

IN-VITRO SUSCEPTIBILITY TO AZTREONAM

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Summary

Aztreonam is a new monobactam antibiotic which is highly active against aerobic Gram negative bacilli. The purpose of this study is to establish the in-vitro activity of aztreonam against recent clinical isolates of aerobic Gram negative bacilli obtained from patients at the Kuala Lumpur General Hospital. Minimum inhibitory concentrations of the antibiotic against the isolates were determined using an agar dilution method. Aztreonam was found to be active against a range of aerobic Gram negative bacilli including those which were resistant to gentamicin. There was however a significant incidence of resistance among *Klebsiella* sp and *Salmonella* sp. Aztreonam could be a valuable alternative in cases of infection due to gentamicin-resistant aerobic Gram negative bacilli.

Keywords: Aztreonam, monobactam, antibiotic sensitivity testing, resistance to aztreonam.

INTRODUCTION

Aztreonam is a new antibiotic with a monocyclic structure. The term monobactam has been applied to this class of antibiotics. Since the days of the development of penicillin, it has been realised that a fused ring structure is essential for antibacterial activity. So far however, attention has been focussed only on compounds with a bicyclic nucleus like penicillins and cephalosporins. In 1979, researchers discovered that certain soil-dwelling microorganisms like *Chromobacterium*, *Glucobacter*, *Acetobacter*, *Agrobacterium*, *Pseudomonas* and *Flexibacter* are capable of producing monocyclic compounds that possess antibacterial activity.²

While such naturally occurring compounds possess rather weak anti-bacterial activity, modifications in their side-chains can lead to very potent antibiotics. An example of this is aztreonam.

The purpose of this study is to establish the in-vitro activity of aztreonam against locally isolated strains of aerobic Gram negative bacilli.

MATERIALS AND METHODS

A total of 365 recent clinical isolates were obtained from patients at the Kuala Lumpur General Hospital over a five month period between 1st January 1987 and 31st May 1987. They comprised 49 strains of *Klebsiella* sp, 49 strains of *E. coli*, 49 strains of *Enterobacter* sp, 48 strains of *Salmonella* sp (including six strains of *S. typhi*), 49 strains of *Proteus*

sp, 49 strains of *Acinetobacter* sp, 49 strains of *Pseudomonas aeruginosa*, 7 strains of *Flavobacterium meningosepticum*, 5 strains of *Shigella* sp, 5 strains of *Alkaligenes* sp, 2 strains of *Achromobacter* sp and 1 strain each of *Citrobacter* sp, *Pasteurella* sp, *Serratia* sp and *Aeromonas hydrophilia* respectively.

An agar dilution method was used to determine minimum inhibitory concentrations of aztreonam against the clinical isolates. The medium used was Diagnostic Sensitivity Test Agar (Oxoid) for all strains except for *Pseudomonas aeruginosa* where Mueller-Hinton agar was used. Overnight broth cultures of the test organisms were diluted a hundred fold to serve as the inocula and plates were inoculated with the inocula using a multipoint inoculator (Dynatech 2000). Each inoculating pin delivered approximately 1.5 microlitres of the inoculum. After inoculation the plates were incubated at 37°C for 18 hours before being read. The minimum inhibitory concentration (MIC) was defined as the minimum concentration of antibiotic that inhibits all visible growth of bacteria on the plate.

RESULTS

The results are expressed as the MIC range, MIC₅₀ and MIC₉₀ (Table 1). The MIC₅₀ and MIC₉₀ are the concentrations of the antibiotic that inhibit 50% and 90% of the strains tested respectively. The *Enterobacteriaceae* were in general very susceptible to aztreonam with the exception

TABLE 1
SUSCEPTIBILITY OF AEROBIC GRAM-NEGATIVE BACILLI TO AZTREONAM

Organism (No. of strains)	MIC Range	MIC50 (all values in mg/l)	MIC90
<i>Ps aeruginosa</i> (49)	2.0–16.0	4.0	16.0
<i>Klebsiella</i> sp (49)	0.06–>32.0	0.06	>32.0
<i>Salmonella</i> sp# (48)	0.06–>32.0	0.06	>32.0
<i>Acinetobacter</i> sp (49)	0.06–>32.0	32.0	>32.0
<i>Proteus</i> sp (49)	0.06–32.0	0.06	0.25
<i>Enterobacter</i> sp (49)	0.06–32.0	0.06	4.0
<i>E. coli</i> (49)	0.06–1.0	0.06	0.12
<i>Shigella</i> sp (5)	–	0.06	0.06
<i>Flavobacterium</i> sp (7)	–	>32.0	>32.0
Others## (11)	0.06–>32.0	1.0	16.0

includes 6 isolates of *S. typhi*

includes 1 *Aeromonas* sp, 1 *Citrobacter* sp, 2 *Achromobacter* sp, 1 *Pasteurella* sp, 1 *Serratia* sp and 5 *Alkaligenes* sp.

of *Klebsiella* sp and *Salmonella* sp (not *S. typhi*) where a significant proportion of strains tested (15 – 20%) were found to be resistant. 33% of *Acinetobacter* sp tested had an MIC of >32.0 mg/l and only 16% were inhibited at 8.0 mg/l. Its activity against *Pseudomonas aeruginosa* was good and 74% of strains tested was inhibited at 8 mg/l. All 7 strains of *Flavobacterium meningosepticum* had MICs of >32 mg/l. The incidence of resistance of the locally isolated strains is summarised in Table 2.

