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Drug resistance and plasmids of local clinical isolates of *Pseudomonas aeruginosa* in Lagos metropolis

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ABSTRACT: Antibiotic susceptibility testing of 50 clinical isolates of *Pseudomonas aeruginosa* from the lagos University Teaching Hospital was carried out. All isolates were resistant to Ampicillin. A large number of isolates were resistant to Nitrofurantoin and tetracycline (96%), and Co-trimoxazole (88%). It is noteworthy that 46% of the isolates were sensitive to Gentamicin. 11 distinct multi-drug resistance patterns were observed in these isolates. 14% harboured plasmid DNA ranging in sizes from 1.51 to 83.18 Mdal. 22 different plasmids of varying molecular weights were found distributed in all the plasmid bearing isolates. A 4.37 Mdal plasmid was found to be common to 6 of the 7 plasmid-bearing strains. The results of this study reveal the emergence of *Pseudomonas aeruginosa* strains with increased resistance to commonly used antibiotics.

Keywords: *Pseudomonas aeruginosa*, Plasmids; Antibiotics susceptibility pattern; Drug resistance.

Introduction

Pseudomonas aeruginosa is an aerobic gram-negative non-fermentative bacilli (1). It is a frequent pathogen that is able to cause infection in virtually any site in immunocompromised patients (2). It is a major cause of nosocomial infections and the leading cause of nosocomial respiratory tract infection, which can be especially serious in the instubated patients in the intensive care unit (3). It has been reported that patients with Pneumonia have a high mortality and require aggressive antimicrobial therapy. *P. aeruginosa* is also associated with nosocomial urinary tract infection and bacteremia (4). Wound infections due to *P. aeruginosa* are p[articularly troublesome in burn patients. The high rate of sepsis following wound infection due to *P. aeruginosa* is responsible for high mortality rate (5). A large body of information is available on the distribution of *P. aeruginosa* in the environment of hospitals including intensive care units. Nevertheless, the ropute of infection and transmission has been controversial (6).

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P. aeruginosa has been known for its resistance to many first generation antimicrobial agents because of their indiscriminate use. It has been reported to be resistant to many antibiotics including –lactams and Quinolones (7). Cases of multiple resistance has since been reported (8). Pseudomonas species have been shown to contain resistant plasmids in their clinical isolates. Plasmid profiling has been used as fingerprints in tracing the epidemiological spread of infection and for resolving problems of classification (9,10,11). In the present report, we describe the plasmid profile and antibiotic susceptibility pattern of our local clinical isolates of *Pseudomonas aeruginosa* in Lagos Metropolis in Nigeria.

Materials and Methods

Source of Clinical Isolates

All 50 isolates used in this study were isolated from clinical specimen obtained from patients, at the Medical Microbiology Department of the Lagos State University teaching Hospital, Idi-Araba, Lagos, Nigeria.

Bacteriology

Primary isolates were stored on Nutrient Agar slants. Subsequent cultures were on Muella Hinton Agar (MHA) at 37°C overnight. The *P. aeruginosa* isolates were speciated biochemically as outlined by Cowan (12). The isolates were slimy with blue-green pigmentation and are oxidase positive.

Antibiotic Susceptibility Testing

Resistance patterns of thye isolates of *P. aeruginosa* were determined by disc diffusion method. Sterile cotton swab was dipped into the MHA broth (Oxoid) suspension, drained and usedfor inoculating 25ml of Muella Hinton Agar (Oxoid) in a 100mm plate (Sterillin, UK). The inoculated plates were air-dried and antibiotic discs (Oxoid), UK) were mounted on them. Six antibiotics that were tested for susceptibility included Ampicillin (10g), Gentamicin (10g), Nalidixic Acid (30g), tetracycline (30g). The plates were inverted in ambient air at 37°C overnight. The zones of inhibition were measured as earlier described (13).

Plasmid DNX Extraction

A modification of the Birnboim and Doly method (14) was found suitable for the extraction of plasmid DNA. All strains were grown on MHA (E. coli K-12 [V517] on Nutrient Agar supplemented with Ampicillin. Pure cultures were suspended in Nutrient broth, grown overnightand harvested by centrifugation. After washing with TEG (50mM glucose, 10mM EDTA, 25mM tris [pH 8.0] buffer, the cells were lysed with 2mg/ml lysozyme at room temperature. This was followed by the addition of 1% SDS (with rapid inversions). 5M Potassium acetate (vortex mixed) and storage on ice. The resulting precipitate was sedimented in the micro centaur (MSE Scientific Instruments) at 13000g and to the supernatant was added Phenyl/Chloroform/Isoamyl alcohol solution, centrifuged and after decanting, 100% Ethanol added. The resulting precipitate was pelleted by centrifugation, washed with 70% Ethanol and air-dried. The pellet (DNA) was re-suspended in TE (10mM Tris [pH 8.0])1.0mM EDTA) buffer.

