CHAPTER 5

DISCUSSION AND CONCLUSION

During the past few years several new beta-lactam antibiotics have been introduced with broader antibacterial spectra. Unfortunately, some of them do not uniformly inhibit Enterobacteriaceae and P. aeruginosa (18), while others are not stable to common plasmid-mediated β-lactamases found in gram negative bacteria (18).

Aztreonam, a total synthetic monocyclic beta-lactam, exhibits a unigue pattern of antimicrobial activity when compared to other beta-lactam and aminoglycoside antibiotics (18,50). It is inactive against gram positive bacteria as well as anaerobic bacteria, but extremely active against a broad range of aerobic gram negative organisms (18). Furthermore, it is not hydrolyzed to any appreciable degree by the common plasmid-mediated beta-lactamases found in Enterobacteriaceae and P. aeruginosa (18,50).

In This study, aztreonam showed good in vitro activity against a broad range of gram negative organisms. The MIC_{90} values against Enterobacteriaceae was not more than 2 μg/ml with the exception of Enterobacter spp. However, the majority of Enterobacter spp. were also highly susceptible with a MIC_{50} value of <0.2 μg/ml. For other gram negative organisms, the activity of aztreonam ranged from highly active against Aeromonas spp., to intermediate for P. aeruginosa , to poor against strains of Acinetobacter spp. The MIC_{90} from this study were similar to the results obtained from the study by Sykes et al. (18) and Paradelis et al. (50). The comparisons of these MIC_{90} are shown in table 13.

Table 13 The comparison of the MIC_{90} of aztreonam to gram negative bacteria.

Organisms	MIC_{90} $\mu g/ml$ (no of strains)		
	Sykes et al	Paradelis et al	This study
Acinetobacter spp.	58.3(25)	-	86(22)
Aeromonas spp.	-	-	0.13(30)
Enterobacter spp.	12.5(29)	12.5(60)	10 (53)
Escherichia coli	0.2(79)	0.2(1,000)	0.11(66)
Klebsiella spp.	0.2(68)	0.3(100)	0.17(52)
P. aeruginosa	12(61)	16(600)	11.9(29)
Proteus spp.	<0.1(25)	<0.1(225)	0.05(19)
Salmonella spp.	0.3(25)	0.4(100)	1.6(28)
Shigella spp.	-	5.3(100)	1.5(32)
Serratia spp.	1.6(113)	1.6(200)	0.77(20)

In the clinical studies aztreonam was used in the treatment of 10 gram negative bacterial infections. Nine cases were children with other compromising underlying conditions, the other case suffered only from gram negative bacterial infection (case No.10). Dose of aztreonam varied from 53 mg/kg/day to 100 mg/kg/day due to patient's renal function and the nature of the infections processes treated.

Overall, the aztreonam treatment was considered to produce a clinical cure in one of 10 patients, improvement in 8 cases and failure in one case constituting a favorable response rate of 90% for the initial infections. Failure was found in case No. 4 whose initial causative organism was eradicated, but developed a subsequent superinfection with Streptococcus group D which required additional therapy. Signs and symptoms were improved when this patient received ceftazoline plus amikacin.

For bacteriological results, all of the initial causative organisms were eradicated, including Klebsiella pneumoniae which was proved to be resist to aztreonam (table 10). This was probably due to high concentration of aztreonam in urine (39), which maybe higher than MIC of this organism. Except superinfection in case No. 4, colonization also occurred in 5 other patients. Case No. 1 developed colonization with C. albican and Streptococcus group D, case No. 3 with E. cloacae, case No. 4 with Streptococcus group D, case No. 8 and 9 with C. albican (table 10). All of 6 patients who developed superinfection or colonization had other compromising underlying conditions (table 8). So, the high incidence of colonization and superinfection in this study probably due to these underlying conditions of the patients.

