CHAPTER IV

RESULTS AND DISCUSSION

1. Development of the HPLC method

Cefoperazone and sulbactam contents were analyzed using a validated HPLC. The HPLC system consists of a C18 column (Hypersil, 250 x 4 mm, 5 μm) with column temperature of 25°C. Column elutes was monitored for cefoperazone at 230 nm wavelength and for sulbactam at 220 nm wavelength. The isocratic mobile phase were acetonitrile: methanol: 5mM tetrabutylammomiumhydroxide (13:9:78), pH=6.4 for cefoperazone and acetonitrile: 5mM tetrabutylammomiumhydroxide (25:75) pH=6.5 for sulbactam. The plasma samples were prepared by liquid-liquid extraction.

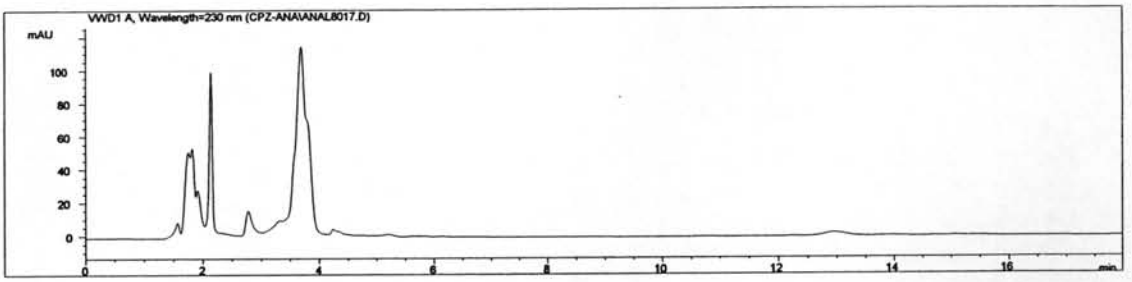
In HPLC system, the separations of the drugs were rapid, needing only 8 min and min for cefoperazone and sulbactam, respectively. The validated method was found to be specific, linear and reproducible. However, these methods are not suitable for the large number of samples because it involves many extraction procedures.

2. Assay validation of cefoperazone and sulbactam in plasma

2.1 Selectivity and specificity

The data from figure 4 shows the characteristic chromatograms of blank plasma, blank plasma spiked with internal standard (enalapril maleate), blank plasma spiked with cefoperazone together with internal standard and blank plasma spiked with cefoperazone and sulbactam together with internal standard, respectively. Peaks of drugs standard were well separated from other interfering peaks from six different blank samples. The mean running times of cefoperazone and enalapril maleate were 8 and 10 min, respectively. There was no effect of sulbactam to those.

Figure 5 represents the chromatograms of blank plasma, the internal standard (rosiglitazone) in blank plasma, and the sulbactam with internal standard in plasma, and the sulbactam and cefoperazone together with internal standard in plasma, respectively. The mean retention times of sulbactam and rosiglitazone were 5.5 and 6.5 min, respectively. There was no interference peaks due to presence of plasma from six different blank plasma samples. There was no interfering from cefoperazone.



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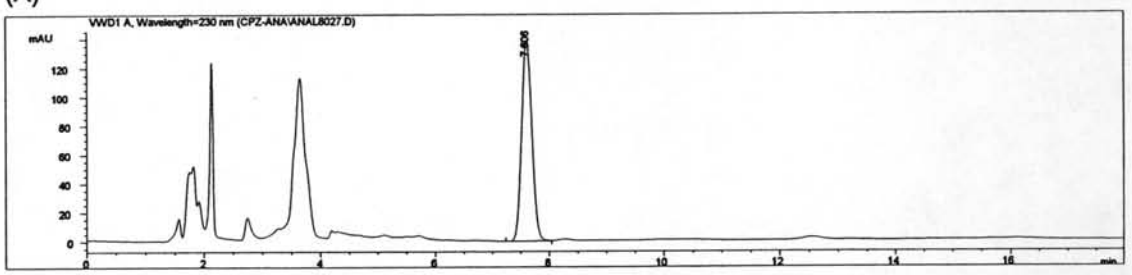

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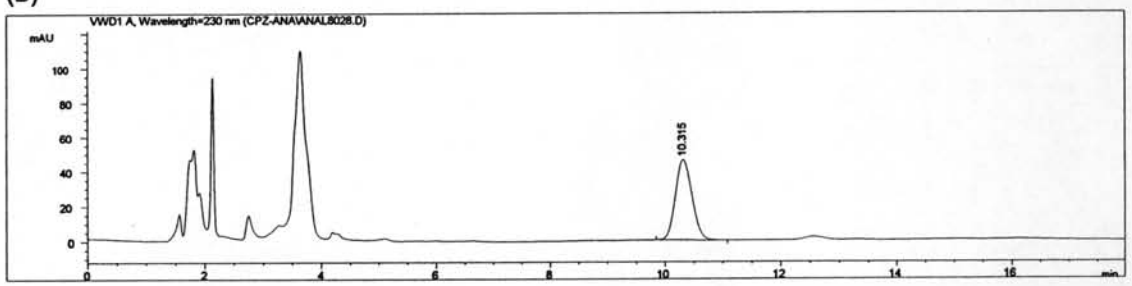

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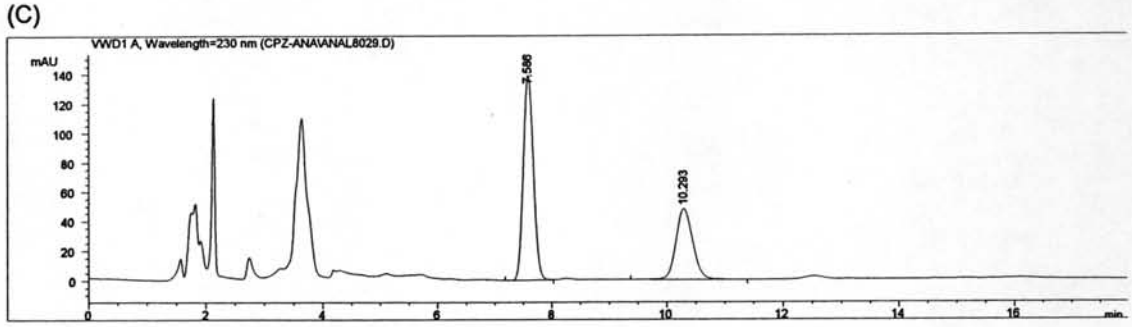
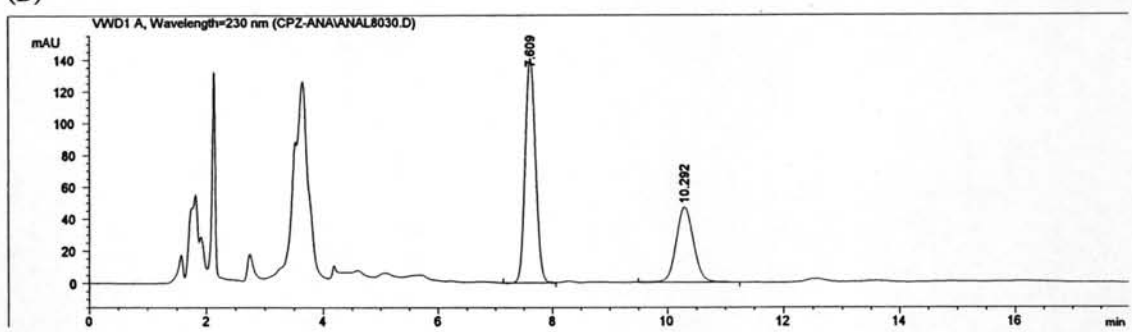


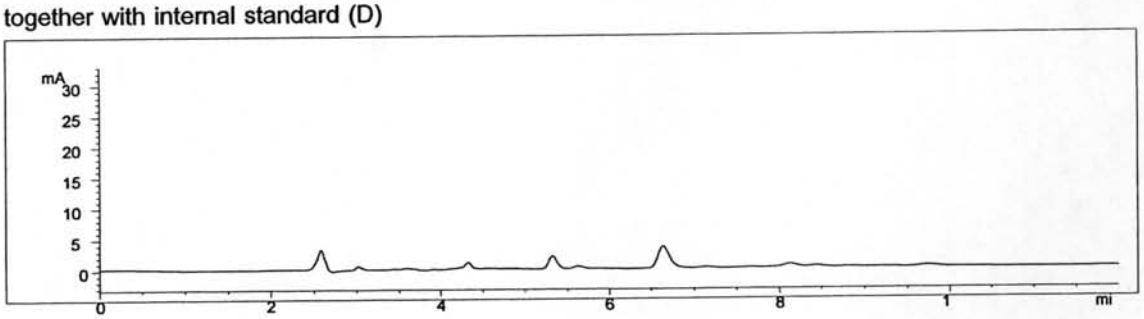
(D)



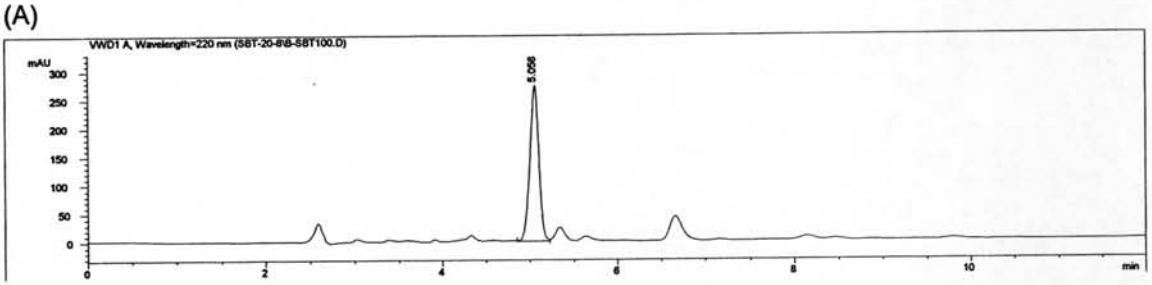
(E)

Figure 4. Chromatogram of blank plasma (A), blank plasma spiked with cefoperazone (B) blank plasma spiked with internal standard (rosiglitazone) (C), blank plasma spiked with cefoperazone

together with the internal standard (C), and blank plasma spiked with cefoperazone and sulbactam together with internal standard (D)



(A)



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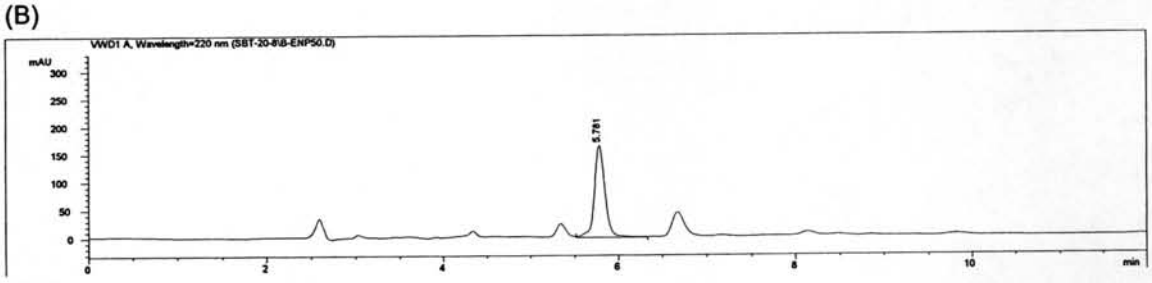

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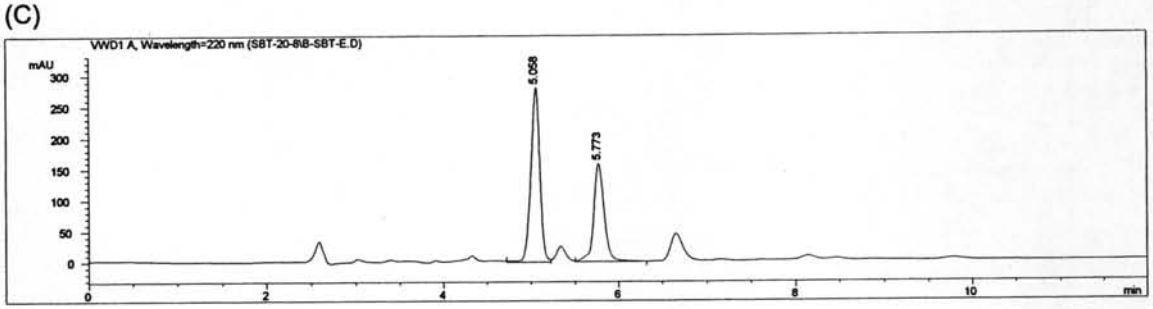

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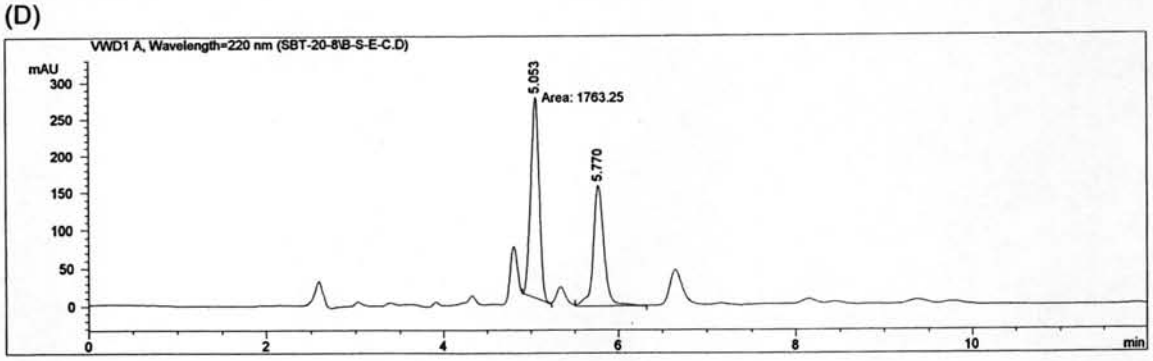


(E)

Figure 5. Chromatogram of blank plasma (A), blank plasma spiked with sulbactam (B) blank plasma spiked with internal standard (Enalapril maleate) (C), blank plasma spiked with sulbactam together with the internal standard (C), and blank plasma spiked with sulbactam and cefoperazone together with internal standard (D)

2.2 The lower limit of quantification (LLOQ)

The lower limit of quantification of the analysis method of cefoperazone was found to be 1.0 μg/ml, and that of sulbactam was 0.5 μg/ml. For cefoperazone, the accuracy at LLOQ was 100.4% with a precision of 8.17%. The accuracy of sulbactam at LLOQ was 106.8% with a precision of 6.3%. Its concentration can be determined with acceptable accuracy (±20 %) and precision (<20%). Thus, these findings were accepted that this level is the lowest on the standard curves. The results are shown in Tables 2 and 3.

Table 2 Lower limit of quantification of analysis method for determination of cefoperazone in plasma

Analysis number	Known concentration (μg/ml)	Estimated Concentration (μg/ml)	% Recovery
1	1.0	1.06	106
2	1.0	0.95	95
3	1.0	1.12	112
4	1.0	0.93	93
5	1.0	0.96	96
Mean		1.004	100.4
S.D.		0.082	8.20
%C.V.		8.17	8.17

Table 3 Lower limit of quantification of analysis method for determination of sulbactam in plasma

Analysis number	Known concentration (μg/ml)	Estimated Concentration (μg/ml)	% Recovery
1	0.5	0.49	98.0
2	0.5	0.52	104.0
3	0.5	0.53	106.0
4	0.5	0.58	116.0
5	0.5	0.55	110.0
Mean		0.534	106.8
S.D.		0.034	6.723
%C.V.		6.29	6.30

Linearity and Standard calibration curve

Concentration ranges for cefoperazone and sulbactam were 1.0–200 μg/ml and 0.5-100 μg/ml, respectively. The calibration curve datas for cefoperazone and sulbactam in plasma are shown in Tables 4 and 5. Standard curves demonstrated linear response over the range of concentrations used in the assay. Linear regressions of peak area ratios versus concentrations give a typical coefficient of determination (r^2) of 0.9999.

Table 4 Linearity of analytical method for determination of cefoperazone in plasma

Analysis number	Known concentration (μg/ml)	Peak area ratio*	Estimated concentration (μg/ml)	S.D.	%C.V.	% Recovery*
1	1.0	0.0087	0.983	0.068	6.92	98.33
2	10.0	0.0867	10.007	0.197	1.97	100.07
3	25.0	0.2145	24.813	0.261	1.05	99.26
4	50.0	0.4339	50.213	0.331	0.66	100.76
5	100.0	0.8640	100.023	0.290	0.29	100.03
6	200.0	1.7271	199.960	0.165	0.08	99.98

* Each data point in mean of triplicate determine

where ; $y = 0.0086X + 0.0003$

$r^2 = 0.9999$, Coefficient of determination

y = Peak area ratio

X = Concentration

Table 5 Linearity of analytical method for determination of sulbactam in plasma

Analysis number	Known concentration (μg/ml)	Peak area ratio*	Estimated concentration (μg/ml)	S.D.	%C.V.	% Recovery*
1	0.5	0.0087	0.530	0.046	8.68	106.00
2	1.0	0.0167	0.990	0.062	6.26	99.00
3	5.0	0.0845	4.857	0.119	2.45	97.20
4	10.0	0.1764	10.117	0.095	0.94	101.20
5	50.0	0.8746	50.033	0.025	0.05	100.06
6	75.0	1.3106	74.963	0.023	0.03	99.95
7	100.0	1.7490	100.013	0.015	0.02	100.01

* Each data point in mean of triplicate determine

where ; $y = 0.0175 X - 0.0006$

$r^2 = 0.9999$, Coefficient of determination

y = Peak area ratio

X = Concentration

2.4 Accuracy

The accuracy of the assay for the cefoperazone and sulbactam were assessed by five replicate analysis of samples containing known amounts of three quality control samples. It showed that percent recovery of cefoperazone was 94.35-99.35%, and that of sulbactam was 99.33 to 100.67%. These results were within acceptance criteria for accuracy (recovery ± 15%). Results are shown in Table 6.

Table 6 Accuracy of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Known concentration (μg/ml)	Estimated concentration ((μg/ml)	S.D.	%C.V.	% Recovery*
Cefoperazone	15.0	14.15	0.35	2.45	94.35
	75.0	73.62	1.70	2.31	98.16
	150.0	149.02	3.10	2.08	99.35
Sulbactam	1.5	1.49	0.01	0.67	99.33
	30.0	30.20	0.19	0.63	100.67
	90.0	89.95	0.69	0.76	99.94

* Results are mean of five replicates.

$$\text{Where; \% Recovery} = \frac{\text{Estimated concentration}}{\text{Known Concentration}} \times 100$$

2.5 Precision

2.5.1 Within-run precision

The within-run precision analysis for cefoperazone and sulbactam were determined by analyzing three quality control samples in five replicates on the same days. Results are shown in Table 7, The % C.V. for within-run precisions of cefoperazone were 1.94 to 2.08, and those of sulbactam were 0.67 to 2.04, respectively These results were within acceptance criteria for precision (% C.V. <15%).

