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Full Length Research paper

Antibiotic susceptibility and plasmid pattern of *Pseudomonas aeruginosa* from the surgical unit of a university teaching hospital in north central Nigeria

A. T. Olayinka[1]*, B. O. Olayinka[2] and B. A. Onile[3]

[1]Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. [2]Department of Medical Microbiology and Parasitology, University of Ilorin, Ilorin, Nigeria [3]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

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This study determined the susceptibility pattern and multiple antibiotic resistance (MAR) index of 92 *Pseudomonas aeruginosa* strains from clinical samples comprising mainly urine (51.1%) and wounds (41.3%) obtained from the surgical units of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria; over a 24-month period. The strains were susceptible to imipenem (94.6%), ciprofloxacin (90.2%), amikacin (89.1%) and ceftazidime (78.3%) but resistant to ofloxacin (82.6%), perfloxacin (58.7%) and gentamicin (35.8%). Analysis of the MAR index of isolates revealed that 60.9% had MAR index of 0.3 and above, which is an indication of probable origin from the hospital environment where antibiotics are extensively used. A strict management of antibiotic policies and a continuous surveillance programme for multidrug resistant pathogens like *P. aeruginosa* in specialised units is advocated.

Key words: Antibiotic resistance, plasmid, *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is a classic opportunistic pathogen especially because of its innate resistance to many antibiotics and disinfectants; and also due to its armoury of putative virulence factors plus additional ac-quired resistance due to plasmids (Govan, 1998; Shahid and Malik, 2004). It is also the most common Gram nega-tive bacterium found in nosocomial infections causing various spectra of infections especially in neutropenic, immunocompromised, burns/tissue injury and cystic fibro-sis patients all over the world (Delden and Iglewiski, 1998; Song et al., 2003; Brown and Izundu, 2004).

Recent advances in medicine such as the advent of more elaborate surgery and intensive care, the use of immunosuppressive drugs, the availability of invasive procedures or instrumentation and the increase in number of immunocompromised patients means there is a rise in patients with impaired immune defences liable to nosocomial infections (Klutymas, 1997; Brown andn Izundu, 2004).

The increasing incidence of infections caused by multidrug resistant organisms have caused attention to be focused on measures for fighting resistance, foremost of which is susceptibility surveillance (Masterton, 2002).

This study therefore determined the prevalence, antibiotic susceptibility and plasmid patterns of *P. aeruginosa* strains from clinical specimens obtained from the surgical units of a University Teaching Hospital in Northern Nigeria.

METHODOLOGY

A sample per patient was studied. A total of 1,452 clinical specimen received from the surgical unit were cultured on blood agar and MacConkey agar plates and incubated at a temperature of 37^0C for 24 h and on Mueller Hinton agar plates to assess pigment production. The culture plates were processed using standard microbiological procedures (Cheesbrough, 1993) . Characterisation and identification of *P. aeruginosa* was carried out using a combination of colonial morphology, Gram stain characteristics, motility tests, pigmentation, oxidation-fermentation tests, catalase and oxidase activity tests and pyocyanin production (Cheesbrough, 1993).

Antibiotic susceptibility was determined on Mueller Hinton aga using the disc diffusion method according to the modified Kirby-r Bauer technique (Vandepitte et al, 1999). All the isolated *P. aerugi-*

*Corresponding author. E-mail: debolaola@yahoo.com.

Table 1. Distribution of *Pseudomonas aeruginosa* strains from various specimens and their antibiotic susceptibility pattern based on site of specimens

	IMP		CIP		AN		CAZ		GN		PEF		OFX		CCL	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Urine(47)	46	1	43	4	40	7	36	11	32	15	17	30	5	42	-	47
Wound(38)	35	3	34	4	36	2	31	7	26	12	18	20	10	28	-	38
Catheter Tip (3)	2	1	2	1	2	1	1	2	-	3	1	2	-	3	-	3
Ear swab(2)	2	-	2	-	2	-	2	-	1	1	1	1	1	1	1	1
Blood(1)	1	-	1	-	1	-	1	-	-	1	1	-	-	1	-	1
Sputum(1)	1	-	1	-	1	-	1	-	-	1	-	1	-	1	1	-
Total	87	5	83	9	82	10	72	20	59	33	38	54	16	76	2	90

