African Journal of Medicine and Medical Sciences

Editor: O.A. Ladipo
Assistant Editors:
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Volume 18
1989

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BLACKWELL SCIENTIFIC PUBLICATIONS
Oxford London Edinburgh Boston Melbourne

Afr. J. Med. med. Sci. (1989) **18**, 63–67.

An in-vitro study on Ciprofloxacin and other anti-microbials against Gram-negative bacteria isolated from patients in Ibadan, Nigeria

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Summary

The high prevalence of common clinical isolates that are resistant to multiple antibiotics calls for regular review of the anti-microbial sensitivity pattern among bacteria of clinical significance in our environment.

In the present study an increasing percentage of common isolates from hospitalized patients have been found to be resistant to Gentamicin and Cefotaxime, which play an important role in the chemotherapy of infections. Of special significance is the finding that over 60% of pseudomonads are now resistant to Gentamicin.

The new fluoroquinolone, Ciprofloxacin, showed strong activity against all the isolates tested, with MIC values within the range of those reported as sensitive from many overseas centres. It should prove to be a valuable agent in the management of infections due to these organisms.

Résumé

La haute prévalence des microbes isolées couramment et qui sont résistantes à plusieurs agents anti-microbiens fait appel à une revue régulière dans cet environnement.

Cette étude a démontré que de plus en plus nombre de nos isolées chez les patients hospitalisés sont résistantes à la Gentamycine et la Céfotaxime, deux anti-microbiens qui jouent un grand rôle dans la chémothérapie des infections. Il est à noter que plus de 60% des pseudomonads sont résistants à la Gentamycine.

La Ciprofloxacine, une nouvelle fluoroquinolone a cependant démontré une grand activité contre toutes les isolées testées avec une CIM comparable à celles rapportées dans des pays étrangers. La Ciprofloxacine s'est montrée un agent anti-microbien utile contre ces isolées.

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Introduction

Nalidixic acid, a 1-8 naphthyridine derivative, which is closely related to the quinolones, was the first of this group of compounds to be introduced into clinical practice. It has been used for many years [1], but suffers a number of disadvantages. It is only active against Gram-negative bacteria, excluding *Pseudomonas aeruginosa*, and although well absorbed from the gastrointestinal tract, is rapidly metabolized and excreted as inactive products in the urine. Because only low serum concentrations can be achieved, its use has been largely restricted to the treatment of urinary tract infections, although it has also been shown to be of value in the management of bacillary dysentery due to multi-resistant strains of Shigellae [2].

Nalidixic acid was followed by oxolinic acid, a true quinolone, which had a slightly broader spectrum of anti-bacterial activity, being active against *Staphylococcus aureus* as well as the Enterobacteriaceae [3,4].

However, the major advance in the use of quinolone derivatives as anti-bacterial agents has resulted from the introduction of the 4-fluoroquinolones [5], which have a wide spectrum of anti-bacterial activity, including most of the Enterobacteriaceae, many Gram-positive organisms, as well as *Ps. aeruginosa* and some Mycobacteria [6,7]. It also has a reasonable activity against *Chlamydia trachomatis* [8], and

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limited action against some anaerobic bacteria [9].

The quinolones act by inhibiting the action of DNAgyrase — in particular by preventing the resealing of breaks in the DNA strand by the alpha subunit of the enzyme [10]. Ciprofloxacin is one of the most recent and active of the quinolones to be introduced, and a wide range of organisms have been shown to be highly susceptible to its bactericidal effects [11,12].

With the introduction of Ciprofloxacin into Nigeria, the need to carry out an in-vitro evaluation of its activity against commonly isolated organisms in hospital practice became apparent. It was anticipated that normally susceptible organisms would be sensitive to the drug, but that information gained at this time would serve as a useful baseline for detection of possible emergence of resistance at some future date.

Materials and methods

Bacterial strains

A total of 138 bacterial isolates were obtained from in-patients admitted to the wards of the University College hospital, Ibadan. Details of isolates and their sources are given in Table 1.

