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# Detection of integron genes in the plasmid DNA of multidrug resistant *Pseudomonas aeruginosa* isolated from surgical wounds of some patients in Benin City, Nigeria

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**Abstract**
***Pseudomonas aeruginosa* is an opportunistic pathogen with the capability to cause serious surgical wound infections and remains a major healthcare problem. Plasmid is an extra chromosomal material in bacterial cells and confers resistance to the cell against many antibiotics. Genetic elements such as integron are implicated in conferring multidrug resistance (MDR) to *P. aeruginosa* . This study aims at investigating the occurrence of integron genes (*int1*, *int2, int3*) in the plasmid DNA and their ability to cause MDR in *P. aeruginosa* . In total, 284 different wound swabs were collected, *P. aeruginosa* isolated and screened using standard laboratory methods. Antibiotics susceptibility tests were carried out using Kirby-Bauer disk diffusion method. Polymerase chain reaction (PCR) was also carried out using P. aeruginosa plasmid DNA as a template to detect the presence/absence of the integron genes using different pairs of specific primers. The results reveal that 34 (54.8%) of the microbes isolated were *P. aeruginosa* . Most of the isolates showed notable resistance to antibiotics, most notably against Ceftazidime, Augmentin, Cefixime and Gentamicin . Eleven isolates harbors the plasmid DNA . PCR amplification showed that 6 (54.5%) of the *P. aeruginosa* isolates harbor integron class 1 genes, non harbors the integron class 2 genes while 3 (27.3%) possess the integron class 3 genes. The isolates with these genes were highly resistant to most of the antibiotics used. *int1* gene was prevalent then *int3.***

**Keywords*:*** Antimicrobial, Wound infection, Integron, Polymerase chain reaction, Plasmid DNA
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## Introduction

*Pseudomonas aeruginosa* is an opportunistic infection causing bacteria with the likelihood to cause severe healthcare-related infections particularly in immune-compromised post surgical wound patients (Strateva and Yordanov, 2009). Generally speaking, surgical wounds via suppression of the body defense system present a suitable site for microorganism multiplication. Therefore, *P. aeruginosa* infection in surgical wound patients is frequent and is seen as one of the leading serious life- threatening conditions in surgical operation units (Church et al., 2006). Moreover, due to the resistance of these

microorganisms to a huge assortment of routinely used antibiotics in recent years, dealing with infections caused by them has been wearisome and has resulted in enhanced mortality (Fair and Tor, 2014). The acquired and intrinsic (low outer membrane Permeability, over expression of efflux pump) antibiotic resistance ability of *P. aeruginosa* makes it difficult for the treatment of *P. aeruginosa* surgical wound infections (Breidenstein et al., 2011).

Bacteria plasmid DNA are second chromosomal material of limited size, frequently firmly inherited in a bacterial cell and potentially able to transfer between strains, species or genera (Alan, et al., 2001). One of the major purposes of plasmid is to transfer genes that confer resistance to drugs used in the treatment of infectious diseases (Alan, et al*.,* 2001).

Integrons are mobile genetic elements which can be found in plasmids, transposons and chromosomal DNA. These are sometimes accountable for the progress in microbial resistance among Gram-negative bacteria pathogens (Fluit and Schmitz, 2004; Gillings, 2014). Integrons are composed of three vital core parts which include the *intI* gene which codes for integrase (*IntI*) necessary for site-specific recombination; attI, the bordering recombination site that is identified by the integrase; and integron related promoter (Pc), which is required for transcription and expression of gene cassettes within the integron. Gene cassettes are genetic components that encode antibiotic resistance, and incorporate a specific site recombination known by integrase which is referred to as attC (Domingues et al., 2012). Due to the type of genes encoded in integrases, integron has been separated into five groups (Cambray et al., 2010; Deng et al*.,* 2015). Integron class 1 type is the most widely distributed integrase gene in drug resistant bacteria isolates (Deng et al., 2015). There is dearth of documented studies due to the significance of plasmid DNA and integron in the wide spread of antibiotic resistance . This study is aimed at investigating the incidence of class 1, 2 and 3 integrons in plasmid DNA of multidrug resistant *P. aeruginosa* isolated from post surgical wound infection in Benin City, Nigeria.