Key:
Imp = Imipenem Cip = Ciprofloxacin
An = Amikacin Caz = Ceftazidime
Gn = Gentamicin Pef = Perfloxacin
Ofx = Ofloxacin Ccl = Chloramphenicol
S = Sensitive R = Resistance

nosa strains and a standard strain of *P. aeruginosa* ATCC 27853 were tested for their sensitivity to the following antibiotics: ceftazidime 30 µg, amikacin 30 µg, gentamicin 10 µg, imipenem 10 µg, ciprofloxacin 5 µg (all from 54932 Bio Merieux SA Marcy L'Etoile France); ofloxacin 5 µg (from B39892 Oxoid Unipath Ltd, Basingstoke, England); perfloxacin 10 µg (152 Pasteur Biological Laboratory, India). Isolates were considered multidrug resistant if they showed resistance to 3 or more of the tested antibiotics.

The multiple antibiotic resistance MAR index was determined for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Krumpernam, 1983).

$$\text{MAR index} = \frac{\text{Number of antibiotics isolate is resistant to}}{\text{Total number of antibiotics tested}}$$

Plasmid DNA isolation was carried out using the modified alkaline lysis procedure described below.

The *P. aeruginosa* strains were grown overnight in Tryptone soya broth. 1.5 ml of each overnight culture was poured into an Eppendorf tube, spun for 10 s in a micro-centrifuge. The supernatant was gently decanted leaving about 50 µl with the pellet. This was then vortexed at high speed to re-suspend the cells completely. 300 µl of TENS (TE buffer +0.1N NaOH +.5% sodium dodecyl sulphate [SDS]) was added and mixed for 2 - 5 s till the mixture became sticky. 150 µl of 3.0N sodium acetate (pH 5.2) was added, then vortexed for 2 - 5 s to mix completely. The contents were then spun for 2 min in a microcentrifuge to separate pellet cell debris and chromosomal DNA. The supernatant was transferred to a fresh eppendorf tube and mixed well with 0.9 ml of absolute ethanol which had been pre-cooled to -20^{0}C. The mixture was then spun for 2 min to pellet plasmid nucleic acid which was rinsed twice with 1ml of 70% ethanol, then dried. The pellet was re-suspended in 20 µl of TE buffer and 1 µl of 0.25%bromophenol blue was added to it. These were then put into wells made in the agarose gel mould, placed in an electrophoretic tank containing Trisborate buffer and electrophoresis ran for one hour at 60volts. The stained gel was visualised under UV light and viewed for the presence of plasmid bands. The plasmids were then photographed using the Polaroid MP-4 camera system (Zhou et al., 1998)

RESULTS

Ninety two strains of *P. aeruginosa* out of 878 positive cultures were recovered from a total of 1,452 clinical specimens obtained from the surgical unit over the 2-year period. The distribution of *P. aeruginosa* strains from various clinical specimens and their antibiotic susceptibility based on the source of the isolates is shown in Table 1. More than 80% of the isolates from wound and more than 70% from urine samples were sensitive to imipenem, ciprofloxacin, amikacin and ceftazidime. Isolates obtained from sputum, blood and ear swab were the least resistant. A total of 51.1% and 41.3% *P. aeruginosa* strains were from urine and wounds respectively. Other recovery rates were from catheter tips (3.3%), ear swab (2.1%), blood (1.1%) and sputum (1.1%)

Figure 1 shows the antimicrobial susceptibility profile of the *P. aeruginosa* to imipenem (94.6%), ciprofloxacin (90.2%) and amikacin (90.2%), while 78.3% were sensitive to ceftazidime and 64.2% to gentamicin and there was high resistance to chloramphenicol (97.8%), ofloxacin 82.6% and perfloxacin 58.7%.

Table 2 shows the MAR index of the *P. aeruginosa* strains Analysis of the MAR index showed that 60.9% had MAR index of 0.3 and above.

