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# Comparative *in-vitro* activities of commonly available quinolones and other antibiotics on bacterial isolates in Ibadan, Nigeria

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**Summary**
The 4-quinolones, many of which are now available in Nigeria under different trade names, have a broad spectrum of activity. An evaluation is made of the comparative in-vitro activities of these quinolones and other antibiotics against 125 strains of bacteria commonly isolated from clinical specimens in University College Hospital, Ibadan, by using the Stroke's disc sensitivity method, and MIC estimation. The quinolones showed greater activity than the cephalosprins against *Klebsiella spp.*, *Proteus spp.* and *Escherichia coli*, but were found to be equipotent against *Pseudomonas spp.* the MIC results revealed ciprofloxacin (Ciprotap) as the most active of the quinolones.

Though some strains of *Klebsiella spp.* and *Pseudomonas spp.* were found to be resistant to all the antibiotics tested, majority of the strains of the Gram-negative bacilli from clinical specimens were highly susceptible to all the quinolones. This emphasizes the need to monitor regularly the emergence of resistance associated with the use of antibiotics in the developing countries.

**Keywords**: *In-vitro activities, quinolones, Ibadan*

**Résumé**
Les 4-quinolones qui sont maintenant disponible au Nigeria tous plusieur nom commerciaux, ont une activité à large spectre. Cette etude faite au Centre Hospitalier Universitaire d'Ibadan, evalue et compare l'activité. In-vitro des 4-quinolones et d'autres antibiotiques sur 125 touches bacterienne isolées au Centre Hospitalier Universitaire d'Ibadan. La methode utilisée pour determine la sensitivite des bacteries aux quinolones et antibiotiques etait celle du disque de stroke. La concentration inhibitrice minimase (MIC) avait été determinée après avoir etablit le mode de sensitivite des bacteries. Les quinolones avaient montre une grande activité par rapport aux cephalosprins contre les *klebsiella spp., proteus spp., et Escherichia Coli*, mais avaient montré une activite equivalent contre le *pseudomonas spp.* Les resultats des concentrations inhibitrice minimale avaient montre que la ciprofloxacin (ciprotals) etait la quinolone la plus active.

Quoique certains souches de *klebsiella spp* et *pseudomona spp.* avaient montre une resistance à tous les antibiotiques testé, la majorite des baciles à a gra negatif des specimen clinique etaient fortement sensible a tous les quinolones. Ces resultats montrent quo il y a un besoin de surveiller l'emergence de la resistance associée à l'utilisation des antibiotiques dans les pays en voie de developement.

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## Introduction

The 4-quinolones are synthetic antibacterial compounds whose principal target of action is the enzyme DNA gyrase, which is responsible for introducing negative super coil into DNA. DNA super coiling plays an important role in bacterial metabolism. It compacts the chromosomes and is involved in the regulation of gene transcription as well as bacterial response to the environment [1]. Nalidixic acid, the first of the quinolones, has been useful for the treatment of urinary tract and enteric infections [1,2].

In the 1980s, compounds such as pefloxacin, norfloxacin, enoxin and ofloxacin with activities against a wider range of bacterial species, became available for clinical use [1]. 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 is known that antibiotics having similar modes of action fall within the same spectrum of activity and are likely to be affected, albeit in certain cases at various degrees, by the same resistance mechanisms (cross-resistance [3]. It was opined that susceptibility to quinolones may remain high in Nigeria as these drugs are expensive and beyond the reach of most individuals, but their use is increasing and resistance may become more problematic in the years to come [4]. Hence an evaluation of the comparative activities of these commonly available quinolones against the local strains of bacteria is therefore desirable to establish a baseline data in our environment. We therefore set out to determine the comparative in-vitro activities of some quinolones against Gram-negative bacilli commonly isolated from clinical specimens in University College Hospital, Ibadan, Nigeria.

## Materials and methods

Fifty-two strains of *Pseudomonas* species, 33 strains of *Klebsiella* species, 22 strains of *Eschericia coli*, 12 strains of *Proteus* species and 6 strains of *Staphylococcus aureus* isolated from clinical specimens (Table I) sent to our laboratory from January to June, 1997 were tested by both disc sensitivity test and minimum inhibitory concentration (MIC).

Antimicrobial disc sensitivity tests were carried out using Stoke's disc diffusion technique [5] on Oxoid Mueller-Hinton agar, using the following antibiotic discs: Pefloxacin 5ug (Peflotab), Pefloxacin 5ug (Abaktal), Ofloxacin 10ug (Tarivid), Ciprofloxacin 5ug (Ciprotab), Ceftazidime 30ug (Fortum), Cetriaxone 30ug (Rocephine) and Gentimicin 10ug. *Escherichia coli* (ATCC25922) was used as control. MIC

estimation was performed by using agar dilution method. Organisms were grown overnight on blood agar, inoculated into Mueller-Hinton broth, and diluted to $10^8$ colony forming units/ml using a McFarland standard. A 1:10 dilution in broth was made and using a multipoint inoculator, $10^4$ organisms were inoculated onto Mueller-Hinton agar containing two-fold dilution of antimicrobials, from 0.0312ug/ml to 256ug/ml. Antimicrobials tested included Pefloxacin ("Peflotab" Fidson &co.), Pefloxacin ("Abaktal" Taylek), Ciprofloxacin "Ciprotab" Fidson & co.), Ofloxacin ("Tarivid" Hoest Nigeria), Ceftazidime ("Fortum" Glaxo), Ceftriaxone ("Rocephine" Roche) and Azithromicin (Pfizer). The plates were examined after overnight incubation at 36°C and the MIC value taken as the lowest antibiotic concentration that prevented growth of the organism.