## Materials and Methods

*Sample collection*

A total of 284 random swab sampling of post operative surgical wounds were collected from both outpatients and inpatients in the University of Benin Teaching Hospital and Central Hospital, Benin City.

*Ethical clearance*

Approval was obtained from the University of Benin Teaching Hospital and Central Hospital, ethical committees and all patients gave their support after being educated of the objectives of the study.

*Bacteriological procedures/identification of isolates*

All wound samples were inoculated as described by Eremwanarue and Shittu (2018). Phenotypic methods were used the in identification of all isolates (Cheesbrough, 2000).

*Antibiotic susceptibility testing*

The Kirby-Bauer disk diffusion method was employed in generating multiple drug resistant isolates with routinely used antimicrobial agents (CLSI, 2011). The antibiotics used include Nitrofurantion (NIT 300µg), Augmentin (AUG, 30µg), Gentamycin (GEN 30µg), Ciprofloxacin (CPR 5µg), Ofloxacin (OFL 5µg), Ceftazidime (CAZ 30µg), Cefixime (CXM 5µg) and Cefuroxime (CRX 30µg). Zones of inhibition were interpreted using the Performance Standards for antimicrobial disk susceptibility tests (CLSI, 2011).

*Bacterial genomic DNA extraction*

The multidrug resistant *Pseudomonas aeruginosa* were sub-cultured overnight in Luria-Bertani broth (Merck, Germany) and plasmid DNA was extracted using Zymopure plasmid DNA extraction kits (Irvine, CA, USA), following the manufacturer's instructions.

*Detection of integron genes in plasmid DNA of Pseudomonas aeruginosa*

The amplification of plasmid DNA containing the integron genes in *Pseudomonas aeruginosa* was carried out using *the* forward and reverse

primers for integron class I, 2 and 3 (*Int1*-F-GCCACTGCGCCGTTACCACC; *Int1*-R-GGCCGAGCAGATCCTGCACG, *IntI2*-F-CACGGATATGCGACAAAAAGGT; *IntI2*-R-GTAGCAAACGAGTGACGAAATG, *IntI3*-F AGTGGGTGGCGAATGAGTG; *IntI3*-R-TGTTCTTGTATCGGCAGGTG) [Sunde, 2005; Mohammadalipour et al, 2017] separately in a ABI9700 thermal cycler PCR machine at Lahor Research Laboratories, Benin City, Nigeria. Quick load PCR Master Mix 2x (New England Biolab, USA) was used in line with the manufacturer's instructions. The PCR was performed in 25 µl reaction volume and PCR conditions as described by Eremwanarue and Shittu (2018), with annealing temperature at 54 °C for 30 seconds. The amplified PCR products (10 µl each) were separated on a 1% agarose gel stained with ethidium bromide in TBE buffer and ran at 90 Volts for 60 minutes. The gel was viewed using a gel documentation system (Wealth Dolphin Doc) and snapped.

## Results and Discussion

The broad spread of antibiotic resistant genes among bacteria as well as *P. aeruginosa* strains is worrisome in the cure for post surgical wound infections. A total of two hundred and eighty four (284) post operative surgical wound swabs specimens from in and out patients were analyzed. Of all the patients studied, 99 (35%) of them had wound infections. is lower than 39.9% previously reported (Mohammed et al., 2006; Oni et al., 2013) but higher than 9.6% reported by Dellinger et al. (2005). Nevertheless, the prevalence rate observed in this study is not in any way different from the rate suggested by the World Health Organization (WHO, 2011). The etiologic agents isolated from the surgical wounds showed that *P. aeruginosa* (62.6%) was the major cause of the wound infection. This is in conformity with the research work of Sulochana et al. (2014) who, as well, reported that *P. aeruginosa* was the main cause of surgical wound infections.