Table 7 Within-run precision of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Known concentration (μg/ml)	Estimated concentration ((μg/ml)	S.D.	%C.V.	% Recovery*
Cefoperazone	1.0	1.03	0.02	1.94	102.76
	15.0	14.15	0.35	2.45	94.35
	75.0	73.92	1.70	2.31	98.88
	150.0	149.02	3.10	2.08	99.35
Sulbactam	0.5	0.49	0.01	2.04	98.00
	1.5	1.49	0.01	0.67	99.33
	30.0	30.20	0.19	0.63	100.67
	90.0	89.95	0.69	0.76	99.94

* Results are mean of five replicates.

$$\text{Where; \% Recovery} = \frac{\text{Estimated concentration}}{\text{Known Concentration}} \times 100$$

2.5.2 Between-run precision

The between-run precision analysis for cefoperazone and sulbactam were determined by analyzing three quality control samples in five replicates on the three different days. Results are shown in Table 8, The % C.V. for between-run precisions of cefoperazone were 1.10 to 8.47%, and those of sulbactam were 0.65 to 6.66%, respectively These results were within acceptance criteria for precision (% C.V. <15%).

Table 8 Between-run precision of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Known concentration (μg/ml)	Estimated concentration ((μg/ml)	S.D.	%C.V.	% Recovery*
Cefoperazone	1.0	1.03	0.06	5.68	103.0
	15.0	14.64	1.24	8.47	97.60
	75.0	75.31	3.09	4.11	100.41
	150.0	151.04	1.65	1.10	100.69
Sulbactam	0.5	0.51	0.02	4.00	102.00
	1.5	1.52	0.01	0.65	101.34
	30.0	30.46	0.31	1.01	101.53
	90.0	87.01	5.79	6.66	96.67

* Results are mean of triplicates.

$$\text{Where; \% Recovery} = \frac{\text{Estimated concentration}}{\text{Known Concentration}} \times 100$$

Extraction recovery

The recovery of extraction for cefoperazone ranged between 102.56 to 108.59% with %C.V. between 1.77 to 2.15% and that of sulbactam ranged between 98.41 to 101.89% with a %C.V. between 1.78 to 6.58%. For internal standards, the recovery of extraction for enalapril maleate and rosiglitazone were 97.94 and 92.71 with %C.V. 3.91% and 0.79%, respectively. According to the Guidance for Bioanalytical Validation (CDER, 2001), recovery of extraction need not be 100%, but the extent of recovery of analyte and internal standard should be consistent, precise, and reproducible. Therefore, these results were acceptable for the purpose of study. The results are presented in Table 9

Table 9 Recovery of extraction of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Known concentration (μg/ml)	Peak area ratio		% Recovery of Extraction	% C.V.
		Extracted	Unextracted		
Cefoperazone	15.0	117.42	109.54	107.19	1.77
	75.0	592.80	578.03	102.56	1.97
	150.0	1268.82	1168.54	108.58	2.15
Rosiglitazone	250.0	985.65	1063.17	92.71	0.79
Sulbactam	1.5	36.58	37.17	98.41	6.58
	30.0	737.19	723.55	101.89	1.78
	90.0	2111.05	2095.16	100.76	2.37
Enalapril maleate	50.0	1102.72	1125.88	97.94	3.91

* Results are mean of five replicates

2.7 Stability studies

Determination of the stability of cefoperazone and sulbactam in plasma were carried out; a short-term room temperature, a long-term, a freeze-thaw and processed samples stability studies.

As presents in Table 10, a short-term room temperature stability of cefoperazone and sulbactam in plasma showed that both of them were not tented to degrade after they were thawed at room temperature and kept at this temperature for 5 hours. The percent deviation of cefoperazone from the zero time was -0.92 to 1.36% and that of sulbactam was -0.23 to -1.39% after keeping at room temperature from 5 hours. These results were with in acceptance criteria for stability (The % deviation of the mean estimated concentration from the zero times should be within ±10%). This indicated that samples could be stable for at least 5 hours at room temperature after thawing. However, the samples should be rapidly extracted and analyzed after thawing at room temperature.

As displayed in Table 11, a long-term stability of cefoperazone and sulbactam in plasma showed that both of cefoperazone and sulbactam were stable for 5 weeks at –40 °C. The percent deviation of cefoperazone from the zero time to 5 weeks was -0.17 to -1.77% and that of sulbactam was -0.63 to -1.39% for 5 weeks. These results were with in acceptance criteria for stability (The % deviation of the mean estimated concentration from the zero times should be within ±15%). Thus, these storage times were sufficient for completing of drug analysis.

The freeze-thaw stability was also determined. Three concentrations of quality control samples were analyzed immediately after finishing three freezing-thawing cycles. As shown in Table 12, the present deviation from the zero time of cefoperazone was -2.8 to -4.70% and that of sulbactam was 1.03 to 4.02%. These results were within acceptance criteria for stability (The % deviation of the mean estimated concentration from the zero times should be within ±10%). This indicated that cefoperazone and sulbactam samples had no tendency of degradation of drugs after three freeze-thaw cycles was observed, referring cefoperazone and sulbactam samples could withstand to this stress condition.

Finally, the stability of the processed plasma samples ready for injection were analyzed after freshly preparing, and after being kept in the autosampler at 24 hours. The results form Table 13 shows that both of cefoperazone and sulbactam samples were stable up to 24 hours after storing in autosampler. The loss was less than acceptance criteria (±10% from the zero time). These results indicated that each run of sample analysis must be finished within 24 hours for cefoperazone and sulbactam.

In this assay validation study indicated that the analysis method of cefoperazone and sulbactam in plasma samples had been proven to be reliable, specific, accurate and precise with the need of internal standard. These findings allowed being successfully applied in a pharmacokinetics study of cefoperazone/sulbactam intravenous injection.

Table 10 Short-term room temperature stability of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Time (hour)	Known concentration (μg/ml)	Estimated concentration (μg/ml)	S.D.	% Deviation*
Cefoperazone	0	15.0	14.15	0.35	-
		75.0	73.62	1.70	-
		150.0	149.02	3.10	-
	5	15.0	14.02	0.14	-0.92
		75.0	74.16	1.42	0.73
		150.0	151.05	1.63	1.36
Sulbactam	0	1.50	1.49	0.01	-
		30.0	30.20	0.19	-
		90.0	89.95	0.76	-
	5	1.50	1.48	0.01	-0.67
		30.0	29.78	0.12	-1.39
		90.0	89.74	0.28	-0.23

* Results are mean of five replicates

where; % Deviation = $\frac{\text{Estimated concentration at time (t)- Estimated concentration at time (0)} \times 100}{\text{Estimated concentration at time (0)}}$

Table 11 Long-term stability of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Time (weeks)	Known concentration (μg/ml)	Estimated concentration (μg/ml)	S.D.	% Deviation*
Cefoperazone	0	15.0	14.15	0.35	-
		75.0	73.62	1.70	-
		150.0	149.02	3.10	-
	2	15.0	14.30	0.33	1.06
		75.0	75.23	2.59	2.18
		150.0	151.13	2.40	1.62
	5	15.0	13.9	0.22	-1.77
		75.0	73.5	1.25	-0.17
		150.0	148.07	0.68	-0.64
Sulbactam	0	1.50	1.49	0.01	-
		30.0	30.20	0.19	-
		90.0	89.95	0.76	-
	2	1.50	1.58	0.07	6.04
		30.0	30.46	0.22	0.86
		90.0	89.64	0.33	0.34
	5	1.50	1.54	0.01	3.35
		30.0	30.10	0.23	-0.33
		90.0	88.92	0.21	-0.80

* Results are mean of five replicates

where; % Deviation = $\frac{\text{Estimated concentration at time (t)- Estimated concentration at time (0)} \times 100}{\text{Estimated concentration at time (0)}}$

Table 12 Freeze-Thaw stability of Analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Cycle	Known concentration (μg/ml)	Estimated concentration (μg/ml)	S.D.	% Deviation*
Cefoperazone	0	15.0	14.15	0.35	-
		75.0	73.62	1.70	-
		150.0	149.02	3.10	-
	3	15.0	13.76	0.19	-2.80
		75.0	69.16	1.40	-6.10
		150.0	142.09	4.75	-4.70
Sulbactam	0	1.50	1.49	0.01	-
		30.0	30.20	0.19	-
		90.0	89.95	0.76	-
	3	1.50	1.55	0.02	4.02
		30.0	30.87	0.20	2.22
		90.0	90.26	0.76	1.03

* Results are mean of five replicates

$$\% \text{ Deviation} = \frac{\text{Estimated concentration at cycle (t)- Estimated concentration at cycle (0)}}{\text{Estimated concentration at cycle (0)}} \times 100$$

Table 13 Post-preparative stability of analytical method for determination of cefoperazone and sulbactam in plasma

Drug	Time (hour)	Known concentration (μg/ml)	Estimated concentration (μg/ml)	S.D.	% Deviation*
Cefoperazone	0	15.0	15.50	0.35	-
		75.0	77.48	1.42	-
		150.0	151.17	0.45	-
	24	15.0	13.98	0.26	-9.80
		75.0	74.48	1.41	-3.87
		150.0	149.05	2.33	-1.40
Sulbactam	0	1.50	1.49	0.01	-
		30.0	30.20	0.19	-
		90.0	89.95	0.69	-
	24	1.50	1.48	0.01	-0.67
		30.0	29.93	0.08	-0.89
		90.0	89.93	0.10	-0.02

* Results are mean of five replicates

where; % Deviation = $\frac{\text{Estimated concentration at time (t)- Estimated concentration at time (0)}}{\text{Estimated concentration at time (0)}} \times 100$

3. Plasma cefoperazone and sulbactam concentration

Cefoperazone and sulbactam concentration in plasma were determined after at least 5 doses of the treatment. All 32 HAP patients (22 male and 10 female) were enrolled and completed the study. Each patient was hospitalized with a serious *A.baumannii* or *P.aeruginosa* hospital-acquired pneumonia. Eight patients (25.0%) were given cefoperazone/sulbactam (500mg/500 mg) 1g I.V. q 12 h. 18 patients (56.3%) and 6 patients (18.8%) were given cefoperazone/sulbactam 2 g I.V. q 12 h and 2g I.V. q 8 h, respectively.

The range age was 17-82 years old (59.36±15.90). The average weight was 53.31 kg (±7.40 S.D.), serum creatinine was 0.92 mg/dl (±0.33 S.D.), creatinine clearance was 71.81 ml/min (±36.50 S.D.). For the liver function test, the average AST level was 44.46 U/L (±23.62 S.D.), ALT level was 41.69 U/L (±29.93 S.D.), serum birilubin level was 0.71 mg/dl (±0.20) and albumin levels was 3.10 g/dl (±0.70 S.D.). Most of the patients were elderly with serious illness (62.1% were ICU or sub-ICU patients) and showed evidence of renal and hepatic compromise. Their demographics data are shown in Tables 14, 15 and 16.

The plasma concentration-time profile of cefoperazone and sulbactam from 32 patients in each regimen are summarized in Tables 17 – 22. Individual and the mean of plasma cefoperazone and sulbactam concentration-time profiles were displayed graphically from figures 6-11.

Table 14 Demographics of HAP patients following cefoperazone/sulbactam (500/500 mg) 1g I.V. every 12 hours

Patient	Age (yr)	Body weight (kg)	Gender	Serum creatinine (mg/dl)	Creatinine clearance (ml/min)	AST (U)	ALT (U)	Alkaline phosphatase (U/liter)	Serum bilirubin (mg/dl)	Albumin (g/dl)	CFZ/SUL trade name
1	53	60	male	0.9	80.55	33	51	109	1.14	2.6	Sulcef
2	71	45	female	0.9	45.27	35	21	73	0.44	3.4	Sulperazone
3	56	50	male	0.8	72.92	26	26	62	0.61	3.3	Sulcef
4	72	45	male	1.3	32.69	25	8	55	0.77	2.4	Sulperazone
5	20	55	female	0.7	111.30	54	102	42	0.91	4.0	Sulcef
6	35	60	male	0.6	145.83	26	42	80	0.68	3.8	Sulcef
7	54	55	female	0.7	79.77	46	96	78	0.72	4.0	Sulcef
8	42	60	male	1.0	81.67	42	33	82	0.84	3.6	Sulperazone
Mean	50.38	53.75	-	0.86	81.25	35.87	47.37	72.62	0.764	3.39	-
S.D.	17.62	6.41	-	0.22	35.45	10.63	34.43	20.20	0.21	0.61	-

Table 15 Demographics of HAP patients following cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 12 h

Patient	Age (yr)	Body weight (kg)	Gender	Serum creatinine (mg/dl)	Creatinine clearance (ml/min)	AST (U)	ALT (U)	Alkaline phosphatase (U/liter)	Serum bilirubin (mg/dl)	Albumin (g/dl)	CFZ/SUL trade name
1	54	60	Male	0.9	79.64	49	67	106	0.6	3.7	Sulperazone
2	69	40	Female	0.8	41.93	52	26	81	0.47	2.5	Sulperazone
3	67	55	Male	0.6	92.94	28	23	51	0.98	3.7	Sulperazone
4	67	50	Male	0.7	71.42	29	25	65	0.76	3.7	Sulcef
5	17	55	Male	0.7	134.23	41	44	71	0.62	4.2	Sulcef
6	57	55	Male	1.1	57.65	24	25	43	0.69	3.0	Sulcef
7	57	60	Male	1.2	57.65	41	58	118	0.47	3.9	Sulperazone
8	43	55	Male	0.4	185.25	46	87	55	1.06	4.7	Sulcef
9	58	70	Male	2.0	39.86	18	13	110	0.65	2.1	Sulcef
10	51	45	Female	0.5	94.57	28	18	94	0.67	2.7	Sulcef
11	60	42	Female	1.0	39.66	27	22	89	0.54	2.3	Sulcef
12	82	55	Male	0.9	49.22	38	46	117	0.41	2.4	Sulperazone
13	71	44	Female	0.7	51.20	50	18	121	0.60	3.0	Sulperazone
14	69	50	Male	1.1	44.82	101	109	118	1.11	2.6	Sulcef
15	77	40	Female	0.8	37.18	46	19	98	0.65	2.6	Sulperazone
16	45	60	Male	1.0	79.16	98	80	104	0.81	2.6	Sulcef
17	78	65	Male	1.4	39.98	28	1	113	0.67	2.9	Sulperazone
18	73	60	Male	1.5	37.22	59	12	110	0.63	3.6	Sulcef
Mean	60.83	53.39	-	0.96	68.53	44.61	38.5	92.44	0.69	3.12	-
S.D.	15.60	8.64	-	0.39	39.16	22.92	30.04	25.48	0.19	0.74	-

Table 16 Demographics of HAP patients following cefoperazone/sulbactam (500/500 mg) 2g I.V. q 8 h

Patient	Age (yr)	Body weight (kg)	Gender	Serum Creatinine (mg/dl)	Creatinine clearance (ml/min)	AST (U)	ALT (U)	Alkaline phosphatase (U/IL)	Serum bilirubin (mg/dl)	Albumin (g/dl)	CFZ/SUL trade name
1	64	50	Male	1.0	52.78	21	11	119	0.57	3.3	Sulcef
2	71	55	Female	0.7	64.0	26	26	79	1.05	2.4	Sulperazone
3	82	60	Male	1.4	34.52	102	55	118	0.82	2.1	Sulcef
4	72	50	Male	0.6	78.70	54	53	95	0.9	2.4	Sulperazone
5	55	55	Male	0.5	129.86	95	87	98	0.61	2.3	Sulperazone
6	58	45	Female	0.8	54.45	33	30	65	0.58	3.5	Sulperazone
Mean	67	52.5	-	0.83	69.05	55.17	43.67	95.67	0.76	2.67	-
S.D.	10	5.24	-	0.33	33.13	35.47	27.05	21.29	0.20	0.58	-

Table 17 Plasma cefoperazone concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 1g I.V. every 12 hour

Patient	Time (hours)				
	0	0.167	2	4	12
1	17.61	73.17	40.91	28.40	17.61
2	3.05	63.69	48.89	33.75	3.05
3	9.91	71.89	33.17	21.53	9.91
4	34.73	112.18	77.53	69.02	34.73
5	3.42	99.37	21.55	10.31	3.42
6	3.11	100.18	22.15	10.77	3.11
7	5.28	72.96	30.26	14.48	5.28
8	5.54	80.21	31.53	12.14	5.54
Mean	10.33	84.21	38.24	25.05	10.33
S.D.	11.01	17.34	18.27	19.76	11.02

Table 18 Plasma cefoperazone concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 2 g I.V. every 12 hour

Patient	Time (hours)				
	0	0.167	2	4	12
1	29.04	149.84	72.36	57.53	29.04
2	55.87	161.30	111.54	84.22	55.87
3	11.80	123.84[a]	50.75	33.02	11.80
4	10.48	96.16[a]	40.78	27.09	10.48
5	5.68	81.94[b]	24.33	14.87	5.68
6	10.65	134.93	49.95	37.74	10.65
7	18.38	107.25	60.77	42.16	18.38
8	6.76	125.98	35.20	21.48	6.76
9	45.37	171.30	101.44	74.22	45.37
10	25.91	163.80	78.64	54.98	25.91
11	12.97	134.90	83.55	44.56	12.97
12	17.23	128.82	85.29	41.45	17.23
13	77.98	193.16	141.16	113.36	77.98
14	82.68	254.27	141.71	125.29	82.68
15	94.67	285.98	169.53	142.57	94.67
16	17.12	105.34	62.25	44.08	17.12
17	35.81	103.24[a]	85.21	71.19	35.81
18	38.48	139.02	74.39	64.36	38.46
Mean	33.16	161.14	81.60	60.79	33.16
S.D.	27.74	52.39	39.27	35.86	27.74

[a] at 20 minutes after administration

[b] at 30 minutes after administration

Table 19. Plasma cefoperazone concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 2 g I.V. every 8 hour

Patient	Time (hours)				
	0	0.167	2	4	8
1	40.42	129.63 [a]	74.81	54.95	40.42
2	30.31	108.80 [b]	51.66	40.22	30.31
3	106.00	192.49	153.96	129.52	106.00
4	39.93	120.90 [a]	62.93	53.28	39.93
5	17.83	152.82	67.29	34.73	17.83
6	48.78	134.57	70.39	50.18	48.78
Mean	47.21	159.96	80.17	60.48	47.21
S.D.	30.68	29.62	37.00	34.72	30.68

[a] at 20 minutes after administration, [b] at 25 minutes after administration

Table 20 Plasma sulbactam concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 1 g I.V. every 12 hour