Agarose Gel Electrophoresis

Electrophoresis of plasmid DNA was carried out on 0.7% agarose slab gels in tris-borate buffer (89mM Tris, 89mM Boric Acid, 25mM [pH 8.0]), using a horizontal slab gel apparatus. The gels were run for 4-5 hours at 87V/cm (constant voltage) at room temperature. The gel was stained after electrophoresis by immersing the gel in water containing ethidium bromide (0.5ug/ml) overnight at room temperature. The stained gel was visualized under Ultraviolet light transilluminator and the photograph of the plasmid bands on the gel taken.

Results

All the 50 isolates were found to be resistant to Ampicillin. However, 23(46%) were found to be sensitive to Gentamicin. Most isolates were resistant to Nalidixic Acid, Co-trimoxazole, Nitrofurantoin and tetracycline with more than 75% resistance. A total of 11 antibiotic susceptibility patterns were observed. 34 (68%) of all the isolates were found to be resistant to all the antibiotics used. 2(4%) of the isolates were associated with resistance to Ampicillin and Tetracycline only (See Tables 1 and 2).

Twenty-two different plasmids with varying molecular weight were found distributed among the plasmid bearing strains. Of the 50 isolates screened for plasmid DNA, 7(14%) were found to carry plasmids of varying sizes ranging from 1.51 to 83.18Mdal. The total number of plasmids encountered was 40. A 4.37Mdal plasmid was found to be common to 6 of the 7 plasmid-carrying strains but one (See Table 3 and Figure 1).

Table 1: Antibiotic Susceptibility Patterns of 0% local isolates of *P. aeruginosa*

Patterns	**% Showing Patterns**
Ap-Ct-Gm-Na-Nt-Tc	34
Ap-Ct-Na-Nt-Tc	30
Ap-Ct-Gm-Nt-Tc	8
Ap-Ct-Nt-Tc	8
Ap-Gm-Nt-Tc	4
Ap-Ct-Gm-Tc	2
Ap-Tc	2
Ap-Na-Nt	2
Ap-Ct-Na-Nt	2
Ap-Gm-Na-Nt-Tc	2
Ap-Gm-Na-Tc	2

Key:
Ap- Ampicillin
Ct- Co-trimoxazole
Gm- Gentamicin
Na- Nalidixic Acid
Nt- Nitrofuranton
Tc- tetracycline

Table 2: Percentage resistance/sensitivity of local clinical isolates of *P. aeruginosa*

Antibiotics	**% Sensitivity**	**% Resistance**
Ampicillin	0	100
Co-trimoxazole	12	88
Gentamicin	46	54
Nalidixic Acid	24	76
Nitrofurantoin	4	96
Tetracycline	4	96