The sixty two *P. aeruginosa* isolated from the surgical wounds were screened to identify antibiotic resistant strains and the presence of *int1, int2 and int3* genes. Thirty four (54.8%) isolates showed multiple drug resistance. On the basis of the antibiotics susceptibility results observed, most isolates showed high resistance to Ceftazidime, Augmentin, Cefixime and gentamicin (54.8%). Figure 1 showed the antibiotic resistance result of the strains of *P. aeruginosa* using eight commonly used drugs. In this study, the antibiotic sensitivity testing revealed that most of our isolates showed resistance (>50.0%) to the commonly used antibiotics. Ehiaghe et al. (2016), reported high resistance of some bacterial isolates from clinical samples to first, second and third generation antibiotics. Yah et al., (2010) reported that bacteria isolated from surgical wounds are mostly resistant to Ceftazidime and Augmentin, which may be due to occurrence of Cephalosporinase and Penicillinase (Fontana et al., 2000).

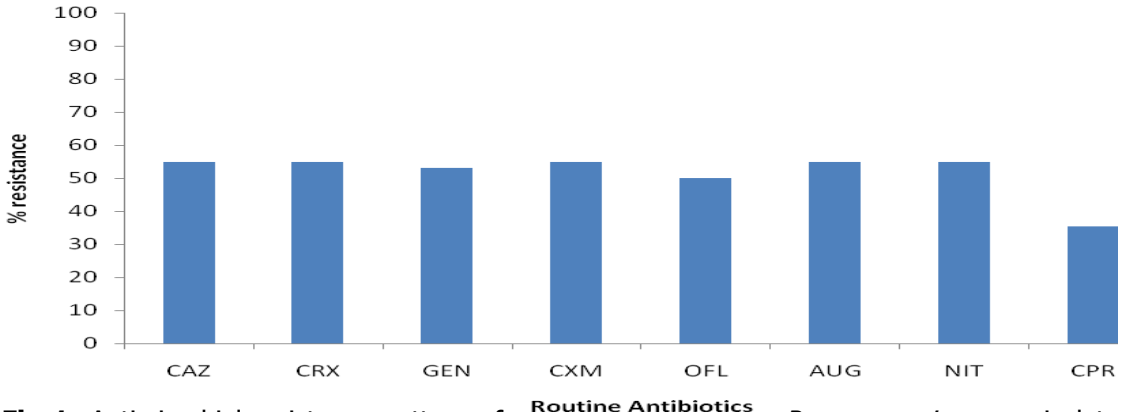


**Fig 1:** Antimicrobial resistance pattern of *P. aeruginosa* isolates. CAZ: Ceftazidime, CRX: Cefuroxime, GEN: Gentamicin, CXM: Cefixime, OFL: Ofloxacin, AUG: Augmentin, NIT: Nitrofuration, CPR: Ciprofloxacin

There are numerous antibiotics resistant gene cassettes within bacteria integrons, which can as well be situated on mobile genetic elements such as plasmids and transposons (Gu et al., 2007). Plasmid DNA profiling of the thirty four multidrug resistant (MDR) *Pseudomonas aeruginosa* strains showed that eleven (32.4%) isolates were found to harbor plasmid genes as shown in plates 1 and 2. Plasmids analysis revealed that eleven MDR *P. aeruginosa* (P20, P31, P33, P60, P78, P80, P32, P38, P75, P17 and P23) had detectable plasmid DNA genes which represents 32.4% of the MDR strains analyzed. Plasmid DNA profiling reveals that the total microbial plasmids are fragmented by electrophoresis. This technique is used to evaluate the wide spread of antibiotics plasmid DNA resistance (Clowers, 2004).

