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# Plasmid determined resistance to quinolones in clinical isolates of Gram-negative bacilli

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**Summary**

A total of 166 strains of Gram-negative bacilli comprising 65 *Klebsiella* species, 22 *Escherichia coli*, 39 *Pseudomonas aeruginosa*, 20 *Proteus* species, 18 other *Pseudomonas* species and 2 *Salmonella typhi*, were isolated from clinical specimens in the University College Hospital, Ibadan. Antimicrobial susceptibility testing and plasmid profiling of the strains were done. Ceftazidine had the highest antibacterial activity of 83.1% compared to the fluoroquinolones followed by ciprofloxacin (78.3%), pefloxacin (69.9%) and ofloxacin (56%) respectively. Of the 166 strains, 44 were found to be resistant to most of the antimicrobial agents tested. Resistance to ofloxacin was common among the resistant strains. The resistant strains harboured plasmids with molecular sizes ranging from 6.6kb to 17.4kb and were grouped into five plasmid profile groups. Transformation experiment showed that 59.2% of the resistant strains carried a common R-plasmid of size 10.7kb. Resistance to ciprofloxacin and pefloxacin were found to be plasmid borne.

**Keywords:** *Plasmid, quinolones, resistance and gram-negative bacilli*

**Résumé**

Au total 966 souches de bacilles de grammes négative compris 65 des espèces de Klebeseille, 22 Escherichia coli, 39 pseudomone aeruginose, 20 proteus, 18 autres pseudomone et 2 samonelle typhi étaient isolés des échantillons cliniques obtenues au centre universitaire hospitalier d Ibadan. Les tests de susceptibilité et le profile des souches de plasmide étaient faite. Le ceftazidine avait d'activité antibactérienne plus élevé 83.1% comparés aux fluoroquinolines, suivit des ciprofloxacines (78.3%), pefloxacine (69.9%) et ofloxacine (56%) respectivement. Sur les 166 souches, 44 étaient résistant à la plupart des agents antibactériens testés. La résistance au ofloxacine était commune parmi les souches résistantes. Ces souches résistantes avaient de souches de plamides de dimension moléculaire de 6.6-17.4 Kb et étaient groupés en 5 catégories. L'expérience en transformation démontrait que 59.2% des souches résistantes portaient un R-plasmide de dimension commune de 10.7Kb. La résistance à la résistance ciprofloxacine et pefloxacine étaient héréditaire au plasmide.

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## Introduction

The fluoroquinolones are relatively newly introduced broad – spectrum synthetic antimicrobial agents. They have greater activity against Gram – positive and Gram – negative bacteria than do the older quinolones analogues, nalidixic acid and oxolinic acid [1,2,3]. Resistance to fluoroquinolones has been formerly limited to chromosomal mutations primarily affecting DNA gyrase and outer membrane permeability [4,5]. Although high level resistance to quinolones in clinical isolates of *Enterobacteriaceae* such as *Escherichia coli* has been reported [6,7,8,9,10,11], but not much has been done on plasmid – mediated resistance to quinolones [12].

Multiple antibiotic resistance in bacterial is most commonly associated with the presence of plasmid which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype. [13,14,15,16,17, 18,19,20].

Quinolones such as ciprofloxacin and Ofloxacin have been introduced into Nigeria, while newer ones like Pefloxacin are just being introduced by some pharmaceutical companies under different trade names. It was considered that susceptibility to quinolones may remain high in Nigeria as these drugs are expensive and beyond the reach of most individual, but their use is increasing and resistance may become more problematic in the years to come. [21,22,23,24,25].

We therefore set out to determine the antibiotic resistance patterns and prevalence of R-plasmids among Gram – negative bacilli commonly isolated from clinical specimens in the University College Hospital, Ibadan, Nigeria.

## Materials and methods

*Strains*

Sixty-five strains of *Klebsiella* species, 22 strains of *Escherichia coli*, 39 strains of *Pseudomonas aeruginosa*, 20 strains of *Proteus* species, 18 strains of other *Pseudomonas* species and 2 strains of *Salmonella typhi* were isolated by standard procedures [26, 27] from clinical specimens sent to the diagnostic laboratory of Medical Microbiology and Parasitology, Department of University College Hospital, Ibadan from May to December 2002.

