

SUSCEPTIBILITY OF NON-FERMENTATIVE GRAM-NEGATIVE ORGANISMS TO UREIDOPENICILLINS AND QUINOLONES

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Summary

The susceptibility of non-fermentative Gram-negative bacteria (excluding *Pseudomonas aeruginosa*) to ureidopenicillins and new quinolones was investigated. The ureidopenicillins were not active against the strains except against *Pseudomonas* species, 90% of which were inhibited by 64 mg/l. The new quinolones, particularly ciprofloxacin, had excellent activity (MIC range 0.06 – 8 mg/l) against these strains which included those resistant to betalactams and aminoglycosides. This suggested a promising alternative group of compounds to be used in chemotherapy of infections caused by non-fermenters.

Keywords: Non-fermenters, ureidopenicillins, quinolones.

INTRODUCTION

Many non-fermentative organisms are ubiquitous in nature. They are able to survive in soil, water and plants as they are not fastidious in their growth requirements. Many of the human strains, such as *Pseudomonas cepacia* and *Ps. fluorescens* have been found within the hospital environment. They have been recovered in specimens from respiratory infections, urinary tract infections, wound infections, arthritis, osteomyelitis and septicæmia. A few strains have also been recovered from mixed infections. Their significance in these infections have been indeterminate, nevertheless they are of importance. Antimicrobial susceptibility of these non-fermenters often show great inter- and intraspecies variation. Strains of *Ps. maltophilia* and *Acinetobacter* often show multiple resistance whilst strains of *Ps. cepacia* are generally susceptible to most antibiotics with the exception of aminoglycosides. The antibiotic testing of each particular strain isolated is therefore important in determining the type of treatment. However, generalisation in the pattern of susceptibility can sometimes be made.

This study recorded the susceptibility pattern of non-fermentative Gram negative organisms (excluding *Ps. aeruginosa*) isolated in the clinical laboratory from patients in the General Hospital, Kuala Lumpur.

MATERIALS AND METHODS

Bacterial strains

The strains were clinical isolates and were identified by using the API 20NE system (API System S.A., France) and additional

complementary tests. The organisms were identified as *Pseudomonas sp* (15 strains) comprising *Ps. maltophilia* (5), *Ps. cepacia* (4), *Ps. acidovorans* (2), *Ps. alcaligenes* (2), *Ps. pseudomallei* (1), *Ps. putida* (1); *Alcaligenes* (16 strains) comprising *Alc. odorans* (15), *Alc. denitrificans* (1); *Achromobacter xyloxidans* (10 strains), *Achromobacter* group VD (1 strain), *Flavobacterium odoratum* (3 strains), *Fl. meningosepticum* (1 strain), *Acinetobacter calcoaceticus var anitratus* (2 strains), and *Aeromonas hydrophilia* (1 strain).

Susceptibility testing

Susceptibility to the ureidopenicillins and the quinolones was determined by the plate dilution method using Sensitest Agar (Oxoid Ltd., UK) and an inoculum size of 10^5 colony forming units. Plates were incubated at 37°C for 18 hours. The minimal inhibitory concentration (MIC) was read as the lowest concentration of the antibiotic which inhibited growth. *Ps. aeruginosa* NCTC 10662 was used as the control organism.

The following antibiotic powders were used : azlocillin (Bayer, Germany), mezlocillin (Bayer, Germany), piperacillin (Lederle, USA) nalidixic acid (Sigma, USA), ciprofloxacin (Bayer, Germany), enoxacin (Warner-Lambert, USA), norfloxacin (Astra, Sweden), ofloxacin (Daichii, Japan) and pefloxacin (May and Baker, UK).

Susceptibility testing to other beta-lactams such as ampicillin, carbenicillin, cefuroxime, cefotaxime, ceftazidime, cefoperazone, aztreonam, and to tetracycline, cotrimoxazole, gentamicin, amikacin and netilmicin was

TABLE 1
ACTIVITY OF UREIDOPENICILLINS AND QUINOLONES (IN MG/L) AGAINST GRAM NEGATIVE NON-FERMENTERS

	Pseudomonas spp (15)*		Alcaligenes spp (16)		Achromobacter spp (11)		Acinetobacter spp (2)		Flavobacterium spp (4)		
	Range	MIC ₅₀ MIC ₉₀	Range	MIC ₅₀ MIC ₉₀	Range	MIC ₅₀ MIC ₉₀	Range	MIC ₅₀ MIC ₉₀	Range	MIC ₅₀ MIC ₉₀	
Ureidopenicillins											
Azlocillin	2 - >128	16 64	1 - >128	8 64	4 - >128	>128	>128	8	>128	16 - >128	64 >128
Mezlocillin	2 - >128	32 64	4 - >128	32 >128	8 - >128	>128	>128	16	>128	32 - >128	64 >128
Piperacillin	2 - >128	32 64	1 - >128	8 32	4 - >128	128	>128	4	>128	64 - >128	128 >128
Quinolones											
Nalidixic acid	8 - >128	64 >128	8 - >128	32 >128	16 - >128	64	>128	4	32	8 - 64	16 64
Ciprofloxacin	0.5 - 8	2 8	0.06 - 8	4 8	2 - 16	2 4	4	1	2	0.12 - 4	2 4
Ofloxacin	0.5 - 8	4 8	0.12 - 16	2 8	2 - 16	8 16	16	0.5	8	0.12 - 4	2 4
Pefloxacin	0.5 - 16	4 8	0.25 - 16	2 8	2 - 32	16 16	16	0.5	1	1 - 8	2 8
Enoxacin	0.5 - 16	4 16	0.12 - 32	4 16	4 - 32	16 32	32	2	4	2 - 16	8 16
Norfloxacin	1 - 64	16 32	0.5 - 64	16 64	4 - 128	32 64	64	8	8	1 - 32	16 32