Streptococcus group D was also reported to be causative organism for superinfection and colonization in many other reports (51,52,53, 54,55). Most of the patients in these reports, like patients in this study, had other underlying conditions. From these data, special attention should

be focused on the role of the underlying diseased and the occurrence of superinfection in subsequent clinical study.

However, colonization that occurred in this study may cause from the microflora which colonized on the skin surface around the wound or the sexual organs. These organisms could contaminate in the samples. Collected pus by swabbing at the wound or collecting urine by using urine bag would accidentally contact these areas. Contaminated by these organisms could easily caused false colonization.

The serum aztreonam concentrations from all of the patients, at $\frac{1}{2}$ - 1 h. after injection, which ranged from 40.8 μg/ml to 85.4 μg/ml exceeded the MIC for 90% of the aerobic gram negative bacteria, including P. aeruginosa (table 6). In addition, serum levels of aztreonam, at 6-8 h. after injection (table 9) were still sufficient to inhibit most of gram negative bacteria (table 6).

Aztreonam were well tolerated in the patients in this study. Only mild side effects (phlebitis, pain at the injection site) occurred in two patients who received aztreonam by i.v. bolus. The side effects were disappeared when the patients received drug by i.v. infusion.

In conclusion, aztreonam is safe and effective for the treatment of most of gram negative bacterial infections, especially urinary tract infections. In patients with compromising underlying diseases, however, superinfections with resistant bacteria should be carefully monitored.

Appendix I

Table 14 Interpretation of reactions on Triple Sugar Iron agar

Reaction	Carbohydrates fermented	Possible organisms
Acid butt Acid slant Gas in butt No H_2S	Glucose with acid and gas Lactose and/or sucrose with acid and gas	Escherichia Klebsiella or Enterobacter** Proteus or Providence Intermediate coliforms
Acid butt Alkaline slant Gas in butt H_2S produced	Glucose with acid and gas Lactose and sucrose not fermented	Salmonella Proteus Arizona (certain types) Citrobacter* (certain types)
Acid butt Alkaline slant No gas in butt No H_2S	Glucose with acid only Lactose and sucrose not fermented	Salmonella*** Shigella Proteus Providence
Acid butt Acid slant Gas in butt H_2S produced	Glucose with acid and gas Lactose and/or sucrose with acid and gas	Arizona Citrobacter*
Alkaline or neutral butt Alkaline slant No H_2S	None	Alcaligenes**** Pseudomonas Herellea

* Formerly Escherichia freundii

** Formerly Aerobacter.

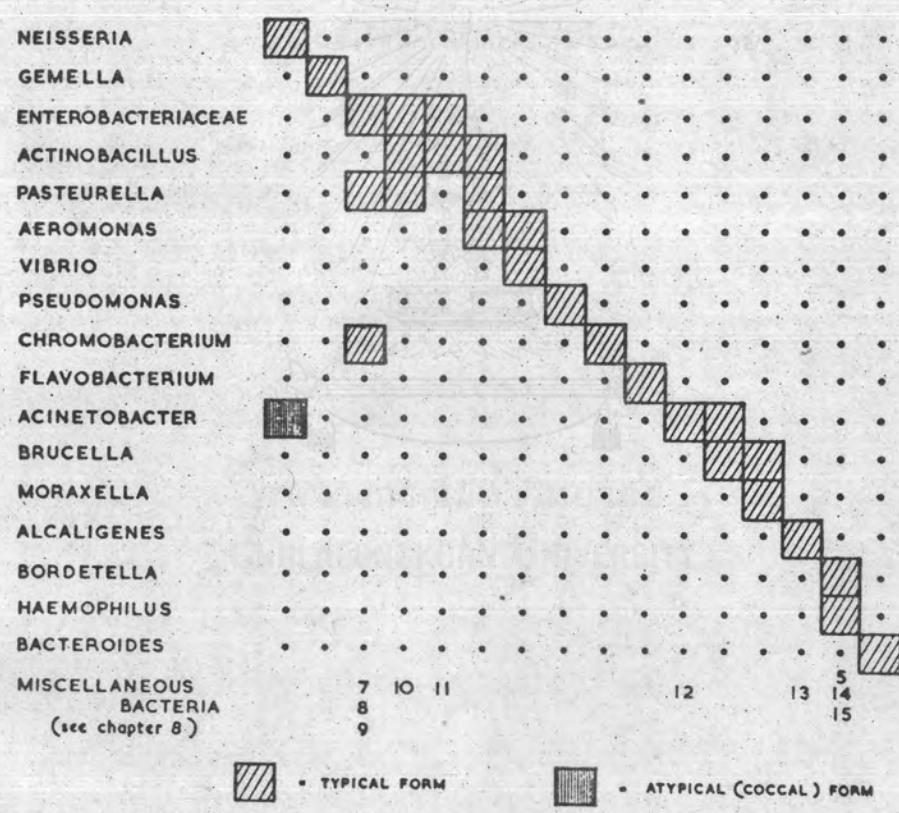
*** Salmonella typhi produces a small amount of H_2S but seldom gas.