**Table 1**: Sources of bacterial isolates

| Body site | Esch. coli | Kleb spp | Proteus spp | Pseudo. aeruginosa | Salm. Typhi | Pseudo spp | Total |
|---|---|---|---|---|---|---|---|
| Ear swab | 0 | 8 | 9 | 19 | 0 | 7 | 43 |
| Wound swab | 13 | 23 | 9 | 13 | 1 | 6 | 65 |
| Throat swab | 1 | 3 | 1 | 0 | 0 | 0 | 5 |
| Conjuctiva swab | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| High vaginal swab | 3 | 9 | 1 | 0 | 0 | 2 | 15 |
| Endocervical swab | 1 | 2 | 0 | 0 | 0 | 0 | 3 |
| Sputum | 0 | 7 | 0 | 6 | 0 | 1 | 14 |
| Urine | 3 | 8 | 0 | 1 | 0 | 0 | 12 |
| Tracheal aspirate | 1 | 2 | 0 | 0 | 0 | 1 | 4 |
| Celebrospinal fluid | 0 | 2 | 0 | 0 | 1 | 0 | 3 |
| Total specimen | 22 | 65 | 20 | 39 | 2 | 18 | 166 |

**Table 2:** Disc sensitivity pattern of bacterial isolates.

| No. of strain n | CIP | | PEF | | GEN | | AMX | | AUG | | COT | | CAZ | | OFX | | CRO | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R | S | R |
| Esch. coli | 16 | 6 | 13 | 9 | 12 | 10 | 2 | 20 | 3 | 19 | 1 | 21 | 17 | 5 | 14 | 8 | 16 | 6 |
| n=22 | (73) | (27) | (59) | (41) | (55) | (45) | (9) | (91) | (13) | (87) | (5) | (95) | (77) | (23) | (64) | (36) | (73) | (27) |
| Klebsiella specie | 49 | 16 | 44 | 21 | 27 | 38 | 0 | 65 | 2 | 63 | 0 | 65 | 55 | 10 | 35 | 30 | 50 | 15 |
| n=65 | (75) | (25) | (59) | (41) | (42) | (58) | (0) | (100) | (3) | (97) | (0) | (100) | (85) | (15) | (54) | (46) | (77) | (23) |
| Proteus species | 17 | 3 | 15 | 5 | 10 | 10 | 4 | 16 | 6 | 14 | 2 | 18 | 18 | 2 | 11 | 9 | 16 | 4 |
| n=20 | (85) | (15) | (75) | (25) | (50) | (50) | (20) | (80) | (30) | (70) | (10) | (90) | (90) | (10) | (55) | (45) | (80) | (20) |
| Pseudomonas aeruginosa | 33 | 6 | 29 | 10 | 17 | 22 | 0 | 39 | 0 | 39 | 0 | 39 | 34 | 5 | 25 | 14 | 30 | 9 |
| n=39 | (85) | (15) | (74) | (26) | (44) | (56) | (0) | (100) | (0) | (100) | (0) | (100) | (87) | (13) | (64) | (36) | (77) | (23) |
| Salm. Typhi | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 1 | 1 | 2 | 0 |
| n=02 | (50) | (50) | (50) | (50) | (50) | (50) | (0) | (100) | (0) | (100) | (0) | (100) | (100) | (0) | (50) | (50) | (100) | (0) |
| Pseudomonas species | 14 | 4 | 14 | 4 | 6 | 12 | 0 | 18 | 0 | 18 | 0 | 18 | 12 | 6 | 7 | 11 | 10 | 8 |
| n=18 | (78) | (22) | (78) | (22) | (33) | (67) | (0) | (100) | (0) | (100) | (0) | (100) | (67) | (33) | (39) | (61) | (56) | (44) |

*Key:*

*AMX rep. the % Amoxycillin*
*AUG rep. the % Augmentin*
*COT rep. the % Cotrimoxazole*
*CAZ rep. the % Ceftazidime*
*CIP rep. the % Ciprofloxacin*
*PEF rep. the % Pefloxacin*
*OFX rep. the % Ofloxacin*
*CRO rep. the % Ceftriaxone*
*GEN rep. the % Gentamicin*
*( ) rep. the %*

