Quinolones Resistance And R-Plasmids Of Clinical Isolates Of *Pseudomonas* Species

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ABSTRACT

Background: There has been reported incidence in the emergence of Quinolones resistance in clinical isolates in Nigeria and the level in resistance has been on the increase.

Objective: To determine the antimicrobial resistance patterns and plasmids profiles of 67 clinical *Pseudomonas* species from a teaching hospital and diagnostic laboratory in Nigeria.

Materials and methods: The *Pseudomonas* species were identified and confirmed by standard procedures. The antibiotic susceptibility patterns were determined by Agar Disk Diffusion method. Plasmids DNA was isolated, separated and stained as previously described.

Results: 30 of these clinical isolates were found to be resistant to 6 quinolones antibiotics with ciprofloxacin being the highest [52.24%] and norfloxacin [38.81%] the lowest. All the strains that were resistant to any antimicrobial agents were also resistant to ciprofloxacin. A total of 54 plasmids ranging in molecular sizes from 3.55kb to 19.95kb were extracted from the resistant strains and grouped into 5 plasmid profiles. Transformation experiment revealed that 66.67% of the resistant strains carried a common R plasmid of size 15.85kb. Plasmid –mediated resistant to ciprofloxacin and levofloxacin were found.

Conclusion: The results highlighted diverse plasmids profile and wide spread antimicrobial resistance patterns of some clinical *Pseudomonas* species from Nigeria.

Key words: Quinolones, R-plasmids, *Pseudomonas*.

Pseudomonadaceae as a family are highly resistant to antibiotics[1-3]. The level of resistance has been on the increase and treatment using the older drugs are gradually failing[4]. Fluoroquinolones are the newest effective antimicrobial agents just being introduced into Nigeria by some Pharmaceutical companies under different trade names. These quinolones are used for various diseases ranging from urinary tract infections, enteric infections, septic wound, septicaemia etc.

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Quinolone resistance is increasing in clinical isolates and has reached a minimum inhibitory concentration(MIC) in *Esecherichia coli* at 265mg\ ml for ciprofloxacin[5,6].

Multiple antibiotic resistance in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype[7-9]. Reported incidence of emergence of resistance to the quinolones by Oni et al[10] and Ozumba[11]has prompted this present study. Thus, this paper describes the antimicrobial susceptibility testing and Plasmid screening of *Pseudomonas* species isolated from clinical specimens in Lagos University Teaching Hospital, Lagos and Pathfinder Diagnostic Laboratory, Festac , Lagos respectively.

METHODOLOGY

Bacteriology

Sixty-seven strains of *Pseudomonas* species isolated by Standard procedures[12] from 710 clinical specimens sent to the diagnostic laboratory of Medical Microbiology and Parasitology laboratory of Lagos University Teaching Hospital, Lagos and Pathfinder Diagnostic Laboratory, Lagos, respectively from January to December 2006.

Antimicrobial Susceptibility Testing

Antimicrobial disc susceptibility tests were carried out on the isolates using Stokes disc diffusion technique[13] on freshly prepared Muller- Hinton agar (Oxoid, England) and Standardized by the method of National Committee for Clinical Laboratory Standard[14], using the following antibiotic discs; Pefloxacin [Pef] 5µg, Ciprofloxacin [Cip] 5µg, Ofloxacin [Ofx] 5µg, Levofloxacin [Luv] 5µg, Norfloxacin [Nfn] 5µg, and Sparfloxacin [Spn] 5µg. *Pseudomonas* (NCTC 10662) obtained from Central Public Health Laboratory, London, was used as control. Plates with antibiotic discs were incubated for 24hours at 37 c and sensitivity pattern was compared with that of the control organism.

Isolation And Separation of Plasmid DNA

Plasmid DNA was isolated, separated and stained as previously described[15]. Plasmid profile groups were constructed by grouping strains possessing the same profile (containing the same number and molecular mass), or part of a profile constituting one plasmid group.

Genetic Transfer

Transformation was done as described by Hanahan[16] using *Escherichia coli* K-12, HB101 (ara-14, galk2, hsd520, lacyl, leu, mt101, proA2, recA13, rps120, supE44, thi xyl-5) as recipient and plasmid pBR322 as the positive control. Co-transformation of resistant character was determined by testing all transformants against all antibiotics to which the donor strains was resistant,

Extracts from transformants were obtained as described above and subjected to Agarose electrophoresis. Transformation was confirmed as positive only when

Resistant transformants were shown to contain a plasmid (s) of a size similar to that found in the original isolate.