(Baily, W.R., and Scott, E.G. Diagnostic Microbiology, 1970, p. 142)

Appendix II

Table 15 First-stage diagnostic table for gram-negative bacteria.

	a		b	c		d		e				f			g	h
	a_1	a_2	b	c_1	c_2	d_1	d_2	e_1	e_2	e_3	e_4	f_1	f_2	f_3	g	h
SHAPE	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
MOTILITY	−	−	+	−	−	−	+	+	+	−	−	−	−	+	−	d
GROWTH IN AIR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
CATALASE	+	−	+	+	−	+	+	+	+	+	+	+	+	+	NT	d
OXIDASE	+	−	−	−	−	+	+	+	−	+	−	−	+	+	d	−
GLUCOSE (acid)	d	+	+	+	+	+	+	+	+	+	+	−	−	−	NT	d
O−F test	O/−	F	F	F	F	F	F	O	O	O	O	−	−	−	NT	NT



Symbols common to all tables:

+ = 100–80% strains positive; d = 79–21% strains positive; − = 20–0% strains positive; F = fermentation; O = oxidation; (•) = delayed reaction; NT = not testable.

Symbols special to Table II:

S = sphere; R = rod.

(Cowan S.T., and Steel K.T. Mannual for the Identification of Medical Bacterie, 1965, p.76)

Table 16 Second-stage for mobile Enterobacteriaceae and similar organisms.

	Salmonella typhi	*S. arizonae*	Other *Salmonella*	*Escherichia coli*	*Citrobacter freundii*	Ballerup-Bethesda	*Hafnia alvei*	*Enterobacter aerogenes*	*E. cloacae*	*E. liquefaciens*	*Serratia marcescens*	*Proteus vulgaris*	*P. mirabilis*	*P. morganii*	*P. rettgeri*	Providence A	Providence B	*Pasteurella pseudotuberculosis*	*Chromobacterium violaceum*	*C. lividum**
Citrate utilization†	−	+	+	−	+	+	+	+	+	+	+	d	d	−	+	+	+	−	d	+
KCN	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	d
Gelatin hydrolysis‡	−	(+)	−	−	−	−	−	d	(+)	+	+	+	+	−	−	−	−	−	+	−
Glucose (gas)	−	+	+	+	+	+	+	+	+	+	d	+	+	+	−	+	−	−	−	−
Arabinose (acid)	−	+	d	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	−
Lactose (acid)	−	d	−	+	+	d	−	+	+	d	d	−	−	−	−	−	−	−	−	−
Sucrose (acid)	−	−	−	d	d	d	d	+	+	+	+	+	+	−	d	d	d	−	d	−
Adonitol (acid)	−	−	−	−	−	−	−	+	d	−	d	−	−	−	+	+	−	−	−	−
Dulcitol (acid)	d	−	+	d	d	d	−	d	d	−	−	−	−	−	−	−	−	−	−	−
Mannitol (acid)	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	d	+	−	−
Inositol (acid)	−	−	d	−	−	−	−	+	−	+	d	−	−	−	+	−	+	−	−	−
Indole	−	−	−	+	−	−	−	−	−	−	−	+	−	+	+	+	+	−	−	−
V–P	−	−	−	−	−	−	d	+	+	d	+	−	d	−	−	−	−	−	−	−
H_2S (in TSI)	+	+	+	−	+	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−
Urease	−	−	−	−	d	d	−	−	d	d	−	+	+	+	+	−	−	+	−	−
Lysine decarboxylase	+	+	+	d	−	−	+	+	−	d	+	−	−	−	−	−	−	−	−	−
Arginine dihydrolase	+	+	+	d	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	d
Ornithine decarboxylase	−	+	+	d	d	d	+	+	+	+	+	−	+	+	−	−	−	−	−	−
Gluconate	−	−	−	−	−	−	+	+	+	+	+	−	d	−	−	−	−	−	−	d
Malonate	−	+	−	−	d	−	+	+	d	−	d	−	−	−	−	−	−	−	d	−
Phenylalanine	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−
Pigment	−	−	−	−	−	−	−	−	d	−	d	−	−	−	−	−	−	−	+	+
ONPG	−	+	−	+	+	d	+	+	+	+	+	−	−	−	−	−	−	+	−	d

