Conjugal Transfer of Antibiotic Resistance Genes in *Pseudomonas aeruginosa*

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Abstract: This study determined the conjugal transfer of antibiotic resistance gene among clinical strains of plasmid carrying *Pseudomonas aeruginosa.* The plasmid sizes range from 800bp to 25000bp while eighteen different plasmid profiles were encountered. 10.8% of the strains (8) presented profile 1, 2.7% of the strains (2) presented profile 2, 4, 8 and 17. 16.2% of the strains (12) presented profile 3, 4.05% of the strains (3) presented profile 5, 9, 11, 14, 15 and 18. 6.8% of the strains (5) presented profile 6 and 10. 8.1% of the strains (6) presented profile 7 and 5.4% of the strains (4) presented profile 12, 13 and 16. The antibiotic resistance patterns transferred from three of the five donor strains to the transconjugants were partial and were associated with the transfer of R plasmids of sizes 24, 23, 4, 2, 25kbp. The frequencies of transfer of R plasmids to the transconjugants ranged from 1.4×10^{-5} to 1.7×10^{-7} per donor strains

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1. Introduction

Horizontal gene transfer, 'the non-genealogical transmission of genetic material from one organism to another is a source of new genes and functions to the recipient of the transferred genetic material. In Bacteria, such types of transfer plays an important role in the acquisition of new phenotypic traits and helps host cells adapt to their environments. These mobile genetic elements (MGEs) largely contribute to evolution and diversity of bacterial genomes through horizontal gene transfer. Among them, Integrative and Conjugative Elements (ICEs) promote their own excision by site-specific recombination, conjugation and integration into a replicon of the recipient cell. MGEs usually encode adaptative functions that may cause drastic changes in the ecological and/or pathogenic properties of bacterial species [1].

Among the several classes of mobile genetic elements are integrative and conjugative elements (ICEs) that, by being excised from genomes, form plasmid-like circular structures, transfer to other cells by conjugation, and reintegrate into the genomes of new hosts [2, 3, 4]. The excision of ICEs from the genome involves the activity of integrase, which excises the circular entity from the genome by recombining the DNA tract found at each end of the ICE. The integrase also catalyzes the integration of the circular form into the genome. In the integration process, the integrase mediates recombination between the attP site on the circular-form DNA and the attB site on the genome of the recipient cell. ICEs are known to encode a variety of phenotypic traits, including pathogenicity, resistance to antibiotics, and xenobiotic degradation [4].

Pseudomonas aeruginosa is responsible for 10–15% of the nosocomial infections worldwide [5]. Often these infections are hard to treat due to the natural resistance of the species, as well as the remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents. *P. aeruginosa* represents a phenomenon of antibiotic resistance, and demonstrates practically all known enzymic and mutational mechanisms of bacterial resistance [6]. These coupled with the possibility of this organisms acquiring other forms of resistance genes through horizontal gene transfer make this bacteria a major public health concerns. This study was therefore aimed at determining the frequency of antibiotic resistance gene transfer among clinical strains of plasmid carrying *Pseudomonas aeruginosa.*

2. Material and Methods

2.1 Bacterial Strains

The bacterial strains used in this study was supplied by Mrs O.D. Popoola of the Department of Microbiology (Medical Microbiology unit), Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria.

2.2 Plasmid Extraction and Profiling

Conjugation experiment was carried out according to Willets [7] using rifampicin-resistant *E. coli* DH5α as the recipient cell and five selected representatives of *Pseudomonas aeruginosa* as donor strains. The initial donor-to-recipient ratio of 1:20 was used for mating. Transconjugants were selected on MH agar plate containing 300 µg/mL of rifampicin and 50 µg/mL of ampicillin. Plasmid extraction of donor strains and transconjugants was done using the alkaline lysis method of Takahashi and Nagano [8]. Plasmid DNA bands were detected by electrophoresis on 0.8% horizontal agarose gel pre-stained with ethidium bromide (0.5 µg/mL) and visualized under UV light. The sizes of the plasmid DNA bands were determined by extrapolation based on the mobilities of Hind III digested λ DNA co-electrophoresed with the plasmid DNA samples [9,10]. Antibiotic susceptibility assay was also carried out on the transconjugants. Plasmid extraction of 74 isolates were carried out using alkaline lysis method of Takahashi and Nagano[8]. Plasmid DNA bands were detected by electrophoresis on 0.8% horizontal agarose gel pre-stained with ethidium bromide (0.5µg/mL) and visualized under UV light. The sizes of the plasmid DNA bands were determined by extrapolation based on the mobilities of Hind III digested λ DNA co-electrophoresed with the plasmid DNA samples [9,10].

