CHAPTER III

RESULTS

A. IN VITRO STUDY

1) The Susceptibility of Gram negative Bacteria to Piperacillin and Other Antimicrobial drugs by Disc Diffusion Method

a) The percentage of antimicrobial susceptibility of isolated gram negative bacteria to piperacillin compared to other antimicrobial drugs was shown in table 2. Piperacillin was highly active against most of *Enterobacteriaceae* (80.93%), excepted *Escherichia coli* (28.57%) and *Enterobacter* (43.47%). For *Pseudomonas aeruginosa*, 52 from 63 strains tested (or 80.95%) were sensitive to piperacillin. For *Acinetobacter spp.*, piperacillin's activity was low only 34.78%.

Comparison of antimicrobial activity of piperacillin with other drugs was shwon in table 2, these drugs included

Ticarcillin : Piperacillin was superior to ticarcillin assayed against all of the species tested.

Gentamicin : Piperacillin was more active than gentamicin against most of tested strains excepted *Klebsiella spp.*, *Salmonella spp.* and *Escherichia coli*. Gentamicin showed higher activity assayed against these strains at 95.6, 100 and 100% of susceptibility, respectively.

Amikacin : Amikacin showed higher activity in *Escherichia coli*, *Enterobacter spp.* and *Acinetobacter spp.* at 100, 82 and 86% susceptibility.

Cefsulodin : Cefsulodin as well as piperacillin, was equally active against most of tested strains but showed little higher activity in *Escherichia coli* and *Salmonella spp.* with no effect against *Indole positive proteus*.

Cefotaxime : Cefotaxime showed higher activity in most of tested strains excepted *Pseudomonas aeruginosa*.

Ceftazidime : Ceftazidime showed higher activity in all of tested strains at high percentage of susceptibility (82-100%).

b) Comparative susceptibility studies of *Pseudomonas aeruginosa* from three hospital centers to piperacillin and other drugs (table 3) showed that the antibiotic resistance among these hospitals showed the same pattern, excepted that of gentamicin. With gentamicin the resistance was 45.55% in Rajvithi and Chulalongkorn hospitals, but only 5% in Ramathibodi hospital. Ceftazidime showed no resistance. Using the chi square method piperacillin resistance of isolated strains from three hospitals showed the same pattern with $\alpha = 0.05$.

c) Susceptibility test of *Pseudomonas aeruginosa* compared to ticarcillin (table 3) was determined by statistic method of linear regression and it showed a cross resistance between piperacillin and ticarcillin with the correlation coefficient (δ) of 0.692 at P value < 0.001.

Table 2 Antimicrobial susceptibility of isolated gram negative bacteria to piperacillin and other antimicrobial drugs.

Percentage of Susceptible Organisms	No.	Piperacillin	Ticarcillin	Gentamicin	Amikacin	Cefsulodin	Cefotaxime	Ceftazidime
Acinetobacter spp.	23	34.8	30.4	39.1	82.6	26.1	52.2	91
Citrobacter spp.	13	84.6	69.2	69.2	84.6	76.9	100	100
Enterobacter spp.	23	43.5	39.1	47.8	86.9	39.1	73.9	82.6
Escherichia coli	21	28.5	23.8	100	100	85.7	100	100
Indole positive proteus	16	93.7	87.5	81.2	81.2	0	100	100
Klebsiella spp.	23	73.9	60.8	95.6	100	95.6	100	100
Proteus mirabilis	26	92.3	80.7	76.9	96.2	80.7	100	100
Ps. aeruginosa	63	80.9	68.3	58.7	93.6	88.6	79.3	100
Ps. pseudomallii	13	100	100	0	100	0	100	100
Salmonella spp.	20	80	65	100	100	100	100	100
Serratia spp.	10	100	100	100	100	70	100	100

Table 3 The susceptibility test of *Pseudomonas aeruginosa* from three hospital centers to piperacillin and other antibiotics

Sensitivity disc	Susceptibility to Piperacillin and other antimicrobials among three hospital of						% (S)
	Ramathibodi		Rajvithi		Chulalongkorn		
	R	S	R	S	R	S	
Piperacillin	4	16	5	18	3	17	80.95
Ticarcillin	5	15	6	17	9	11	68.25
Gentamicin	4	16	11	12	11	9	58.73
Amikacin	1	19	1	22	2	18	93.6
Cefsulodin	2	18	4	19	2	18	88.68
Cefotaxime	6	14	4	19	3	17	79.36
Ceftazidime	0	20	0	23	0	20	100
	N = 20		N = 23		N = 20		

R = Resistance

S = Susceptible

N = Total number of test organisms

% (S) = Percent of susceptible organisms

2) Determination of Minimum Inhibitory Concentration (MICs) and Mininum Bactericidal Concentration (MBCs) by Broth Dilution Technique

a) Table 4 and 5 showed the activity of piperacillin against gram negative bacteria in cumulative percentage of MICs and MBCs (µg/ml). At concentration of 8 µg/ml, piperacillin inhibited more than 80% of *Enterobacteriaceae* excepted *Enterobacter spp.* (42%) and *Escherichia coli* (48%). 84% of *Pseudomonas aeruginosa* (77 isolated organisms) had MIC of less than 64 µg/ml and was inhibited by 61% at 8 µg/ml of piperacillin. The MICs of *Acinetobacter* and *Enterobacter spp.* was high (128 µg/ml), with 76 and 55% of inhibition respectively. 58 Isolated *Pseudomonas pseudomallii* was 100% inhibited at 2 µg/ml of piperacillin

Results of cumulative percentage at MBCs were higher than MICs in most of *Enterobacteriaceae.*(Table 5). For example, 75% of *Pseudomonas aeruginosa* was inhibited by MIC of 16 µg/ml while its MBC was 64 µg/ml.

b) Results in 2.a showed only the differences in MICs and MBCs of some strains. Comparisons between the values of MIC and MBC in detail were shown in Figures 2-12.

These relative values (MICs and MBCs) were analyzed by statistic method of variance ratio.

All tested strains showed no differences in variance ratio with significant value of 0.05 ($\alpha = 0.05$) [all the VR_{cal} were less than VR_{table}]. This meant that the MICs of all tested strains were either equal or less than the MBC values.

c) Activity of piperacillin against gram negative bacteria in $MIC_{50,90}$ and $MBC_{50,90}$ values was shown in table 6. The MIC_{90} and MBC_{90} of *Acinetobacter spp.*, *Citrobacter spp.*, *Escherichia coli* and *Enterobacter spp.* appeared to be more than 256 µg/ml. *Pseudomonas aeruginosa* had MIC_{90} of 112.52 µg/ml, while MBC_{90} proceeded to more than 256 µg/ml.

d) Figure 13 and 14 showed the comparative activity of piperacillin in MIC and MBC (µg/ml) against *Pseudomonas aeruginosa* from three hospital centers. Data were analyzed by statistic test of analysis of variance (ANOVA) with CRBD methods. No differences in the variance ratio (V.R.) at $\alpha = 0.05$ were found among these hospital centers.

e) Activity of piperacillin in MICs and MBCs to *Pseudomonas aeruginosa* and *Pseudomonas pseudomallii* with larger inculum of 10^6 CFU/ml and 10^7 CFU/ml was shown in table 7. It revealed that both organisms had the inoculum effects. MIC_{50} of *Pseudomonas aeruginosa* (at 10^7 CFU/ml) was fourteen fold higher than MIC_{50} (at 10^5 CFU/ml) and MIC_{90} raised from 82.28 µg/ml (at 10^5 CFU/ml) to > 256 µg/ml (at 10^7 CFU/ml).

Pseudomonas pseudomallii had the same inoculum effect, its MICs and MBCs were more than 256 µg/ml with the large inoculum of 10^7 CFU/ml.

Table 4 Cumulative percentage of MIC (µg/ml) of gram (-) bacteria to piperacillin

Organism	No of Test	Cumulative percentage of Isolated strains Inhibited at concentrations (µg/ml) of												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Acinetobacter spp.	41			2		5	7	20	39	56	61	76	78	100
Citrobacter spp.	12			8	17	50	67			75		83		100
E. coli	52		2	8	15	37	42	48	50	54	62	67	79	100
Enterobacter spp.	53		2	6	13	30	38	42	47	49	51	55	60	100
Klebsiella spp.	47		2		4	26	57	68	70		77	79	85	100
Indole positive Proteus	16	13	63				75	81	94			100		
Proteus mirabilis	26	15	38	54	69	81	85	92			100			
Ps. aeruginosa	92				1	10	41	66	74	79	84	92	100	
Ps. psedomallii	58			38	98	100								
Salmonella spp.	53				32	64	74	81	91	96	100			
Serratia spp.	10	10	20	60	80	90	100							

Table 5 Cumulative percentage of MBC (µg/ml) of gram (-) bacteria to piperacillin

Organism	No of Test	Cumulative percentage of Isolated strains Bactericided at concentrations (µg/ml) of												
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Acinetobacter spp.	41				2	5		10	34	49	59	63	66	100
Citrobacter spp.	12			8	17	42	50	58	67					100
E. coli	52		2	6	13	37	42	48	50	54	58	62	71	100
Enterobacter spp.	53			4	11	26	36	38	45	47	49		55	100
Klebsiella spp.	47		2		6	23	57	68	70		74	79	85	100
Indole positive Proteus	16	6	44	50	63		75	81	94			100		
Proteus mirabilis	26	12	35	50	69	77	81	92			100			
Ps. aeruginosa	92				1	2	25	46	54	62	74	82	85	100
Ps. pseudomallii	58			7	91	100								
Salmonella spp.	53				25	62	77	81	92	96	100			
Serratia spp.	10		20	60	80	90	100							

