

## Original Research Article

<https://doi.org/10.20546/ijcmas.2018.706.021>**Plasmid Profile of Bacteria**O.V. Olagoke<sup>1,2\*</sup>, O.O. Oyewale<sup>3</sup>, M.O. Olufehinti<sup>3</sup> and B.S. Adesina<sup>3</sup><sup>1</sup>Department of Microbiology, Obafemi Awolowo University, Ile-Ife<sup>2</sup>Department of Agricultural Development Management (ADEM), <sup>3</sup>Department of Rural Development and Gender Issue (RUDEG), Agricultural and Rural Management Training Institute (ARMTI) Ilorin*\*Corresponding author***ABSTRACT****Keywords**

Plasmid, Multi-resistant and HIV seropositive

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Plasmid induces the production of extra-cellular enzymes called  $\beta$ -lactamases, and also, encoded resistance genes which are often located within genetic elements. This study evaluated the plasmid profile of bacterial isolates cultured from urine samples of HIV seropositive pregnant women that attended antenatal clinic of the Ondo State Specialist hospital, Akure. Plasmid analysis of bacterial isolates was also done by alkaline method. The resistance gene (plasmid) was detected in *E. coli* and *Pseudomonas aeruginosa* isolates used. The study concluded that appearance of the resistance genes in some of these bacterial isolates could help the isolates to be multidrug resistant which may lead to a serious public health challenges.

**Introduction**

Healthcare associated infections are a problem globally (Mbim *et al.*, 2016). The widespread use of antibiotics continues to influence this menace giving rise to antibiotic-resistant bacteria in the hospital setting and among immunocompromised patients (Bereket *et al.*, 2012).

Also, Studies done by Olagoke *et al.*, (2017) clarified that most of the bacterial isolates cultured from urine samples of HIV seropositive pregnant women were multi-resistant to different antibiotics tested *in vitro* but some of the isolates were sensitive to ciprofloxacin. The environment of the hospital

is an obvious important focus for the selection and spread of multi-resistant bacteria and a possible direct source of nosocomial infections (Russotto *et al.*, 2015; Betteridge *et al.*, 2013). The development of resistance by bacteria species against these agents through various mechanisms including the acquisition of extra- chromosomal elements called plasmids which induces the production of extra-cellular enzymes called  $\beta$ -lactamases (Elizabeth *et al.*, 2016). Most of integrons are located within transposons that contribute to the vertical transmission, favouring their mobilization between plasmids and the bacterial chromosome by transposition events (Carattoli *et al.*, 2001). Resistant genes are also known to be located on extra-

chromosomal genetic elements or in segments inserted within the chromosome that originate from other genome. However, resistance genes encoded in plasmids are often located within genetic elements (Carattoli, 2003). There are speculations that resistance to  $\beta$ -lactam agents has influenced resistance of bacteria to other classes of antibiotics such as the quinolones and aminoglycosides with its attendant effect more devastating most especially on immune compromised patients (Amaya *et al.*, 2012). Therefore, this study is aimed at determining the plasmid profile of selected multidrug resistant bacteria isolates recovered from Urine Sample of HIV Seropositive Pregnant Women.

## Materials and Methods

### Sample collection

Bacterial isolates (*Escherichia coli* and *Pseudomonas* spp) cultured from the urine samples of HIV seropositive pregnant women attending antenatal clinic at the Ondo State Specialist Hospital, Akure, South western, Nigeria were used for the study. Each bacterial isolate was verified using cultural morphology, Gram's staining, selective media and differential media as well as biochemical tests to authenticate each isolate's identity.

### Plasmid analysis

Plasmid analysis of selected bacterial isolates was done by method of Kado and Liu, 1981. 600  $\mu$ l of nucleic lysis solution was used to suspend the bacterial and centrifuged for 5 min. Thereafter, protein extraction was done by added 200  $\mu$ l of phenol/chloroform (1:1) together with 3  $\mu$ l of 5 M sodium chloride to the lysate for precipitation of chromosomal DNA and incubated on ice for 4 h. The mixture was centrifuged for 10 min. Thereafter, 150  $\mu$ l of supernatant was transferred into new tube and added with 300  $\mu$ l of cold 80 % ethanol and spun 20 min at