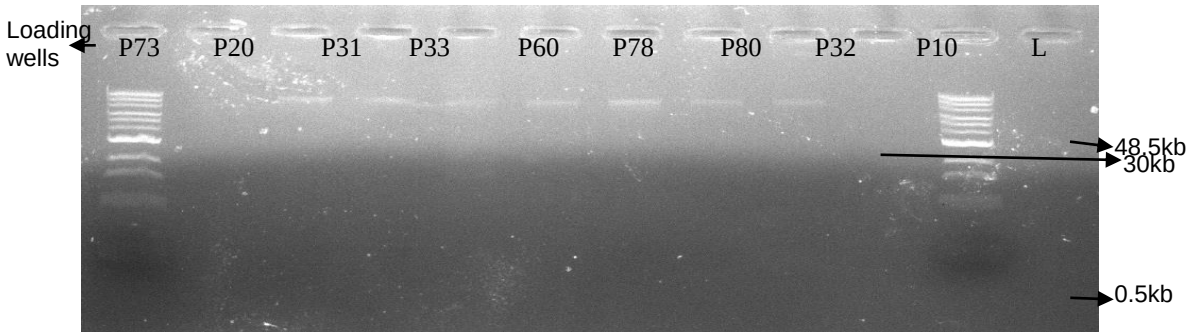


**Plate 1:** Plasmid profile of cured multiple drug resistant *Pseudomonas aeruginosa* strains. L is 0.5kb-48.5kb DNA ladder, isolates P20, P31, P33, P60, P78, P80 and P32 harbors plasmid genes with bands at 30kb, P73 and 10 did not harbor plasmid genes.

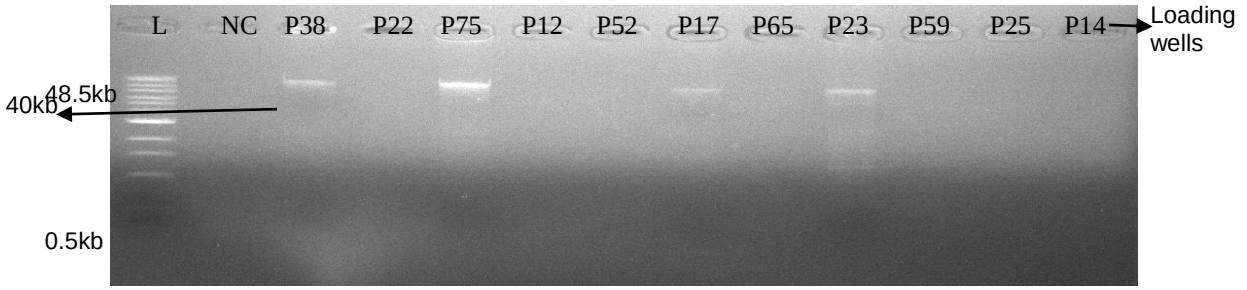


**Plate 2:** Plasmid profile of cured multiple drug resistant *Pseudomonas aeruginosa* strains. L is 0.5kb-48.5kb DNA ladder, isolates P38, P75, P17 and P23 harbors plasmid genes with bands at 40kb, P22, P12, P52, P65, P59, P25 and 14 did not harbor plasmid genes. NC: is a negative control.

In the present study, PCR amplification was carried out to detect the three classes (*int1, int2, int3*) of integron genes in the plasmid DNA isolated from the MDR *P. aeruginosa.* The results revealed that 6 (54.5%) of the plasmid- positive MDR *P. aeruginosa* strains harbor integrase class 1 genes ( Plate 3), non (100%) of the plasmid- positive isolates was found to contain class 2 integrase genes ( Plate 4) and only 3 (27.3%) of them carried *int3* gene ( Plate 5).