Plasmid Curing:

The curing of the resistant plasmids of the clinical *Pseudomonas* isolates was done as described by Vivyan et al[17].

RESULTS

The sources of the clinical *Pseudomonas* isolates are shown in Table 1.

The isolates were from various body sites. Of the 67 clinical *Pseudomonas* strains isolated, 30 were resistant to most of the antimicrobial agents tested.

The frequency of susceptibility to Norfloxacin was the highest (61.19%), while the sensitivity to other fluoroquinolones was shown in Table 2. A total of 54 different plasmids with molecular mass ranging from 3.55kb to 19.95kb were observed in the antibiotic resistant strains. Plasmids were not detected in 10 of the resistant strains indicating that their resistance was probably chromosomally borne. Five different plasmid profile groups for the antibiotic resistant strains could be defined. The number of strain per plasmid profile group vary from 4-6 (Table3).

The most common antimicrobial resistance pattern was Luv, Pef, Nfn, Spn, Cip, Ofx. This was followed in decreasing order of occurrence by the R-types resistant patterns: Luv, Pef, Cip, Spn, Ofx; Luv; Pef; Luv,Pef; Pef, Nfn; Luv, Cip, Nfn; and Luv, Nfn, Spn, Cip, Ofx, harboured the highest number of plasmids, while no plasmid was found in the strains with these resistance patterns viz Luv, Nfn, Spn, Cip, Ofx and Luv, Cip, Nfn.

Table 1: Sources of the Bacterial isolates.

Clinical Specimen	Luth		Pathfinder Laboratory		Total	
	N	n	N	n	N	N
Wound swab	197	22	32	7	229	29
Urine	82	5	69	2	151	7
Sputum	42	5	29	3	71	8
Ear swab	25	3	17	4	42	7
Throat swab	10	-	16	1	26	1
Catheter	6	1	-	-	6	1
Aspirate	7	1	-	-	7	1
Semen	39	1	17	-	56	1
Urethral Discharge	14	1	14	1	28	2
Eye swab	25	2	3	-	28	2
High vaginal swab	40	5	26	3	66	8
TOTAL	487	46	223	21	710	67

KEY: N = Total Number of Clinical specimens, n = Total Number of *Pseudomonas* species, LUTH = Lagos University Teaching Hospital, Lagos

Table 2: Antibiotic sensitivity pattern of clinical isolates of *Pseudomonas*

Antibiotics	Number sensitive	% sensitive	% resistant
Levofloxacin	33	49.25	50.75
Pefloxacin	38	56.72	43.28
Ciprofloxacin	32	47.76	52.24
Norfloxacin	41	61.19	38.81
Ofloxacin	37	55.22	44.78
Sparfloxacin	39	58.21	41.79

TABLE 3: Plasmid Profile Groups of Antibiotic Resistant Clinical *Pseudomonas* Strains

Plasmid Profile	No Of Strains	Molecular Mass(Kb) Of Plasmids
0	10	NO Plasmids
1	6	3.55, 3.98, 15.85
2	4	3.55, 3.98, 4.47, 15.85
3	6	3.55,3.98, 4.47, 15.85, 17.78, 19.95
4	4	3.55, 3.98,4.47, 5.o1,15.85,19.95

Transformation experiment showed that 66.67% of the resistant strains that harboured plasmids were able to transfer their resistance plasmids to *E.coli* K-12 HB101. Plasmid- determined resistance to ciprofloxacin and levofloxacin was found. It is noteworthy that all the R-plasmids isolated in this study have a common molecular size of 15.85kb (Table 5). All the strains harbouring R-plasmids were cured of their plasmids upon treatment with sodium dodecyl sulphate (SDS) with resultant loss of their plasmid-associated properties. This indicates that the antibiotic resistant genes of the *Pseudomonas* strains used in this study were plasmid borne.