* Included only for comparison; its attack on carbohydrates is oxidative.
† Citrate utilization in Koser's or Simmons' medium.
‡ Gelatin hydrolysis: + = positive within 7 days at 22° C;
(+) = positive after 7 days.

(Cowan S.T., and Steel K.T. Mannual for the Identification of Medical Bacteria, 1965, p. 78)

Table 17 Second-stage table for Shigella, Klebsiella, Pasteurella and Acinitobacillus.

	Shigella dysenteriae 1	*S. dysenteriae* 2 +	*S. flexneri* & *S. boydii*	*S. sonnei*	A-D group	*Klebsiella aerogenes*	*K. pneumoniae*	*K. edwardsii* v. *edwardsii*	*K. edwardsii* v. *atlantae*	*K. ozaenae*	*K. rhinoscleromatis*	*Salmonella gallinarum*	*S. pullorum*	*Pasteurella multocida*	*P. pestis*	*P. pseudotuberculosis*	*Actinobacillus lignieresii*	*A. equuli*
Catalase	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	d
Citrate utilization*	−	−	−	−	−	+	+	d	+	d	−	−	d	−	−	−	−	−
Growth on MacConkey	+	+	+	+	+	+	+	+	+	+	+	+	d	−	+	+	+	d
KCN	−	−	−	−	−	+	−	+	+	+	+	−	−	+	−	−	−	−
Gelatin hydrolysis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glucose (gas)	−	−	−	−	−	+	+	−	+	d	−	−	+	−	−	−	−	−
Lactose (acid)	−	−	−	(+)	d	+	+	(+)	(+)	(+)	−	−	−	−	−	−	d	+
Sucrose (acid)	−	−	−	(+)	d	+	+	+	+	d	+	−	−	(+)	−	−	+	+
Dulcitol (acid)	−	−	−	−	d	d	+	−	−	−	−	+	−	d	−	−	−	−
Mannitol (acid)	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole	−	d	d	−	d	−	−	−	−	−	−	−	−	+	−	−	−	−
MR	+	+	+	+	+	−	+	d	+	+	+	+	+	−	+	+	−	−
V–P	−	−	−	−	−	+	−	+	d	−	−	−	−	−	−	−	d	−
H_2S (in TSI)	−	−	−	−	−	−	−	−	−	−	−	+	+	d	−	−	+	−
Urease	−	−	−	−	−	+	+	+	+	d	−	−	−	−	−	+	d	+
Lysine decarboxylase	−	−	−	−	d	+	+	+	+	d	−	+	+	−	−	−	−	−
Arginine dihydrolase	−	−	−	d	d	−	−	−	−	−	−	d	+	−	−	−	−	−
Ornithine decarboxylase	−	−	−	+	d	−	−	−	−	−	−	−	+	+	−	−	−	−
Gluconate	−	−	−	−	−	+	d	+	d	−	−	−	−	−	−	−	−	−
Malonate	−	−	−	−	−	+	+	d	−	−	+	−	−	−	−	−	−	−
ONPG	+	d	d	d	d	+	+	+	+	+	−	−	−	−	+	+	+	+
Christensen's citrate	..	..	..	−	+	..	..	..	..	..	..	..	..	..	..	..	..	..