Table 1: Plasmid profiles of the studied *Pseudomonas aeruginosa* strains

Plasmid profiles	Molecular weight of plasmids	Frequency of plasmids occurrence
1	24	8(10.8)
2	25, 1	2(2.7)
3	25	12(16.2)
4	25, 6.5,1	2(2.7)
5	25,1.5	3(4.05)
6	25,2	5(6.8)
7	25,3	6(8.1)
8	25, 5	2(2.7)
9	0.8	3(4.05)
10	23.5	5(6.8)
11	23, 4, 2	3(4.05)
12	23	4(5.4)
13	23, 2	4(5.4)
14	20, 23.5	3(4.05)
15	23.5, 2	3(4.05)
16	24, 2	4(5.4)
17	23.5, 4, 3	2(2.7)
18	24, 23.5	3(4.05)
Total	33	74(100)

Table 2: Conjugal transfer of antibiotic resistance and plasmids to Escherichia coli DH 5α by some of the selected bacterial isolates

Donor strains	ATB	PF	ATB to EC	RP to EC	CF
Pseudomonas aeruginosa 1	Amp, Ami, Cip, Gent	24	Amp, Ami, Cip,	24	2.3×10^{6}
Pseudomonas aeruginosa 11	Car, Cef, Cot, Imi	23, 4, 2	Car, Cot	23,4	1.7×10^{7}
Pseudomonas aeruginosa 2	Gen, Imi, Kan, Cot	25, 1	en, Kan,	25	1.4×10^{5}
Pseudomonas aeruginosa 12	PT, Cip, Cef, Cefti	23	PT, Cip	-	1.3×10^{7}
Pseudomonas aeruginosa 14	Cef, Amp, Car, Ami	20,	23.5	-	-

3.0 Results And Discussions

Of the 74 strains analyzed, none was found without plasmid, 32 (43.2%) had one plasmid (Table 1), 37 (50%) had two plasmids while the remaining five carried three plasmids each. The sizes of the extracted plasmid range from 800bp to 25000-bp. Eighteen different profiles were encountered, 10.8% of the strains (8) presented profile 1, 2.7% of the strains (2) presented profile 2, 4, 8 and 17. 16.2% of the strains (12) presented profile 3, 4.05% of the strains (3) presented profile 5, 9, 11, 14, 15 and 18. 6.8% of the strains (5) presented profile 6 and 10. 8.1% of the strains (6) presented profile 7 and 5.4% of the strains (4) presented profile 12, 13 and 16. The plasmid profiles in *Pseudomonas aeruginosa* have been studied [10] where a high diversity of profiles was observed [11]. Plasmid profiling has been proven useful to differentiate between *Pseudomonas aeruginosa* strains [12] but their discriminatory power has also been questioned [13]. Tables 2 depict the outcomes of the conjugation experiment. Conjugal transfer of resistance to ampicillin, amikacin, ciprofloxacin, gentamicin, carbenicilllin, cotrimoxazole, kanamycin, piperacillin and tazobactam was detected in the transconjugants after the mating experiment. The antibiotic resistances were transferred from four of the five selected donor strains. The antibiotic resistance pattern transferred by these donor strains was partial and was associated with the transfer of R plasmids of sizes 24, 23, 4,2,25kb kb from three of the five transferable strains (tables 6). The frequencies of transfer of antibiotype or R plasmids to the transconjugants ranged from 1.4×10^{-5} to 1.7×10^{-7} transconjugants per donor strain. This study documented the involvement of plasmids as factors responsible for antibiotic resistance in some of the recovered pathogens since these resistances were partly transferred to *E. coli* DH5α by conjugation. Therefore, our findings suggest the emergence and active transfer of antibiotic resistance and R plasmids among the studied strains of *Pseudomonas aeruginosa*. In this study, antibiotics such as ampicillin, amikacin, ciprofloxacin, carbenicillin, cotrimoxazole, gentamicin, kanamycin and combination of piperacillin and tazobactam were easily transferred from a multi drug resistant *Pseudomonas aeruginosa* to *E. coli*. The co-transfer of plasmids of sizes 23 and 4kb suggests that these extrachromosomal DNAs are R plasmids. The minimum size of a plasmid with an efficient conjugation system has been reported to be >15 kb [14]. Therefore, the presence of the 5.0-kb plasmids in the transconjugants implies that the larger molecular size plasmids (i.e., 25, 24 and 23kb) might serve as vehicles for the transfer of lower molecular weight plasmids such as 4 kb to *E. coli* DH5α. This also implies that the mechanism of plasmid mobilization among the studied pathogens may entail the use of larger sized plasmids as vehicles in addition to the classical transfer systems such as conjugation that require considerable genetic information, as previously reported by Smith and Linggood [15], Achmith and Helmuth [16] and Christiansen et al. [17]. The result from the conjugation experiment also revealed that the varied drug-resistant exconjugants arose at a frequency range of 10^{-5}–10^{-7} per donor cell. These evolution rates howver are similar to those of previously reported epidemiological and clinically important pathogens in Nigeria.

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