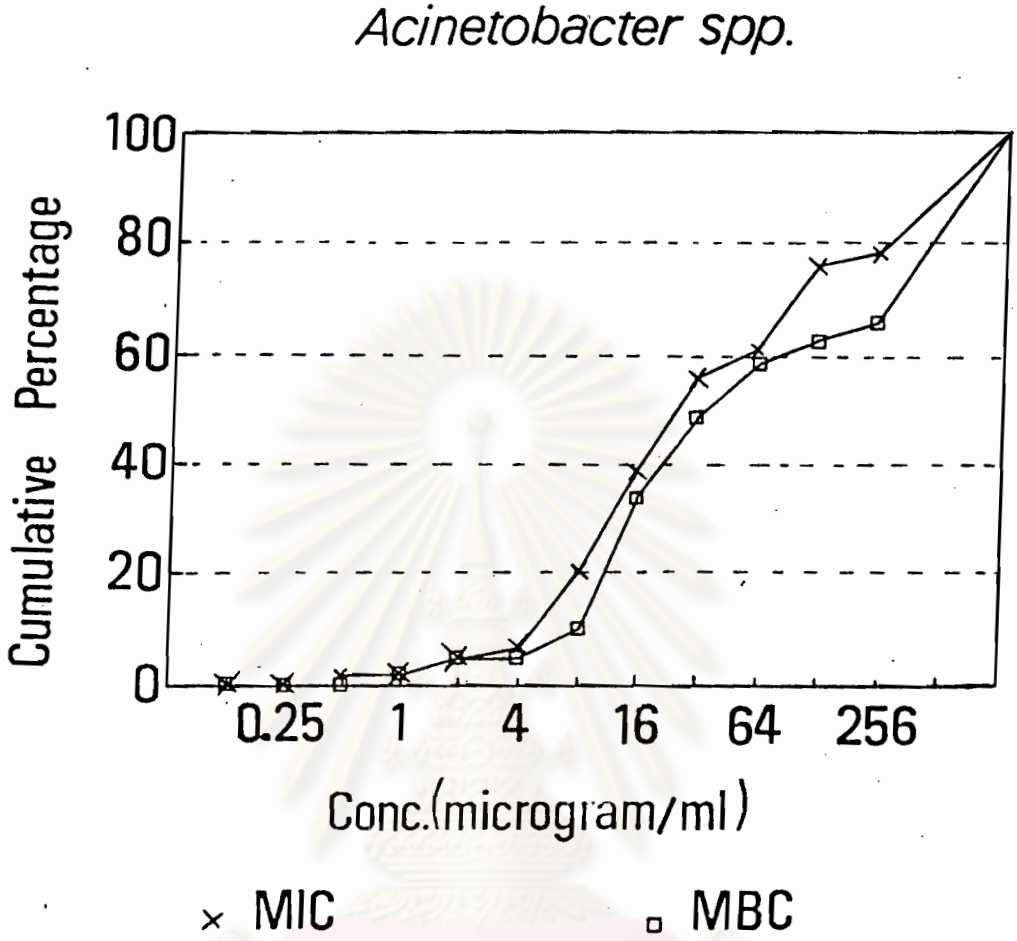

Figure 2 Relative values of MIC and MBC (µg/ml) of *Acinetobacter spp.*

$N = 41$

$S^2_{MIC} = 10183$

$S_{MIC} = 100.91$

$S^2_{MBC} = 12522.72$

$S_{MBC} = 111.904$

$VR_{cal} = 1.23$

$VR_{table} = 1.69$

S^2 = variance

S = Standard deviation

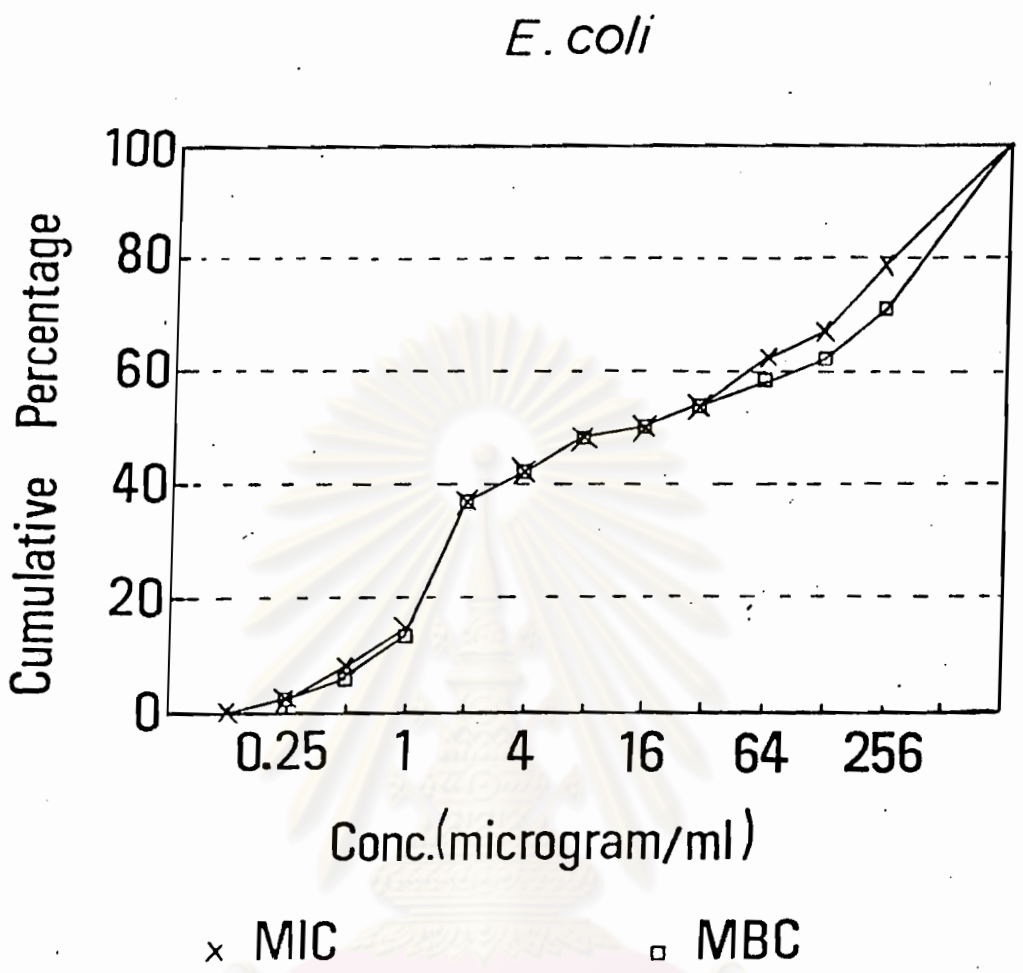

Figure 3 Relative values of MIC and MBC (µg/ml) of *E. coli*

N = 52

S^2_{MIC} = 13568

S = 116.49

S^2_{MBC} = 14899.5

S_{MBC} = 122.6

VR_{cal} = 0.910

VR_{table} = 1.69

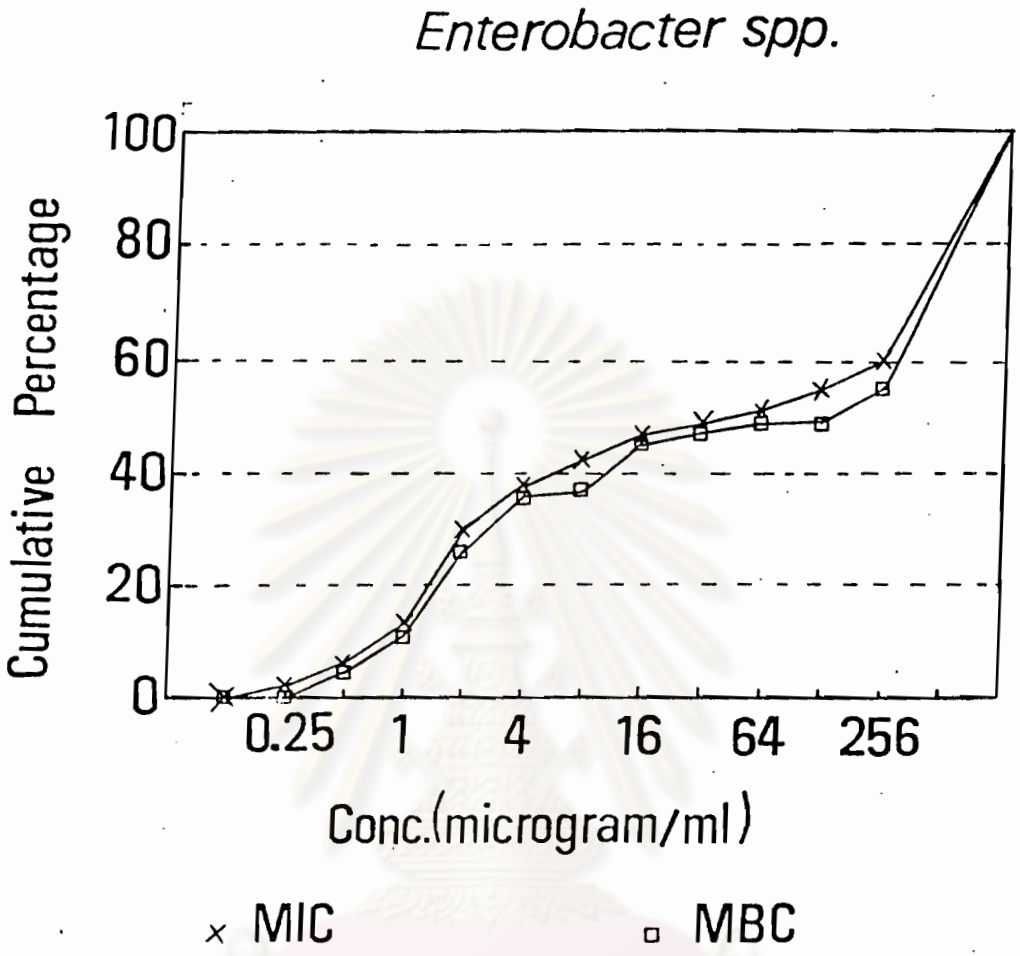

Figure 4 Relative values of MIC and MBC (µg/ml) of *Enterobacter spp.*

$N = 53$

$S^2_{MIC} = 10770.98$

$S_{MIC} = 103.78$

$S^2_{MBC} = 11998.87$

$S_{MBC} = 109.53$

$VR_{cal} = 0.94$

$VR_{table} = 1.69$

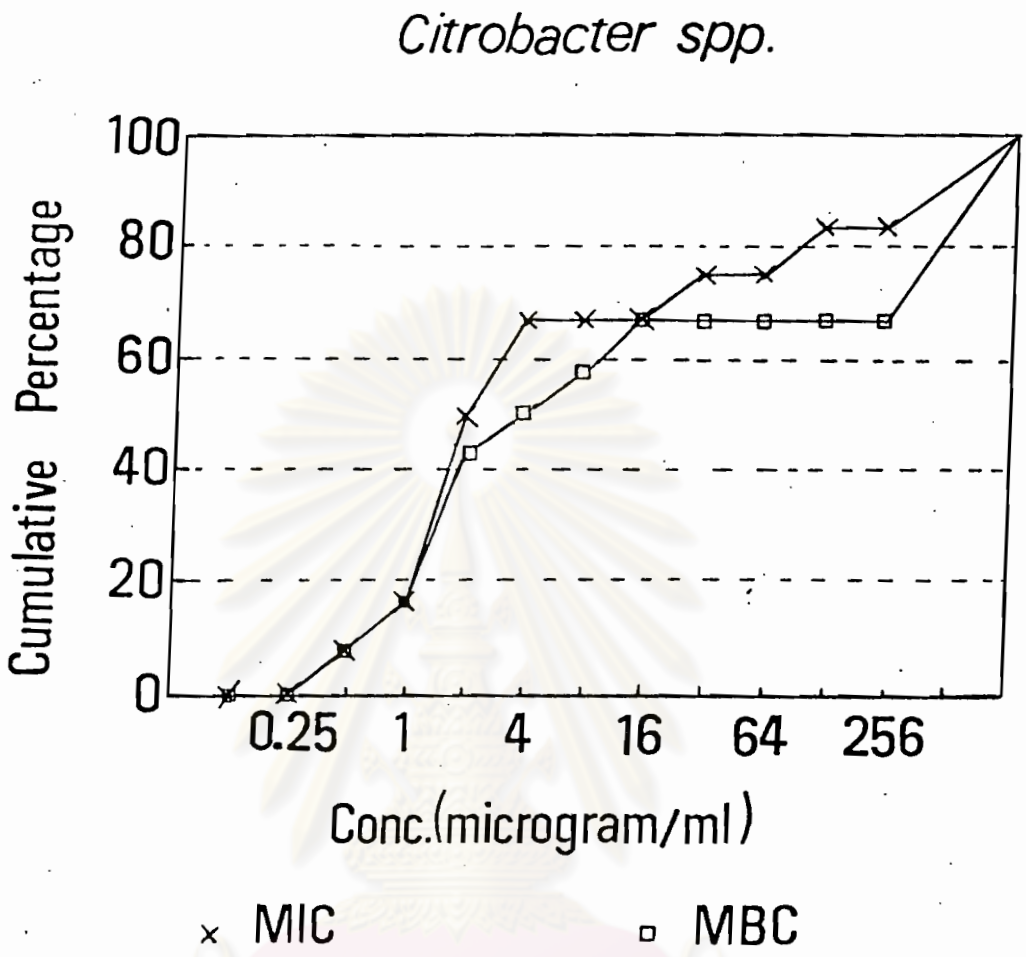

Figure 5 Relative values of MIC and MBC (µg/ml) of *Citrobacter spp.*

N = 12

S^2_{MIC} = 3246.06

S_{MIC} = 56.97

S^2_{MBC} = 15481

S_{MBC} = 124.42

VR_{cal} = 0.457

VR_{table} = 2.79

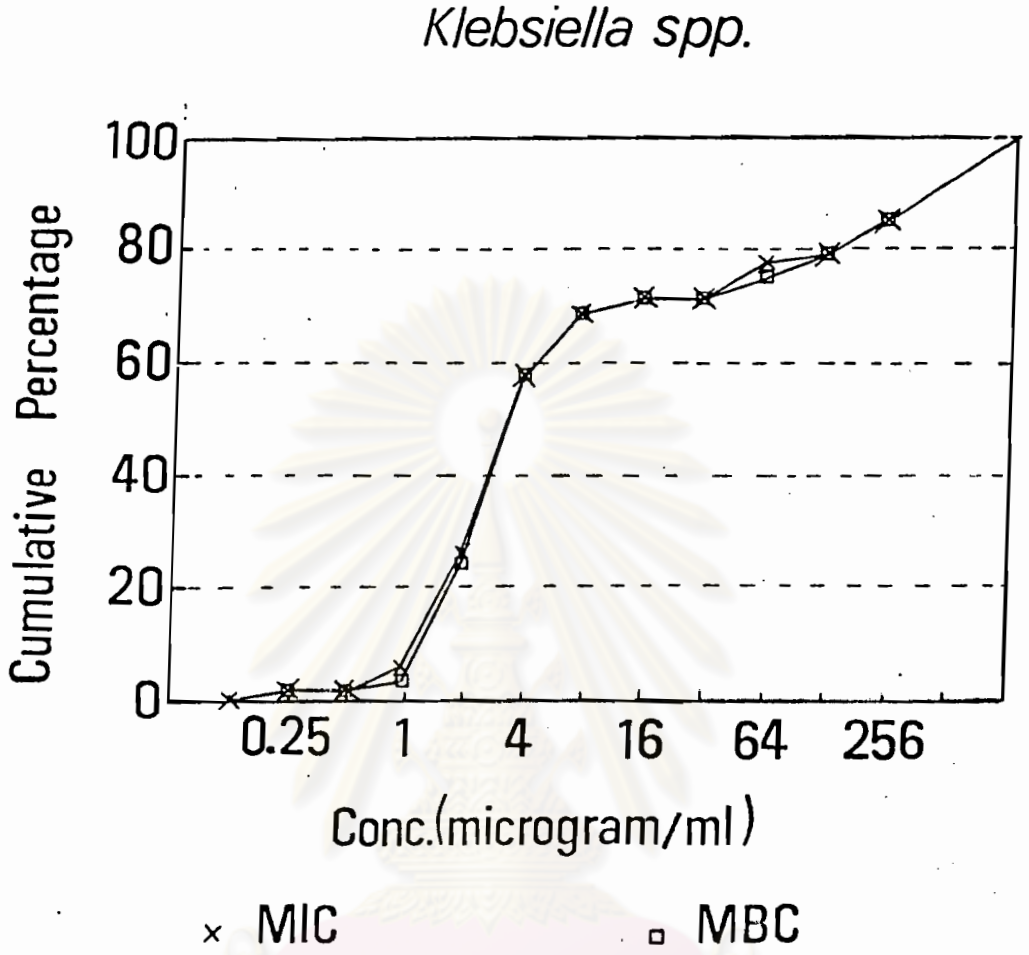

Figure 6 Relative values of MIC and MBC (µg/ml) of *Klebsiella spp.*

$N = 47$

$S^2_{MIC} = 10736.55$

$S_{MIC} = 103.61$

$S^2_{MBC} = 10835.43$

$S_{MBC} = 104.09$

$VR_{cal} = 0.99$