* Citrate utilization in Koser's or Simmons' medium.

(Cowan S.T., and Steel K.T. Mannual for the Identification of Medical Bacteria, 1965, p.79)

Table 18 Second-stage table for Pseudomonas, Chromobacterium, Flavabacterium and Acinetobacter species.

	Pseudomonas aeruginosa	*P. fluorescens*	*P. pseudomallei*	*Chromobacterium lividum*	*Flavobacterium meningosepticum*	*Acinetobacter mallei*	*A. antiratus*
Motility	+	+	+	+	–	–	–
Oxidase	+	+	+	–	+	–	–
Growth at 42° C	+	–	+	–	–	–	d
Growth at 37° C	+	d	+	–	+	+	+
Growth at 5° C	–	+	–	+	–	–	–
Citrate utilization	+	+	+	+	–	d	+
Growth on MacConkey	+	+	+	d	d	–	+
KCN	+	d	+	d	–	d	+
Gelatin hydrolysis	+	d	+	–	+	–	d
Starch hydrolysis	–	–	+	–	–	–	–
Mannitol (acid)	d	d	+	–	(+)	d	–
Indole	–	–	–	–	d	–	–
Nitrate reduction	+	d	+	+	–	+	–
Urease	+	d	d	–	–	d	d
Gluconate	+	d	–	d	–	–	–
Pigment	+	+	d	+	+	–	–
Diffusion of pigment	+	+	–	–	–	–	–
Arginine dihydrolase	+	+	+	d	–	+	–

Table 19 Second-stage for Alkaligenes, Acinetobacter, Brucella, and Moraxella species.

	Alcaligenes faecalis	*Alcaligenes bronchisepticus*	*Acinetobacter lwoffii*	*Acinetobacter parapertussis*	*Brucella* spp.	*Moraxella* spp.
Motility	+	+	–	–	–	–
Oxidase	+	+	–	–	d	+
Growth on nutrient agar	+	+	+	+	+	d
Citrate utilization	d	d	+	d	–	–
Growth on MacConkey	+	+	+	–	d	–
KCN	+	+	–	–	–	–
Gelatin hydrolysis	–	–	–	–	–	d
Nitrate reduction	d	+	–	–	+	d
H_2S	d	d	–	–	d	–
Urease	–	+	–	+	+	–

(Cowan S.T., and Steel K.T. Mannual for the Identification of Medical Bacteria, 1965, p.81)

Appendix III

Blood agar base

Ingredients per liter	
Beef heart infusion	500 g
Bacto-Tryptose	10 g
Sodium chloride	5 g
Bacto-Agar	15 g

pH 6.8

Sterilize in the autoclave for 15 minutes at 15 pounds. Cool to 50°C and add 5% sterile defrinated blood.

MacConkey agar

Ingredients per liter	
Bacto-Peptone	17 g
Proteose peptone Difco	3 g
Bacto-Lactose	10 g
Bacto-Bile salt No.3	15 g
Sodium chloride	5 g
Bacto-Agar	13.5 g
Bacto-Neutral red	0.03 g
Bacto-Crystal violet	0.001 g

pH 7.1

Sterilize in autoclave for 15 minutes at 15 pounds pressure.

Malonate Broth

Ingredients per liter	
Ammonium Sulfate	2.0 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium chloride	2.0 g
Sodium Malnoate	3.0 g
Bacto-Brom thymol blue	0.025 g

pH 6.7

Sterilize in the autoclave for 15 minutes at 15 pounds.