*Determination of Antimicrobial susceptibility of the Bacterial isolates*

Antimicrobial disc diffusion tests were carried out using Stoke's disc diffusion technique [28] on freshly prepared oxoid Muller–Hinton agar, and standardized by the method of National Committee for Clinical Laboratory Standard (NCCLS) [29], using the following antibiotic discs: Pefloxacin 5ig (Peflotab), Ofloxacin 30g (Tarivid), Ciprofloxacin 5ig (Ciprotab), Ceftazidime 30ig (Fortum), Ceftriaxone 30ig (Recephine), Gentamicin 10ig, Amoxycillin 25ig, Augmentin 30ig, Cotrimoxazole 30ig (Septrin).

*Isolation and Separation of Plasmid DNA*

Plasmid DNA was isolated, separated and stained as previously described [18].

*Plasmid Profile Groups*

Plasmid profile groups were constructed by grouping strains possessing the same profile (constituted of the number and molecular mass of different plasmids) or parts of a profile constituting a core profile. Bacterial strains that carried no plasmid were regarded as constituting non-plasmid profile group.

**Table 3:** Antimicrobial resistance patterns of 44 clinical bacterial strains in relation to plasmid contents

| Antimicrobial resistance pattern | No showing pattern | % showing pattern | No with Plasmid |
|---|---|---|---|
| Cip Pef Ofx Gen Amx Aug Cot Caz Cro | 9 | 20.5 | 6 |
| Pef Ofx Gen Amx Aug Cot Caz Cro | 8 | 18.2 | 3 |
| Cip Ofx Gen Amx Aug Cot Caz Cro | 1 | 2.3 | 1 |
| Cip Pef Ofx Amx Aug Cot Caz Cro | 11 | 25.0 | 7 |
| Cip Pef Ofx Gen Amx Aug Cot Cro | 4 | 9.1 | 4 |
| Cip Pef Ofx Gen Amx Aug Cot | 6 | 13.6 | 4 |
| Ofx Amx Aug Cot Gen | 5 | 11.4 | 2 |

***Key:***

*Cip - Ciprofloxacin.*
*Pef - Pefloxacin.*
*Ofx - Ofloxacin.*
*Gen – Gentamicin*
*Amx - Amoxycillin.*
*Aug - Augmentin*
*Cot - Cotrimoxazole*
*Caz - Ceftazidime*
*Cro - Ceftriaxone.*

*Genetic Transfer*

Transformation was done as described by Hanahan [30] using *Escherichia coli* k–12 HB 101 (ara–14, hsd 520, lacyl, len, mtl–1, pro A2, rec A13, rps L20, sup E44, thii xyl–5) as recipient and plasmid PBR 322 as the positive control. Co-transformation of resistant characters was determined by testing al transformants against all antibiotics to which the donor strain was resistant. Extract from transformants were obtained as described above and subjected to 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.

## Results

The sources of the clinical bacterial isolates are shown in Table 1. The isolates were from various body sites. Of the 166 clinical bacterial strains isolated, 44 were resistant to most of the antimicrobial agents tested (Table 2). A total of 27 different plasmids with molecular masses ranging from 6.6 – 17.4kb was observed in the antibiotic resistant strains. Plasmids were not detected in seventeen of the resistant strains indicating that their resistance was chromosomally borne.