The resistance pattern of the *P. aeruginosa* strains is shown in Table 3; with combined resistance to ceftazidime, gentamicin, perfloxacin and ofloxacin resistance being most prevalent.

Screening for the presence of plasmids in these multiresistant strains displayed the presence of plasmids in 14 of the MDR strains. Figure 2 shows the agarose gel electrophoretogram of the extracted plasmids. Eight of the isolates had similar plasmid band patterns, harbouring between 1-3 plasmid bands. Most of the bands were of low to intermediate molecular weights

DISCUSSION

This reports that the prevalence of *P. aeruginosa* was 10.5% of the total bacterial pathogens isolated from the

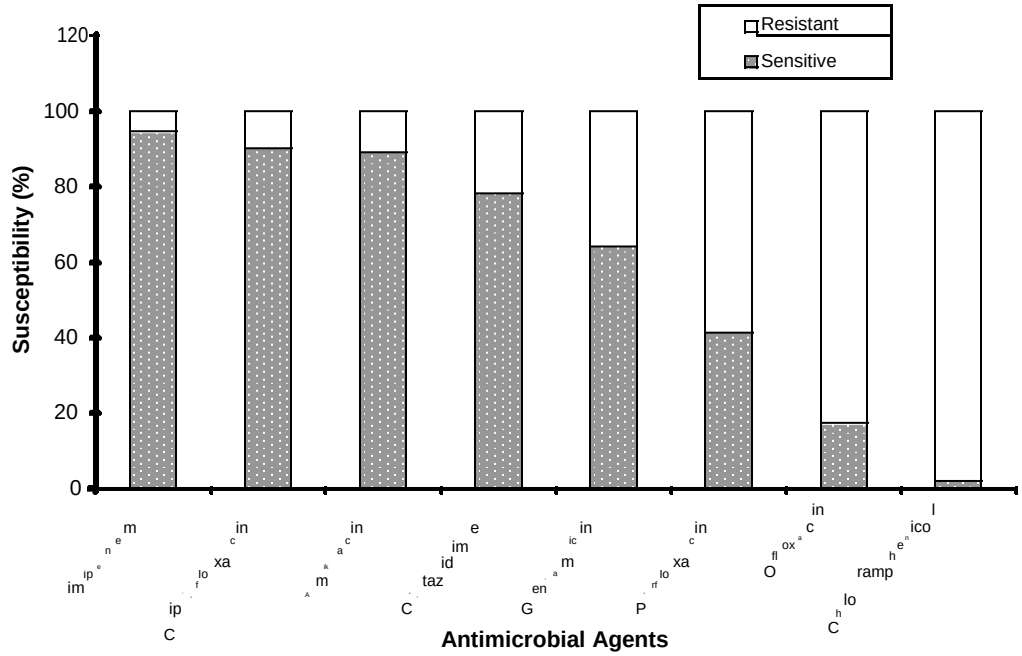

Figure 1. Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* isolates.

Table 2. Multiple Antibiotic Resistance (MAR) index of *Pseudomonas aeruginosa* isolates

MAR index	No of isolates	Percentage
0	13	14.1
0.1	23	25.0
0.3	21	22.8
0.4	13	14.1
0.6	11	11.9
0.7	9	9.9
0.9	1	1.1
1.0	1	1.1

Table 3. Resistance pattern of multi-resistant strains of *Pseudomonas aeruginosa*

Resistance attern	Number of isolates
Cip^R An^R Gn^R Pef^R Ofx^R	1
Caz^R Gn^R Pef^R Ofx^R	5
Cip^R Caz^R Gn^R Ofx^R	2
Cip^R Gn^R Pef^R Ofx^R	1
An^R Gn^R Pef^R Ofx^R	1
Gn^R Pef^R Ofx^R	2
Caz^R Gn^R Ofx^R	2
Cip^R Prf^R Ofx^R	2
Cip^R An^R Gn^R	1
An^R Gn^R Ofx^R	1

Key:
Imp^R = Imipenem resistant Cip^R = Ciprofloxacin resistant
An^R = Amikacin resistant Caz^R = Ceftazidime resistant
Gn^R = Gentamicin resistant Pef^R = Perfloxacin resistant
Ofx^R = Ofloxacin resistant R = Resistance
S = Sensitive