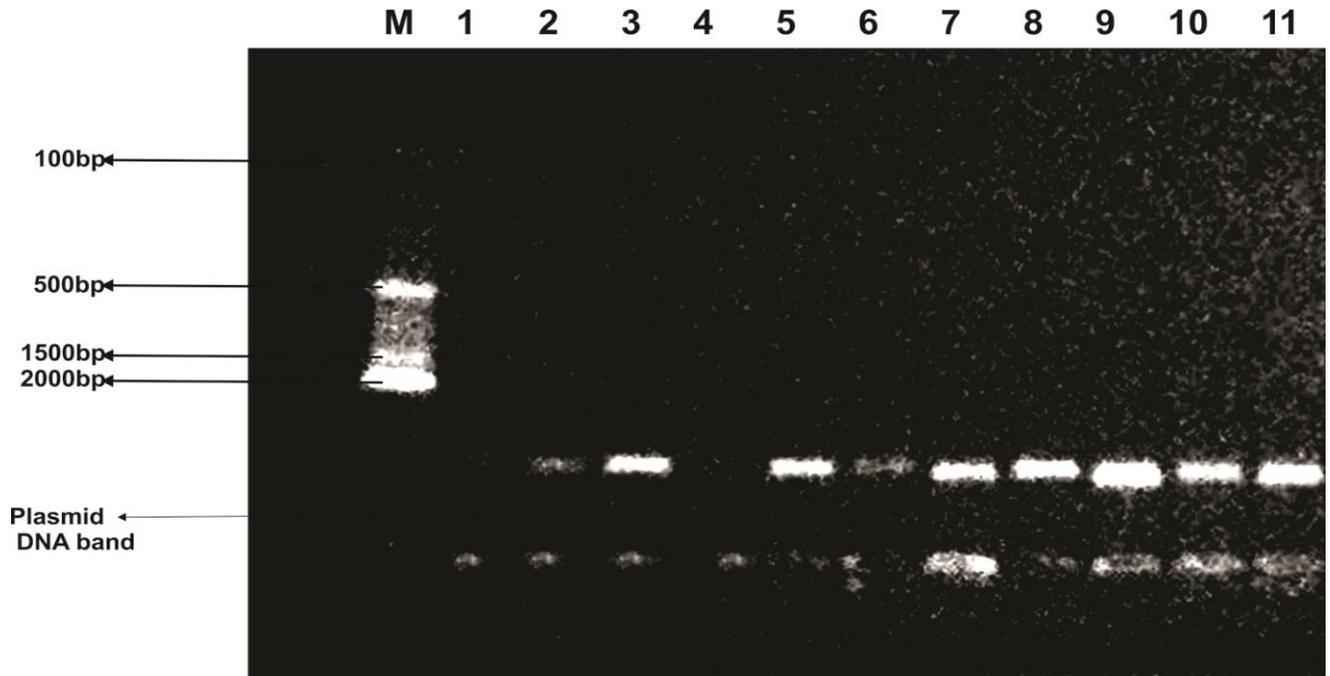
13,000 rpm. The desired lysate (supernatant) was removed and washed the pellet with 100  $\mu$ l of cold 80 % ethanol and then left to air-dry before suspended in TAE with RNase (1:100) dilution. All preparations were visualized by combining 2  $\mu$ l of loading dye with 8  $\mu$ l of plasmid preparations and electrophoresed on a 0.8 % agarose gel for 1 min at 200v before bringing the voltage down to 100v for the next 59 min. The gels were seeded with ethidium bromide solution for 30 min and destained for about 1 h with distilled water. Thereafter, the bands were visualized and photographed under Ultra-violet illumination.

## Results and Discussion

Antimicrobial drug resistance can occur by point mutations in the bacterial genome or through horizontal transfer of genetic elements carrying resistance genes. Resistance may be disseminated through clonal expansion of drug-resistant strains or through horizontal transfer of genetic elements coding for resistance determinants.