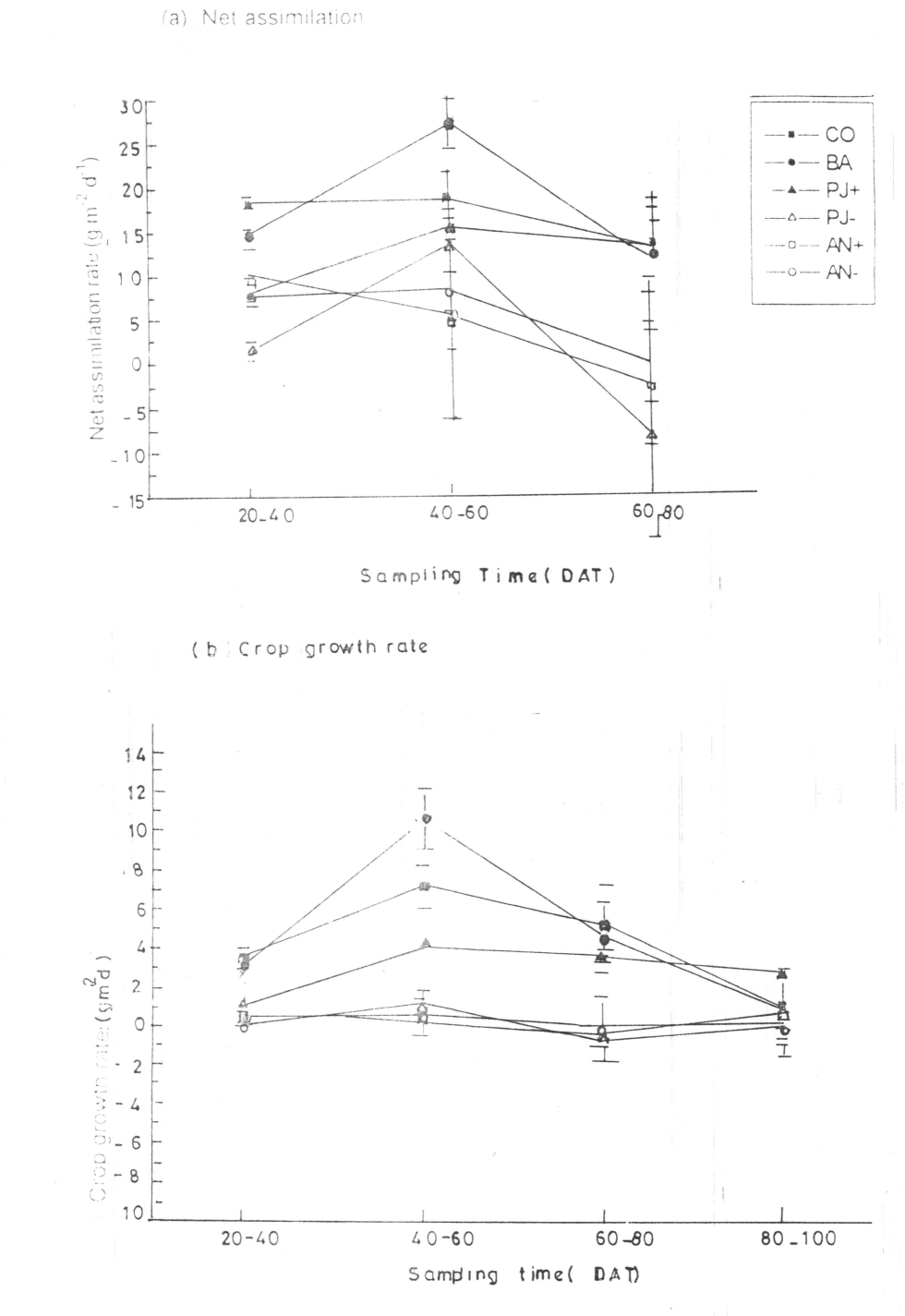

Fig. 1: Effect of unpruned *B. aegyptiaca* (BA), pruned *P. juliflora* (PJ+), unpruned *P. juliflora* (PJ-), Pruned *A. nilotica* (AN+), unpruned *A. nilotica* (AN-) and control on net assimilation rate and crop growth rate of dry season sorghum.

Table 3: Plasmids of local clinical isolates of *P. aeruginosa*

Strain	**Molecular Weight (Mdal)**
LU1295	4.37, 4.17, 3.80, 2.82, 2.63, 1.58
LU1401	28.5, 4.37, 3.98, 3.63, 2.75, 2.48, 1.51
LU1822	4.37, 3.98, 2.82, 2.54, 1.58
LU2280	83.18, 4.37, 4.17
LU1023	35.48. 4.37, 3.98, 3.63, 2.95, 2.82, 2.75, 2.48, 1.51
UC818	4.37, 3.98, 3.63, 2.95, 2.75, 2.48, 1.51
LU1438	4.77
E. Coli (V517)	35.8, 4.8, 3.7, 2.6, 2.0, 1.8, 1.4

Discussion

In this study, we carried out antibiotic susceptibility test on 50 clinical isolates of *P. aeruginosa*. The resistance of the isolates studied, to antibiotics is so high as more than 50% of all the isolates were resistant to the entire antibiotics used. This result is similar to those obtained elsewhere (15, 16). The high percentages of resistance to Ampicillin and teteracycline are a result similar to the ones earlier described (17,8). This study also supports earlier reports from Eke and Rotimi (18) on *P. aeruginosa* to Ampicillin. They also reportewd Gentamicin to be potent against gram-negative bacteria isolates from clinical infections, at attainable serum concentrations. Our findings indicate however a decrease in the percentage susceptibility (from 90 to 40%) to Gentamicin. This evidently indicates the emergence of more Gentamicin-resistant strains in the Lagos Metropolis. The usefulness of a high dosage of Gentamicin in the treatment of serious *P. aeruginosa* infections in this part of the world still needs to be ascertained.

Resistance to Ampicillin and other β-lactans antibiotics has been severally reported, which resistance could either be chromosomally or plasmid borne (19,20,21). The presence of a 4.37Mdal plasmid in 6 of the 7 plasmid-carrying strains is noteworthy. The strains were found to be resistant to both Ampicillin and tetracycline. There is therefore a likelihood that the 4.37Mdal plasmid may code for resistance against these antibiotics. The most common plasmids encountered in this study were less than 5Mdal in size. The numerous nature of the plasmids is probably due to the presence in multi-copy number. Plasmid profiling has been shown to be a good epidemiological tool in investigating epidemics of bacteria disease (22).

We did not find any of the antibiotic tested to which all the isolates were sensitive. In addition, most of the plasmid carrying isolates contains more than one plasmid. The indiscriminate use of antibiotics coupled with these findings is likely to compound the problems of antibiotic therapy in our environment. The varied resistance patterns gotten and the plasmid analysis may be an adjunct in epidemiological studies of the bacteria in Nigeria.

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