Patient	Time (hours)				
	0	0.167	2	4	12
1	0.86	31.21	19.90	6.14	0.86
2	0.19	34.04	26.56	10.03	0.19
3	0.09	31.26	6.21	2.77	0.09
4	7.44	35.44	28.83	12.47	7.44
5	0.16	43.15	4.10	1.99	0.16
6	0.94	41.95	3.95	1.16	0.94
7	0.78	34.61	15.29	3.74	0.78
8	0.88	37.89	10.83	4.82	0.88
Mean	1.42	36.19	14.46	5.39	1.42
S.D.	2.46	4.49	9.87	3.99	2.46

Table 21 Plasma sulbactam concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 2 g I.V. every 12 hour

Patient	Time (hours)				
	0	0.167	2	4	12
1	1.72	63.45	24.76	13.77	1.72
2	4.42	67.45	29.61	17.67	4.42
3	0.11	55.56[a]	10.92	3.83	0.11
4	0.37	42.81	18.50	7.27	0.37
5	0.15	26.37[b]	6.44	0.85	0.15
6	0.41	67.47	17.16	6.58	0.41
7	0.13	59.22	11.82	3.75	0.13
8	0.11	52.45	5.84	1.57	0.11
9	28.38	70.93[b]	50.73	46.16	28.38
10	3.13	67.58	31.73	16.52	3.13
11	3.50	63.77	23.09	14.61	3.50
12	1.23	67.56	24.02	9.37	1.23
13	6.35	68.36	30.89	19.94	6.35
14	9.11	129.61	63.22	41.86	9.11
15	29.41	160.55	74.68	57.91	29.41
16	1.41	58.73	14.98	5.37	1.41
17	20.44	79.11[b]	58.64	47.44	20.44
18	1.64	37.80	30.85	16.05	1.64
Mean	6.22	71.92	29.33	18.36	6.22
S.D.	9.60	34.32	20.02	17.67	9.60

[a] at 20 minutes after administration [b] at 25 minutes after administration

Table 22 Plasma sulbactam concentrations (μg/ml) of HAP patients following cefoperazone/sulbactam (500/500 mg) 2g I.V. every 8 hour

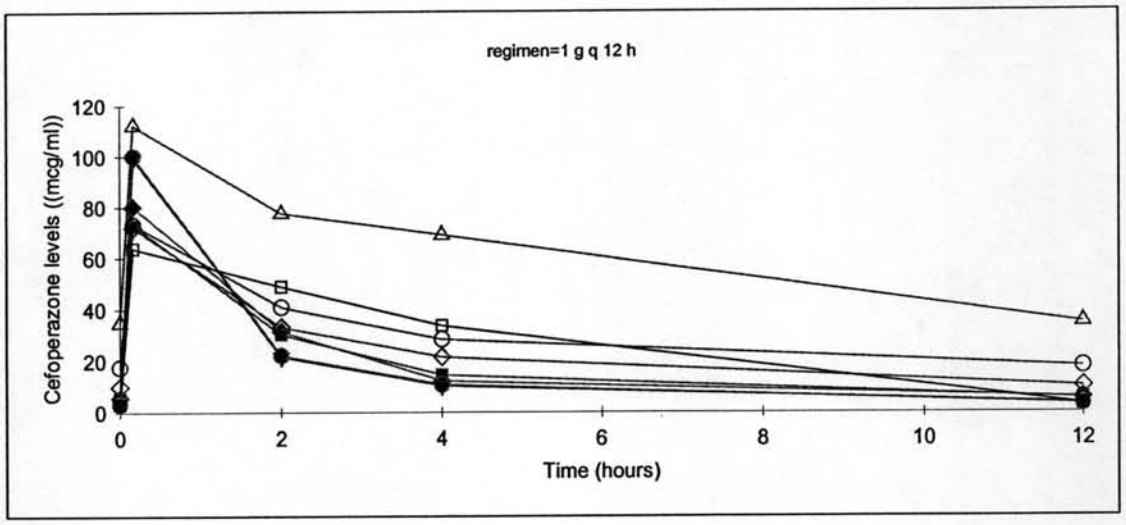
Patient	Time (hours)				
	0	0.167	2	4	8
1	5.76	59.00[a]	28.59	15.21	5.76
2	2.70	50.36[b]	16.34	6.57	2.70
3	22.70	100.75	64.55	46.42	22.70
4	2.21	41.16[a]	17.51	4.29	2.21
5	0.38	91.92	14.33	2.87	0.38
6	3.21	50.52	23.43	5.86	3.21
Mean	6.16	81.06	27.46	13.54	6.16
S.D.	8.29	26.82	18.91	16.68	8.29

[a] at 20 minutes after administration, [b] at 25 minutes after administration.

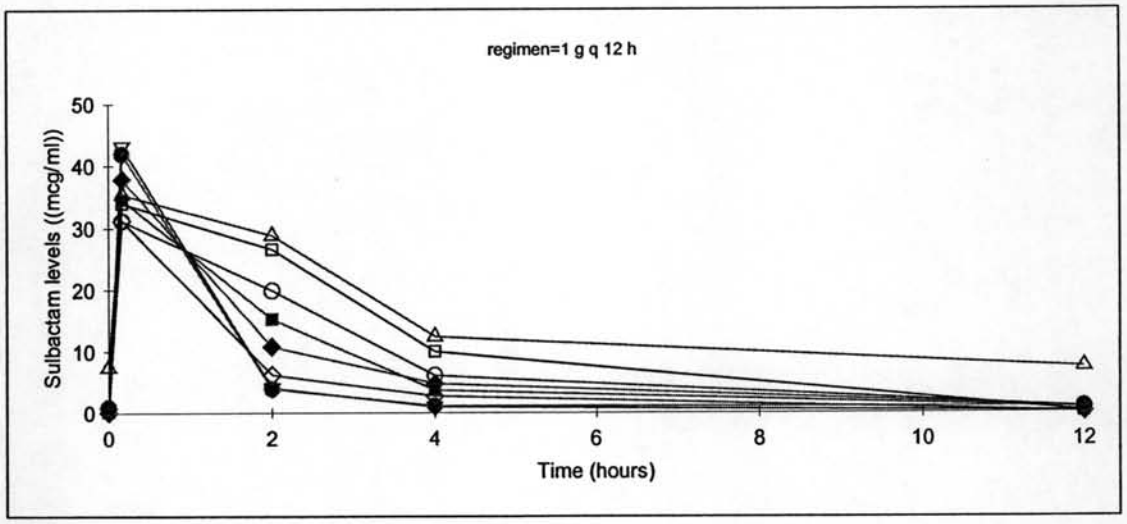
Figure 6 Plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 1g I.V. every 12 h

A: Cefoperazone

B: Sulbactam



(A)

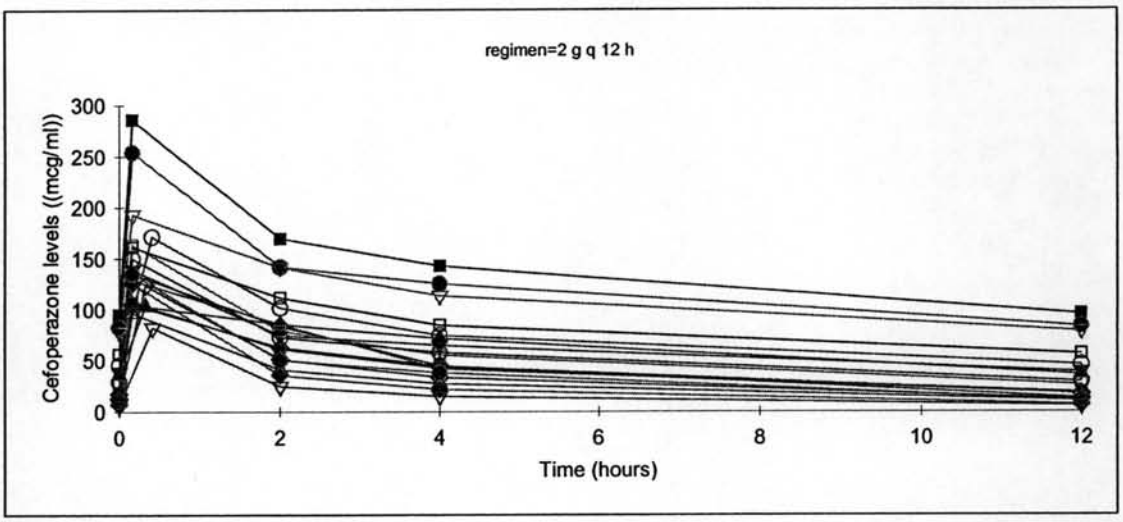


(B)

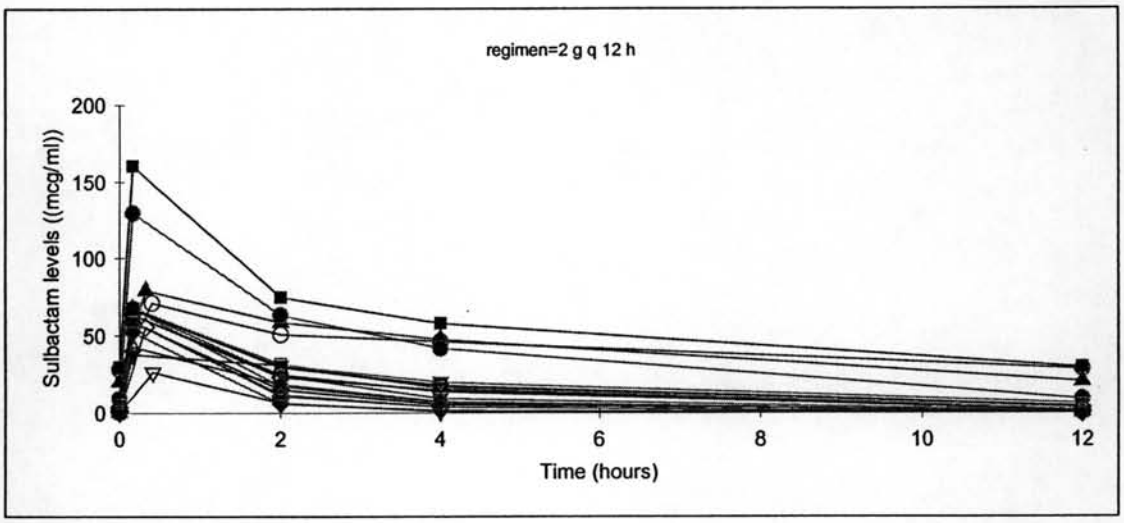
Figure 7 Plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 2g I.V. every 12 h

A: Cefoperazone

B: Sulbactam



(A)

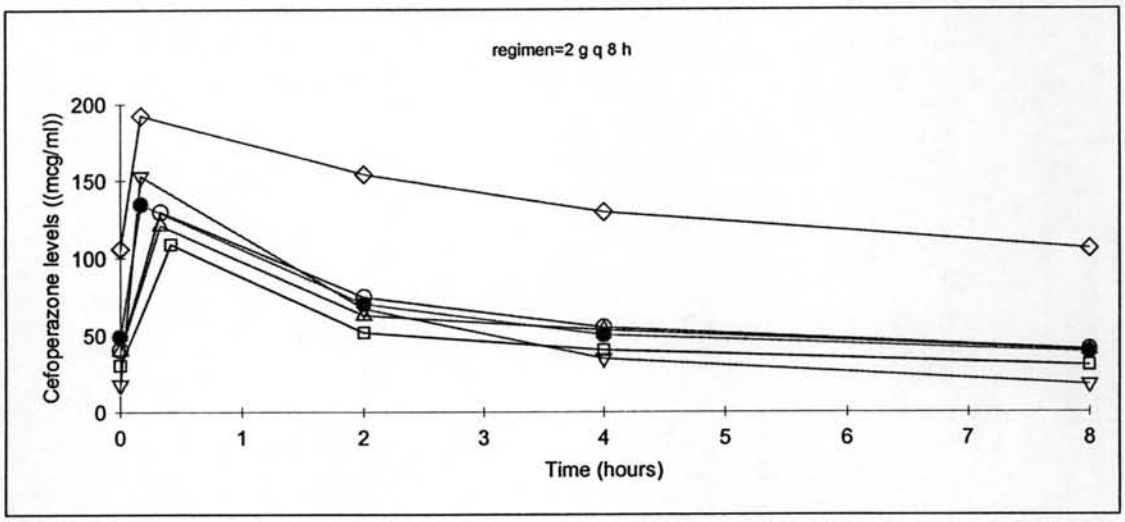


(B)

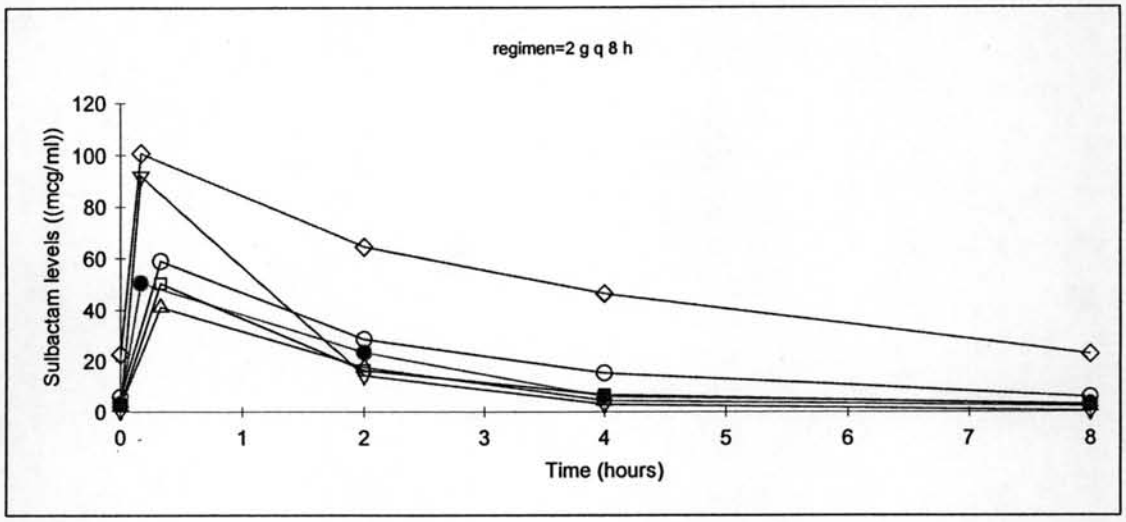
Figure 8 Plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 2g I.V. every 8 h

A: Cefoperazone

B: Sulbactam



(A)

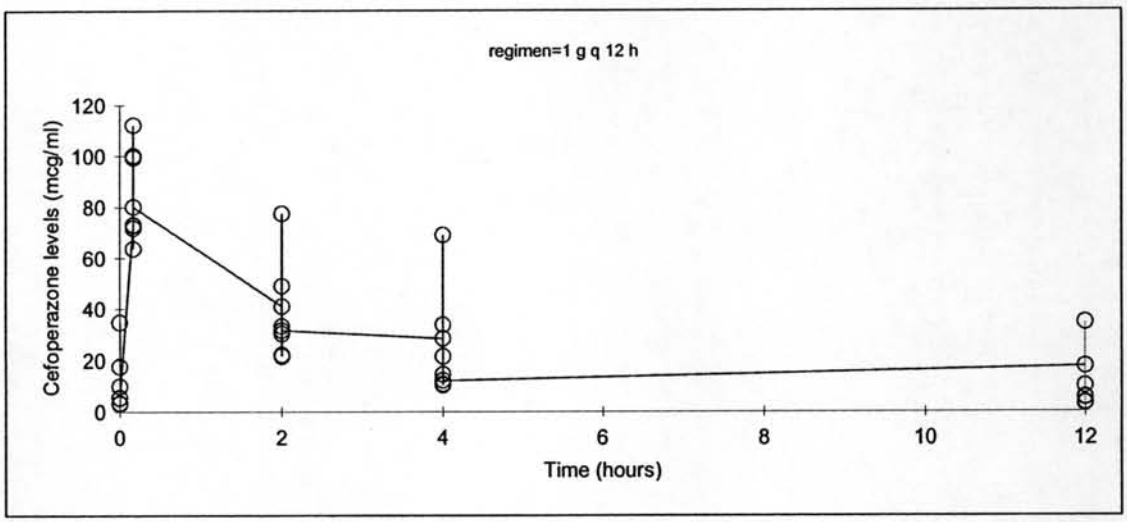


(B)

Figure 9 Mean plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 1g I.V. every 12 h

A: Cefoperazone

B: Sulbactam



A

B

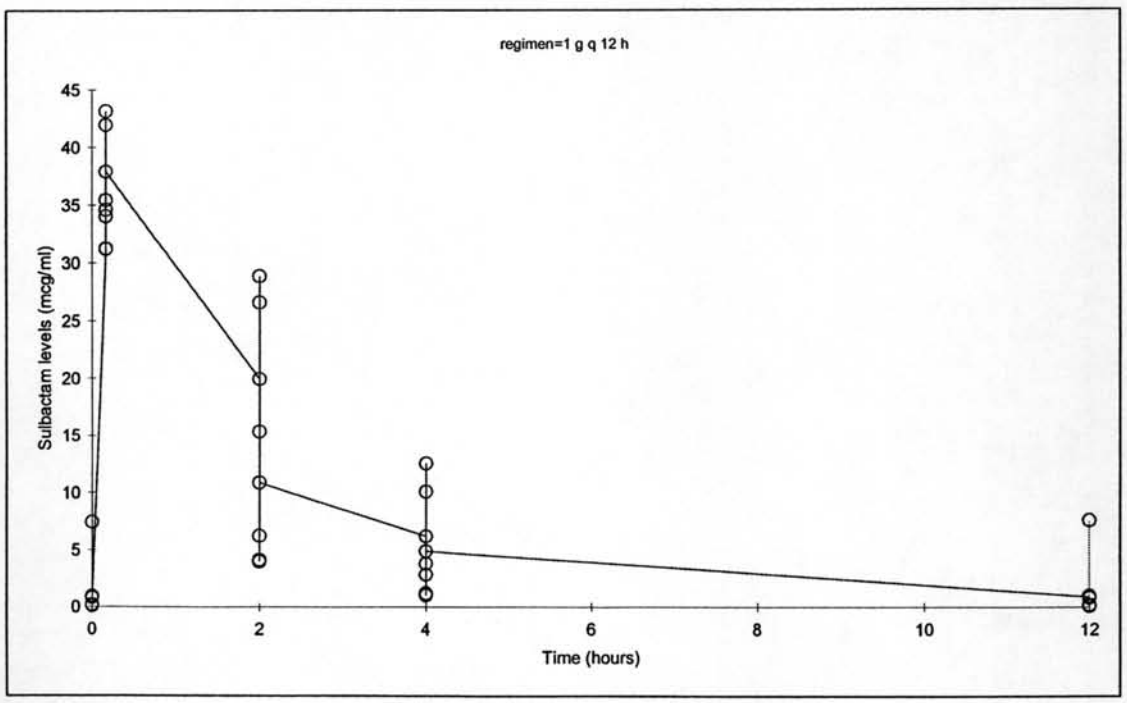
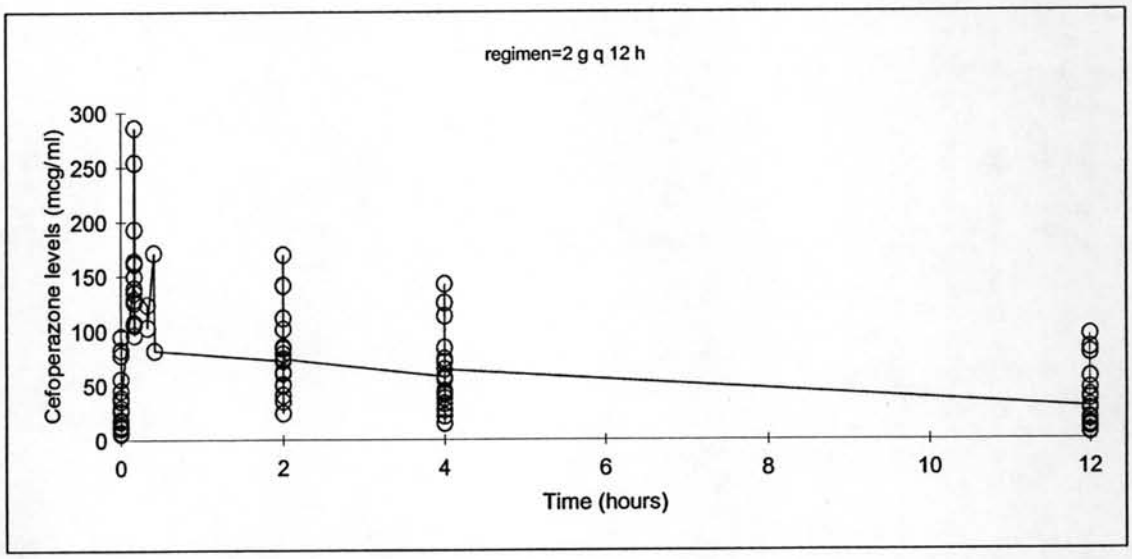