Table 4: Antimicrobial Resistance Patterns of 30 Clinical *Pseudomonas* Isolates In Relation To Plasmid Contents

Antimicrobial Resistance Patterns	No Of Isolates	%	No With Plasmids
Luv, Pef,Nfn,Spn,Cip,Ofx	15	50	12
Luv,Nfn,Spn,Cip, Ofx	1	3.3	NIL
Luv,Pef, Cip,Spn,Ofx	4	13.3	1
Luv,Cip,Nfn	1	3.3	NIL
Pef, Nfn	2	6.7	1
Luv, Pef	2	6.7	1
Pef	2	6.7	2
Luv	3	10	3

KEY: Luv= Levofloxacin, Pef = Pefloxacin, Nfn = Norfloxacin, Spn = Sparfloxacin, Cip =Ciprofloxacin Ofx = Ofloxacin

Table 5: Characteristics of Some of the Clinical *Pseudomonas* R- Plasmids

Pseudomonas Isolate (Code)	Plasmids Molecular Ssize(Kb)	Antibioticgene Transferred To E.Coli Hb101	Transformant R-Plasmid Size (Kb)
35	3.55,3.98,15.85	Cip, Luv	15.85
24	3.55,3.98,15.85,19.95	Cip, Luv	15.85
38	3.55, 3.98, 4.47 15.85	Cip, Luv	15.85
32	3.55, 3.98, 15.85	Cip,Luv	15.85
45	3.98, 15.85	Cip, Luv	15.85
41	3.98, 4.47, 15.85 19.95	Cip,Luv	15.85
42	3.55, 3.98, 15.85	Cip ,Luv	15.85
17	3.98, 15.85	Cip, Luv	15.85
28	3.55, 15.85	Cip, Luv	15.85
33	3.98, 14.47,5.01,15.85	Cip, Luv	15.85
30	3.98, 4.47,15.85	Cip,Luv	15.85
14	3.55, 3.98, 15.85	Cip, Luv	15.85
15	15.85	Luv	15.85
6	15.85	Luv	15.85
12	15.85	Cip, Luv	15.85
39	15.85	Cip, Luv	15.85
43	3.55, 3.98, 15.85	Cip, Luv	15.85
20	3.98, 4.47 ,15.85	Cip, Luv	15.85
5	3.98, 4.47, 15.85	Cip, Luv	15.85
31	3.98, 15.85,17.78, 19.95	Cip, Luv	15.85

KEY: Cip = Ciprofloxacin, Luv = Levofloxacin

Discussion

In this study, most of the *Pseudomonas* aeruginosa were isolated from surgical wound infections (12.66%), sputum(11.27%), and urinary specimen (4.64%). This is consistent with earlier findings[18] for the three sites, and Daini et al.[1] as regards wound infection. *Pseudomonas aeruginosa* is a well – known community acquired wound infections even in immuno

competent patients and are multi- resistant to commonly used antimicrobial agents[19,20]. The comparative disc sensitivities shown in Table2 is similar to that obtained by the author and others[1,11]. The increasing resistance of *Pseudomonas* strains to fluoroquinolones [table2] is in agreement with previous studies[8,10,21].

The antimicrobial resistance pattern revealed a total of eight patterns. This indicates the emergence of resistance to the quinolones in our environment. Also these patterns depict the occurrence of multi-resistant strains. This is similar to that obtained by Odugbemi et al[8,22,23]. All the strains that were resistant to any antimicrobial agents were also resistant to ciprofloxacin. Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids[12,24,25].

The most common plasmids encountered were 15.85kb in size. This is slightly higher than that reported in the litarture[1,26].66.67% of the drug resistant strains carried R-plasmids. Plasmid determined resistance to ciprofloxacin and levofloxacin was found. The emergence of R-plasmids in this study could be due to over zealous desire to treat every infection, diagnosed or undiagnosed and to the over the counter availability of antibiotics[4,27,28]. A different plasmid profile could be seen for each of the 20 R-plasmids ,and plasmids of the same molecular could be found in different strains. Thus the plasmid profile of these strains was diverse in nature. Plasmid profiling analysis distinguished more strains than the antimicrobial susceptibility patterns in agreement with the findings elsewhere[8,29]. Plasmid profiling has been shown to be a good epidemiological tool in investigating epidemics or out breaks of bacterial diseases[25,30,31]. The transformation experiment enabled us to detect non-self transmissible plasmids while curing of the resistant strains of the R-plasmids with SDS showed that their antimicrobial – resistant genes were plasmid borne[6,31 8,9].

Conclusion:

The results of our study highlighted diverse plasmid profiles and widespread antimicrobial resistance patterns of some clinical *Pseudomonas* species from Nigeria and we hope that this information from this locality would be a useful base line for further epidemiological studies.

Acknowledgement: We wish to thank the Consultants and staff of Lagos University Teaching Hospital, Lagos as well as the staff of Pathfinder Diagnostic Laboratory, Lagos, for their assistance.

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