$VR_{table} = 1.69$

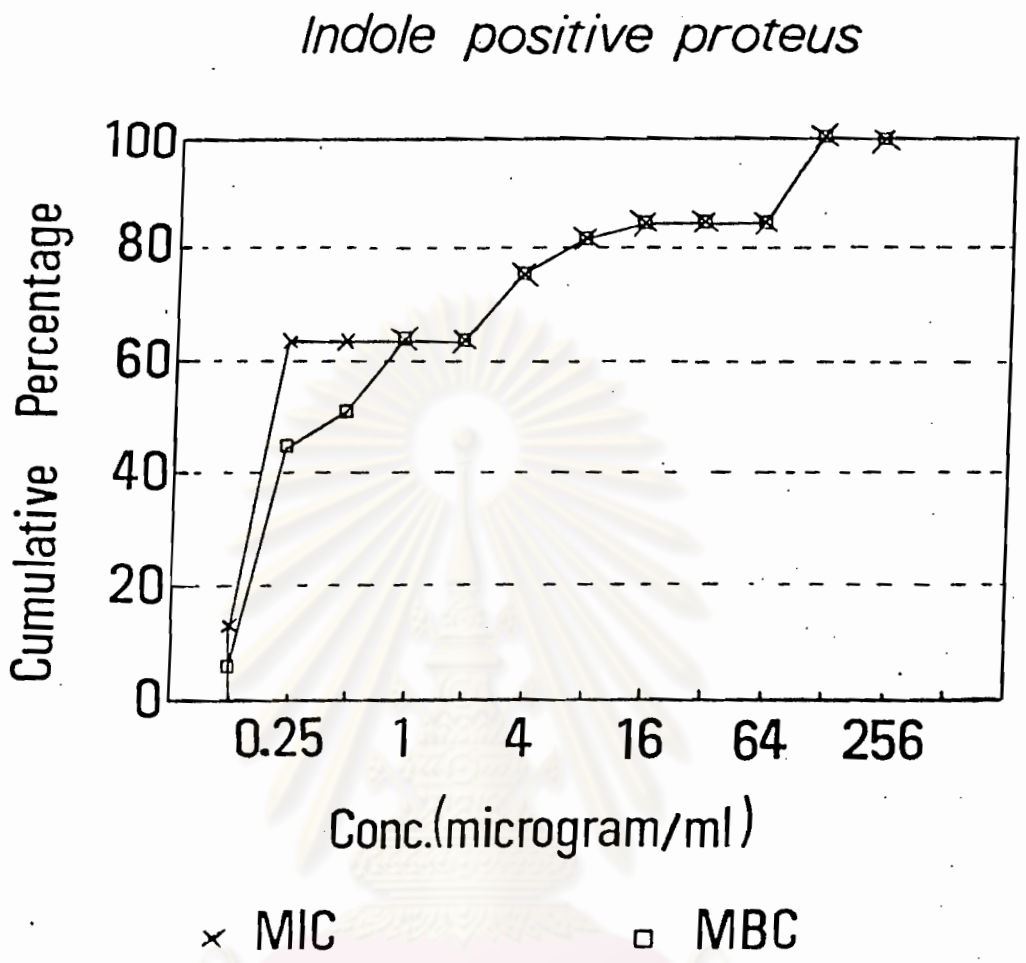

Figure 7 Relative values of MIC and MBC (µg/ml) of *Indole positive proteus*

N = 16

S^2_{MIC} = 1000.44

S_{MIC} = 31.62

S^2_{MBC} = 997.78

S_{MBC} = 31.58

VR_{cal} = 1.002

VR_{table} = 2.40

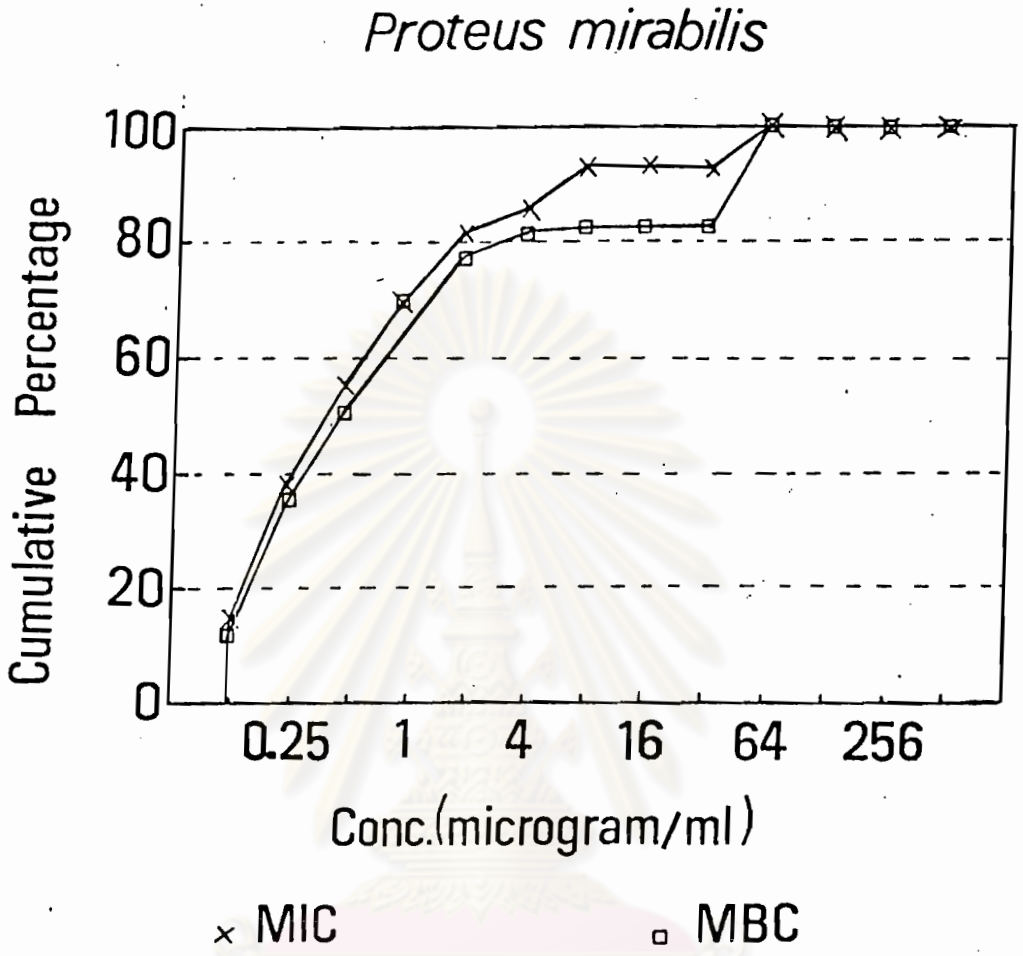

Figure 8 Relative values of MIC and MBC (µg/ml) of *Proteus mirabilis*

$N = 26$

$S^2_{MIC} = 293.76$

$S_{MIC} = 17.139$

$S^2_{MBC} = 292.70$

$S_{MBC} = 17.108$

$VR_{cal} = 1.002$

$VR_{table} = 1.96$

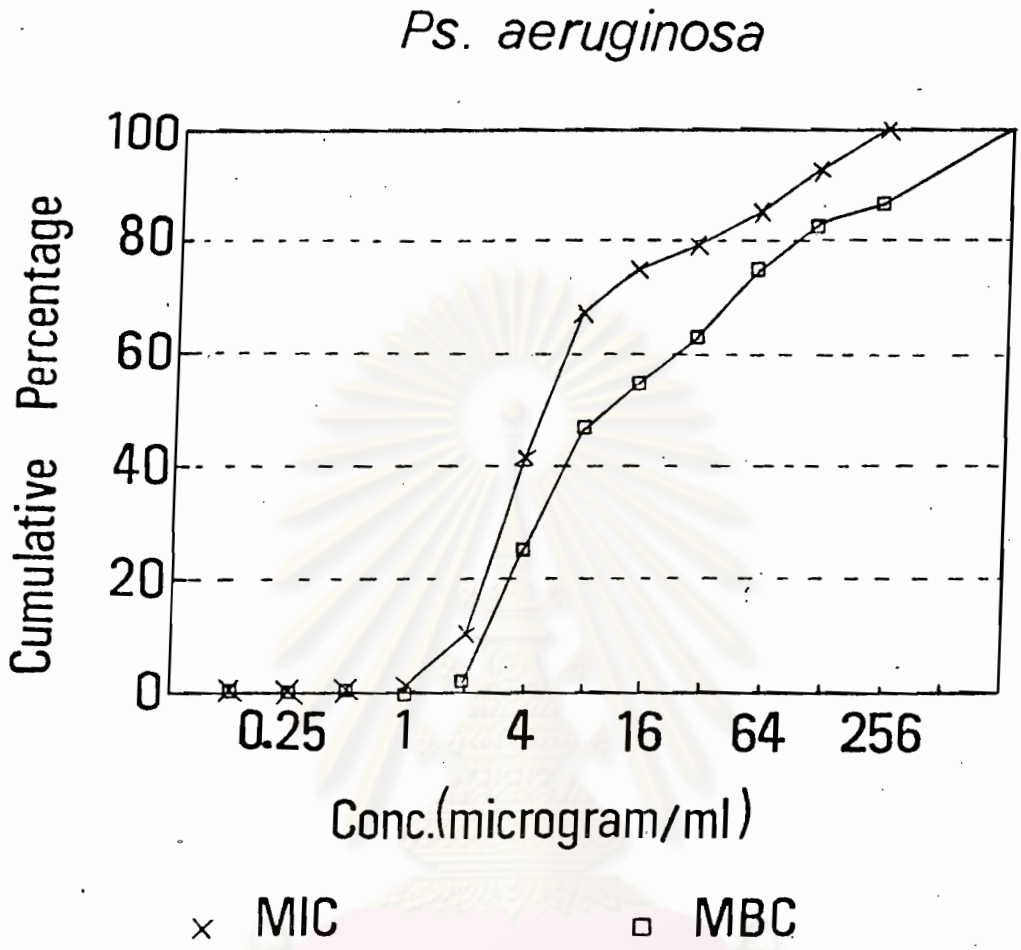

Figure 9 Relative values of MIC and MBC (µg/ml) of *Ps. aeruginosa*

N = 92

S^2_{MIC} = 5159.16

S_{MIC} = 71.82

S^2_{MBC} = 9061.81

S_{MBC} = 95.19

VR_{cal} = 0.754

VR_{table} = 1.35

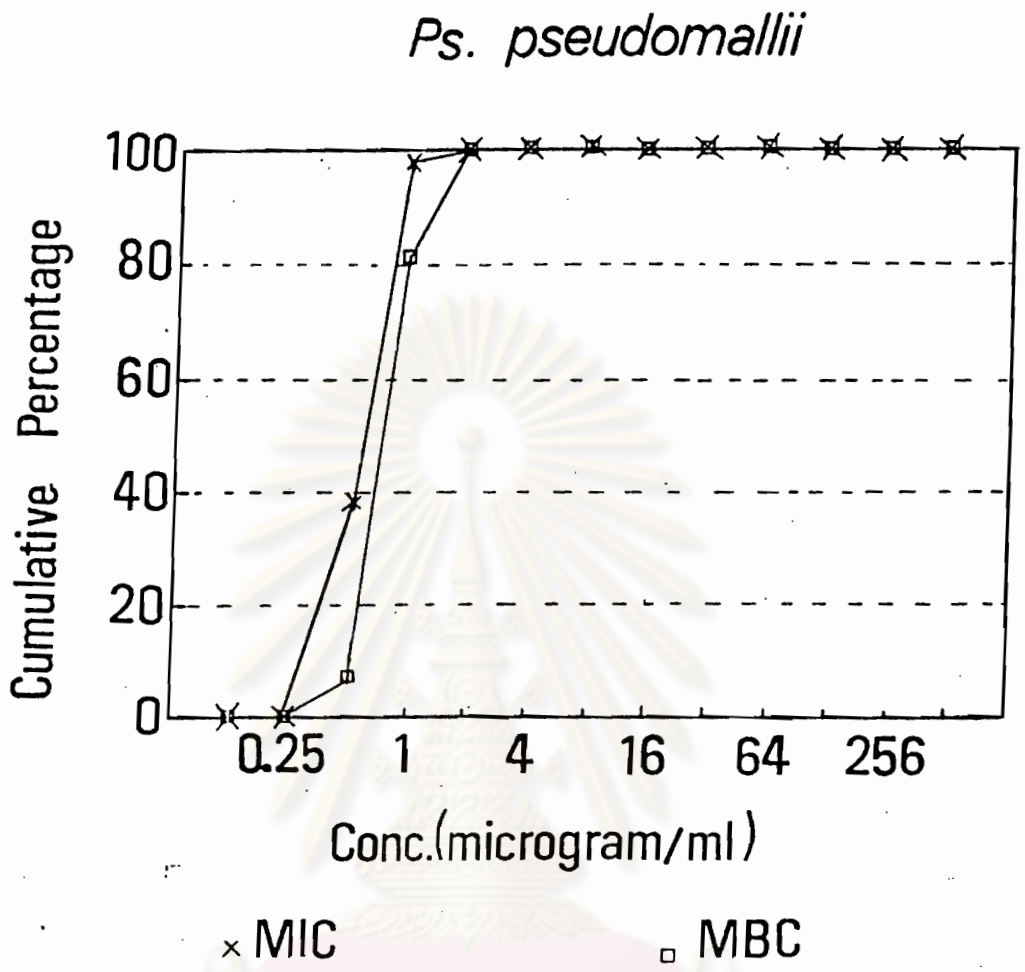

Figure 10 Relative values of MIC and MBC (µg/ml) of *Ps. pseudomallii*

$N = 58$

$S^2_{MIC} = 0.083$

$S_{MIC} = 0.289$

$S^2_{MBC} = 0.629$

$S_{MBC} = 0.793$

$VR_{cal} = 0.13$

$VR_{table} = 1.53$

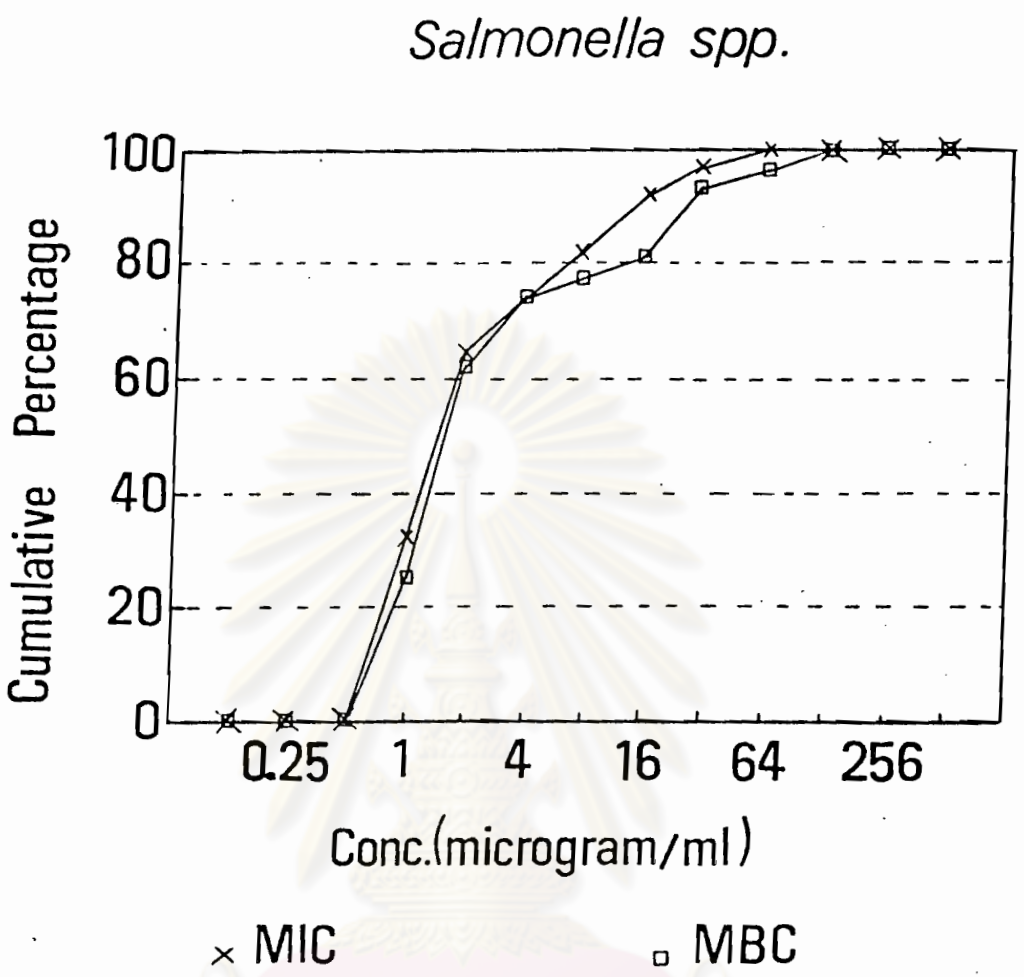

Figure 11 Relative values of MIC and MBC (µg/ml) of *Salmonella spp.*

$N = 53$

$S^2_{MIC} = 189.22$

$S_{MIC} = 13.75$

$S^2_{MBC} = 743.48$

$S_{MBC} = 27.26$

$VR_{cal} = 0.50$

$VR_{table} = 1.53$