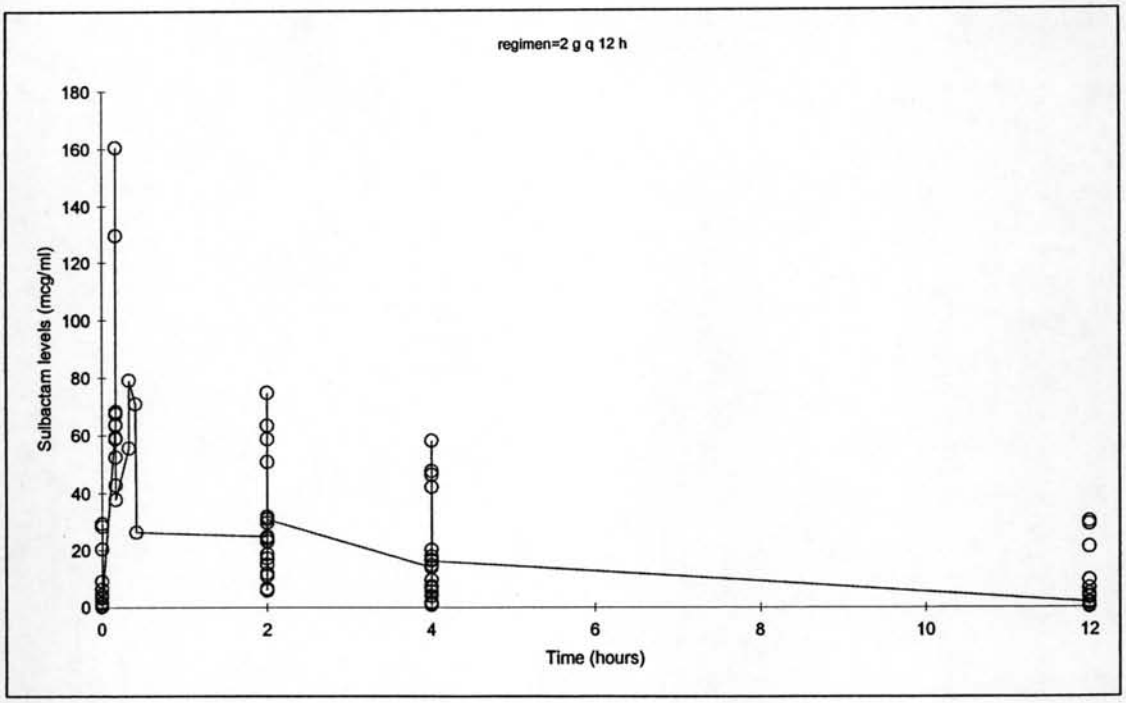
Figure 10 Mean plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 2g I.V. every 12 h

A: Cefoperazone

B: Sulbactam



A

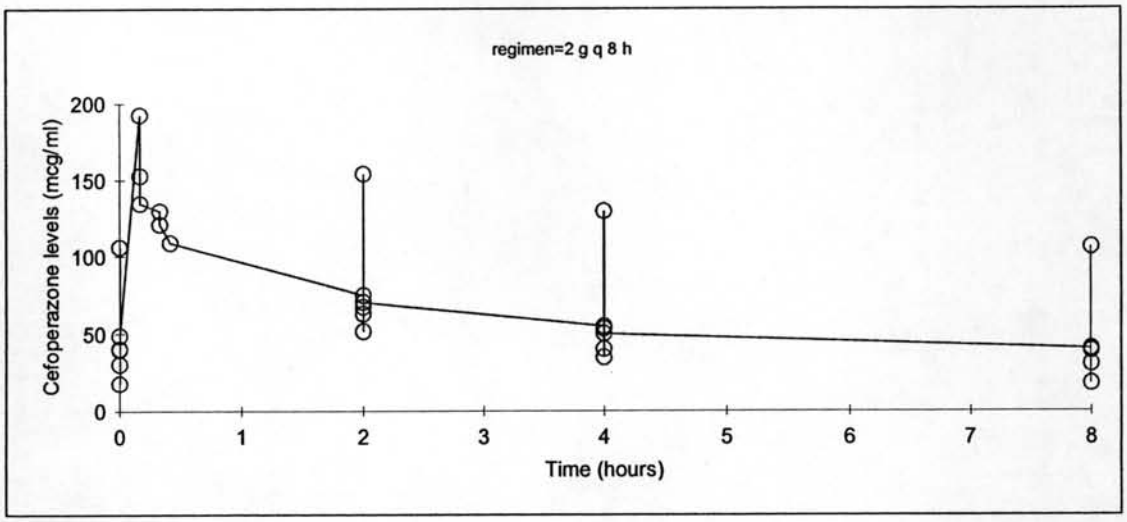


B

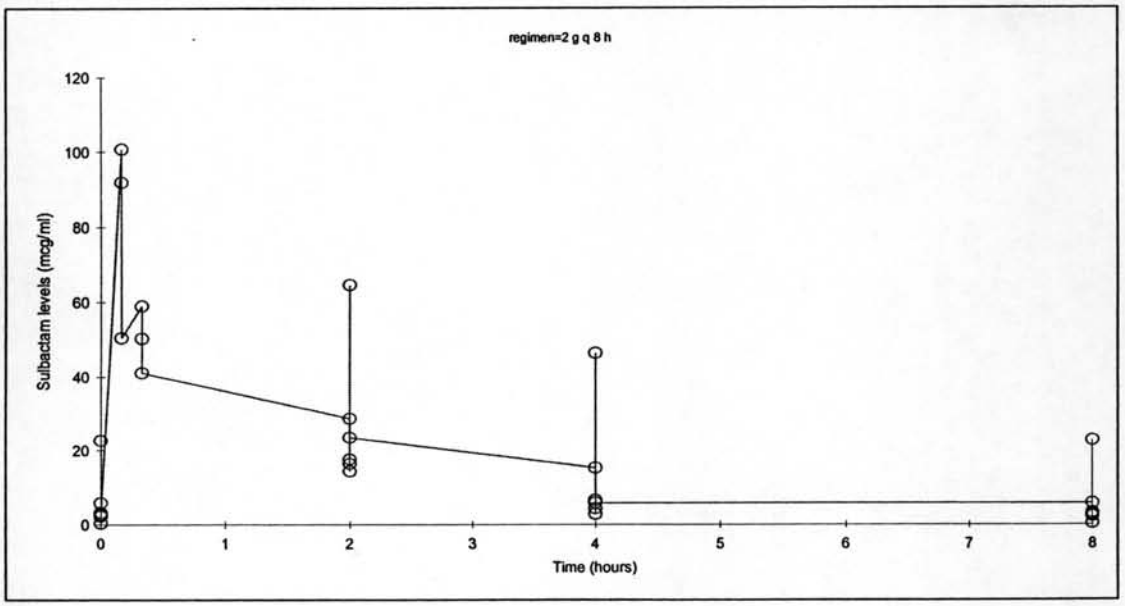
Figure 11 Mean plasma concentrations vs. time profiles of HAP patients following cefoperazone-sulbactam (500 mg/500 mg) 2g I.V. every 8 h

A: Cefoperazone

B: Sulbactam



A.



B

4. Pharmacokinetics analysis

Non-compartment methods were used to estimate pharmacokinetic parameters. Agreement between non-compartment and model-dependent analysis methods has been previously reported for a cephalosporin given to critically illness patients.[73]

Pharmacokinetics parameter estimates of cefoperazone and sulbactam after multiple intravenous injection of 500/500 mg cefoperazone/sulbactam of each dosage regimen for all subjects are summarized in Table 23-25. Wide inter-subject variability was seen with coefficients of variation from 29.12-55.72% for cefoperazone pharmacokinetic parameters and 36.21-77.61% percent for sulbactam. As predicted from laboratory abnormalities and the greater physiologic variability by these HAP patient compared with normal subjects, several observations are supportive of greater inter-subject pharmacokinetic variability in patients as well. The Coefficient variation for CL in these patients was approximately double for cefoperazone (CL = 1.82$\pm$0.81 L/h) and sulbactam (6.80$\pm$4.3) compared with that previously reported normal subjects (CL= 4.55$\pm$0.79, CL =18.07$\pm$2.75, respectively)[107] Therefore, the physiologic alteration and variability imposed by serious infection patients may be manifested, as well as altered mean pharmacokinetic parameter values.

Following dose 1 g I.V. q 12 h, 2 g I.V q 12 h and 2 g I.V. q 8 h, the mean terminal eliminate half-life was 5.19, 7.45, and 7.72 h, respectively for cefoperazone and 2.62, 2.64, and 2.32 h, respectively for sulbactam. The volume of distribution was 12.31, 15.92, and 20.67 L, respectively for cefoperazone and 21.45, 25.25, and 22.39 L, respectively for sulbactam. The total clearance was 1.82, 1.77, and 1.97 L/h, respectively for cefoperazone, and 6.11, 6.89 and 7.48 L/h, respectively for sulbactam. Both of cefoperazone and sulbactam, the pharmacokinetic parameters, CL, Vd, Ke, T1/2 were not significant different when comparing between dosing regimens. A comparison of the AUC during the dosing interval in each dosing regimens showed a statistically significant (p=0.06 for cefoperazone and p=0.024 for sulbactam). The results are shown in Table 26.

Table 27-28 compares the pharmacokinetic parameters of cefoperazone and sulbactam obtained from this study with those of previous studies. HAP patients exhibited a cefoperazone and sulbactam half life which was approximately 3-4 times, and 2 times, respectively longer than that for healthy subjects.[108] Compared with healthy volunteers, our HAP patients also showed slower clearance and larger Vd value than that for healthy subjects. Schwartz et al.[109] observed these same differences to a larger degree in a group of elderly, serious ill patients. Four of their six patients had impaired renal function, and two patients had a serum bilirubin concentration > 17.1 μmol/L. Similar to our study included patients with elderly, serious hospital-acquired pneumonia, decreased renal function and

abnormalities liver function test. Therefore, while the differences observed may be the result of abnormal organ function.

Cefoperazone is cleared primarily by hepatobiliary excretion, with approximately 30% of the dose recovered in the urine of normal volunteers,[110] also a much greater proportion is eliminated by the renal route in biliary obstruction or hepatic dysfunction. In our study, we excluded patients who had history of biliary obstruction, cholestasis or had extremely increased serum bilirubin and alkaline phosphatase, but some of them had abnormally AST and ALT levels (within 3 time upper normal limit) and also had mild to moderate renal impairment. This result provides an explanation for changes in cefoperazone CL, which may have been dynamically changing in concordance with the disease state. However, cefoperazone also exhibited a 30% larger Vd in HAP patients (15.59 ± 5.89) compared with normal volunteer (11.3 ± 1.4), contributing quantitatively to the prolonged T1/2.

When constant tissue binding is assumed, the effect of altered serum protein binding on distribution may be attenuated serum protein binding by the relative mass of drug in the extravascular volume. One possible explanation for the larger cefoperazone Vd in HAP patients compared with normal subjects stems from that cefoperazone is normal extensively bound to serum protein, exhibiting a bound fraction of approximately 90%.[111] Therefore, the change of cefoperazone serum protein binding would be expected to result in a more alteration in Vd, since the fraction of total cefoperazone in the extravascular volume under normal conditions is relatively smaller and the fraction available for loss from the central compartment is relatively larger. Sulbactam is only 38% bound to serum protein. As was observed, compared with cefoperazone, sulbactam would not be expected to show relatively large increase in Vd when protein binding is impaired.

An increase in cefoperazone Vd may be consistent with an elevated free fraction perhaps due to the marked reduction in albumin levels typically observed in these nutritionally compromised patients. However, since free fraction is expected to be related to the logarithm of albumin concentration, albumin must be depleted to values under 2 g/dl before extensive protein binding alteration would be expected. Another explanation for a binding defect is the presence of exogenous or endogenous competitors for binding sites. This hypothesis is supported by the mild to moderate renal impairment in these patients and the reported defect in protein binding of drugs in uremia. However, in volunteers with mild to moderate renal failure, no alteration in cefoperazone Vd was described in a previous study.[112] Therefore, the presence of competitors in serum would probably be related to hepatic impairment or the other physiologic alterations imposed by serious illness. For example, Shimizu[109] reported that bilirubin can displace cefoperazone from serum protein; this observation may be

particularly relevant for the patients who had experienced hyperbilirubinemia. Unfortuantely, we did not measure cefoperazone free fraction, although a change in free fraction has been previously reported for the cephalosporin; cefmemoxime when given to critically ill, elderly patients.

Sulbactam pharmacokinetics was also altered by serious HAP patients. Patients exhibited a sulbactam halfl-life which was 2.6 times longer than that observed for healthy subject.[112] While sulbactam CL for patients was slower than that for normal subjects. Because sulbactam undergoes predominantly renal elimination, CL_R has represented approximately 80% of total clearance, it was not surprising that subjects with renal impairment exhibited a sulbactam half-life longer than the healthy subjects and also decreased in CL.

Table 23. Pharmacokinetic parameter and variability for cefoperazone/sulbactam (500/500mg) 1 g I.V. q 12 h.

Patient	Cefoperazone					Sulbactam				
	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)
1	365.48	17.70	0.08	1.37	8.97	103.56	15.89	0.30	4.83	2.28
2	338.59	5.20	0.28	1.48	2.44	135.87	7.44	0.49	3.68	1.40
3	283.58	15.47	0.11	1.76	6.08	57.38	20.51	0.42	8.71	1.63
4	747.69	8.17	0.08	0.67	8.46	183.42	21.07	0.13	2.73	5.36
5	206.19	14.19	0.17	2.42	4.06	56.61	29.76	0.30	8.83	2.34
6	209.18	12.95	0.18	2.39	3.76	59.15	33.81	0.25	8.45	2.77
7	224.91	13.83	0.16	2.22	4.31	85.80	19.00	0.31	5.83	2.26
8	223.96	11.00	0.20	2.23	3.42	86.34	24.13	0.24	5.79	2.89
Mean	324.95	12.31	0.16	1.82	5.19	96.02	21.45	0.31	6.11	2.62
S.D.	181.25	4.05	0.07	0.62	2.41	44.54	8.13	0.11	2.35	1.22
%C.V.	55.78	32.87	42.76	33.90	46.41	46.39	37.92	36.21	38.55	46.58

Table 24 Pharmacokinetic parameter and variability for cefoperazone/sulbactam (500/500mg) 2 g I.V. q 12 h.