16151413121110 9 8 7 6 5 4 3 2 1

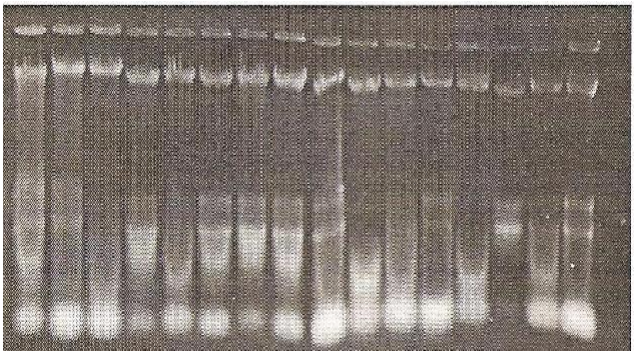

Figure 2. Agarose gel elctrophoretogram of extracted plasmids from multiresistant strains. There were no plasmids detected in lanes 6 and 14, while 8 of the isolates had similar plasmid bands.

surgical unit over a period of 2 years. This rate is lower than what was obtained in 2 similar studies (Oduyebo et al., 1997; Brown, 2004) in which the percentage prevalence rates were 17.5 and 14.4% respectively. This difference may be as a result of the differences in the study population and the larger number of specimens in the previous studies which covered all specimens from every clinical unit within the respective hospitals (Oduyebo et al., 1997; Brown, 2004).

The *P. aeruginosa* strains from urine, accounted for 51.1% of the total strains in this study which differs from other studies where majority of the Pseudomonas strains isolated were either from surgical site samples or wound swabs (Stark and Maki, 1984; Henwood et al., 2001).

The high rate obtained from urine is not surprising considering the fact that most patients going in for major surgery tend to get catheterised. Catheter associated urinary tract infections (CAUTIs) are said to comprise the largest institutional reservoir of nosocomial antibiotic resistant pathogens (Stark and Maki, 1984; Liu et al., 1992; Henwood et al., 2001). The current study included patients from the urology unit most of whom had been on catheter for a considerable long period of time which may also justify the higher recovery rates of *P. aeruginosa* strains from urine specimens.

Out of all the 92 isolates of *P. aeruginosa* strains, 35.8% were resistant to gentamicin which used to be traditionnally considered as a first-line drug against Gram negative bacterial infections in the hospital setting (Shanson, 1989; Oduyebo et al., 1997). In this study, 1.9% of the *P. aeruginosa* strains were reportedly resistant to amikacin, while from the southern part of the country, less than 5% were resistant and in Jamaica, amikacin was the only antibiotic to which all the *Ps. aeruginosa* strains were susceptible. This is worrisome since amikacin is considered a potent agent in the treatment of infections caused by multi-resistant *P. aeruginosa* and those strains that have shown resistance to gentamicin and tobramycin (Vanhoof et al., 1993; Oduyebo et al., 1997; Gerding, 2000; Lambert et al., 2001; Brown and Izundu, 2004).

Generally most strains of *P. aeruginosa* are known to be sensitive to ceftazidime (Vanhoof et al., 1993; Zemelman et al., 1993; Bonfiglio et al., 1998). While in this study, 11.9% of the isolates were resistant to ceftazidime (a 3rd generation cephalosporin) useful in the treatment of pseudomonal infection. In Belgium and Jamaica a lower level of resistance was found whereas the level was higher in Lagos (Vanhoof et al., 1993; Oduyebo et al., 1997; Brown and Izundu, 2004). This comparatively lower rate of resistance may be due to the relative high cost of the drug and the poor socio-economic status of majority of the people in this environment. As frequent use of drugs tend to induce selective pressure on multi resistant strains.

Resistance to imipenem has been found to be independent of –lactamase production and in *P. aeruginosa* has been attributed to diminished expression of certain outer membrane proteins (Buscher et al., 2000) . More than 80% of isolates in this study were sensitive to imipenem (94.6%). Compared with results of a study conducted at the Lagos University Teaching Hospital (LUTH) in which 12.5% were resistant to imipenem, in this study only 5.4% Pseudomonas strains were resistant. Imipenem is a drug that is not readily available in our environment and its cost is also prohibitive.