A significant proportion of the strains tested (111 isolates altogether) were multiply resistant strains including resistance to gentamicin (MIC of 4 mg/l or greater). At a concentration of 8 mg/l, aztreonam was active against 60% of the gentamicin-resistant Gram negative bacilli. It was active against all gentamicin-resistant strains of *Proteus* sp and *Enterobacter* sp. It was however active against only two thirds of gentamicin-resistant *Klebsiella* sp and *Salmonella* sp strains. Less than a fifth of gentamicin-resistant strains of *Acinetobacter* sp were inhibited at this concentration (8 mg/l). The susceptibilities

of the gentamicin-resistant strains to aztreonam are summarised in Table 3.

DISCUSSION

The pattern of susceptibility to aztreonam among the local clinical isolates is generally in keeping with that which has already been described by Barry et al in the United States.³ Based on the pharmacokinetic characteristics of aztreonam, the MIC breakpoints which have been suggested by them were ≥ 32 mg/l for the resistant category and ≤ 8 mg/l for the sensitive category. Organisms with MICs which fall in between are classified as intermediate in sensitivity. The local strains of *Enterobacteriaceae* were highly susceptible with the exception of *Klebsiella* sp and *Salmonella* sp. All the strains of *Salmonella typhi* tested were however sensitive. The activity of aztreonam against local strains of *Pseudomonas aeruginosa* and *Acinetobacter* sp were also in conformity with that reported by Barry et al. Aztreonam was found to be inactive against *Flavobacterium meningosepticum*.

There appeared to be a higher incidence of resistance among local strains of *Klebsiella* sp and *Salmonella* sp. This is rather interesting as aztreonam has not been used in Malaysia before. Although aztreonam is stable to most plasmid mediated betalactamases, it is moderately unstable to a few chromosomal mediated betalactamase like the K1 betalactamase of *Klebsiella oxytoca*.⁴ It would be interesting to know the mechanism of resistance of these strains and a study is currently being carried out.

Although the number of strains studied

were few, some conclusions may be derived. Aztreonam has good activity against *Enterobacteriaceae*. From the in-vitro results it appears that the 1 gm tds regimen would be adequate for treating these infections since it had been shown that the mean serum level 4 hours after a 1 gm intravenous injection is 13.2 mg/l.⁵ For *Pseudomonas aeruginosa* and sensitive *Acinetobacter* sp a 2 gm tds regimen would be more appropriate since these strains have in general higher MICs. After a 2 gm intravenous injection the mean serum level after 4 hours has been shown

TABLE 2
INCIDENCE OF RESISTANCE TO AZTREONAM

Organism	Percentage of strains inhibited at		
	2.0 mg/l	8.0 mg/l	32.0 mg/l
<i>Salmonella</i> sp	86	86	86
<i>Proteus</i> sp	96	96	100
<i>Klebsiella</i> sp	73	76	78
<i>Enterobacter</i> sp	86	98	100
<i>E. coli</i>	98	98	100
<i>Shigella</i> sp	100		—
<i>Acinetobacter</i> sp	14	16	67
<i>Ps aeruginosa</i>	10	74	100

TABLE 3
SUSCEPTIBILITY OF 111 GENTAMICIN RESISTANT GRAM NEGATIVE BACILLI
TOWARDS AZTREONAM

Organism (No. of strains)	Percentage inhibited by aztreonam at		
	2 mg/l	8 mg/l	32 mg/l
<i>E. coli</i> (7)	86	86	86
<i>Proteus</i> sp (12)	92	100	
<i>Enterobacter</i> sp (11)	55	100	—
<i>Klebsiella</i> sp (30)	60	66	66
<i>Salmonella</i> sp (3)	66	66	66
<i>Acinetobacter</i> sp (29)	17	17	48
<i>Ps aeruginosa</i> (19)	11	68	100
Total (111)	45	60	69

to be 26 mg/l.⁵ Resistance to aminoglycosides is a major problem in our hospital. It is gratifying to note that aztreonam is active against almost two thirds of our gentamicin-resistant aerobic Gram negative bacilli. Aztreonam would be a valuable alternative in the treatment of infections due to gentamicin-resistant Gram negative bacilli.

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