Double Disk Synergy Test

Double disk synergy test as described by Jarlier et al*.,* [15] was used to confirm ESBL production. Test isolate was swabbed on the surface of Mueller Hinton agar, then placement of a ceftazidime disk close (20 or 30 mm) to an

amoxicillin-clavulanate disk on a plate inoculated with the test organism. A clear extension of inhibition of the zone of inhibition towards the centrally placed amoxicillin-clavulanate disk indicates the production of ESBL. This extension occurred because the clavulanic acid present in the amoxicillin-clavulanate disc synergistically potentiated the ESBL produced by the test organism.

Inoculation was also done on CHROMagar ESBL, a completely new and innovative chromogenic medium designed specifically for the Screening of extended spectrum ß-Lactamase (ESBL) producing bacteria [16]. Incubation was done for 18-24hrs. *Escherichia coli* produced pink to burgundy colouration of ß-glucuronidase-producing colonies *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC): green/blue to browny-green colouration of ß-glucosidase-producing colonies. *Proteeae (Proteus, Providencia, Moraganella)* produced dark to light brown colouration.

2.3 Plasmid Isolation and Profiling

Plasmid isolation was done using a commercial plasmid isolation kit (plasmid miniprep kit, zymogen co. LTD UK.) according to the manufacturer's instruction.

2.4 Gel Electrophoresis

Electrophoresis of the DNA was carried out on 0.8% w/v agarose gel in 0.5XTBE for 1.5 hour at 75 Volts/cm.

3. RESULTS

Chi square test, the level of statistical significance was <0.05. Key: DDST: Double Disk Synergy Test.

Table 1. Distribution pattern of isolates recovered from wound swabs

Isolates	Frequency (%)
P. aeruginosa	50 (63)
P. mirabilis	20 (25)
K. pneumonia	10 (12)
	80 (100)

4. DISCUSSION

The 100 wound swabs from different patients yielded 80 different isolates. The percentage frequencies of the isolates were as follows; *Pseudomonas aeruginosa* (63%), *P. mirabilis* (25%) and *K. pneumonia* (12%) as shown in Table 1. The DDST and CHROMagar ESBL test methods revealed more numbers of ESBL producers at 23% and 57% as shown in Table 3. According to the type of wound swabs types and frequencies, the highest were from diabetic wound swabs followed by wounds from burn with 38% and 25% respectively (Table 4). The Agarose gel electrophoresis of plasmids recovered from bacterial isolates, *P. aeruginosa* 5,3,6 and 7 revealed that these were not plasmid mediated but chromosomally mediated, as shown in Table 5.

The study revealed that 63% prevalence rate of *P. aeruginosa* as a cause of wound infection in this health care facility as shown in Table 1. This finding is higher than what was reported in another study with infection rate of 35% for *P. aeruginosa* in Egypt [17]. The relatively high incidence of wound infection in our study could be due to either intrinsic organism in wounds that are contaminated as reported in some other study by Gad Gf et al [18]. Our study revealed a significantly high incidence of 38% of isolates in diabetic wound ulcer, this is in contrast with the study in Iran in which a low incidence of 5.2% was reported for diabetic foot ulcer [19].

Table 2. Antibiotic susceptibility pattern of isolates

Antimicrobial µg	*P. aeruginosa* (n=50)		*P. mirabilis* (n=20)		*K. pneumonia* (n=10)	
	S (%)	R (%)	S	R	S	R
CTX (30)	10(20)	40(80)	5(25)	15(75)	3 (30)	7(70)
OFX (5)	15 (30)	35(70)	5(25)	15(75)	3(30)	7 (70)
CAZ (30)	10 (20)	40(80)	2(10)	18 (90)	4 (40)	6 (60)
ATM (30)	5(10)	45(90)	5(25)	15(75)	5(50)	5 (50)
IPM (30)	40(80)	10(40)	18(90)	2 (10)	5 (50)	5(50)
AUG (30)	39(78)	11(22)	15(75)	5 (25)	5(50)	5 (50)
AK (30)	10(20)	40(80)	8(40)	12 (60)	3 (30)	7 (70)

Table 3. ESBL detection by DDST and CHROMagar ESBL

Bacterial isolates	N	DDST n (%)	CHROMagar	P-value
P. aeruginosa	50	15 (30)	35(70)	0.05195
P. mirabilis	20	5 (25)	15 (75)	
K. pneumoniae	10	3 (30)	7 (70)	
Total	80	23 (28.8)	57 (71.2)	

A high incidence of multidrug resistance (MDR) was detected in 90% of the isolates. The increase in MDR has been observed in many studies in Egypt [20,21]. This high level of resistance could be due to the lack of proper infection control measures and the extensive abuse of different antimicrobials that seems to be the setting in our settings. *P. aeruginosa* is naturally resistant to beta-lactams including broad spectrum cephalosporins, quinolones, chloramphenicol mainly because of the very low permeability of their cell wall [22]. Gram negative organisms have been reported to easily acquire drug resistant properties particularly extended spectrum beta lactamase at a higher frequency than gram positives [23]. This tendency puts the afflicted patient at a high risk of developing multidrug resistant infection particularly if adequate and effective laboratory investigations and follow up care are not giving to the infected wound site.