Patient	Cefoperazone					Sulbactam				
	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)
1	694.75	16.06	0.09	1.44	7.73	186.78	20.22	0.26	5.35	2.62
2	1024.31	15.25	0.06	0.98	10.82	230.60	23.40	0.19	4.34	3.74
3	431.21	16.45	0.14	2.32	4.92	95.21	23.07	0.46	10.50	1.52
4	352.56	21.65	0.13	2.84	5.29	116.13	22.32	0.39	8.61	1.80
5	223.75	32.32	0.14	4.47	5.01	42.78	57.11	0.41	23.38	1.69
6	462.85	13.89	0.16	2.16	4.46	134.93	20.25	0.37	7.41	1.89
7	509.57	17.05	0.12	1.96	6.02	101.15	22.36	0.44	9.89	1.57
8	328.45	19.14	0.16	3.04	4.36	71.94	36.70	0.38	13.90	1.83
9	915.27	14.55	0.08	1.09	9.24	512.13	33.15	0.06	1.95	11.78
10	695.22	13.54	0.11	1.44	6.53	223.77	19.87	0.22	4.47	3.08
11	570.79	9.12	0.19	1.75	3.61	195.36	27.55	0.19	5.12	3.73
12	569.89	10.72	0.16	1.75	4.24	165.47	21.22	0.28	6.04	2.43
13	1348.92	13.31	0.06	0.74	12.43	253.19	25.66	0.15	3.95	4.50
14	1489.93	12.59	0.05	0.67	13.00	497.27	10.43	0.19	2.01	3.59
15	1710.32	10.40	0.06	0.58	12.33	713.32	15.44	0.09	1.40	7.64
16	514.95	15.41	0.13	1.94	5.50	120.05	38.46	0.22	8.33	3.20
17	764.50	15.12	0.09	1.31	8.02	509.05	18.64	0.11	1.96	6.58
18	760.52	20.07	0.07	1.31	10.59	183.87	18.68	0.29	5.44	2.38
Mean	742.65	15.92	0.11	1.77	7.45	241.83	25.25	0.26	6.89	3.64
S.D.	413.78	5.23	0.04	0.97	3.18	187.68	10.60	0.12	5.32	2.64
%C.V.	55.72	32.86	38.42	54.90	42.63	77.61	42.00	47.45	77.13	72.50

Table 25 Pharmacokinetic parameter and variability for cefoperazone/sulbactam (500/500mg) 2g I.V. q 8 h

Patient	Cefoperazone					Sulbactam				
	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)	AUC (μg.h/ml)	Vd (L)	Ke (h^{-1})	CL (L/h)	$T_{1/2}$ (h)
1	519.27	19.47	0.10	1.93	7.01	169.56	22.37	0.26	5.90	2.63
2	388.96	29.79	0.09	2.57	8.03	105.90	32.67	0.29	9.44	2.40
3	1096.97	15.07	0.06	0.91	11.46	411.02	13.92	0.17	2.43	3.96
4	482.67	27.51	0.08	2.07	9.21	90.95	28.81	0.38	11.00	1.82
5	423.12	11.07	0.21	2.36	3.25	128.79	13.14	0.59	7.76	1.17
6	501.65	21.12	0.09	1.99	7.35	119.69	23.40	0.36	8.35	1.94
Mean	568.77	20.67	0.11	1.97	7.72	170.99	22.39	0.34	7.48	2.32
S.D.	263.40	7.14	0.05	0.57	2.72	120.56	7.81	0.14	3.00	0.95
%C.V.	46.31	34.56	50.66	29.12	35.21	70.51	34.90	41.86	40.14	40.93

Table 26 Summary of pharmacokinetic parameter and variability for cefoperazone and sulbactam used in Monte Carlo simulation

Dosage regimen	Individual estimates and measures of dispersion			
	Cefoperazone		Sulbactam	
	Mean (S.D.)	%C.V.	Mean (S.D.)	%C.V.
1 g I.V. q 12 h				
AUC (μg.h/ml)	324.95(181.25)[a]	55.78	96.02(44.54)[a]	46.39
Vd (L)	12.31(4.05)	32.87	21.54(8.13)	37.92
Ke (h^{-1})	0.16(0.07)	42.76	0.31(0.11)	36.21
CL (L/h)	1.82(0.62)	33.90	6.11(2.35)	38.55
$T_{1/2}$ (h)	5.19(2.41)	46.41	2.62(1.22)	46.58
2 g I.V. q 12 h				
AUC (μg.h/ml)	742.65(413.78)[a]	55.72	241.83(187.68)[a]	77.61
Vd (L)	15.92(5.23)	32.86	25.25(10.60)	42.00
Ke (h^{-1})	0.11(0.04)	38.42	0.26(0.12)	47.45
CL (L/h)	1.77(0.97)	54.90	6.89(5.32)	77.13
$T_{1/2}$ (h)	7.45(3.18)	42.63	3.64(2.64)	72.50
2 g I.V. q 8 h				
AUC (μg.h/ml)	568.77(263.40)[a]	46.31	170.99(120.56)[a]	70.51
Vd (L)	20.67(7.14)	34.56	22.39(7.81)	34.90
Ke (h^{-1})	0.11(0.05)	50.66	0.34(0.14)	41.86
CL (L/h)	1.97(0.57)	29.12	7.48(3.00)	40.14
$T_{1/2}$ (h)	7.72(2.72)	35.21	2.32(0.95)	40.93

[a] Significant difference between study dosage regimens ($p < 0.05$)

Table 27 Cefoperazone pharmacokinetic parameters from published studies

REF.	Type of patients	No.	Dose[a]	Study day	CL (ml/min)	Vd (L)	T1/2 (h)	AUC (mg.h/L)
Reitberg et al.	Healthy volunteer	14	3 g/1.5g	1	67.6±10.2	10.4±1.3	1.82±0.33	756±121.6[b]
				7	75.8±13.1	11.3±1.4	1.76±0.30	679.2±121.6[c]
Schwartz et. al.	Elderly, serious ill	6	2 g/1g	1	29±9	13.1±4.5	7.0±3.5	1247±353[b]
				5	34±10	14.4±4.2	4.0±1.7	1062±372[c]
Johnson et.al.	CAPD	6	2g/1g	Single dose	71.7±33.4	10.5±1.5	2.08±0.82	564.9±254.0[c]
Reiberg et al.	Healty volunteer	6	2g/1g	Single dose	95±14	10.2±1.0	1.6±0.3	356±52[b]
	Functionally anephric	4	2g/1g		97±30	13.1±1.7	2.4±1.1	672±33.3[b]
Present study	HAP	8	0.5/0.5g	≥5th dose	30.33±10.3	12.31±4.1	5.19±2.41	324.95±181.2[c]
		18	1g/1g[e]		29.5±16.16	15.92±5.2	7.45±3.18	742.65±413.8[c]
		6	1g/1g[f]		32.83±9.5	20.67±7.1	7.72±2.72	568.77±2633.4[d]

[a] dose represented as cefoperazone/sulbactam, [b] AUC0-α was used in this study, [c] AUC0-12h was used in this study, [d] AUC0-8 h was used in this study, [e] dose interval as every 12 h, [f] dose interval as every 8 h

Table 28 Sulbactam pharmacokinetic parameters from published studies

REF.	Type of patients	No.	Dose[a]	Study day	CL (ml/min)	Vd (L)	T1/2 (h)	AUC (mg.h/L)
Reitberg et al.	Healthy volunteer	14	3 g/1.5g	1	297.8±71.3	28.1±12.9	1.06±0.26	88.6±21.8[b]
				7	301.1±45.8	27.6±6.0	1.06±0.16	85.0±14.3[c]
Schwartz et. al.	Elderly, serious ill	6	2 g/1g	1	97±61	18.9±10.5	3.4±1.2	228±115[b]
				5	94±47	15.4±5.7	2.5±0.5	217±105[c]
Johnson et.al.	CAPD	6	2g/1g	Single dose	33.4±5.3	19.4±2.7	6.86±1.67	521.9±86.5[b]
Reiberg et al.	Healty volunteer	6	2g/1g	Single dose	267±49	18±10	1.0±0.2	64±11[b]
	Functionally anephric	4	2g/1g		26±10	18.6 ±2.9	9.7±5.3	709 ±271[b]
Present study	HAP	8	0.5/0.5g	≥5th dose	101.8±39.2	21.5±8.1	2.65±1.2	96.02±44.5[c]
		18	1g/1g[e]		114.8±88.7	25.3±10.7	3.64±2.64	241.8±187.7[c]
		6	1g/1g[f]		124.7±49.8	22.4±7.8	2.32±0.95	171±120.6[d]

[a] dose represented as cefoperazone/sulbactam, [b] AUC0-α was used in this study, [c] AUC0-12h was used in this study, [d] AUC0-8 h was used in this study, [e] dose interval as every 12 h, [f] dose interval as every 8 h

5. Determination of MIC

We test 149 isolate of *A.baumannii*, and 215 isolates of *P.aeruginosa* against cefoperazone/sulbactam. All resultant isolates were obtained from true sputum or tracheal secretion of patients in Maharaj Nakorn Chiang Mai hospital. Over a 6 month period, MIC data for each isolates were determined by the E-test method. Multiple isolates of the same species from the same patient were excluded. Central microbiology laboratory identify the species by means of colony morphology or simple biochemical tests.

A.baumannii were isolated from the following ward of admission; 22.8% of general internal medical, 38.6% of ICU internal medical ward, 7.4% of general surgical ward, 22.3 of ICU surgical ward, and 8.9% of the other wards. Susceptible rate is shown in Table 27 and Figure 12. Less than 50 percent of isolates were susceptible to each of the following antibiotics including co-trimoxazole, gentamicin, amikacin, netilmicin, piperacillin-tazobactam, ceftazidime, cefpirome, imipenem, meropenem and ciprofloxaxin. Cefoperazone/sulbactam and colistin were the only two commonly used antibiotics to which approximately 60% and 99% isolates, respectively were susceptible. For cefoperazone/sulbactam, we found 58.3%% for susceptible, 29.2%% for intermediate and 12.5% for resistance of isolates. MIC frequency values for *A.baumannii* are listed in Table 29 and figure 12. The MICs of cefoperazone/sulbactam ranged from 1 to 256 μg/ml for *A.baumannii*. The percentage of isolates at MIC < 16 μg/ml were 54.2 % (susceptible), 16-48 μg/ml were 31.40 % (intermediate) and >64 μg/ml were 14.4 % (resistant).

P.aeruginosa were isolated from general internal medical 22.1%, ICU internal medical ward 24.2%, general surgical ward 15.4% , ICU surgical ward 12.1% , private ward 14.1% and the other wards 12.1%. Susceptible rate is listed in Table 27 and Figure 13 shows the susceptibility of all isolates of *P.aeruginosa* to various antibiotics. Most of recommended antibiotic were susceptible to *P.aeruginosa* more than 50%. *P.aeruginosa* was highly susceptible to colistin, with 97.8% of isolates. It was susceptible to cefoperazone/sulbactam 53.0% of isolates. MIC frequency values for *P.aeruginosa* are listed in Table 30 and figure13. The MICs of cefoperazone/sulbactam ranged from 1.0 to 256 μg/ml for *P.aeruginosa*. The percentage of isolates at MIC <16 μg/ml were 60.4% (susceptible), 16-48 μg/ml were 21.5 % (intermediate) and >64 μg/ml were 18.1 % (resistant) .These results related to the susceptibility data from the disk-diffusion test method.

MIC distributions were built for each population of bacteria based on the MIC frequencies in the pharmacodynamic study using Crystal ball software.

Table 29 Antibiotic susceptibility against *A. baumannii* and *P. aeruginosa* pulmonary isolates from Maharaj Nakorn Chiang Mai hospital. (Disk test method)

Antibiotic	*A.baumannii* (n=215, %)			*P.aeruginosa* (n=149, %)		
	susceptible	intermediate	resistant	susceptible	intermediate	resistant
Cotrimoxazole	19.8	0.5	79.7	-	-	-
Colistin	99.5	0.5	0	97.8	0	2.2
Gentamicin	22.1	2.7	74.7	55.6	4.8	39.7
Netilmicin	32.9	2.9	64.3	61.9	9.5	28.6
Amikacin	25.7	3.4	70.9	67.1	4.1	28.8
Piperacillin/tazobactam	19.9	1.0	79.1	82.6	0.7	16.7
Cefoperazone/sulbactam	58.3	29.2	12.5	53.0	20.8	26.2
Ceftazidime	17.5	1.5	81.1	54.1	2.7	43.2
Cefirome	17.9	1.5	80.6	47.4	4.5	48.1
Cefepime	18.4	3.9	77.8	59.2	2.1	34.2
Imipenem	23.9	0	76.1	63.7	2.1	34.2
Meropenem	23.9	0.5	75.6	62.9	4.1	33.3
Ciprofloxacin	20.2	0	79.8	57.1	4.1	38.8

Figure 12 The susceptibility of *A. buamannii* isolates to various antibiotics.

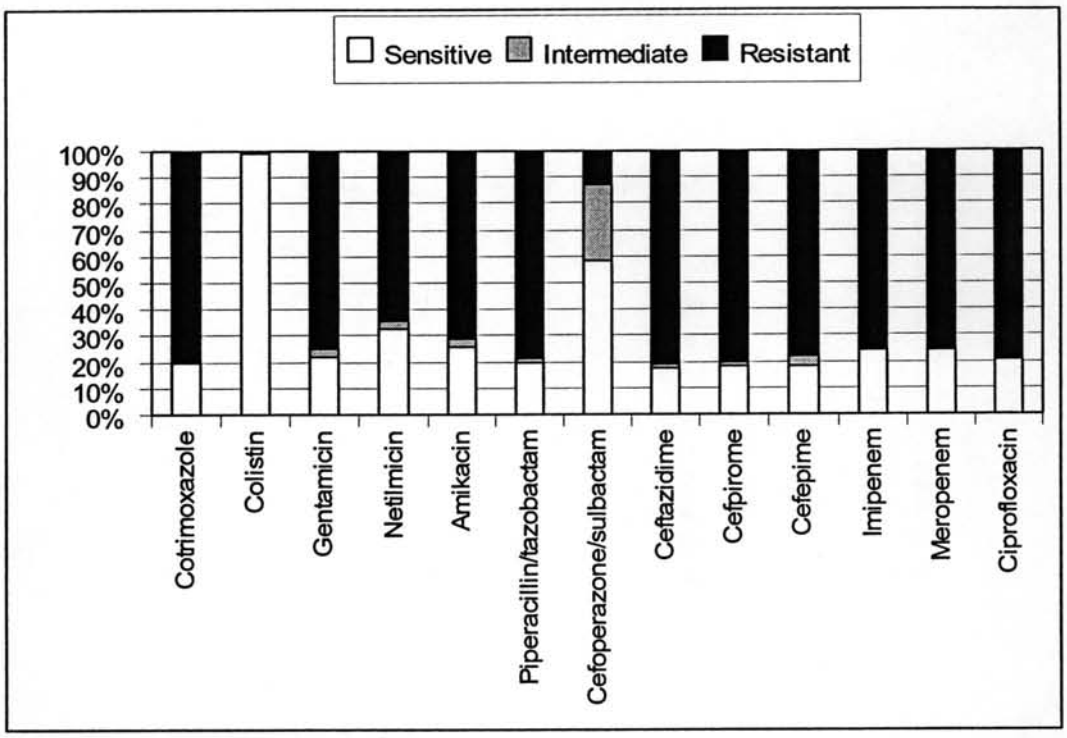


Figure 13 The susceptibility of *P. aeruginosa* isolates to various antibiotics

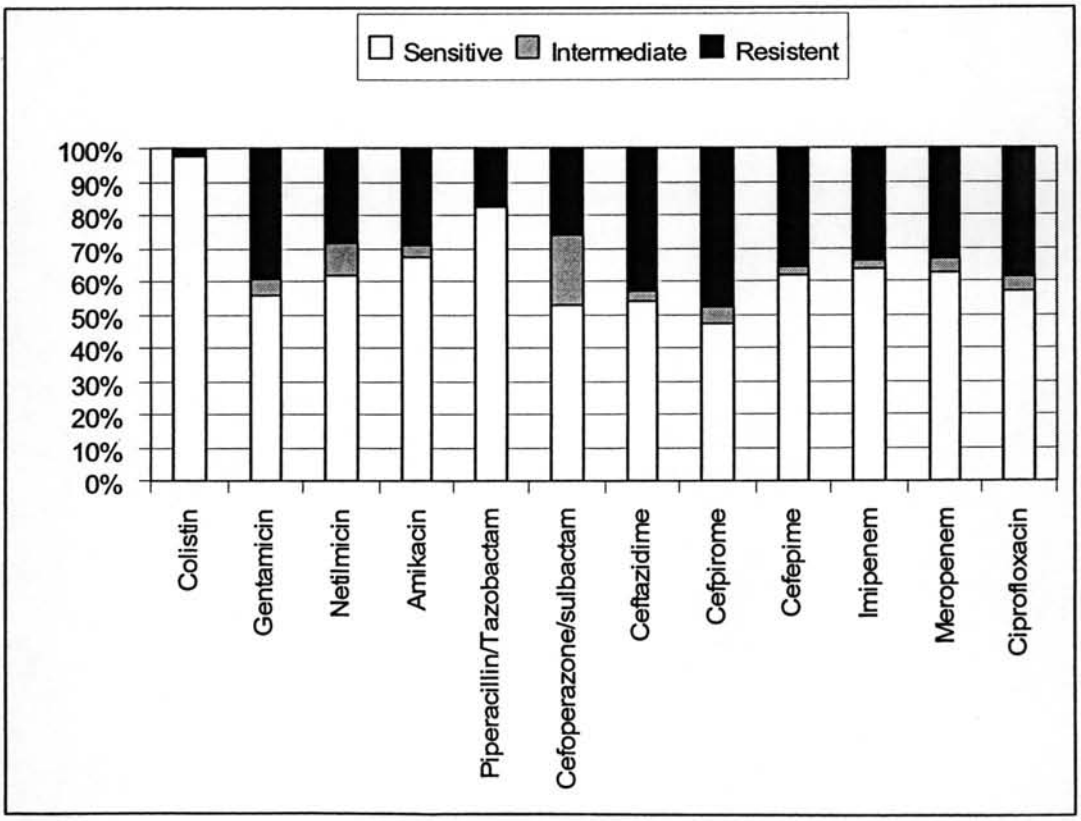


Table 30 MIC distribution for cefoperazone/sulbactam against *A.baumanni* and *P.aeruginosa* pulmonary isolates from Maharaj Nakorn Chiang Mai hospital. (E-test method)

MIC (μg/ml)	% of isolates susceptible at MIC of :	
	A.baumannii (n=215)	*P.aeruginosa* (n=149)
1	5.1	0.7
1.5	12.0	0
2	2.8	2.0
3	0.9	26.8
3.5	0	1.3
4	1.4	14.1
6	0.5	2.7
8	12.0	2.7
12	19.4	10.1
16	3.7	10.1
24	6.9	4.7
32	12.0	4.7
48	8.8	2.0
64	8.3	3.4
96	3.2	1.3
192	0.5	1.3
256	2.3	12.1

Figure 14 Cefoperazone/sulbactam MIC distribution for *A.baumannii* at Maharaj Nakorn Chiang Mai hospital.

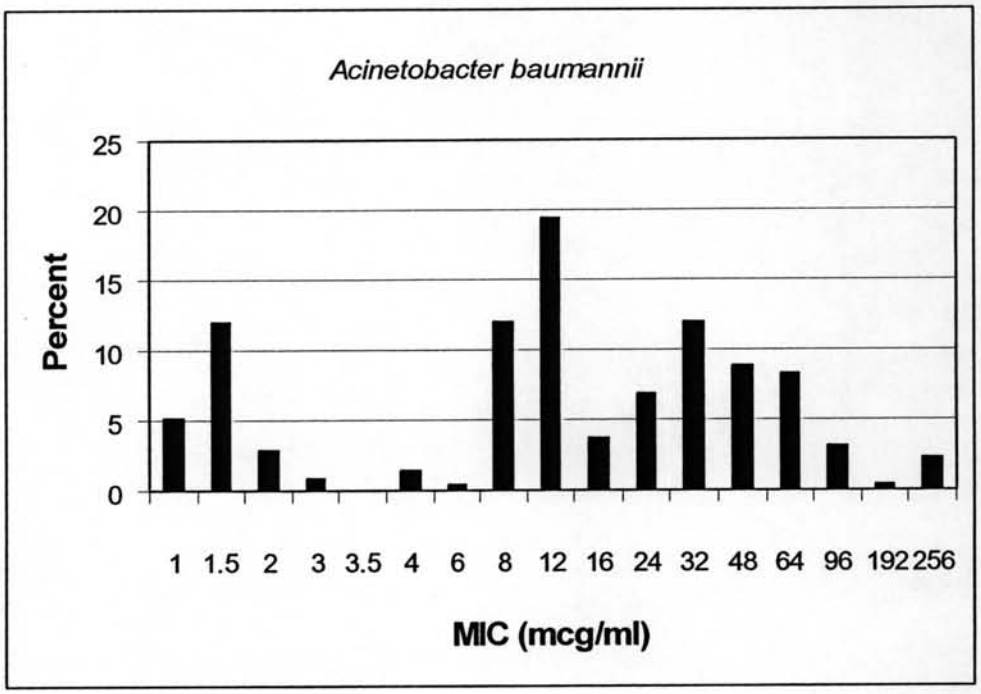
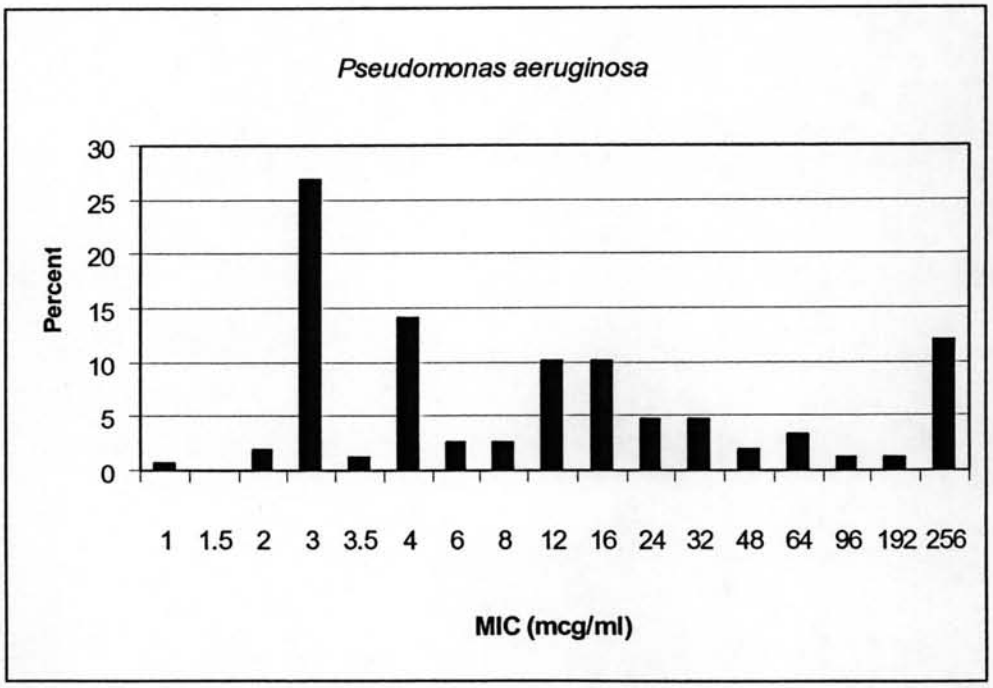


Figure 15 Cefoperazone/sulbactam MIC distribution for *P.aeruginosa* at Maharaj Nakorn Chiang Mai hospital.