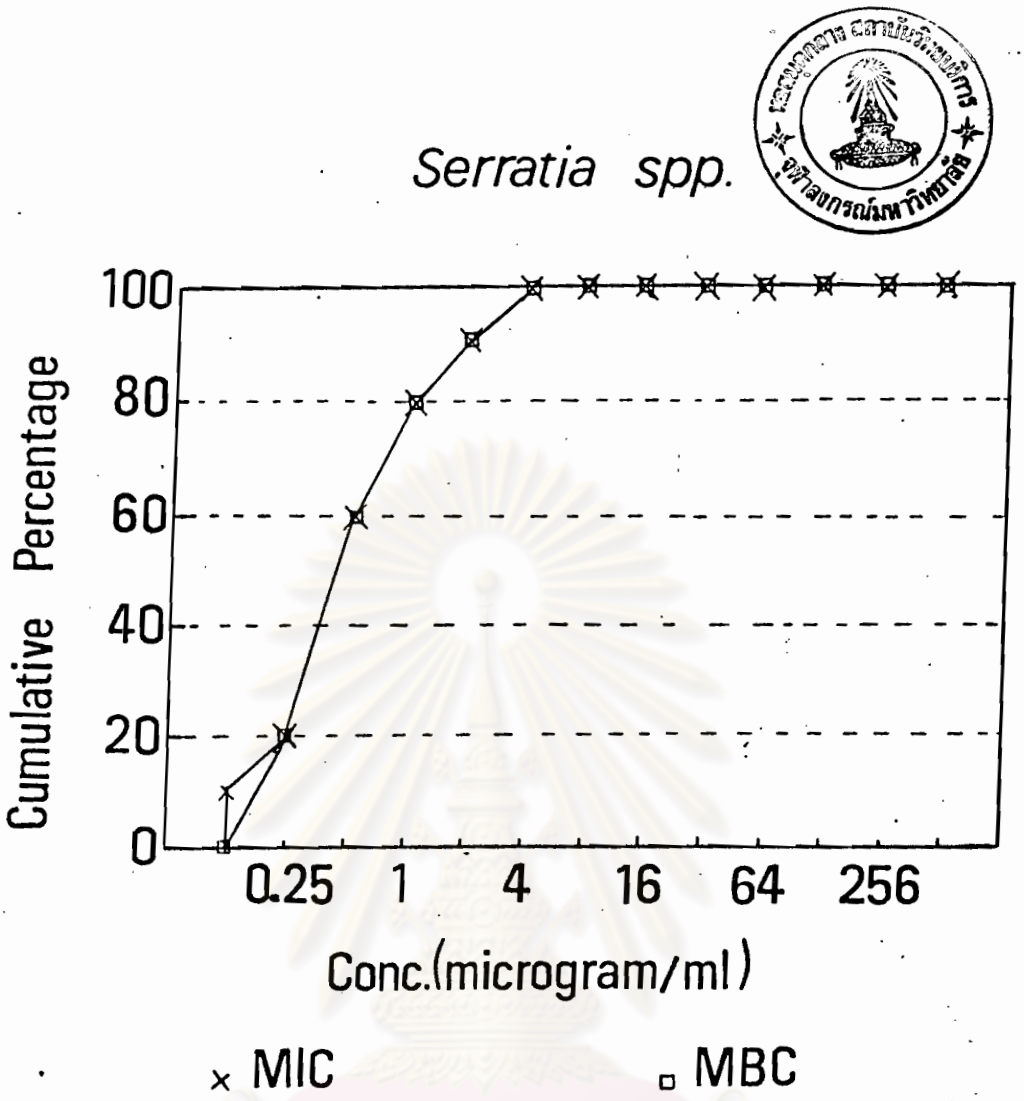

Figure 12 Relative values of MIC and MBC (µg/ml) of *Serratia spp.*

N = 10

S^2_{MIC} = 1.367

S_{MIC} = 1.169

S^2_{MBC} = 1.344

S_{MBC} = 1.159

VR_{cal} = 1.02

VR_{table} = 2.98

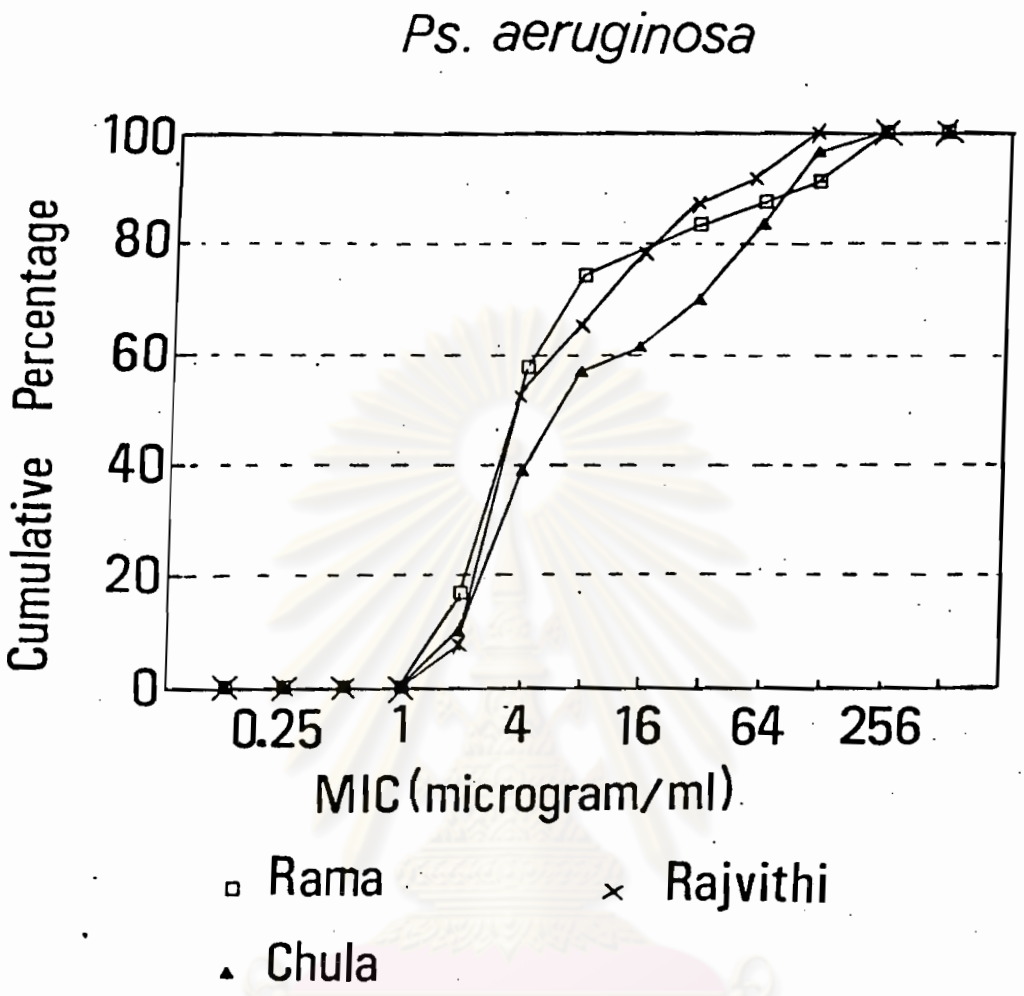

Figure 13 Relative values of MICs (µg/ml) of *Ps. aeruginosa* from three hospital centers

VR_{cal} = 0.013

VR_{table} = 3.74

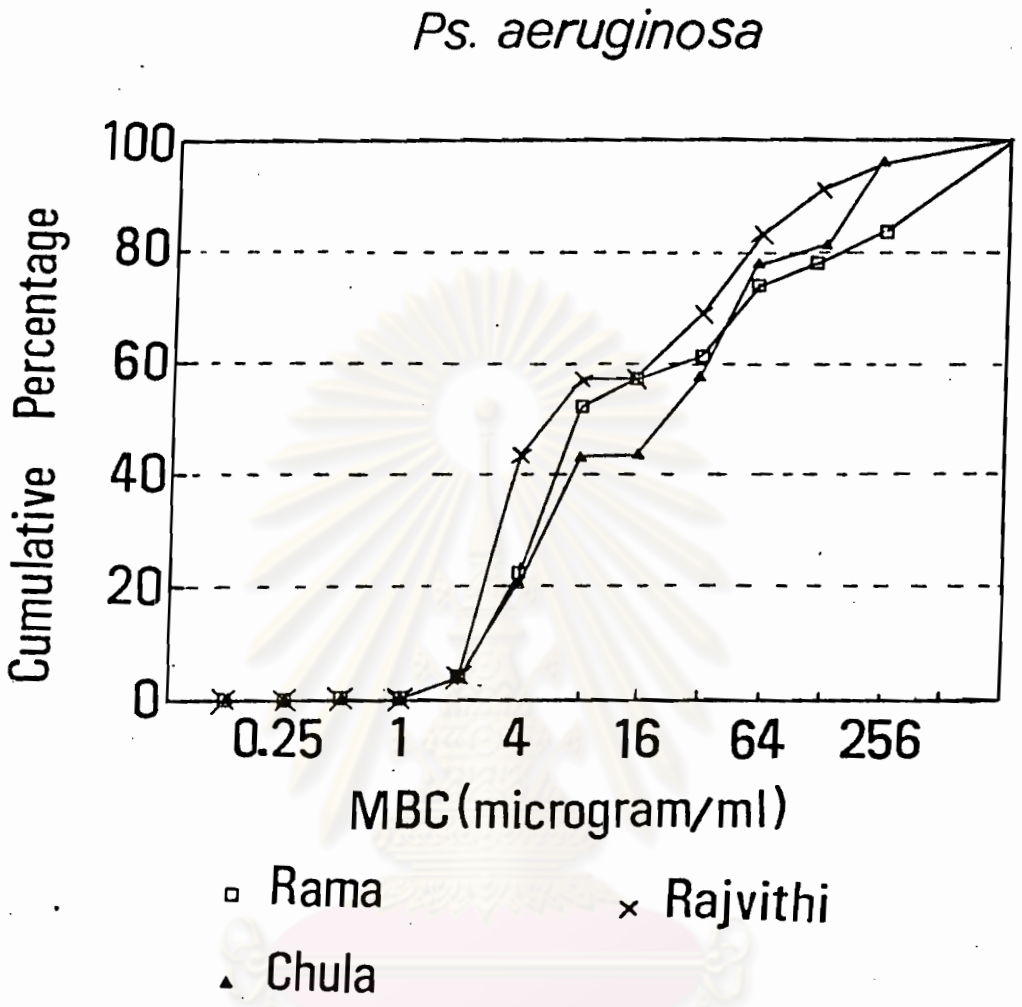

Figure 14 Relative values of MBCs (µg/ml) of *Ps. aeruginosa* from three hospital centers

VR_{cal} = -3.01

VR_{table} = -3.74

Table 6 Activity of piperacillin against gram negative organisms in the values of $MIC_{50,90}$ and $MBC_{50,90}$

Organisms	Activity of piperacillin			
	MIC_{50}	MIC_{90}	MBC_{50}	MBC_{90}
Acinetobacter spp.	27.00	> 256	35.76	> 256
Citrobacter spp.	2.00	> 256	4.00	> 256
E. coli	16.00	> 256	16.00	> 256
Enterobacter spp.	49.6	> 256	152.06	> 256
Klebsiella spp.	3.67	> 256	3.71	> 256
Indole positive proteus	0.23	13.63	0.50	13.63
Proteus mirabilis	0.45	5.11	0.50	7.05
Ps. aeruginosa	5.67	112.52	14.85	> 256
Ps. pseudomallii	0.64	0.95	0.88	0.98
Salmonella spp.	1.64	15.26	1.77	25.07
Serratia spp.	0.45	2.00	0.45	0.98