Sensitivity tests

Disc diffusion tests. Disc diffusion tests were performed using Oxoid Mueller Hinton Medium (CM337), and the following discs: 10 µg Ampicillin, 25 µg Cotrimoxazole, 30 µg Tetracycline, 100 µg Carbenicillin, 30 µg Cefotaxime, 30 µg Nalidixic acid, 10 µg Gentamicin, and 5 µg Ciprofloxacin.

Inhibition zone diameters were measured after 18 h incubation at 36°C, and compared with those of control strains *E. coli* (ATCC 25922) and *Klebsiella edwardsii* (NCTC 10896). Resistance of isolates in relation to control organisms was determined as described by Collins and Lyne [13].

MIC estimations. The MIC values for Cefotaxime, Gentamicin and Ciprofloxacin were estimated using the agar dilution technique, with a multipoint inoculator which delivered approximately 10,000 colony forming units per inoculum. Results were read after incubation for 18 h at 36°C. The MIC was taken as the lowest concentration of anti-microbial at which growth was inhibited.

Results

Disc sensitivities

Fifty strains of *E. coli* were tested: 48 were resistant to Tetracycline, 45 to Ampicillin, 38 to Cotrimoxazole, 12 to Gentamicin, six to Nalidixic acid, four to Cefotaxime and none to Ciprofloxacin.

Twenty-seven strains of *Klebsiella* spp. were examined: 26 were resistant to Tetracycline, 23 to Cotrimoxazole, nine to Gentamicin, four to

Table 1. Sources of bacterial isolates examined

Sources	Bacterial species (nos)				
	Escherichia coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Proteus* spp.	*Citrobacter* spp.
Urine	40	16	18	10	12
Wounds	6	4	8	5	0
Blood	0	3	4	0	2
Cerebrospinal fluid	4	2	1	0	1
Osteomyelitis	0	1	0	0	0
Ascitic fluid	0	1	0	0	0
Total	50	27	31	15	15

The organisms were isolated and identified using standard methods [13].

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Table 2. Disc sensitivity pattern of hospital isolates of Gram-negative bacilli

Organisms (No. strains)	No. resistant (%) to:							
	Ampicillin	Tetracycline	Cefotaxime	Gentamicin	Cotrimoxazole	Carbenicillin	Nalidixic acid	Ciprofloxacin
Escherichia coli ATCC 25922 (6 replicates)	0 (−)	0 (−)	0 (−)	0 (−)	0 (−)	0 (−)	0 (−)	0 (−)
Klebsiella edwardsii NCTC 10896 (6 replicates)	6 (100)	0 (−)	0 (−)	0 (−)	0 (−)	6 (100)	0 (−)	0 (−)
Escherichia coli (50)	45 (90)	48 (96)	4 (8)	12 (24)	38 (76)	NT (−)	6 (−)	0 (0)
Klebsiella spp. (27)	NT (−)	26 (96)	1 (4)	9 (33)	23 (85)	NT (−)	4 (15)	0 (0)
Pseudomonas spp. (31)	NT (−)	NT (−)	24 (77)	20 (65)	NT (−)	27 (87)	NT (−)	0 (0)
Indole +ve *Proteus* spp. (9)	8	NT	1	6	6	NT	5	0
Indole −ve *Proteus* spp. (6)	6	NT	2	5	5	NT	2	0
Citrobacter spp. (15)	15 (100)	15 (100)	2 (13)	7 (46)	11 (73)	NT (−)	4 (27)	0 (0)

NT = not tested.

Nalidixic acid, one to Cefotaxime, and none to Ciprofloxacin.

Thirty-one strains of *Pseudomonas* spp. (29 of which were *Ps. aeruginosa*) were tested: 27 were resistant to Carbenicillin, 20 to Gentamicin, 24 to Cefotaxime, and none to Ciprofloxacin. Strains were not examined for sensitivity to Ampicillin, Nalidixic acid, Tetracycline or Cotrimoxazole, to each of which pseudomonads show intrinsic resistance.