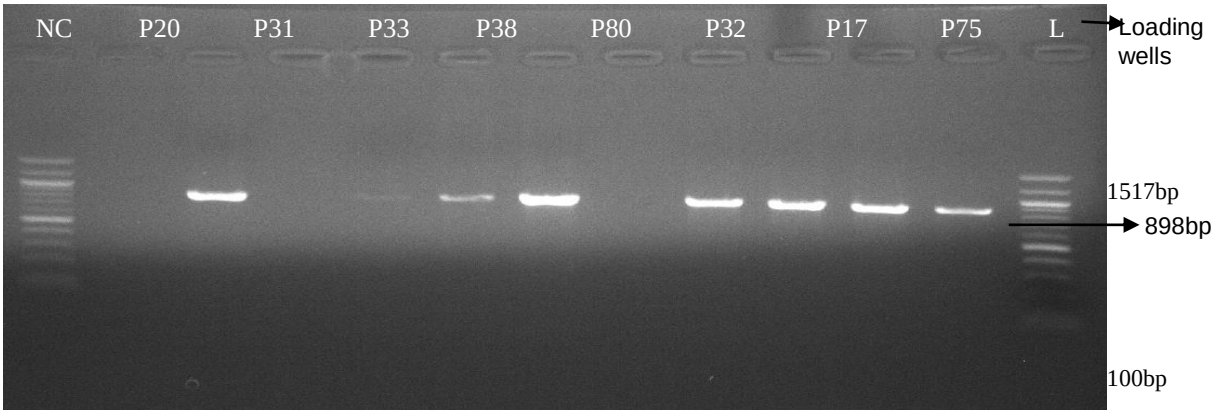


**Plate 3.** PCR detection of integrase class 1 genes in plasmid DNA of *Pseudomonas aeruginosa* strains shows that isolates P20, P31, P33, P80, P32, P17 and P75 harbors *int1* genes with bands at 898bp while isolates P38 and others were didn't have *int1* genes. NC: is a negative control.

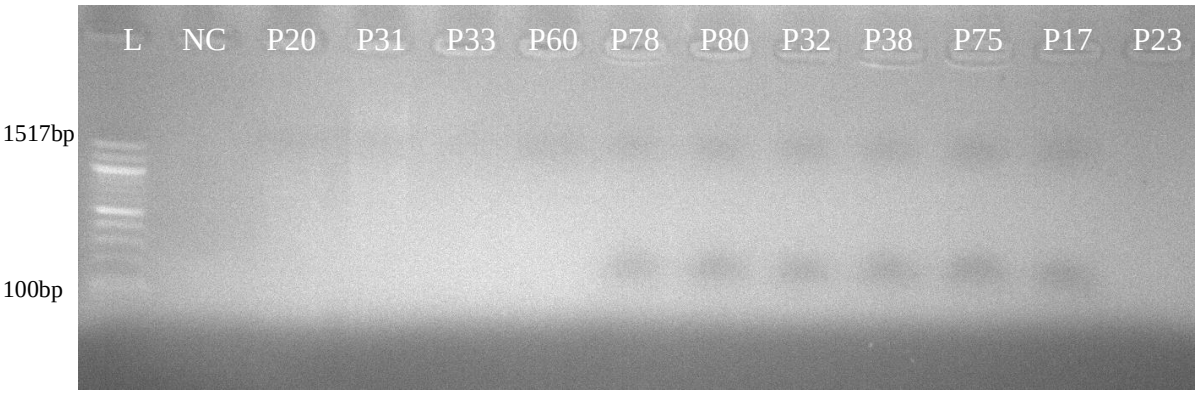


**Plate 4.** PCR detection of integrase class 2 genes in plasmid DNA of *Pseudomonas aeruginosa* strains shows that all isolates P20, P31, P33, P60, P78, P80, P32, P38, P75, P17 and P23 did not harbors *int2* genes. NC: is a negative control.