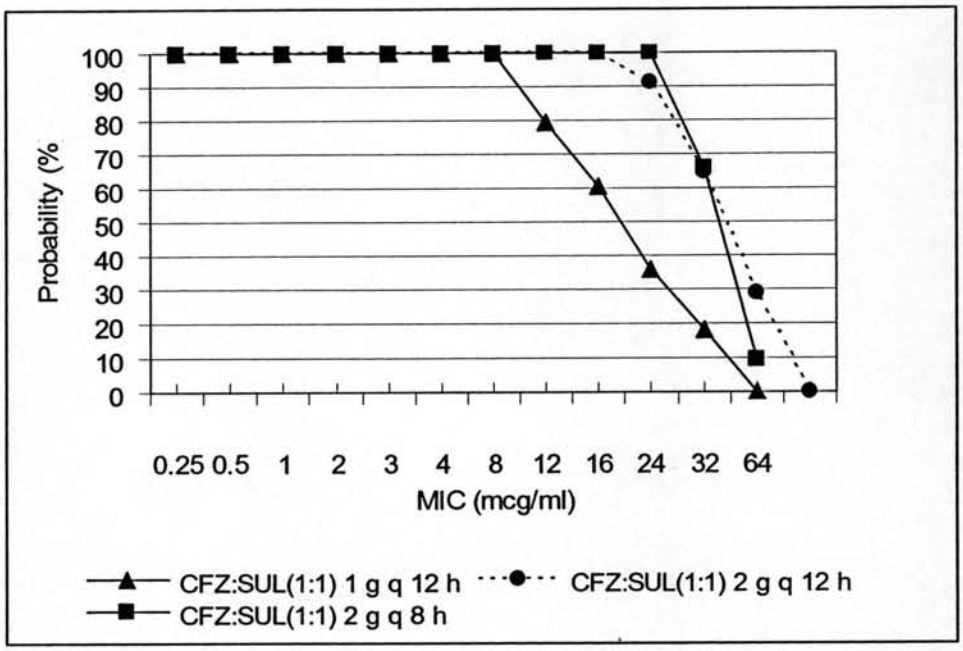
6. Monte Carlo simulations

A 5,000-patient Monte Carlo simulation was conducted to calculated estimates of %T>MIC for each cefoperazone/sulbactam regimen against *A.baumannii* and *P.aeruginosa* using a formula equation 5. The %T>MIC was calculated according to an intravenous bolus model that permitted variation in the volume distribution and half life were assumed to follow log-normal probability distribution during simulations. Probability of achieving pharmacodynamic target (PTA) or Cumulative fraction of response (CFR) was calculated over the MIC distributions using weighted summation as described by Drusano et al.[115] Bactericidal pharmacodynamic targets of cefoperazone/sulbactam were defined as 50% T>MIC[116]. A regimen that achieved >90% CFR against a population of organism was considered optimal for empirical treatment.[117]

6.1 CFR of recommended dosage regimens: According to total drug concentration

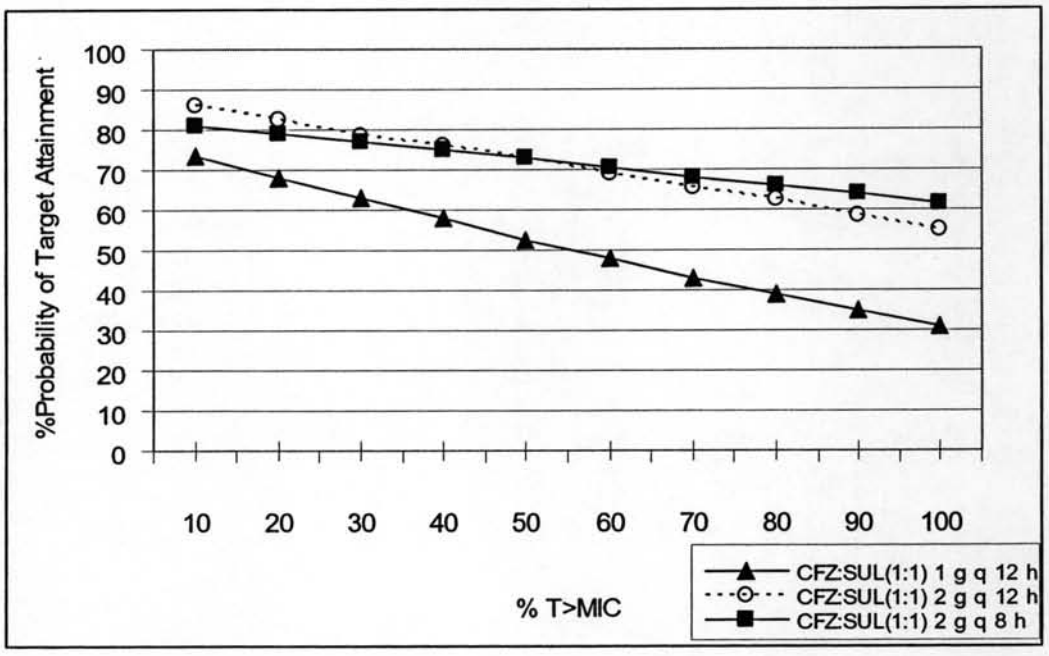
Figure 16 shows the probability of attaining target for each cefoperazone/sulbactm dosing regimens at 50% T>MIC as a function MIC. The highest MIC for which the target is attained by at least 90% of the simulated subjected is defined as PK-PD breakpoint. For doses of cefoperazone/sulbactam 1 g q 12 h the highest MIC was 10 μg/ml. Furthermore, both of the regimens 2 g q 12 h and 2 g q 8 h achieved greater than 90% probability at MICs of 24 μg/ml. Considering that the susceptibility breakpoint for *A.baumannii*, and *P.aeruginosa* is <16 μg/ml. Doses of 2 g q 12 h and 2 g q 8 h achieve the highest attainment for all susceptible isolates.

Figure 16 Target attainment rate for 50% time above the minimum inhibitory concentration (MIC) for each cefoperazone/sulbactam dosage regimen at each MIC.

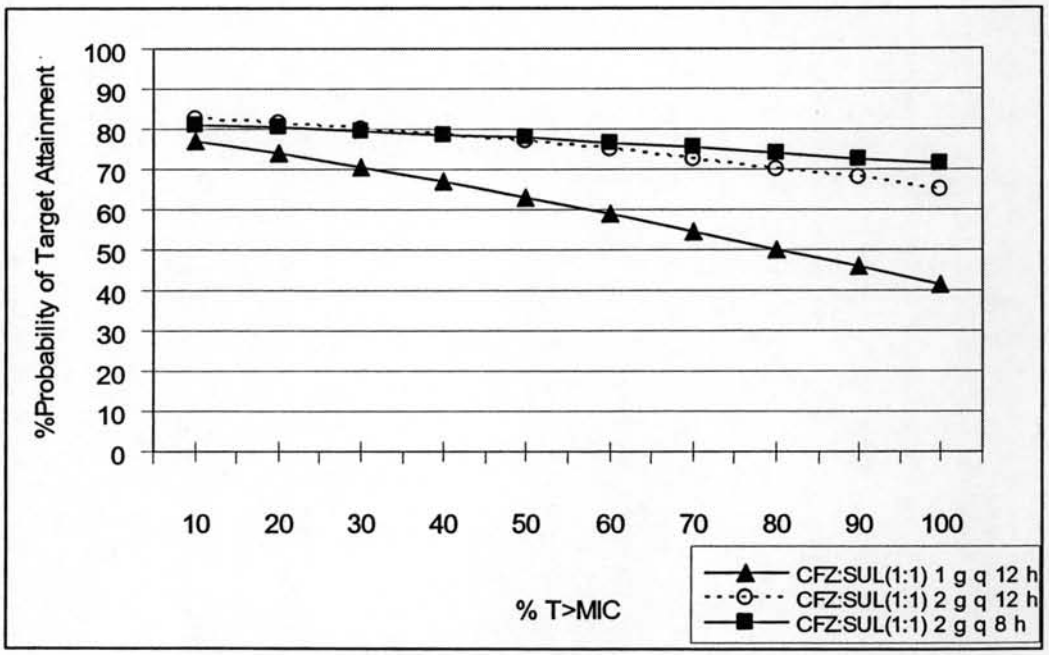


Figures 17(A) and 16(B) indicate the probability of target attainment for cefoperazone/sulbactam dosing regimens over the range of pharmacodynamic at T>MIC exposures (%T>MIC 10,20,30,40,50,60,70,80,90,100) against *A.baumannii* and *P.aeruginosa*, respectively. Target attainment for all of dosage regimens decreased as the targeted exposure was increased. This decrease was fastest for the cefoperazone/sulbactam (500mg/500mg) 1 g q 12 h against both *P.aeruginosa* and *A.baumannii*. The probability of target attainment at various T>MIC exposures were significant different between three dosing regimens (p=0.004 for *A.baumannii* and p=0.002 for *P.aeruginosa*). However, there were not significant different between cefoperazone/sulbactam 2 g q 12 h and 2 g q 8 h (p=0.825 for *A.baumannii* and p=0.431 for *P.aeruginosa*). For comparative purpose, bacteriocidal were defined as drug concentration above the MIC for $\geq$50% of the dosing interval for cefoperazone. Regimens that had a $\geq$90% likelihood of achieving target exposures were considered optimal. The results are shown in Table 31.

Figure 17 Probability of target attainment at various %T>MIC for recommended cefoperazone/sulbactam dosage regimens against *A.baumannii* (A) and *P.aeruginosa* (B)



(A)



(B)

Table 31 Cumulative fraction of response for recommended cefoperazone/sulbactam dosage regimens against *A.baumanni* and *P.aeruginosa*

Dosage regimen	Probability of achieving pharmacodynamic target (%) [a]	
	A.baumannii	*P.aeruginosa*
Cefoperazone/sulbactam (500mg/500mg) 1 g I.V. q 12 h (500 mg cefoperazone)	52.58	63.06
Cefoperazone/sulbactam (500mg/500mg) 2 g I.V. q 12 h (1000 mg cefoperazone)	72.76	76.84
Cefoperazone/sulbactam (500mg/500mg) 2 g I.V. q 8 h (1000 mg cefoperazone)	72.84	77.78

[a] Pharmacodynamic target (%T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

As shown in Table 31, there are no regimens were able to attain 90% CFR against this population of *A.baumannii* and *P.aeruginosa*. The greatest CFRs were attained by cefoperazone/sulbactam (500mg/500mg) 2 g I.V. q 8 h and 2 g I.V. q 12 h, 72.84% and 72.76 CFR, respectively for *A.baumannii* and 77.78 and 76.84% CFR, respectively for *P.aeruginosa*. This study would support limiting the use of cefoperazone/sulbactam 1 g (500 mg) I.V. q 12 h to HAP patients as empirical treatment, and the CFR could be improve by increasing the dose 2 g (1000mg cefoperazone) I.V. q 12 h to 2 g (1000mg cefoperazone) I.V. q 8 h. The simulations in this study demonstrated that the recommended; cefoperazone/sulbactam (500mg/500mg) 2 g q 12 h and 2 g q 8 h dosage regimens provide a high probability target attainment. However, there were not able to attain 90% CFR. Therefore, cefoperazone/sulbactam in recommended dosing regimens should not be a single empirical drug treatment for *A.baumannii* and *P.aeruginosa* infection in our hospital, clearly indicating the need for combination therapy when empirically treating in this pathogen. According to ATS and IDSA guidelines for patients with HAP, the guidelines cite several risk factors for multi-drug resistant (MDR) pathogens, including prior antimicrobial therapy, extended hospitalization, and a high frequency of resistance in the community and immunosuppressive

disease or therapy. For treatment of patients with HAP plus risk factors for MDR pathogens, the guidelines recommended an antipseudomonal β-lactam, or β-lactam/β-lactamase inhibitor, plus an antipseudomonal fluoroquinolone or aminoglycoside[23]. Unfortunately, the fluoroquinolone and aminoglycoside in our hospital were susceptible to *P.aeruginosa* approximately 50% and *A.baumannii* lesser than 30%.

CFR of alternative dosage regimens.

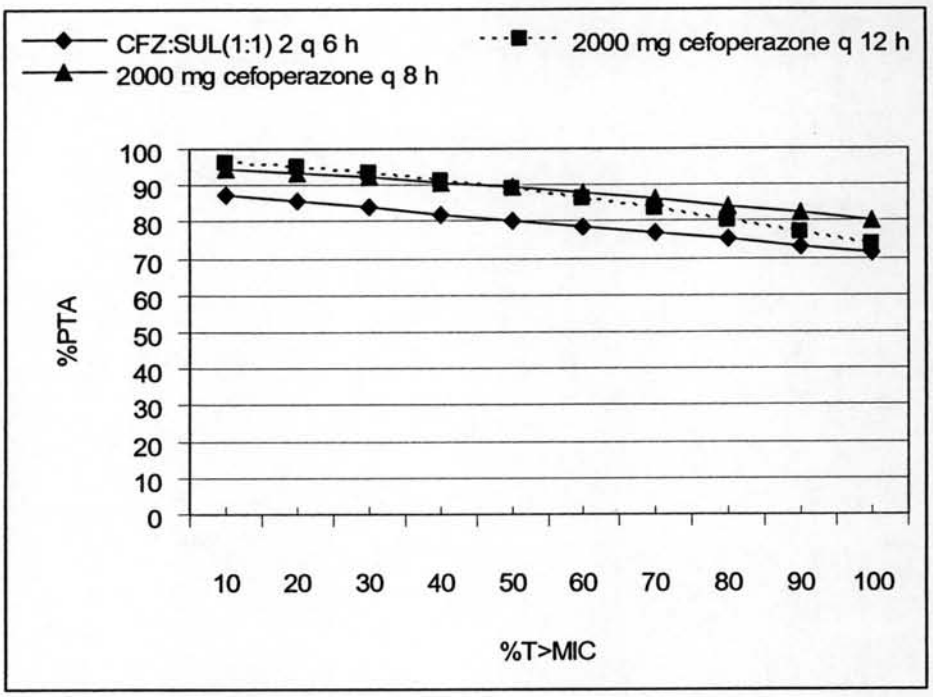
Alternative dosage regimens were simulated from pharmacokinetic parameters in dosage regimens of 1000 mg cefoperazone every 12 h and 1000 mg cefoperazone every 8 h. Alternative dosage regimens were simulated by increasing the dose or dosing interval while maintaining the IV bolus injection. When the cefoperazone/sulbactam was increased to 1000 mg of cefoperazone injection every 6 h, 2000 mg of cefoperazone injection every 12 h and every 8 h, the bactericidal CFR increased to 80.4, 88.88, and 89.69%, respectively for *A.baumannii* and 76.88, 82.78, and 83.16%, respectively for *P.aeruginosa* organism. The results are shown in Table 32 and Figure 18. Although the bactericidal were increased, but there were no significant different between the recommended dosing regimens (cefoperazone/sulbactam (500mg/500mg) 2 g q 12 h and 2 g q 8 h) and the alternative dosing regimen ($p<0.05$).

Table 32 Cumulative fraction of response for alternative cefoperazone/sulbactam dosage regimens against *A.baumanni* and *P.aeruginosa*

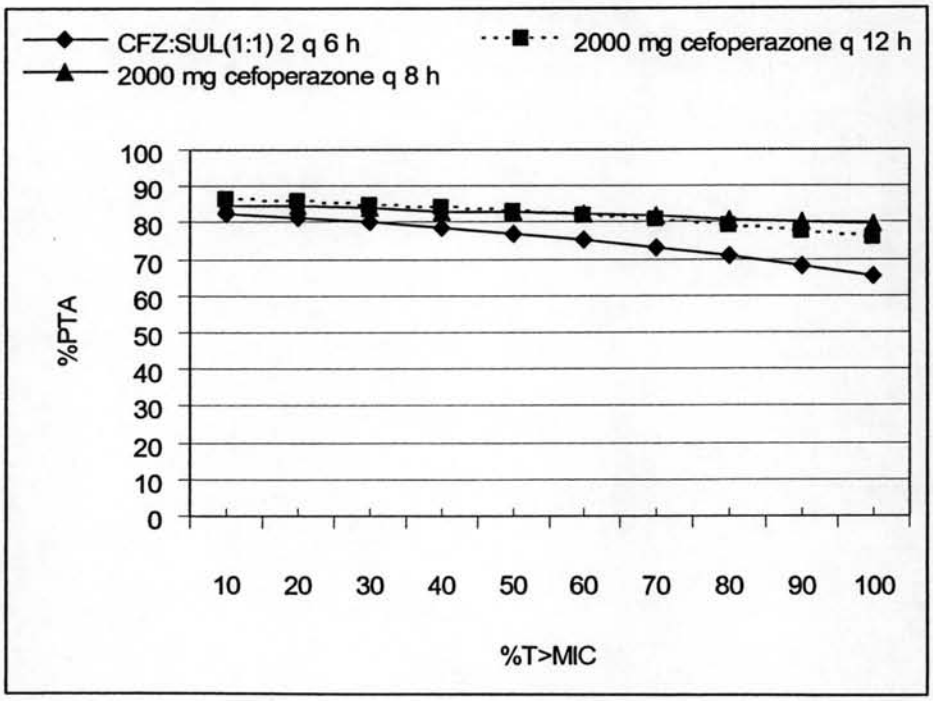
Dosage regimen	Probability of achieving pharmacodynamic target (%)[a]	
	A.baumannii	*P.aeruginosa*
Cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 6 h (1000mg cefoperazone/dose)	80.4	76.88
cefoperazone/sulbactam 2000 mg cefoperazone q 12 h	88.88	82.78
cefoperazone/sulbactam 2000 mg cefoperazone q 8 h	89.68	83.16

[a] Pharmacodynamic target (%T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

Figure 18 Probability of target attainment at various %T>MIC for alternative cefoperazone/sulbactam dosage regimens against *A.baumannii* (A) and *P.aeruginosa* (B)



(A)



(B)

6.3 Targeted therapy

In clinical practice, cefoperazone/sulbactam is frequently used as empiric therapy for patient with suspected hospital-acquired pneumonia. However, physician will consider continuing or changing cefoperazone/sulbactam treatment when the susceptibility for cefoperazone/sulbactam data is reported. As the results, in our trial was also simulated by using MIC distribution data when susceptible were reported. For *P.aeruginosa* and *A.baumannii*, the MIC of susceptible strain ranged from 1.0-16.0 μg/ml.