Table 7 The Inoculum effect of two different organisms on MICs (µg/ml) and MBCs (µg/ml) of piperacillin

Organisms (No. of strains)	MICs(µg/ml) and MBCs(µg/ml) with inocula CFU/ml of														
	10^5 CFU/ml					10^6 CFU/ml					10^7 CFU/ml				
	MIC_{50}	MBC_{50}	MIC_{90}	MBC_{90}	Mean (MIC) (MBC)	MIC_{50}	MBC_{50}	MIC_{90}	MBC_{90}	Mean (MIC) (MBC)	MIC_{50}	MBC_{50}	MIC_{90}	MBC_{90}	Mean (MIC) (MBC)
Ps. aeruginosa (39)	13.13	193.87	82.28	> 256	(34.30) (179.33)	31.56	> 256	98.23	> 256	(58.35) (> 256)	186.29	> 256	> 256	> 256	(186.69) (> 256)
Ps. pseudomallii (58)	0.627	0.872	0.956	0.995	(0.81) (1.04)	0.924	1.647	1.71	2.738	(1.31) (2.12)	> 256	> 256	> 256	> 256	(> 256) (> 256)

B. IN VIVO STUDY

1. Pharmacokinetics of Piperacillin after 2 and 4 g Intravenous Bolus Injection in 7 Normal Subjects and Determination of Serum Drug Level in Patients with Doses of 200-300 mg/kg/day

a) In normal subjects

After two doses of piperacillin, serum level declined in bioexponential maner (Figure 15). The mean pharmacokinetic parameters estimated from serum and urine data were given in table 8. The average concentrations immediately at the end of injection were 342.31 ± 37.97 and 599.38 ± 68.08 µg/ml. Mean concentration at 6 h were 0.75 ± 0.52 and 2.19 ± 1.08 g/ml, respectively.