Nine strains of indole-positive *Proteus* spp. were examined: all were sensitive to Ciprofloxacin, eight to Cefotaxime, four to Nalidixic acid, three each to Gentamicin and Cotrimoxazole, and one to Ampicillin.

Of the six indole-negative *Proteus* spp. none were sensitive to Ampicillin, one each were sensitive to Gentamicin, Cotrimoxazole, and four each to Nalidixic acid and Cefotaxime. All were sensitive to Ciprofloxacin.

Fifteen strains of *Citrobacter* spp. were examined: all were resistant to Tetracycline and Ampicillin. Eleven were resistant to Cotrimoxazole, seven to Gentamicin, four to Nalidixic acid, two to Cefotaxime and none to Ciprofloxacin. Details of these results are given in Table 2.

MIC estimations

The results for MIC(50), MIC(90), and the MIC ranges for the different organisms and anti-microbial agents are shown in Table 3.

Discussion

As we pointed out in 1981 [14] the results of our surveillance of bacterial sensitivity patterns over nearly two decades [15] have shown a progressive increase in the emergence of strains of commonly isolated organisms that are resistant to many chemotherapeutic agents.

The present study again emphasizes these observations: where similar organisms and antibiotics have been tested, a substantial increase in resistance has been observed since our last report in 1981. At present, for example, a substantial proportion of hospital strains of *E. coli, Klebsiella* spp., and *Proteus* spp. are now resistant to one of the third generation cephalosporins, as well as the frequently used Gentamicin. In addition, there has been a very marked increase in the prevalence of Gentamicin-resistant strains of *Ps. aeruginosa* in the hospital since 1981.

Table 3. MICs for a cumulative percentage of isolates with inocula of 10,000 cfu

Organisms (No. strains)	Antimicrobial	MIC (μg/ml)		
		MIC(50)	range	MIC(90)
Escherichia coli (50)	Cefotaxime	0.5	0.062–>16	>16
	Gentamicin	1.0	0.25–>32	>32
	Ciprofloxacin	0.016	0.008–0.512	0.062
Klebsiella spp. (27)	Cefotaxime	0.062	0.031–>16	16.0
	Gentamicin	4.0	0.25–>32	>32
	Ciprofloxacin	0.062	0.016–0.512	0.256
Pseudomonas spp. (31)	Cefotaxime	>16	0.062–>16	>16
	Gentamicin	8.0	0.5–>32	>32
	Ciprofloxacin	0.256	0.128–1.024	0.512
Proteus spp. (15)	Cefotaxime	4.0	1.0–>16	16.0
	Gentamicin	2.0	1.0–>32	32.0
	Ciprofloxacin	0.031	0.016–0.128	0.128
Citrobacter spp. (15)	Cefotaxime	0.062	0.031–16.0	16.0
	Gentamicin	1.0	0.25–>32	>32
	Ciprofloxacin	0.031	0.016–0.256	0.256

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Ciprofloxacin was introduced into the Nigerian market in mid-1987, and has as yet been little used. Our study shows that all strains of the common Gram-negative bacilli circulating in this hospital are presently sensitive to this agent, including strains of *Ps. aeruginosa*. The MIC values for the various bacterial genera are closely similar to those previously summarized from many centres throughout the world [16].

Although we have not detected any evidence of Ciprofloxacin resistance at the present time, it is known that chromosomal resistance can develop, although it is thought that the more dangerous plasmid mediated form of resistance is less likely to occur among the fluoroquinolones than among other groups of antimicrobial agents [17]. While Ciprofloxacin is considered to be one of the most effective of the new fluoroquinolones [18], there have already been isolated reports of resistance developing, particularly during treatment of infections due to *Ps. aeruginosa* [19,20].

Thus, although Ciprofloxacin will be a potentially effective new agent for the treatment of infections in Nigeria where multiple antibiotic resistance is common, its use should be undertaken with due regard to the problems of developing antibiotic resistance.

Acknowledgment

We are grateful to Bayer (Nigeria) Ltd for financial support to enable us to undertake this study.

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(*Accepted* 4 *January* 1988)

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