Table 4. Types of wound and frequencies

Types of wound	Isolates (%)
Diabetic Ulcers	30 (38)
Burns	20 (25)
Superficial lesion	15 (18.5)
Wound bite	15 (18.5)
Total	80 (100)

In this study *P. aeruginosa* had a high level (80%) of resistance against several antibiotics including cefotaxime and amikacin, but sensitive to imipenem (80%) and amoxcicillin/clavulanic acid (78%) (Table 2); which in contrast to another study by Yoon et al., [24], 56% of Korean *Pseudomonas aeruginosa* isolates were multidrug resistant (MDR) out of which 44% showed resistance to five or more antibiotics. This high level of resistance has been described to be due to the presence of plasmids [25,26]; and this appears to be the issue in our current study because of the detection of plasmids in the Pseudomonas isolates.

Double disc susceptibility testing (DDST) detected approximately 29% of the ESBL producing isolates while CHROMagar ESBL detected a significantly higher rate of 71%. DDST is described as a reliable technique for ESBL detection [27]. The differences in sensitivity results in DDST may be due to the fact that optimal substrate profile varies from one ESBL enzyme to another [28]. DDST is an easy procedure although with subjective interpretation of result [29], while CHROMagar ESBL has a high sensitivity and specificity making it reliable for ESBL detection. This medium allows for easy differentiation of different bacteria based on colony colouration (Table 3).

Table 5. Plasmid profile of ESBL producing isolates

Bacterial isolates	Plasmid size, bp
P. aeruginosa-6	1000, 2000, 2500
P. aeruginosa-1	1000
P. aeruginosa-2	1000, 2000, 2500
P. aeruginosa-5	-
P. aeruginosa-3	-
P. aeruginosa-6	-
P. aeruginosa-7	-
P. aeruginosa-10	-
P. mirabilis-2	1000
P. mirabilis-10	1000
P. mirabilis-8	1000
P. mirabilis-7	1000
P. mirabilis-5	1000

The plasmid analysis revealed that there were detectable plasmids 45(90%) out of the 50 isolates of *P. aeruginosa* while 5(10%) had no plasmid bands. This could be due to the fact that many of the genera of Gram negative bacteria possess a naturally occurring chromosomally mediated beta-lactamases [30]. This result agrees with the findings reported by Marhova K et al. [31]. The involvement of plasmids as a factor responsible for antibiotic resistance in the ESBL isolates further suggests the emergence and active transfer of antibiotic resistance and R plasmids among the circulating strains causing pseudomonas wound infection in this part of the country.

All isolates that had plasmids were resistant to cefotaxime, ofloxacin, ceftazidime and amikacin (Fig. 1). The sizes of the plasmids ranged

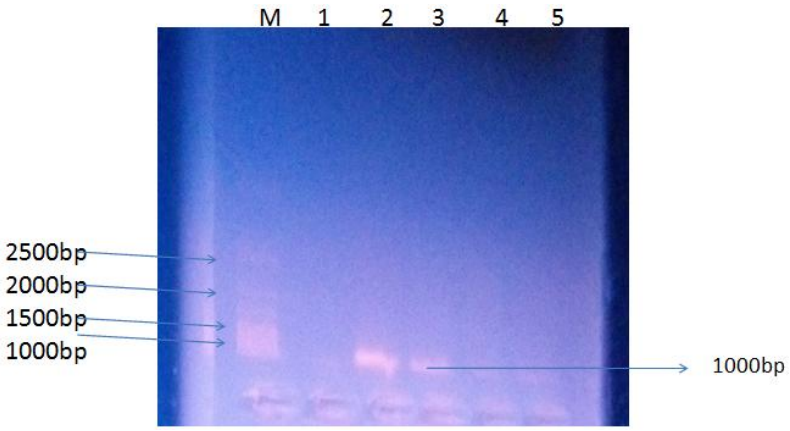

Fig. 1. Agarose gel electrophoresis of plasmids recovered from ESBL producing bacterial isolates. Lane M: 1kb DNA ladder, lanes 3, 4 = *P. aeruginosa* 6 and 2

from 3,5 and 10kb. Plasmid mediated resistance to various infections caused by antibiotic-resistant bacteria antimicrobial drugs have been demonstrated by various studies and these researchers have highlighted these diverse plasmid profiles [32,33,34].

5. CONCLUSION

The CHROMagar ESBL showed better results in the detection of ESBL production and should be recommended as a detection method. The antibiotic resistance by *P. aeruginosa* was increasingly very high in wound infections and this appears to be linked to or mediated by the presence of plasmid and ESBL enzymes.

CONSENT

As per international standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethics committee of State Ministry of health, gave ethical clearance for the study; Ref: MH/ PRS/99/VOL.IV/200. Participants' privacy and confidentiality were ensured (no names were used, only serial numbers) and all data and results were handled and treated high degree of confidentiality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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