Mean $t_{\frac{1}{2}}/\alpha$, $t_{\frac{1}{2}}/\beta$ for these doses did not show the prolonged $t_{\frac{1}{2}}/\beta$ value when increasing doses from 2 g to 4 g (Table 8).

Mean area under the concentration time curve ($AUC_{0-\alpha}$) were 203.13 ± 6.55 and 456.58 ± 67.47 which reasonably proportinated to the administered dose in particular the higher doses (2 g to 4 g).

The Vd_{area} of piperacillin was not significantly altered when increasing doses. Mean values (litre/1.73 m^2) were 17.64 ± 4.13 and 16.71 ± 4.16 with the Vd at steady state of 10.78 ± 1.30 and 13.21 ± 2.29, respectively.

The renal excretion of piperacillin in 24 h amounted from 80.59 ± 10.43 to 86.87 ± 4.18% of these doses. Renal clearance (Cl_R) of piperacillin was more rapid with the low dose (2 g). Mean clearance rate adjusted to the body surface area (1.73 m^2) were

160.99 ± 54.10 and 150.29 ± 49.80 ml/min/1.73 m^2)(Table 8).

b) In the patients

Serum levels after the intravenous administration of 200 mg/kg/day (mean 45.23 mg/kg/dose) piperacillin in three patients were given in table 9. At 10 min of injection, serum levels were 95.00, 82.75 and 75.50 µg/ml, respectively and 0.90, 0.45 and 0.31 µg/ml at 6 h of injection.