Methyl Red-Voges Proskauer medium

Ingredients per liter

Buffered peptone	7 g
Dipotassium phosphate	5 g
Bacto-Dextrose	5 g

pH 6.9

Sterilize in the autoclave for 15 minutes at 15 pounds.

Motility test medium

Ingredients per liter

Bacto Tryptose	10 g
Sodium chloride	5 g
Bacto Agar	5 g

pH 7.2

Sterilize in autoclave for 15 minutes at 15 pounds.

Nutrient Agar

Ingredients per liter

Bacto - Beef Extract	3	g
Bacto - Peptone	5	g
Bacto - Agar	15	g

pH 6.8

Sterilize in antoclave for 15 minutes at 15 pounds pressure

Nutrient Broth

Ingredients per liter

Bacto - Beef Extract	3	g
Bacto - Peptone	5	g

pH 6.8

Sterilize in antoclave for 15 minutes at 15 pounds pressure

Phenol red tartrate agar

Ingredients per liter

Bacto-Peptone	10	g
Sodium potassium tartrate	10	g
Sodium chloride	5	g
Bacto-Agar	15	g
Bacto-Phenol red	0.024	g

pH 7.6

Sterilize in the autoclave for 15 minutes at 15 pounds.

Phenylalanine broth

Ingredients per liter		
Ammonium sulfate	2.0	g
Dipotassium phosphate	0.6	g
Monopotassium phosphate	0.4	g
Sodium chloride	2.0	g
Sodium malonate	3.0	g
Bacto-Brom thymol blue	0.025	g
Phenylalanine	2.0	g

pH 6.7

Sterilize in the autoclave for 15 minutes at 15 pounds.

Potassium Cyanide broth base

Ingredients per liter

Proteose peptone No.3 Difco	3	g
Disodium phosphate	5.64	g
Monopotassium phosphate	0.225	g
Sodium chloride	5	g

pH 7.6

Prepare a 0.5% solution of potassium cyanide in distilled water.

Add 1.5 ml of solution of potassium cyanide to each 100 ml basal medium.

Simmons Citrate agar

Ingredients per liter		
Magnesium sulfate	0.2	g
Ammonium dihydrogen phosphate	1	g
Dipotassium phosphate	1	g
Sodium citrate	2	g
Bacto-Agar	15	g
Bacto-Brom thymol blue	0.068	g

pH 6.8

Sterilize in the autoclave for 15 minutes at 15 pounds.

Triple Sugar Iron agar

Ingredients per liter	
Bacto-Beef extract	3 g
Bacto-Yeast extract	3 g
Bacto-peptone	15 g
Proteose peptone Difco	5 g
Bacto Dextrose	1 g
Bacto Lactose	10 g
Saccharose Difco	10 g
Ferrous sulfate	0.2 g
Sodium chloride	5 g
Sodium thiosulfate	0.3 g
Bacto Agar	12 g
Bacto-Phenol red	0.024 g

pH 7.4

Sterilize in the autoclave for 15 minutes at 15 pounds.

Urease agar

Ingredients per liter		
Bacto - Peptone	1	g
Bacto - Dextrose	1	g
Sodium Chloride	5	g
Potassium Phosphate, Monobasic	2	g
Urea, Difco	20	g
Bacto Phenol Red	0.012	g

pH 6.8

Suspend 29 g of the medium in 100 ml distilled water and mix thoroughly to dissolve completely and filter sterilize (Urea agar Base Concentrate). Dissolve 1.5 g of Bacto - Agar in 90 ml distilled water and sterilize in the autoclave for 15 minutes at 15 pounds. Cool to 50 - 55°C and add the contents of one tube of the concentrate (10 ml) under aseptic conditions. Mix thoroughly and distribute in sterile tubes. Slant the tubes so as to have a butt of about one inch in depth.