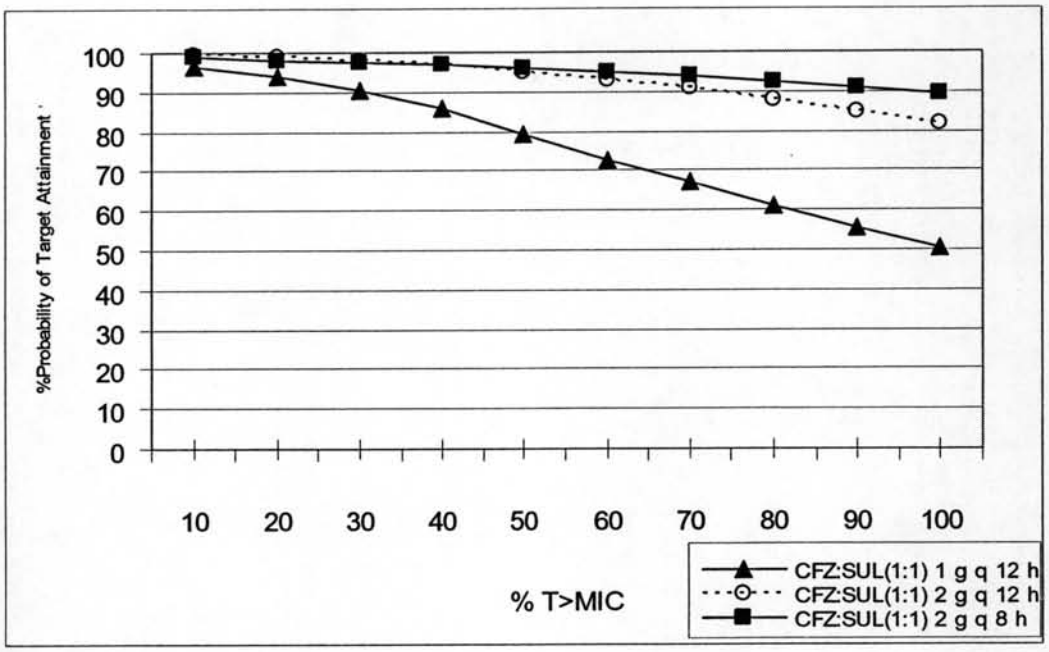
The CFRs for each recommended dosage regimen against susceptible *P.aeruginosa* and *A.baumannii* which were ranged from MIC 1.0-16.0 μg/ml are shown in Table 33 and Figure 19. Cefoperazone/sulbactam (500/500 mg) 2 g q 12 h and 2 g q 8 h were able to achieve > 90% CFRs against *A.baumannii* (94.98 and 99.0%, respectively) and For *P.aeruginosa*, all of regimens were able to achieve > 90% CFR (93.06-99.34%). As a result, these regimens should be optimal for treatment when susceptible was reported.

Table 33 Cumulative fraction of response for recommended cefoperazone/sulbactam dosage regimens against *A.baumanni* and *P.aeruginosa* : Using MIC susceptible distribution

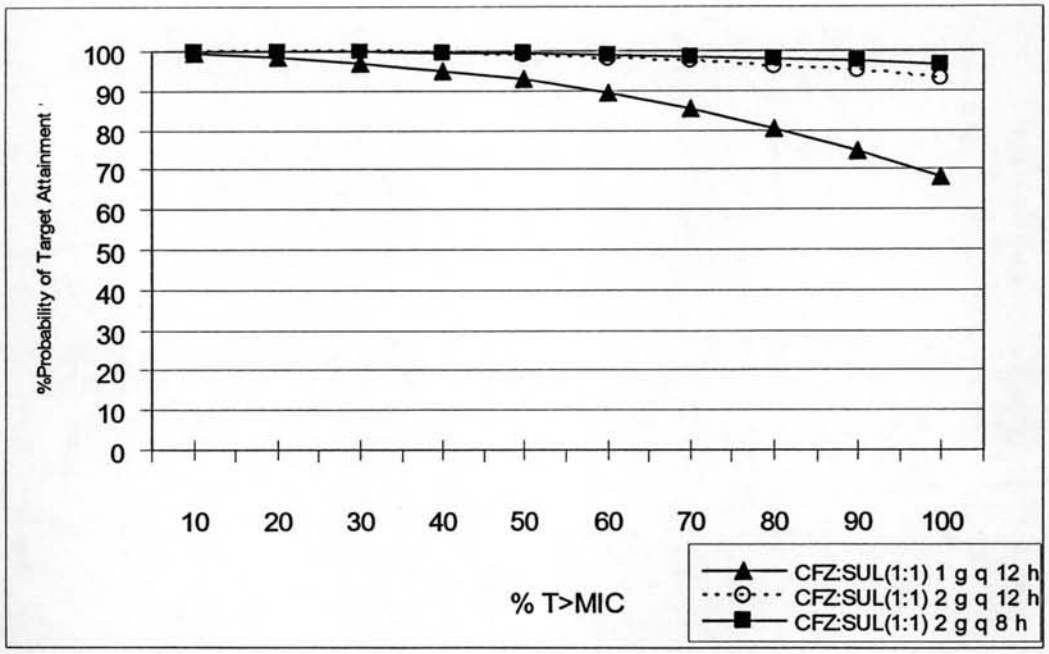
Dosage regimen	Probability of achieving pharmacodynamic target (%)[a]	
	A.baumannii	*P.aeruginosa*
cefoperazone/sulbactam (500/500 mg) 1 g I.V. q 12 h (500 mg cefoperazone)	79.46	93.06
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 12 h (1000 mg cefoperazone)	94.98	99.0
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 8 h (1000 mg cefoperazone)	95.94	99.34

[a] Pharmacodynamic target (%T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

Figure 19 Probability of target attainment at various %T>MIC for recommended cefoperazone/sulbactam dosage regimens against *A.baumannii* (A) and *P.aeruginosa* (B): Using MIC susceptible distribution.



(A)



(B)

6.4 CFR of recommended dosing regimen: According to free drug concentration.

Although total drug concentrations in blood or plasma are often used as a guide to dosage adjustment or evaluation, free (unbound) drug concentrations in blood are more closely related to drug effect. For the β-lactams, in vitro and animal studies have demonstrated that the amount of time in which the free or unbound protein drug concentration exceeds the MICs (*f*T>MIC) is the best predictor of bacterial and microbiologic response. Unfortunately, this study was not determining free cefoperazone levels because it is more complicate and difficult. However, If a total drug concentration measurement is available, the unbound concentration can be estimated by determining the direction and probable degree of alteration in the patient's unbound plasma fraction (as determined from the literature).

In this study, A 5,000-patient Monte Carlo simulation was also conducted to calculated estimates of % free T>MIC for each cefoperazone/sulbactam regimen against *A.baumannii* and *P.aeruginosa* using a formula equation 6. The %free T>MIC was calculated according to an intravenous bolus model that permitted variation in the volume distribution and half life were assumed to follow log-normal probability distribution during simulations. The fraction unbound drug was 0.1, and assumed to follow a uniform distribution. Probability of achieving pharmacodynamic target (PTA) or cumulative fraction of response (CFR) was calculated over the MIC distributions using weighted summation. Bactericidal pharmacodynamic targets of cefoperazone/sulbactam were also defined as 50% free T>MIC. A regimen that achieved >90% CFR against a population of organism was considered optimal for empirical treatment.

Target attainment rate of achieving 50% free time above MIC are listed in Table 34. There were very low CFR achieved the bactericidal target in any recommended dosage regimens. Cefoperazone/sulbactam (500/500 mg) 1 g q 12 h, 2 g q 12 h and 2 g q 8 h, the CFRs were 12.68, 20.64 and 20.72%, respectively for *A.baumannii*, and 4.24, 25.36, and 26.1%, respectively for *P.aeruginosa*. In summary, no recommended regimen was able to attain 90% CFR against this population of *A.baumannii* and *P.aeruginosa*.

Table 34 Cumulative fraction of response for recommended cefoperazone/sulbactam dosage regimens against *A.baumannii* and *P.aeruginosa* (%*f*T>MIC)

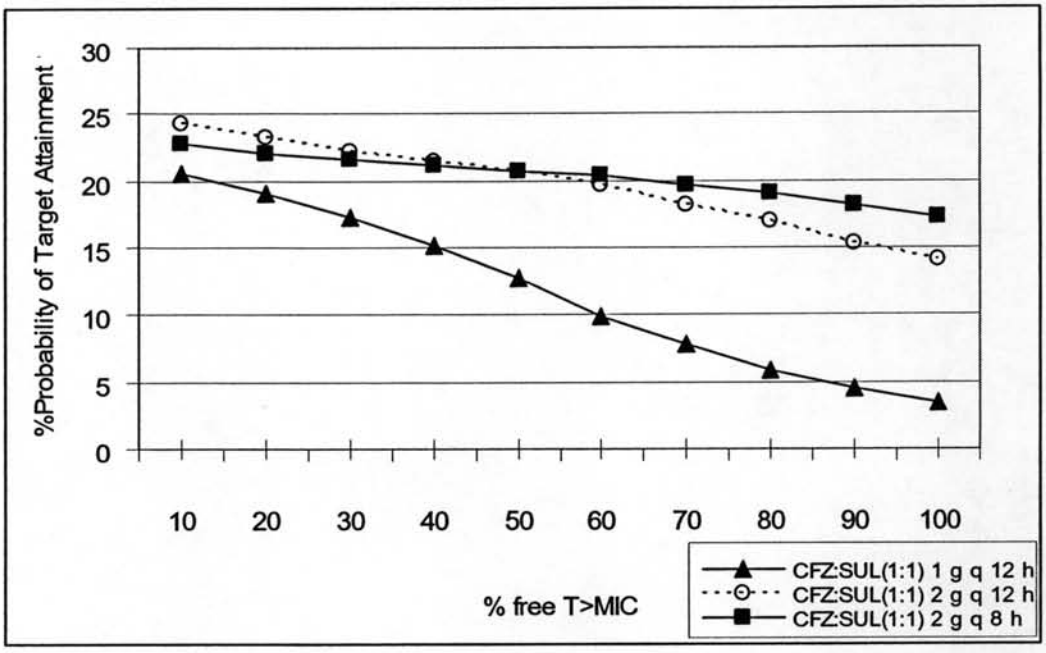
Dosage regimen	Probability of achieving pharmacodynamic target (%)[a]	
	A.baumannii	*P.aeruginosa*
cefoperazone/sulbactam (500/500 mg) 1 g I.V. q 12 h (500 mg cefoperazone)	12.68	4.24
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 12 h (1000 mg cefoperazone)	20.64	25.36
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 8 h (1000 mg cefoperazone)	20.72	26.1

[a] Pharmacodynamic target (free %T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

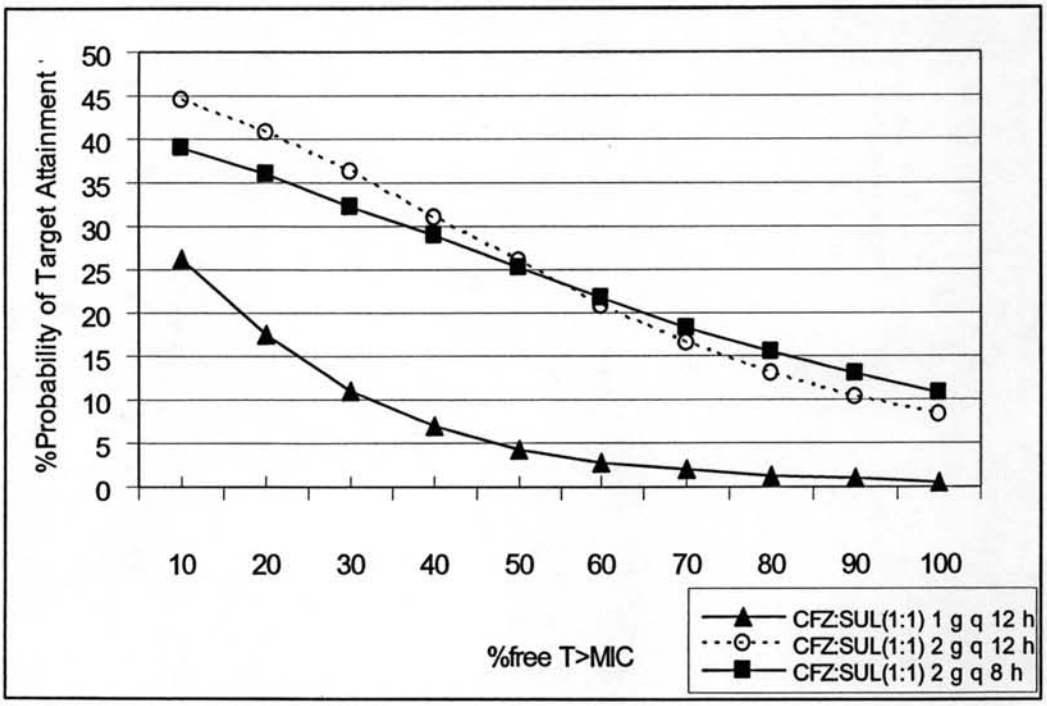
Figures 19 (A) and (B) indicate the probability of target attainment for cefoperazone/sulbactam dosing regimens over the range of pharmacodynamic at T>MIC exposures (%T>MIC 10,20,30,40,50,60,70,80,90,100) against *A.baumannii* and *P.aeruginosa*, respectively. Target attainment for all of dosage regimens decreased as the targeted exposure was increased. This decrease was fastest for the cefoperazone/sulbactam (500mg/500mg) 1 g q 12 h against both *P.aeruginosa* and *A.baumannii*.

As a results in Table 34 and Figure 20, According to 50% free T>MIC, the recommended dosing regimens presented a very low probability target attainment. It should not recommend being an empiric treatment for *A.baumannii* and *P.aeruginosa*, despite combined with the other antibiotics. However, in clinical practice, we found that cefoperazone/sulbactam in these recommended dosing regimen had widely used and effective in treating nosocomial pneumonia.[47,]

Figure 20 Probability of target attainment at various %*f*T>MIC for recommended cefoperazone/sulbactam dosage regimens against *A.baumannii* (A) and *P.aeruginosa* (B)



(A)



(B)

Because the recommended regimens provide a very low of CFR, alternative dosage regimens were also simulated from pharmacokinetic parameters in dosage regimens of 1000

mg cefoperazone every 12 h and 1000 mg cefoperazone every 8 h. Alternative dosage regimens were simulated by increasing the dose or dosing interval while maintaining the IV bolus injection. For cefoperazone/sulbactam 1000 mg every 6 h, the bactericidal CFR was 22.3, and 26.26% for *A.baumannii*, and *P.aeruginosa*, respectively. When cefoperazone was increased to 2000 mg of cefoperazone injection every 12 h and every 8 h, the bactericidal CFR increased to 45.7% and 46.5%, respectively for *P.aeruginosa* organism, and slightly increasing to 27.98 and 29.13%, respectively for *A.baumannii* organism.(Table 35). Alternative dosage regimens did not significantly increase target attainment. Therefore, no cefoperazone/sulbactam regimens achieved sufficiently high enough attainment against the *A.baumannii* and *P.aeruginosa* to warrant its use empirically as monotherapy.

Table 35 Cumulative fraction of response for alternative cefoperazone/sulbactam dosage regimens against *A.baumannii* and *P.aeruginosa*. (%*f*T>MIC)

Dosage regimen	Probability of achieving pharmacodynamic target (%)[a]	
	A.baumannii	*P.aeruginosa*
Cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 6 h (1000mg cefoperazone/dose)	22.3	26.26
Cefoperazone/sulbactam 2000 mg cefoperazone q 12 h	27.98	45.7
Cefoperazone/sulbactam 2000 mg cefoperazone q 8 h	29.14	46.5

[a] Pharmacodynamic target (free %T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

Consequently, when antimicrobial sensitivity data for *P.aeruginosa* and *A.baumannii* are unavailable and monotherapy with cefoperazone/sulbactam is selected, higher dosage regimen should be used for empirical treatment. However, these infections often are treated with at least two agents because addition of a second agent often lowers the MIC of cefoperazone/sulbactam by synergism.[118] Therefore %*f*T>MIC of cefoperazone/sulbactam will be increased by combination therapy in clinical practice.