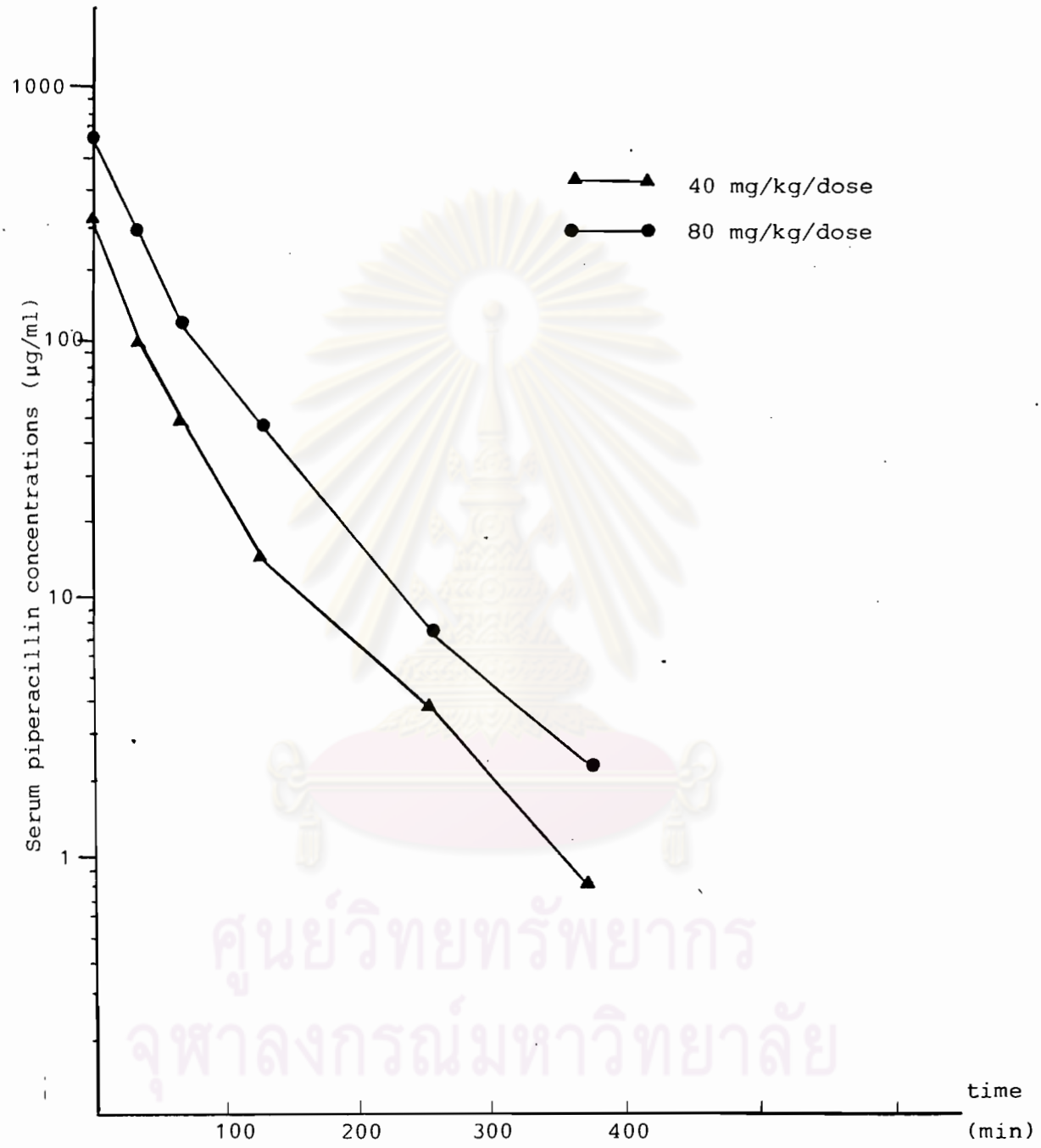

Figure 15 Regression Lines from serum concentration time Profile after two doses of Piperacillin

Table 8 Pharmacokinetic data of piperacillin in normal subject after IV bolus injection

Dose (N=7)	Serum concentration at the end of injection (μg/ml)	at the end of 6 h	A (μg/ml)	B (μg/ml)
2 g (Range 32.78 - 45.45 mg/kg/dose)	342.31 ±37.97	0.75 ±0.52	258.49 ±35.23	86.0 ±15.66
4 g (Range 65.57 - 90.90 mg/kg/dose)	599.38 ±68.08	2.19 ±1.08	362.35 ±72.43	221.14 ±38.02

Dose	α (h^{-1})	β (h^{-1})	$t_{\frac{1}{2}}\alpha$ (h)	$t_{\frac{1}{2}}\beta$ (h)	$AUC_{0-\alpha}$ (μg/ml-h)
2 g	3.25±0.20	0.82±0.46	0.25±0.03	0.84±0.01	203.13±6.55
4 g	3.50±0.45	0.84±0.11	0.23±0.03	0.83±0.05	456.58±67.47

	Volume of distribution (litre/1.73 m^2)			
Dose	V_1 (central compartment)	V_2 (peripheral compartment)	Vd (Steady state)	Vd (area)
2 g	6.97±0.30	3.80±0.57	10.78±1.30	17.64±4.13
4 g	8.97±0.11	4.55±1.16	13.21±2.29	16.71±4.16

Table 8 (Continued)

	Intercompartmental rate constant		
	k_{12} (h^{-1})	k_{21} (h^{-1})	k_{el} (h^{-1})
2 g	0.76±0.42	1.41±0.06	1.90±0.24
4 g	0.93±0.26	1.80±0.17	1.60±0.16

Dose (N=7)	Cl_{Tot} (ml/min/1.73 m^2)	Cl_R (ml/min/1.73 m^2)	Cl_{NR} (ml/min/1.73 m^2)
2 g	201.91 57.98	160.99 54.10	40.90 55.20
4 g	193.14 63.90	150.29 49.80	28.56 62.10

	% dose recovered in urine (unchange form) at
Dose	0 - 24 h
2 g	80.59±10.43
4 g	86.87± 4.18

Table 9 The plasma values from patients after IV bolus piperacillin administration (200 mg/kg/day)

Dose (mg/kg/day) (Mean 45.23 mg/kg/dose)	serum level at 10 min	serum level at 6 h	blood level at		
			$\frac{1}{2}$ h - 1 h		2 - 4 h
35.71 (1 g $\bar{q}$ 6 h)	95.00	0.90	45.5 (28 min)		3.45 (2.55 h)
50 (150 mg $\bar{q}$ 6 h)	82.75	0.45	37.8 (45 min)		4.2 (3.3 h)
50 (500 mg $\bar{q}$ 6 h)	75.50	0.31	40.0 (30 min)	4.7 (2 h)	1.2 (4 h)

2. Study for Clinical Efficacy and Bacteriological Response of Piperacillin

a) Table 10 and 11 were the overall collected data of piperacillin

b) Evaluation of the clinical efficacy and bacteriological response of piperacillin were shown in table 12 to 16

A total 15 courses of piperacillin therapy in 14 children were reported age varied from 1 morth - 13 years. All of patients recieved drugs, intravenously. Causative bacteria was known such as *Pseudomonas aeruginosa* and other gram-negative bacteria.

From 14 patients treated with piperacillin, causative bacteria was known in 13 cases, excepted the one that could not find the cause of infection. Sites of infection included pulmonary system (7 cases), urinary system (5 cases), skin and soft tissue (2 cases), blood system (1 case), central nervous system (1 case), mastoid and middle ear (1 case) and gastro-intestinal system (1 case).

Dose of piperacillin varied from 200-300 mg/kg/day, dutration of piperacillin therapy varied from 2 days to 21 days with the average of 11.57 ± 3.90 days. Seven of 14 cases were treated with piperacillin alone, the others recieved concomittant antibiotics.

Table 10 Sex, age, weight, diagnosis, causative organism, site of infection, bacteriological response, clinical response of paedriatic patients treated with piperacillin

Case No.	Code	Sex	Age (yr)	Weight (kg)	Diagnosis	Causative organism	Site of infection	Bacteriological response	clinical response
1	T.V.	M	1	10	- Pneumonia - Ventricular septal defect	*Ps. aeruginosa* (TSC.)	Pulmonary system	Eradication of *Ps. aeruginosa*, Superimposed of *E. clocaea*	Failure
2	O.N.	F	13	23.3	- Chronic myelocytic leukemia with blastic crisis	Unknown	Unpredicted	Indeterminate	Not evaluate
3	S.P.	M	$2\frac{3}{4}$	13	- Acute lymphocytic leukemia - Urinary tract infection	*Ps. aeruginosa* (urine c/s) *E. coli*, *Streptococcus gr. D.*	Urinary tract Gastrointestinal tract	Marked reduction	Cure

Table 10 (continued)

Case No.	Code	Sex	Age (yr)	Weight (kg)	Diagnosis	Causative organism	Site of infection	Bacteriological response	Clinical response
					- Gastrointestinal tract infection	*Non-enterococci* (RSC)			
4	T.C.	M	7	16	- Acute lymphocytic leukemia - Pneumonia	*Ps. aeruginosa*, *Klebsiella spp.*, *Citrobacter spp.* (urine c/s) *Few Neisseria spp.*, *Streptococcus viridan* (TSC)	Urinary tract, Pulmonary-system	Marked reduction	Improvement
5	P.L.	M	3/12	6	- Acute bronchiolitis - Pneumonia	*Ps. aeruginosa* (TSC)	Pulmonary system	Marked reduction	Cure

Table 10 (Continued)

Case No.	Code	Sex	Age (yr)	Weight (kg)	Diagnosis	Causative organism	Site of infection	Bacteriological response	Clinical response
6	P.N.	F	25/365	3.3	- Pneumonia	*Ps. aeruginosa* (TSC)	Pulmonary system	Persistence	Failure
7	V.R.	F	10	20	- Transverse mylitis - Urinary tract infection	*Ps. aeruginosa*, *E. coli* (urine c/s)	Urinary tract	Eradication of *Ps. aeruginosa*, Persistence of *E. coli*	Improvement
8	S.Y.	M	8	16	- Bilateral UPJ obstruction with hydronephrosis	*Ps. aeruginosa*	Urinary tract	Marked reduction	Improvement
9	P.S.	F	3/12	2.8	- Bronchopulmonary dysplasia post measles	*Ps. aeruginosa* *Acinetobacter spp.*	Pulmonary system	Eradication of *Ps. aeruginosa*	Improvement