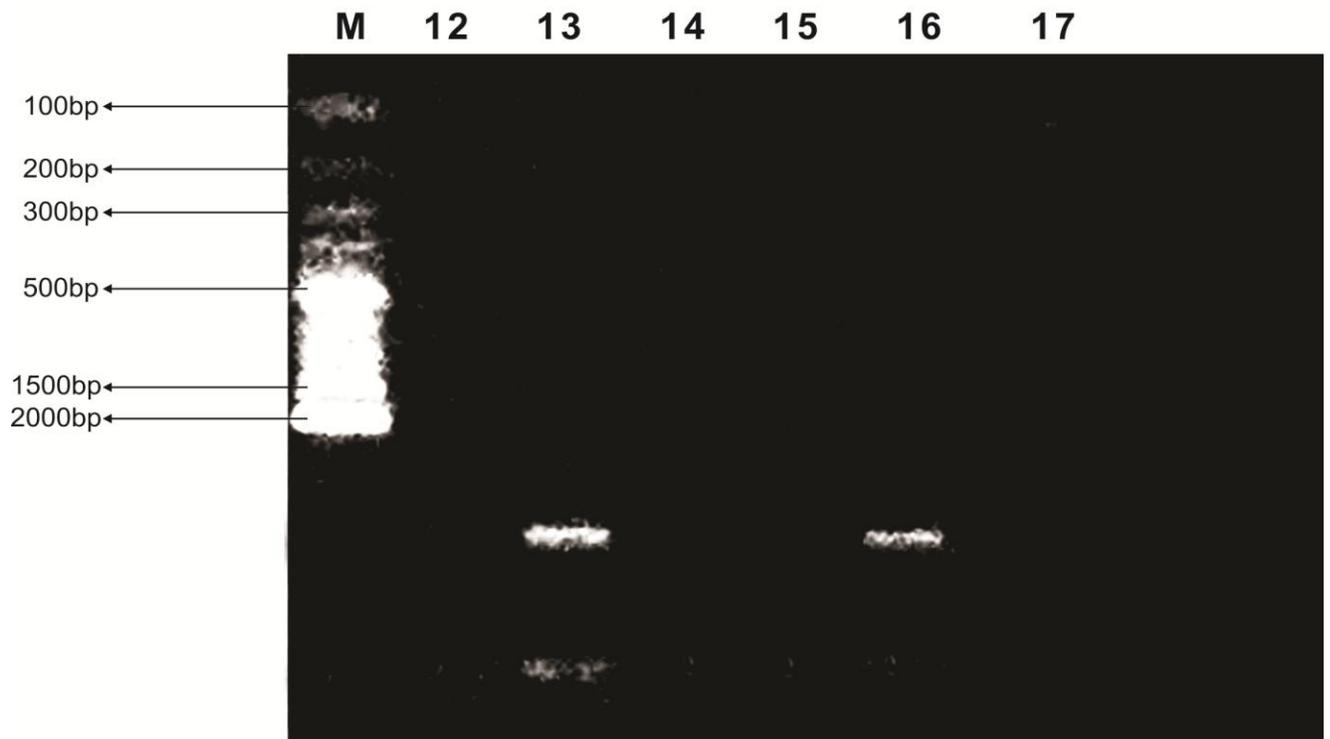
Plasmid is an important feature contributing to the dissemination of antibiotic resistance among bacteria most especially Gram negative bacteria. In this study, ten (10) *E. coli* isolates were used for plasmid analysis, nine were found to have plasmid; two of the twenty (20) *Pseudomonas* spp (*Pseudomonas aeruginosa*-13 and *Pseudomonas fluorescens*-7) isolates tested also produced plasmid while *S. aureus* isolates screened for plasmid was found to be plasmidless. The reason for this may be that resistance in *S. aureus* used may be chromosomal borne. Studies have shown that *S. aureus* acquire resistance through horizontal gene transfer and this acquisition especially the *MecA* genes which are on mobile genetic elements called Staphylococcal cassette chromosome which code for penicillin binding proteins (PBPs) (Giguere, 2006) (Fig. 1 and 2).

Fig.1 Gel for *E. coli* plasmid DNA band



Plasmid analysis profile. Lane M: 1kb DNA Ladder; Lane 1: E.Coli Control (NCIB 86); Lane 2 to 11: E.Coli samples recovered from urine culture of HIV seropositive pregnant women

Fig.2 Gel for *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* plasmid DNA band



Plasmid analysis profile. Lane M: 1kb DNA ladder; Lane 12: Pseudomonas aeruginosa control (NCIB 950); Lane 13 to 17 : Pseudomonas sp samples recovered from urine culture of HIV seropositive pregnant

**Table.1** Profile of plasmid DNA recovered from bacterial isolates

S/N	Code	Organisms	Plasmid Size (bp)
1	NCIB 86	<i>Escherichia coli</i> (control)	ND
2	A39b+	<i>Escherichia coli</i>	6595
3	A43a+	<i>Escherichia coli</i>	6595
4	A34a+ca	<i>Escherichia coli</i>	ND
5	A34a+	<i>Escherichia coli</i>	7129
6	A5b+	<i>Escherichia coli</i>	6595
7	A33a+	<i>Escherichia coli</i>	9004
8	A20a+mac	<i>Escherichia coli</i>	7706
9	A31c+	<i>Escherichia coli</i>	7706
10	A40a+	<i>Escherichia coli</i>	9004
11	A2a+	<i>Escherichia coli</i>	7706
12	NCIB 950	<i>Pseudomonas aeruginosa</i> (control)	ND
13	B4c+	<i>Pseudomonas aeruginosa</i>	9004
14	A38b+mac	<i>Pseudomonas fluorescens</i>	ND
15	A37a+	<i>Pseudomonas aeruginosa</i>	ND
16	B17b+	<i>Pseudomonas aeruginosa</i>	9004
17	A16a+ca	<i>Pseudomonas fluorescens</i>	ND

The studies done by Stuart (2002) shown that the appearance of the resistance genes in a clinical isolate and in a clinical setting is a serious warning to clinicians to control use of the new drug. The presence of this plasmid profile confirmed the amplification of these isolates pathogenicity factor gene and Salmonella invasive factor gene. The study concluded that appearance of the resistance genes in some of these bacterial isolates could help the isolates to be multidrug resistant which may lead to a serious public health challenges (Table 1).

This work was carried out in collaboration among all authors. Authors OVO, OOO, MOO and SBA designed the study; authors OVO wrote the protocol and interpreted the data, anchored the field study, gathered the initial data and performed preliminary data analysis. While authors OVO and OOO managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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