The CFRs for each recommended and alternative dosage regimen also simulated against susceptible *P.aeruginosa* and *A.baumannii* which were ranged from MIC 1.0-16.0

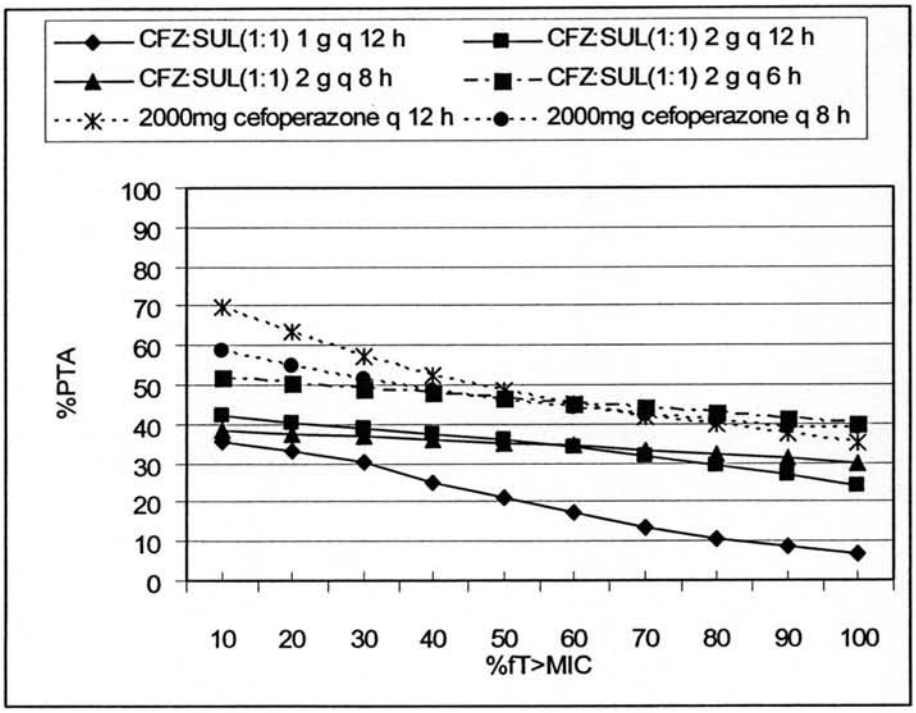
μg/ml. For recommended regimens, all of dosages regimens could not achieve 90% CFRs. However, cefoperazone/sulbactam (500mg/500mg) 2 g q 8 h was superior to the other regimens against *A.baumannii* and *P.aeruginosa* (35.24% and 45.38%, respectively). This decrease was fastest for the cefoperazone/sulbactam (500mg/500mg) 1 g q 12 h against both *P.aeruginosa* and *A.baumannii* and achieved very low CFR in any reasons of treatment. For alternative regimens, both of 2000 mg cefoperazone I.V. every 12 and 8 h regimens were achieved high CFRs against *P.aeruginosa* (80.32% and 81.36%), which was considered an optimal regimen for treatment susceptible *P.aeruginosa* in our hospital as a monotherapy treatment. However there were also achieved higher CFR against *A.baumannii* than the other cefoperazone/sulbactam regimens but should not be recommended as a single therapy. The results are shown in Table 36 and Figure 21.

Table 36 Cumulative fraction of response for alternative cefoperazone/sulbactam dosage regimens against *A.baumannii* and *P.aeruginosa* (%*f*T>MIC) : Using MIC susceptible

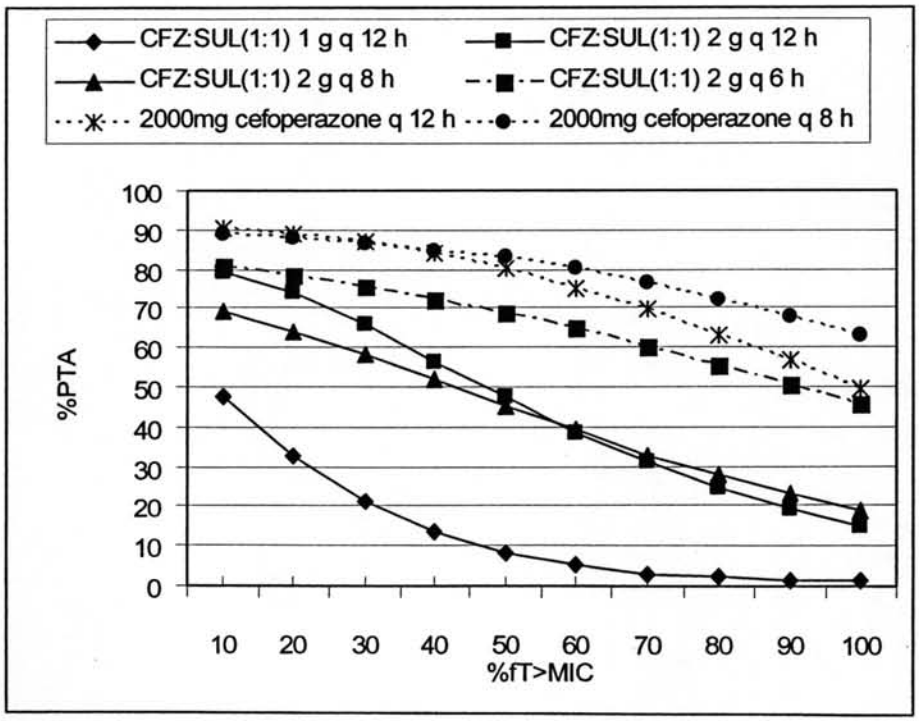
Dosage regimen	Probability of achieving pharmacodynamic target (%)[a]	
	A.baumannii	*P.aeruginosa*
cefoperazone/sulbactam (500/500 mg) 1 g I.V. q 12 h (500 mg cefoperazone)	21.1	8.24
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 12 h (1000 mg cefoperazone)	35.86	47.78
cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 8 h (1000 mg cefoperazone)	35.24	45.38
Cefoperazone/sulbactam (500/500 mg) 2 g I.V. q 6 h (1000mg cefoperazone/dose)	46.78	68.6
cefoperazone/sulbactam 2000 mg cefoperazone q 12 h	48.38	80.32
cefoperazone/sulbactam 2000 mg cefoperazone q 8 h	45.92	81.36

[a] Pharmacodynamic target (free %T>MIC), bacteriocidal exposure for cefoperazone/sulbactam defined as 50%T>MIC

Figure 21 Probability of target attainment at various %*f*T>MIC for alternative cefoperazone/sulbactam dosage regimens against *A.baumannii* (A) and *P.aeruginosa* (B): Using susceptible MIC distribution



(A)



(B)

7. Clinical outcome

28 cases of hospital-acquired pneumonia patients were identified. 26 cases (93.1%) had *A.baumannii* infection and 2 cases (6.9%) had both of *P.aeruginosa* and *A.baumannii* infection. The characteristics of the 28 cases of HAP studied were presented in Table 35. The average age was 61.11(±15.91) years. The male to female ratio was 20:8. Seven patients who were admitted to general internal medical ward and twenty-one patients were admitted to medicine sub ICU and medicine ICU ward. 60.7 percent (17/28) of patients had cerebrovascular disease. 32.1% (9/28) of patients had cardiovascular disease. 28.6% (8/28) of patients had chronic lung disease. Most of patients, 26 of 28 patients had mechanical ventilator. 13 patients had co-infection with HAP, which were urinary tract infection and septicemia 20.68 % and 13.79%, respectively. Cefoperazone/sulbactam was given on average 11.38 days (±3.58 days). Antibiotic pharmacokinetic parameters, MIC data and pharmacodynamic indices are summarized in Table 37. Treatment failure was documented in 32.1% (9/28). Clinical cure and improvement was achieved in 67.8% (19/28). Antibiotic combinations were documented in 60.7% (17/28). Combination regimens consisted of cefoperazone/sulbactam plus colistin (3/28), ciprofloxacin (6/28), netilmicin (1/28), meropenem (2/28) or vancomycin (5/28). All eligible patients were assessed for a clinical and microbiological response. All evaluations were performed on day 1, day 3, day 7 or the end of cefoperazone-sulbactam treatment.

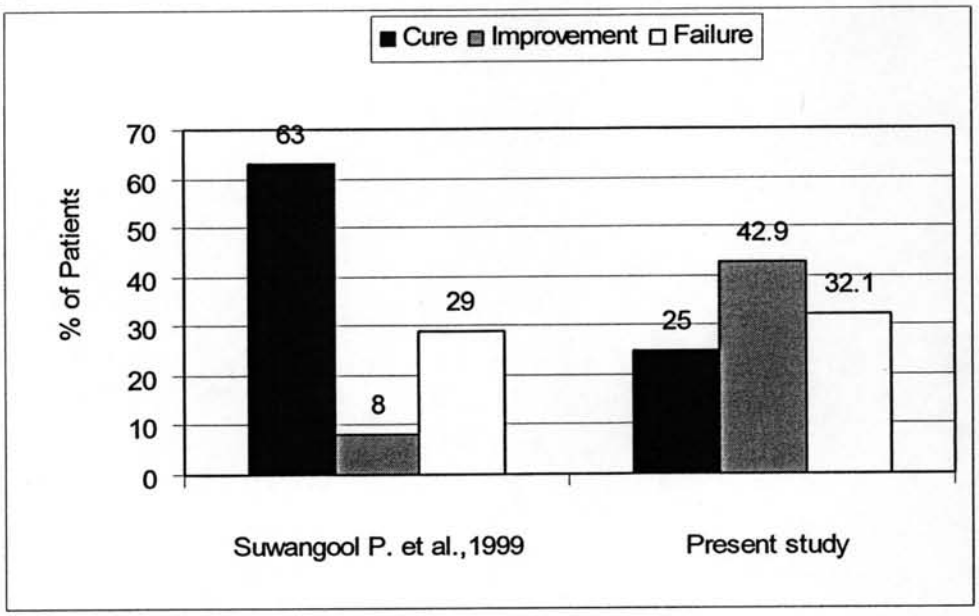
At the end of treatment, clinical cure was note in 7 (25.0%), improvement in 12 (42.9%), 9 patients (32.1%) had clinical failure. During the treatment, Microbiology outcome, microbiological eradication was note in 13 (46.43%) of the patient, while 11 (39.28%) of the patient had the organism persistence in their sputum, however, these patients had either clinical improvement or clinical failure. 4 patients (14.29%) had the new infection organism or superinfection with *P.aeruginosa, K.pneumoniae,* MRSA. In 1999, Suwangool P et al[47]., performed a study in three hospital in Thailand to assess the activity of cefoperazone/sulbactam treatment in 24 patients with nosocomial pneumonia showed the similar results. The most common causative agent was *P.aeruginosa* (37.5% of cases), followed by *K.pneumoniae* and *A.baumannii* (16.7% each). The patients were treated with cefoperazone/sulbactam 1-2 g twice daily for mean duration of 13 days. The results of therapy were encouraging, with response being seen in 71% of patients (63% cure, 8% improvement) The microbiologic response showed eradication 67%, persistence in 29% and superinfection in only one patient. Our study, showed the lower success rate of treatment compared to those study. It may explain by the drug resistance subsequently has become a problem after a number of year of use. The result are shown in Figure 22

Table 37 Patient demographics for 28 cases of *A.buamanni* and *P.aeruginosa* hospital-acquired pneumonia

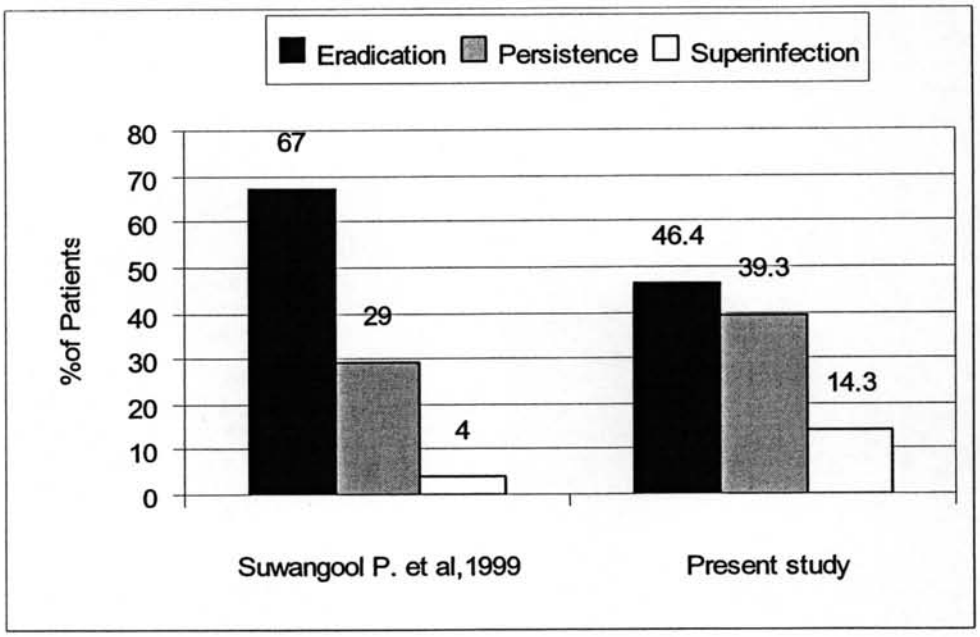
Characteristic	Mean ± S.D. or No. (%) patients
Age (years)	61.11±15.91 (17-82)
Gender (male)	20 (69%)
Weight (kg)	53.13±74.15
Ward of admission	
General medical ward	7(24.1%)
Medical sub ICU or ICU ward	21(62.1%)
Co-morbidity	
Cardiovascular disease	10(35.7%)
Cerebrovascular disease	9(32.1%)
Chronic lung disease	8(28.6%)
Diabetes	2(7.1%)
Malignancy	2(7.1%)
Co-infection	
Urinary tract infection	6(20.68%)
Septicemia	4(13.79%)
Vascular catheter/skin infection	4(13.79%)
Antibiotic combination	
Single antibiotic	11(39.3%)
Co-administration	17(60.7%)
Inotropes	3(10.3%)
Mechanical ventilation	26(92.6%)
Duration of cefoperazone/sulbactam treatment (days)	11.38(±3.58)
CPIS score (day 1)	6.83(±1.39)

Figure 22 Efficacy of cefoperazone/sulbactam in the treatment of nosocomial pneumonia.

(A) Clinical outcome (B) Microbiology outcome



(A)



(B)

Table 38 Antibiotic pharmacokinetic parameters and pharmacodynamic indices in patients with *A.baumannii* and *P.aeruginosa* hospital acquired pneumonia.

Pt.	regimen	Vd (L)	T1/2 (h)	MIC (μg/ml)	50% fT>MIC	50% T>MIC	Clinical outcome	Microbiological outcome
1	2 g q 12h	16.0	7.73	16	N	Y	Improve	Eradicate
2	2 g q 12h	15.25	10.82	24	N	Y	Failure	New infection
3	2 g q 12h	16.44	4.91	12	N	Y	Cure	Eradicate
4	2 g q 12h	21.40	5.29	16	N	Y	Cure	Eradicate
5	2 g q 8h	19.47	7.00	16	N	Y	Cure	Persistent
6	2 g q 12h	13.88	4.45	12	N	Y	Cure	Eradicate
7	1 g q 12h	9.18	4.14	32	N	N	Failure	New infection
8	1 g q 12h	5.19	2.44	64	N	N	Failure	Eradicate
9	2 g q 12h	17.04	6.02	16	N	Y	Improve	New infection
10	2 g q 12h	19.13	4.35	16	N	N	Cure	Persistent
11	2 g q 12h	15.67	6.78	32	N	Y	Improve	Eradicate
12	2 g q 8h	21.75	7.69	12	N	Y	Failure	Eradicate
13	2 g q 12h	13.54	6.52	32	N	Y	Improve	Eradicate
14	1 g q 12h	15.46	6.08	12	N	Y	Improve	Persistent
15	2 g q 12h	9.12	3.61	32	N	N	Failure	Persistent
16	2 g q 12h	10.71	4.23	16/48	N	N	Improve	Persistent
17	2 g q 12h	11.81	5.23	12/16	N	Y	Improve	Persistent
18	2 g q 12h	12.59	12.99	4	Y	Y	Improve	Eradicate
19	2 g q 12h	11.50	7.51	24	N	Y	Failure	Persistent
20	2 g q 12h	15.41	5.50	24	N	Y	Improve	Persistent
21	2 g q 8h	15.07	11.46	24	N	Y	Cure	Persistent
22	1 g q 12h	8.17	8.46	32	N	Y	Failure	New infection
23	1 g q 12h	14.18	4.06	32	N	N	Improve	Persistent
24	2 g q 12h	32.32	5.01	16	N	Y	Cure	Eradicate
25	2 g q 8h	27.51	9.21	48	N	N	Failure	Persistent
26	2 g q 12h	15.04	8.01	32	N	Y	Failure	Persistent
27	2 g q 12h	20.07	10.59	12	N	Y	Improve	Eradicate
28	2 g q 8h	11.07	3.25	8	N	Y	Improve	Eradicate

Y as achieved pharmacodynamic target at 50%, N as not achieved pharmacodynamic target at 50%

In chi-square analyses in Table 37, 50% of total cefoperazone/sulbactam concentration exceed the MIC (50% T>MIC) and age less than 60 year old were significantly associated with clinical response (p=0.041, (OR= 6.8) and p= 0.01 (OR=13.71), respectively). Meanwhile, the 50% free T>MIC was not significantly associated with clinical response. However many previous studies, demonstrated that free T>MIC is the best predictor for outcome of β-lactams treatment. It may be explained by drugs that are highly bound in plasma, as cefoperazone, are most likely to show wide variations among patients in the unbound plasma fraction and it is recognized that protein binding is a rapid process that produces a reversible interaction between antibiotic and protein. In HAP patients have many condition associated with altered protein binding, such as, elderly patients, hypoalbuminemia, stress, infection, etc. Thus, in more complex cases, effect of protein binding depends upon the extent of plasma protein binding and the relative affinity to plasma protein and bacterial receptor sites. The free fraction unbound as 0.1 may not suitable to use directly in the equation for % free T>MIC. This may also explain why ceftriaxone (95% protein bound) provides activity at below its MIC, even though failure would be predicted. The effect of protein binding continues to be more investigation for the pharmacodynamics of β-lactams. Although 50% of total cefopeazone/sulbactam above MIC was associated clinical response, cefoperazone may be requires a larger T>MIC when calculations are based on the total drug concentration because it has a highly protein binding.

In our study, combination of antibiotics was given to the major of patients. There were no difference in treatment outcome between patients who received monotherapy and those who were given a combination of antibiotics. According to ATS and IDSA guidelines for treatment of patients with HAP plus risk factors for MDR pathogens, the guidelines recommended an antipseudomonal β-lactam, or β-lactam/β-lactamase inhibitor, plus an antipseudomonal fluoroquinolone or aminoglycoside. However, given that in Thailand, the fluoroquinolone and aminoglycoside in our hospital were susceptible to *P.aeruginosa* approximately 50% and *A.baumannii* lesser than 30% that may explained why combination therapy in our study have not observed significant advantage with the use of multiple antibiotic

Table 39 Chi-square analyses of factor associated with clinical response in patients with *A.baumannii* and *P.aeruginosa* hospital acquired pneumonia.

Variable	No.(%) patient, mean ± S.D.		
	Clinical cure/improvement (n=19)	Clinical failure (n=9)	*P* value
Age less than 60 years	12	1	0.010[a]
Chronic lung disease	4	4	0.686
Cardiovascular disease	7	3	0.856
Cerobrovascular disease	8	1	0.101
Diabetes mellitus	1	1	0.575
Malignancy	1	1	0.575
Co-infection	9	5	0.657
Inotropes	2	1	0.407
Mechanical ventilation	17	9	0.312
Use of combination antibiotics	11	6	0.657
Achieved 50% *f*T>MIC for cefoperazone/sulbactam	17	5	0.041[a]
Achieved 50%T>MIC for cefoperazone/sulbactam	0	1	0.483

[a] significantly difference $p < 0.05$