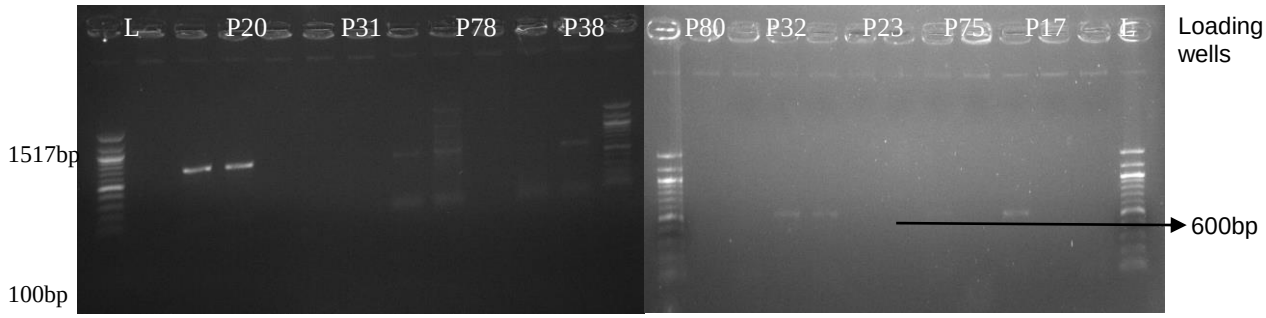


**Plate 5.** PCR detection of integrase class 2 genes in plasmid DNA of *Pseudomonas aeruginosa* strains shows that isolates P31, P78, and P23 harbors *int3* genes with bands at 600bp while isolates P20, P38, P80, P32, P75 and P17 did not harbors *int3* genes.

To the best of our knowledge, there is no documented information on the presence of integron genes in the plasmid DNA of MDR *P. aeruginosa*. The frequency of *int1* gene in the genomic DNA of clinical isolates of *P. aeruginosa* has been acknowledged in numerous similar research work from around the world, ranging from 43% to 56.3% (Nikokar et al., 2013; Sun et al., 2014). In this study, none of the MDR *P. aeruginosa* harbored *int2* gene. This was in concordance to the investigation conducted by Khosravi et al. (2017), who also reported zero occurrence of *int2* gene in the genomic DNA of all the *P. aeruginosa* they screened , although other researchers reported the occurrence of class 2 integron gene in the genomic DNA of the bacteria to be 19.5% and 2.7% (Xu et al 2009; Moazami and Eftekhar, 2015). Integron class 3 genes were detected in the plasmid DNA of three (27.3%) of the multidrug resistant *P. aeruginosa* strains. Zero occurrence of *intl3* gene in the genomic DNA of the bacteria was reported by Mohadeseh Zarei et al. (2018). The presence and proliferation of class 3 integrons genes could carry an assortment of resistant gene cassettes which can make the plasmid DNA- positive bacterial multiple drug resistance more worrisome worldwide (Shibata et al., 2003).

All the plasmid- positive isolates in our study were multidrug resistant isolates. Integrons are eminently related to multidrug resistance, particularly integron class 1 which was broadly disseminated in *P. aeruginosa*. We found high antibiotics resistance among the *P. aeruginosa* isolates with plasmid DNA that harbors integron genes, especially those with *int1.* As stated formally , researchers had recognized that multidrug resistance is strongly connected with the presence of integrons, and a greater part of our MDR isolates comprise *int1* gene. However, the presence of integron genes in the plasmid DNA of some *P. aeruginosa* MDR isolates will further increase the resistance capacity of these isolates. Furthermore, our findings revealed the presence of *int1* and *int3* genes in the plasmid DNA from one particular strain of *P. aeruginosa* which, to the best of our knowledge, is uncommon. Considering the results from this study, it appears that a greater part of the antimicrobial agents employed are unsuitable for the treatment of the causative agents isolated from the surgical wounds which may be due to the presence of integrons in their plasmids . Of all the antibiotics used, Ciprofloxacin recorded the highest sensitivity and may possibly be the drug of choice.

## Conclusion

The presence of *Int1 and Int3* integron genes in the plasmid DNA of the multidrug resistant *P. aeruginosa* bacteria isolates is of serious healthcare concern , as they can be transferred to other non- MDR bacteria. We found a high antibiotics resistance among the *P. aeruginosa* isolated from the surgical wounds in our study. The results will assist to develop control strategies for the bacterial infections. The presence of integron genes in the plasmid DNA of the bacteria can assist in making most of the plasmid- positive isolates become MDR. Thus, efforts to maintain the correct use of antibiotics are supreme in order to avoid therapeutic failure, and is indispensable for choosing the appropriate therapy, routine antibiotic surveillance programs, and management of surgical wound infection control practices. However, more studies need to be carried out to examine the gene cassettes present in the plasmid DNA of somMDR and prevalence of class 1, 2 and 3 integrons in other bacteria plasmid in recent years. The result will assist to develop control strategies for bacterial infections.

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