*() number of organisms

TABLE 2
RESISTANCE (NO. OF STRAINS) OF GRAM NEGATIVE NON-FERMENTERS TO OTHER ANTIBIOTICS

	Pseudomonas spp (15)*	Alcaligenes spp (16)	Achromobacter spp (11)	Acinetobacter spp (2)	Flavobacterium spp (4)	Total resistance (%)
Ampicillin	—	3	9	2	4	42.8
Carbenicillin	3	3	9	2	3	40.0
Cefuroxime	9	16	11	2	4	85.7
Cefotaxime	1	1	9	1	2	28.5
Ceftazidime	2	1	2	1	4	20.4
Cefoperazone	3	3	2	1	4	26.5
Aztreonam	4	11	10	2	4	63.3
Tetracycline	8	9	10	1	2	61.2
Cotrimoxazole	7	5	0	1	3	32.6
Gentamicin	13	3	4	1	3	49.0
Netilmicin	9	2	2	1	3	34.7
Amikacin	10	0	0	1	3	28.6

* () number of strains tested

performed by the disk method on Sensitest Agar (Oxoid Ltd., UK). The inoculum which resulted in a semi-confluent growth was standardised by a spectrophotometric method¹ and zone sizes were measured after incubation at 37°C. Results were interpreted as sensitive or resistant by comparing the zone sizes obtained to those of the control strain.²

RESULTS

Table 1 shows the susceptibility of the strains to ureidopenicillins and the quinolones. The ureidopenicillins were not very active against the non-fermenters with MIC₉₀ exceeding 128 mg/l against most strains. MIC₅₀ of ≤ 32 mg/l were only seen against *Pseudomonas*, *Alcaligenes* and *Acinetobacter* spp.

The new quinolones were more active than nalidixic acid. The MIC₉₀ of these compounds did not exceed 64 mg/l. Ciprofloxacin, ofloxacin and pefloxacin inhibited *Pseudomonas* and *Alcaligenes* at 8 mg/l; only a few strains had to be inhibited by 16 mg/l. *Acinetobacter* strains were inhibited by ciprofloxacin and pefloxacin at 2 mg/l. Norfloxacin and enoxacin were slightly less active against these strains with MIC₉₀ of 4-64 mg/l. The quinolones were also less active against *Achromobacter* and *Flavobacterium* strains with MIC₉₀ of 4 to 64 mg/l. Ciprofloxacin, however, was active against *Achromobacter* sp (MIC₉₀ of 4 mg/l). The *Aeromonas* strain was susceptible to the new quinolones with MIC ranging from 0.06 – 1 mg/l.

The non-fermenters were mostly resistant to cefuroxime (42/49 strains), by disc testing (Table 2). These strains consisted mainly the *Achromobacter* and *Alcaligenes* species. *Achromobacter* strains also showed across resistance to ampicillin, carbenicillin and cefotaxime and aztreonam. These strains were however susceptible to amikacin. Resistance to other antibiotics were evenly distributed among the strains. Many of the *Pseudomonas* strains were resistant to the aminoglycosides.

DISCUSSION

Azlocillin and piperacillin had been demonstrated to have good anti-pseudomonal activity.³ In this study, their activity against non-fermenters was poor with MIC₉₀ of ≥ 128 mg/l except against *Pseudomonas* and *Alcaligenes* spp (MIC₉₀ of 64 mg/l). Other studies have shown azlocillin to be fairly active against *Ps. aeruginosa* inhibiting about 98% of strains at 64 mg/l.⁴ Mezlocillin, like azlocillin, has a wide spectrum activity against many Gram negative organisms.⁵ Mezlocillin

however was not active against the non-fermentative organisms. It does appear that the ureidopenicillins has limited use against the non-fermenters except against *Pseudomonas* and *Alcaligenes* strains where easily achievable blood levels of 64 mg/l would be inhibitory. Resistance to the ureidopenicillins and to other beta-lactams did not reflect the presence of specific beta-lactamases, except that perhaps an oximase-type enzyme may be more prevalent in strains of *Alcaligenes*, *Achromobacter* and the *Pseudomonas* tested.

Recently developed quinolones of many types have improved activity over many agents against Enterobacteriaceae and *Ps. aeruginosa*.^{6,7} This study showed that the new quinolones were more active than nalidixic acid with MIC₉₀ not exceeding 32 mg/l. Ciprofloxacin was 4 – 8 times more active than ofloxacin, pefloxacin and enoxacin although all have excellent activity against many of the strains, including those which were multiply resistant. Ciprofloxacin-resistant strains were not observed in this study although they had been found within the Enterobacteriaceae and some Gram positive organisms.^{8,9} The high *in vitro* activity of the new quinolones suggests that there is a role for these new drugs in the treatment of infections caused by non-fermentative gram negative organisms particularly when they are resistant to other antibiotics such as the beta-lactams and the aminoglycosides. Development of resistance to these drugs however need to be monitored and analysed closely.

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