Except in the case of ciprofloxacin in which, 90.2% of the Pseudomonas strains were susceptible, relatively lower susceptibility rates were recorded against some of the quinolone antibiotics. Just over 40% and about 17% of the Pseudomonas strains were recorded as being susceptible to perfloxacin and ofloxacin respectively.

Generally, most strains of *P. aeruginosa* are sensitive to ciprofloxacin (Zemelman et al., 1993; Brown and Izundu, 2004; Oduyebo et al., 1997). The difference in the resistance pattern to the various quinolones is similar to a study in Turkey where a wide range of resistance status against various quinolones was also recorded (Algun et al., 2004). The main mechanism of resistance to fluoroquinolones has been reported to be the decrease in binding of the target quinolones to enzymes as a result of changes in DNA gyrase and or topoisomerase enzymes. Mutations occur in gyr A and par C genes. This is usually against all quinolones. However, resistance due to mutations of gyr B, though less common may not be against all quinolones (Algun et al., 2004). Other mechanisms of resistance are; the decrease in the amount of quinolones entering the cells because of defect in the function of porin channels and various efflux systems in the bacterial membrane which pump out the drug from the bacteria (Livermore, 2004).

The susceptibility pattern of the multi resistant strains of *P. aeruginosa* showed that, 5 of the 18 strains were resistant to ceftazidime, gentamicin, pefloxacin and ofloxacin. All multi resistant strains were however sensitive to imipenem, almost all the strains, that is 16 out of 18 were resistant to gentamicin. It has been said that there is generally an excess of resistance among isolates from hospitalised patients compared with those from out patients (Livermore, 2004).

Analysis of the MAR index of the Pseudomonas strains showed that 60.9% had MAR index of 0.3 and above. MAR index higher than 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used (Krumpernam, 1983; Paul et al., 1997). The practical significance of the index may however be lost in Nigeria and other 3rd world countries where antibiotic use and abuse is rampant since the cut-off point was determined in countries with tight antibiotic control protocols. The MAR values can however be viewed as an indication of the extent of microbial exposure to antibiotics used within the community.

Plasmid analysis of the multi-resistant strains showed that 14 of the *Pseudomonas* strains harboured plasmids, eight of which had similar plasmid band patterns of 1-3 plasmid bands having low to intermediate molecular weights. Plasmid prevalence was higher in the strains from catheter tips and urine. Acquisition of mobile genetic elements is known to be the main mechanism for short term accumulation of resistance determinants in bacterial genomes (Liu et al, 2000).

In a study at LUTH, resistance to gentamicin, tobramycin and carbenicillin were attributed to transferable plasmids (Rotimi et al, 1984). In another study done in Greece, plasmids isolated from multi-resistant *P. aeruginosa* strains were found to encode high level resistance to gentamicin and tobramycin (Tsakris et al, 1992).

In a few cases of outbreaks in Korea, Japan and Turkey, plasmids encoding potent -lactamases together

with aminoglycoside-modifying enzymes were disseminated among *P. aeruginosa* strains rendering control even more difficult (Livermore, 2004).

When strains have multiple antibiotic resistance, the choice of therapy is limited and difficult. The tremendous therapeutic advantages afforded by the introduction of new antimicrobial agents will always be threatened by the emergence of increasingly resistant bacteria pathogens (File, 1999; Sexton, 2000). This is especially true in specialised units (e.g. surgery, intensive care units) where invasive procedures disrupt natural barriers to bacterial invasion and catheters may act as conduits for infection. These units may also provide foci of infection for other areas within the hospital. To prevent the spread and selection of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented and continuously studied (Elliot and Lambert, 1999). In the meantime, it is desirable that the antibiotic susceptibility pattern of bacterial pathogens like *P. aeruginosa* in specialised clinical units be continuously monitored and the results readily made available to clinicians so as to maximize the possibility of administering an effective therapeutic agent whenever there is a need to so do.

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