## Results

The sources of the bacterial isolates are shown in Table 1. The isolates were from various body sites. The comparative disc sensitivities are shown in Table 2. Considering *Klebsiella* species, *Proteus* species and *Escherichia coli*, a greater percentage of strains were susceptible to the quinolones than to the cephalosporins. However, against Pseudomonas species, the quinolones were more or less equipotent with the cepalosporins. 15% of the strains of *Pseudomonas* species were resistant to pefloxacin, ofloxacin, ceftazidime and ceftriaxone, while 77% of strains were resistant to gentamicin.

**Table 1:** Sources of the bacterial isolates

| Body sites | Bacteial isolates | | | | | |
|---|---|---|---|---|---|---|
| | Kleb. Spp. | Pseud. Spp. | Esch. coli | Proteus Spp. | Staph. aureus | Total |
| Ear Swab | 1 | 15 | 2 | 4 | 0 | 22 |
| Endwocervical-Swab | 0 | 2 | 4 | 0 | 0 | 6 |
| Wound Swab | 8 | 21 | 3 | 0 | 3 | 35 |
| Conjunctival Swab | 0 | 2 | 0 | 0 | 3 | 5 |
| Urine | 6 | 3 | 12 | 6 | 0 | 27 |
| Sputum | 8 | 9 | 0 | 0 | 0 | 17 |
| Throat Swab | 8 | 0 | 0 | 0 | 0 | 8 |
| Pleural Aspirate | 2 | 0 | 0 | 0 | 0 | 2 |
| Urethral Swab | 0 | 0 | 1 | 1 | 0 | 2 |
| Bone Biopsy | 0 | 0 | 0 | 1 | 0 | 1 |
| Total Biopsy | 33 | 52 | 22 | 12 | 6 | 125 |

The MIC, expressed as $MIC_{50}$, $MIC_{90}$ and range are shown in Table 3. Considering the Gram-negative bacilli, ciprofloxacin has the best sensitivity result. This is followed by ofloxacin and then pefloxacin. For Staphylococcus aureus ofloxacin has the best sensitivity result. The $MIC_{90}$ of the quinolones were 2 – 4 folds higher than their $MIC_{50}$.

## Discussion

The role of Gram-negative organisms in the aetiology of severe infections have continued to generate much interest. Most of the Gram-negative bacilli especially Klebsiella species and

**Table 2:** Disc sensitivity pattern of hospital isolates

*Isolates*

| No. of Strains | Pefloxacin | | Ofloxacin | | Ceftazidime | | Ceftriaxone | | Ciprofloxacin | | Gentamicin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | R | S | R | S | R | S | R | S | R | S | R |
| Klebsiella spp.(33) | 30 (91) | 3 (9) | 31 (94) | 2 (6) | 22 (67) | 11 (33) | 30 (91) | 3 (9) | 26 (79) | 7 (21) | 7 (21) | 26 (79) |
| Pseudomonas spp.(52) | 38 (73) | 14 (27) | 37 (71) | 15 (29) | 38 (73) | 14 (27) | 38 (73) | 14 (27) | 44 (85) | 8 (15) | 12 (23) | 40 (77) |
| Escherichia coli (22) | 20 (91) | 2 (9) | 21 (95) | 1 (5) | 12 (55) | 10 (45) | 18 (82) | 4 (18) | 22 (100) | 0 | 6 (27) | 16 (73) |
| Proteus spp. (12) | 12 (100) | 0 | 12 (100) | 0 | 6 (50) | 6 (50) | 11 (92) | 1 (8) | 9 (75) | 3 (25) | 4 (33) | 8 (67) |
| Staph aureus(6) | 6 (100) | 0 | 6 (100) | 0 | 2 (33) | 4 (67) | 5 (83) | 1 (17) | 5 (83) | 1 (17) | 2 (33) | 4 (67) |