The most common antimicrobial susceptibility pattern was Cip Pef Ofx Amx Aug Cot Caz Cro. This was followed in decreasing order of occurrence by the R-types resistance patterns viz:

Cip Pef Ofx Gen Amx Aug Cot Caz Cro,
Pef Ofx Gen Amx Aug Cot Caz Cro,
Cip Pef Ofx Gen Amx Aug Cot Cro,
Ofx Amx Aug Cot Gen,

**Table 4:** Characteristics of some of the bacterial R- plasmids

| Bacterial Strain | Plasmid Molecular Size (Kb) | Antibiotic Gene Transferred to E. coli HB101 | Transformant Plasmid Size(kb) |
|---|---|---|---|
| $MMB_{8}$ - Klebsiella species | 10.7 | Pef | 10.7 |
| $MMB_{10}$ – Pseudomonas aeruginosa | 10.7 | Cip | 10.7 |
| $MMB_{11}$ - Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{16}$ – Escherichia coli | 10.7 | Cip/Pef | 10.7 |
| $MMB_{17}$ – Pseudomonas aeruginosa | 10.7 | Cip/Pef | 10.7 |
| $MMB_{25}$- Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{26}$- Pseudomonas aeruginosa | 10.7 | Cip/Pef | 10.7 |
| $MMB_{28}$- Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{30}$ - Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{32}$ - Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{35}$- Proteus species | 10.7 | Pef | 10.7 |
| $MMB_{36}$ - Klebsiella species | 10.7 | Cip/Pef | 10.7 |
| $MMB_{37}$ - Proteus species | 10.7 | Pef | 10.7 |
| $MMB_{41}$ - Pseudomonas aeruginosa | 10.7/6.6 | Cip/Pef | 10.7 |
| $MMB_{42}$ - Klebsiella species | 10.7 | Pef | 10.7. |
| $MMB_{43}$ -Pseudomonas aeruginosa | 10.7 | | |

***Key:***

*CIP - Ciprofloxacin*
*PEF - Pefloxacin*

Cip Pef Ofx Gen Amx Aug Cot Cro.
Cip Ofx Gen Amx Aug Cot Caz Cro (Table 3)

Pseudomonas, strains showing the resistance pattern Cip Pef Ofx Amx Aug Cot Caz Cro harboured the highest number of plasmids while the lowest number was found in the single strain (Klebsiella species) with the resistance pattern Cip Ofx Gen Amx Aug Cot Caz Cro. Transformation experiment showed that 59.2% of the resistant strains that harboured plasmids were able to transfer their resistance plasmids to *Escherichia coli* K-12 HB 101. The R-plasmids isolated in this study have a common size of 10.7kb (Table 4).

## Discussion

The resistance of bacteria to antibiotics particularly those used for first line therapy is an increasing cause for concern [11,16,19,24,25,31]. Most of the Gram- negative bacilli especially *Klebsiella* species and *Pseudomonas* species are intrinsically resistant to most antibiotics, a situation which favours their continued existence in hospital environment [18,21,22,23,25]. This fact greatly contributes to the high incidence of these agents in patients.

The ceftazidime has the highest antibacterial activity of 83.1% compared to the fluoroquinolones, followed by ciprofloxacin (78.3%), pefloxacin (69.9%) and ofloxacin (56.0%) respectively. This is in agreement with findings of Oni *et al* [23,24] Ozumba [25]. The antimicrobial susceptibility pattern revealed the emergence of resistance to the quinolones in our environment. This is similar to that obtained by Oni *et al* [23,24], Threfall *et al* [11], Livermore *et al* [31]. The most common plasmids encountered were 10.7kb in size. This is in agreement with the findings of Moller *et al* [33], Diani *et al* [13], Olukoya *et al* [19]. Resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids [13, 15,17,19,20,32].

59.2% of the drug-resistant strains carried R-plasmids. Plasmid-determined resistance to ciprofloxacin and pefloxacin was found. The emergence of R-plasmids in this study could be due to the indiscriminate and widespread use caused by the over-the-counter availability of antibiotics as well as the higher exposure of people to enteric flora in places with poor sanitation [13,14,18,19]. A different plasmid profile was seen for each of the 16 R-plasmids and plasmids of the same molecular weight 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 of Diani *et al* [13], Levy *et al* [17], Senerwa *et al* [34]. Plasmid profiling has been shown to be a good epidemiological tool in investigating epidemics or outbreaks of bacterial diseases [33, 35]. The use of transformation enabled us to detect non-self-transmissible plasmids.

In view of the occurrence of multiple antibiotics resistant strains coupled with the emergence of the fluoroquinolones resistant plasmids, considerable effort must be made to establish an antibiotic policy for the hospital and the country.

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