Table 10 (Continued)

Case No.	Code	Sex	Age (yr)	Weight (kg)	Diagnosis	Causative organism	Site of infection	Bacteriological response	Clinical response
					- Pneumonia	*E. coli* (TSC)		Persistence of *Acinetobacter spp.*, and *E. coli*	
10	C.B.	M	4 $\frac{1}{2}$	7	- Pneumonia - Urinary tract infection	*Ps. aeruginosa*, *E. coli strain* I,II (direct tracheal secretion c/s)	Pulmonary system	Persistence	Improvement
11	P.C.	M	2	10	- Meningoencephalitis	*Ps. pseudomallii*	Central nervous system Skin and soft tissue	Eradication	Cure

Table 10 (Continued)

Case No.	Code	Sex	Age (yr)	Weight (kg)	Diagnosis	Causative organism	Site of infection	Bacteriological response	Clinical response
12	K.R.	F	10	23	- chronic otitis media - Mastoiditis	*Proteus mirabilis* (PUS c/s)	Mastoid and middle ear	Eradication	Improvement
13	P.Y.	M	13	28	- Aplastic anemia - Cellulitis	*Ps. aeruginosa* (PUS c/s)	Blood Skin and soft tissue	Persistence	Failure
14	M.V.	F	13	28	- Post encephalitis - Pneumonia	*Ps. aeruginosa* (TSC)	Pulmonary system	Marked reduction	Improvement

Table 11 Dosing interval, Duration, Previous antibiotic and Concomitant antibiotic in children treated with piperacillin

Case No.	piperacillin dose mg/kg/day	Duration of treatment (days)	Previous antibiotic	Concomitant antibiotic
1	171.42	18,14	Ampicillin Amikacin Cefotaxime Ceftazidime	-
2	257.51	7	Cloxacillin Gentamicin	Amikacin
3	200	10	Cefazolin	Gentamicin
4	300	9	Amoxil PGS. Gentamicin	Bactrim Amikacin
	300	14	Ampicillin Gentamicin Cloxacillin Amikacin	-
6	303.03	10	Cefotaxime Amikacin	Amikacin
7	200	21	PGS. Gentamicin	Cloxacillin

Table 11 (Continued)

Case No.	piperacillin dose mg/kg/day	Duration of treatment (days)	Previous antibiotic	Concomitant antibiotic
8	250	8	Neomycin Erythromycin Gentamicin Netilmicin	-
9	214.2	14	Cloxacillin Amikacin Cefotaxime	-
10	200	2	PGS. Gentamicin	-
11	200	14	Gentamicin	Bactrim
12	391.30	10	PGS. Chloram Gentamicin	-
13	285.7	13	PGS. Gentamicin Ticarcillin Metronidazole	Amikacin
14	260.86 173.91	10 21	Amikacin Cefamicin	- Tobramicin

Bacteriological results : A total of 30 causative organisms were isolated from 18 infection sites out of 14 patients (table 12). *Pseudomonas aeruginosa* strains were found in 11 cases. 32.14% of Bacteria (9 out of 30) came from urinary tract and 36.66% (11 out of 30) came from pulmonary system.

Determination of bacteriological response was available for 25 isolated organism : 5 organisms were eradicated, 8 were persisted and 12 were markedly reduced. Generally, most of the markedly reduced strains were the strains of *Pseudomonas aeruginosa* (5), *Escherichia coli* (1), *Non-enterococci* (1), *Streptococcus gr.* D. (1), *Klebsiella spp.* (1), *Citrobacter spp.* (1), *Neisseria spp.* (1) and *Streptococcus viridan* (1). The bacteriological response to *Pseudomonas aeruginosa* showed that 3 out of 11 strains were persisted (table 13). The overall bacteriological response was shown in table 14.

Table 12 Causative organisms and infection sites in patients treated with piperacillin

Causative organisms	Infection sites							
	UT	RT	skin and soft tissue	blood	CNS	Mastoid & Middle ear	GI	total
Ps. aeruginosa	5	6	2	1			1	15
Ps. psedomallii			1		1			2
Escherichia coli	2	2					1	5
Proteus mirabilis						1		1
Acinetobacter		1						1
Non-enterococci							1	1
Streptococcus gr. D.							1	1
Klebsiella spp.	1							1
Citrobacter spp.	1							1
Neisseria spp.		1						1
Streptococcus viridan		1						1
	9	11	3	1	1	1	4	30

UT = Urinary tract

RT = Respiratory tract

CNS = Central nervous system

GI = Gastrointestinal system

Table 13 In vitro activity of piperacillin against *Pseudomonas aeruginosa* compared to bacteriological response

Patient No.	Invitro results	Bacteriological response			
		Eradication	Marked reduction	Persis-tence	Indeter-mirat
VI	S			√	
V	S		√		
X	R			√	
XIII	-			√	
VIII	S		√		
XIV	S		√		
I	S	√			
III	-		√		
IV	-		√		
VII	S	√			
IX	S	√			

R = resistance S = sensitive

Piperacillin can eradicate or marked reduce other gram negative bacteria with good effect in *Ps. aeruginosa.* (eradication : 3 out of 11 cases, marked reduction : 5 out of 11 cases, persistance : 3 out of 11 cases and indetermination : not found).

Table 14 The overall bacteriological response for 25 causative organisms

Causative organisms	Bacteriological response			
	Eradication	Marked reduction	Persis-tence	Indeter-minate
Ps. aeruginosa	3	5	3	-
Ps. pseudomallii	1			-
E. coli		1	4	-
Proteus mirabilis	1			-
Acinetobacter spp.			1	-
Non enterococci		1		-
Streptococcus gr.		1		-
Klebsiella spp.		1		-
Citrobacter spp.		1		-
Neisseria spp.		1		-
Streptococcus viridan		1		-
total	5	12	8	-

A total of 11 isolated organisms were obtained from 14 patients. Bacteriological response was available : 12 organisms were markedly reduced, 5 out of 25 organisms were eradicated and 8 organisms were persisted.

Table 15 The overall clinical responses

Clinical response	No. of
Cure	3
Improvement	8
Failure	3
Not evaluate	1

Clinical response of 14 out of 15 courses of treatment given, were evaluated. A complete clinical resolution of infection occured in 3 cases (20%). 8 cases (53.33%) resulted in a marked clinical improvement. A favorable clinical response was therefore 11 courses of piperacillin (73.33%), while unfavorable clinical response (failure) occured in 3 cases (20%)(Table 15).

The overall responses for specific infections were evaluated. All sites of infection, both clinical and bacteriological responses were observed in those patients receiving piperacillin alone and other antibiotics given concomitantly. The relationship of clinical and bacteriological responses to site of infection for evaluable cases was shown in table 16.

Table 16 Clinical and bacteriological responses according to infection sites of evaluable cases

	Infection sites						
	UT	RT	skin and soft tissue	blood	CNS	Mastoid & Middle ear	GI
Clinical response							
Cure	1	1	1		1		1
Improve	4	4				1	
Fail		2	1	1			
Not evaluate							
Bacteriological response							
Eradication	1	2	1		1	1	
Marked reduction	3	6					1
Persistence	2	3	1	1			
Indeterminate							

UT = Urinary tract

RT = Respiratory tract

GI = Gastrointestinal system

CNS = Central nervous system

Urinary tract : Five cases of urinary tract infection treated with piperacillin had the satisfied clinical and bacteriological responses. 2 Strains were persisted and 3 strains were markedly reduced.

Respiratory tract : Piperacillin was used to treat respiratory tract infections. Most of case were improved. Failure was found only in two cases, both of which were pneumonia and the patient recieved amikacin, cefotaxime and ceftazidime prior to piperacillin.

Skin and soft tissue infection : Skin and soft tissue infections comprised in 2 cases and 1 case was evaluated. Satisfied clinical response was obtained.

Blood + GI : There was one case of septicemia treated with piperacillin and the result was failed. The patient was compromised host (aplastic anemia) and many drugs had been treated but not effective. The other one of GI tract infection caused by strains of *E. coli* and *Streptococcus gr.*D. was improved and the bacteriological response was markedly reduced.

Mastoid and Middle ear : There was one case of otitis media with mastoiditis due to Proteus mirabilis. After treatment with piperacillin, patient was clinically improved, and the bacteriological response was eradicated.

Central nervous system : There was one case of meningoencephalitis due to *Pseudomonas aeruginosa*. The in vitro result showed that this strain was eradicated by piperacillin. Fever according to this drug was noticed and decreased after piperacillin was discontinued.

3. Study for Adverse Drug Reactions

All patient were tolerated to piperacillin. Adverse reactions occured in some cases.

Fever : Fever occured in 5 cases after treatment with piperacillin. General clinical findings was stable during the high fever.

Allergic reactions : Two patients generated sensitivity reaction to piperacillin during treatment. In one case, patient received the combination of piperacillin and amikacin and the noticeble reaction developed after the discontinuation of these drugs. Therefore, piperacillin might not be the cause of this reaction.

Gastrointestinal reaction : Stomachache occured in one case during drug treatment. This reaction appeared at the same time with high fever in the case of chronic leukemia $\bar{c}$ blastic crisis. Body pain was the chief compliance of this case.

Nephrotoxicity : No nephrotoxicity due to piperacillin was found in all studied cases. Laboratory data for creatinine were in normal range (1-2 mg %), BUN value was also in the range of 8-16 mg %. the microscopy was negative and 0-1 of WBC and RBC casts.

Other adverse reactions : The disturbance of platelets function was not found. For the electrolyte imbalance, k^+ value was noticed in one case who had been using this drug for a long period of time. After the discontinuation of piperacillin, the k^+ was increased from normal range of 3.5-5.3 mEq/L to 7.3-7.7 mEq/L, and Na^+ decreased from normal range of 135-148 mEq/L to 120 mEq/L.