**Table 3:** MICs of a cumulative percentage of isolates with inocula of 10000cfu

| Organisms (No of Strains) | Antimicrobial Agents | MIC ug/ml $MIC_{50}$ | MIC Range | $MIC_{90}$ |
|---|---|---|---|---|
| Klebsiella spp. (33) | "Peflotab" | 2 | 0.125 – 32 | 8 |
| | "Ciprotab" | 1 | 0.0625 – 2 | 2 |
| | Abaktal" | 1 | 0.0625 – 32 | 32 |
| | Ofloxacin | 1 | 0.0311 – 16 | 1 |
| | Ceftazidime | 2 | 0.0625 – 128 | 8 |
| | Ceftriaxone | 1 | 0.125 – 32 | 4 |
| | Azithromycin | 16 | 1 – 128 | 64 |
| Pseudomonas spp. (52) | Peflotab" | 4 | 1 – 8 | 8 |
| | "Ciprotab" | 2 | 0.0625 – 2 | 2 |
| | "Abaktal" | 1 | 0.0625 – 32 | 4 |
| | Ofloxacin | 0.125 | 0.0311 – 4 | 1 |
| | Ceftazidime | 0.25 | 0.0311 – 16 | 0.25 |
| | Ceftriaxone | 0.5 | 0.0311 – 8 | 8 |
| | Azithromycin | 8 | 1 – 256 | 64 |
| Escherichia coli (22) | Peflotab" | 4 | 2 – 32 | 8 |
| | "Ciprotab" | 0.5 | 0.125 – 2 | 2 |
| | "Abaktal" | 1 | 0.5 – 32 | 4 |
| | Ofloxacin | 0.25 | 0.0311 – 4 | 1 |
| | Ceftazidime | 1 | 0.25 – 64 | 8 |
| | Ceftriaxone | 0.25 | 0.125 | 0.25 |
| | Azithromycin | 8 | 2 – 128 | 64 |
| Proteus spp. (12) | Peflotab" | 8 | 1 – 8 | 8 |
| | "Ciprotab" | 0.125 | 0.125 – 2 | 1 |
| | Abaktal" | 2 | 0.0625 – 32 | 32 |
| | Ofloxacin | 1 | 0.0311 – 4 | 2 |
| | Ceftazidime | 0.5 | 0.25 – 64 | 8 |
| | Ceftriaxone | 0.25 | 0.0311 – 4 | 1 |
| | Azithromycin | 8 | 4 – 256 | 128 |
| Staph aureus (6) | Peflotab" | 2 | 2 – 8 | 8 |
| | "Ciprotab" | 0.125 | 0.062 – 0.5 | 0.5 |
| | "Abaktal" | 2 | 0.125 – 32 | 32 |
| | Ofloxacin | 0.0625 | 0.0311 – 0.05 | 0.05 |
| | Ceftazidime | 4 | 0.125 – 8 | 8 |
| | Ceftriaxone | 0.125 | 0.0625 – 0.25 | 0.25 |
| | Azithromycin | 4 | 2 - 8 | 8 |

Pseudomonas species are intrinsically resistant to most antibiotics, a situation which favours their continued existence in hospital environment [4,6].

Dilution methods are used to determine the MIC of antibiotics and are generally accepted as the reference method (the 'gold standard"), for antimicrobial susceptibility testing. The most extensive use of MIC method is in the comparative testing of new antimicrobial agents [7]. The MIC breakpoints for defining ciprofloxacin susceptibility have been proposed: 1ug/ml, susceptible; 2ug/ml, moderately susceptible; 4ug/ml, resistant [8].

This in-vitro study suggests that the majority of strains of Gram-negative bacilli isolated from clinical specimens were highly susceptible to all quinolones. Ciprofloxacin had the lowest MIC and was thus the most active of all the drugs. Peflaxacin (Peflatab and Abaktal) had the highest MIC.

Some strains of *Klebsiella* species and *Pseudomonas* species were resistant to the quinolones (MICs >8ug/ml). This is quite different from the report of Odugbemi *et al.* which documented a 100% sensitivity of *Pseudomonas aeruginosa* to Ciprofloxacin in 1994 in Lagos [4], but similar to those reported in other populations [8,9]. This emergence of resistance to quinolones has also been reported in patients with complicated urinary infection with *Pseudomonas aeruginosa* [9].

In this study there is good susceptibility of *Staphylococcus aureus* to the quinolones a picture that is similar to the findings of Okesola *et al.* in the same center in 1996 [10].

The lifetime of an antimicrobial agent can be drastically shortened if resistance develops among initially susceptible microorganisms [11]. Unfortunately the development of resistance is often inadequately assessed especially in the developing countries, so that the potential for resistance to shorten the lifespan of anti-microbials is often unknown. The results of this study emphasize the need to analyse closely the emergence of resistance associated with the use of any antibiotic. This will reduce the financial burden of pa' :nts and relations in the purchase of "inactive" antimicrobi.. agents.

These in-vitro observations support the use of these agents for treatment of clinical bacterial infections, when other agents cannot be used due to antimicrobial resistance since more gaps occur in Gram-negative spectrum of Ceftazidime and Ceftriaxone. There is weakness in the activity of Ceftazidime against all bacteria from clinical specimens and a marked weakness of Ceftriaxone against Pseuduomonas species. However since less susceptible strains are detectable among anaerobes [12], it is advisable to combine these quinolones with metronidazole when considering empirical treatment.

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