CHAPTER IV

RESULTS

1. Prevalence of *K. pneumoniae*

During the 6 - month of study period, among 22,278 clinical specimens from patients admitted at Siriraj Hospital, 75 (0.83%), 140 (2.94%) and 185 (2.21%), *K. pneumoniae* were isolated from blood, sputum, and urine respectively. Thirty - six (36 %) isolates were recovered from feces of 100 normal persons. The details of the results were shown in the Table 4.1. In addition, from the total 436 isolates obtained in this part of study, 17.2 % of the isolates were from blood, 32.1% from urine and 42.4 % from sputum as shown in the Table 4.2.1.

2. Detection of the suspicious extended spectrum β - lactamase (ESBL) producing *K. penumoniae* by initial screen test (NCCLS, 2000) (76)

All 436 *K. pneumoniae* isolates were examined for extended spectrum β - lactamase producing strains by initial screen test according to NCCLS, 2000. Hundred and twenty- three isolates were accepted as ESBL producing *K. pneumoniae*. Among them 26.7% were isolated from blood, 37.9 % were from sputum, and 27 % were from urine. None of them was isolated from feces of healthy adults. as shown in the Table 4.2.1 and 4.2.2, accordingly.

There were 123 suspicious ESBL producing isolates according to NCCLS criteria for detection using either one out of the three recommended cephalosporins.. The number of suspicious ESBL producing *K. pneumoniae* isolated from the clinical specimens in relation to the antimicrobial agents used in the test were shown in the Table 4.3.

The positive test for suspicious ESBL producing isolates tested by only one drug either ceftazidime or cefotaxime or ceftriaxone were observed in 11 isolates while the 8 isolates were recovered by only two drugs which included either ceftazidime and cefotaxime or ceftazidime and ceftriaxone or cefotaxime and ceftriaxone. 104 isolates were positive to all three drugs which included ceftazidime, cefotaxime and ceftriaxone. According to NCCLS, total 123 isolates were suspicious ESBL production.

3. Extended spectrum β - lactamase (ESBL) producing *K. penumoniae* phenotypic confirmatory test (NCCLS,2000) (76)

The 123 suspicious ESBL producing *K. pneumoniae* were confirmed for the ESBL producing isolates using the NCCLS phenotypic confirmatory test (The combination disk method). None of the 11 isolates which were screened by one antimicrobial agents for the suspicious ESBL producing *K. pneumoniae* were confirmed as ESBL producer and neither did the 8 isolates which were screened by

only two antimicrobial agents. In contrast, 100 out of 104 isolates were confirmed as ESBL producer. Therefore, the most concordant result was obtained between the initial screen test using all 3 antimicrobial agents and the phenotypic confirmatory test as shown in the Table 4.4. There was only 4 suspicious ESBL producing organisms by initial screen test that could not be confirmed by the phenotypic confirmatory test while the 11 and 8 isolates from the test using only one or two antimicrobial agents, respectively, were not confirmed as ESBL producing *K. pneumoniae.*

3. Confirmed Extended spectrum β - lactamase (ESBL) producing *K. penumoniae* from the E - test ESBL screen

Number of the confirmed extended spectrum β - lactamase producing *K. pneumoniae* detected by E - test ESBL screen as compared with the initial screen test were shown in the Table 4.5

The number of ESBL producing *K. pneumoniae* detected by E - test ESBL strip were not absolutely concordant with the number of suspicious for ESBL producing *K. pneumoniae* detected by initial screen test using less than three drugs. The results were partially agreed when the three drugs initial screen test was used. There were only 71 out of 104 suspicious ESBL producing *K pneumoniae* were confirmed by this method. In addition, the detection by E - test ESBL strip showed

the limitation in the isolates from sputum and urine that the MIC values were undetectable in 33 isolates as shown in the Table aIII6, aIII9 in the appendix. III

5. Percentage of the antimicrobial susceptibility of the extended spectrum β - lactamase producing *K. pneumoniae*

The percentage of the susceptibility of extended spectrum β - lactamase producing *K pneumoniae* isolates against 11 antimicrobial agents were tested. The MICs values were detected by E - test against ceftazidime , cefotaxime, ceftriaxone, cefuroxime , cefoxitin and imipenem. The agar disk diffusion method was used to determined the susceptibility of the other groups of antimicrobial agents. The result were shown in the Table 4.6 and 4.7.1, respectively. From the MICs values, only 5% of the ESBL producing isolates were susceptible to ceftazidime while 87% were still susceptible to cefotaxime, 79% to ceftriaxone, 30% to cefuroxime, 90% to cefoxitin and 100% were susceptible to imipenem. The results from the Kirby-Bauer paper-disk test, showed that 50% of the isolates were susceptible to gentamicin, 71% to amikacin, 16% to tobramycin, 67% to ciprofloxacin and 38% to trimethoprim-sulfamethoxazole. Additional result was obtained in the Table 4.7.2 which showed that *K. pneumoniae* isolated from feces (non ESBL producer) were all susceptible to all of tested antimicrobial agents.

6. Suggestive phenotypes of extended spectrum β - lactamase producing *K. pneumoniae* according to their activities

Three different groups of ESBL producing by *K. pneumoniae* isolates based on the MIC values of ceftazidime and cefotaxime were shown in the Table 4.8. The criteria for typing of the ESBL were recommended by Livermore, DM and Williams, JD (80) as shown in the Table aIV1 of the appendix IV. The details of the MICs of each isolates were also shown in the Table aIV3, aIV5, aIV7 in the appendix IV. There were as high as 32 isolates with the MICs of ceftazidime and cefotaxime were $\geq$ 32 μg/ml suggesting that such isolates produced "Broad" type activity. The other 13 isolates could possibly produced the "ceftazidimase" because they showed obviously high resistance against ceftazidime (MICs $\geq$128 μg/ml) but showed small reductions in susceptibility to cefotaxime (MICs $\leq$ 4 μg/ml). None of the isolates produced ESBL with marginal activity. The last group of isolates including 55 isolates produced ESBL with "undetermined" activity. There were 2 groups of isolates which were confused as undetermined activity type I (MIC of both CAZ and CTX were < 32 μg/ml) and type II (MIC of CAZ $\geq$ 32 μg/ml while CTX < 32 μg/ml). There were 5 isolates in undetermined activity type I and as many as 50 isolates with undetermined type II. Therefore, further study on the enzymes ' characteristics should be performed before more definite conclusions could be made.

7. Genotypes of extended spectrum β - lactamase producing *K. pneumoniae* by pulsed - field gel electrophoresis (PFGE)

All 100 extended spectrum β - lactamase producing *K. pneumoniae* were typed by PFGE as shown in the Table 4.9. There were as many as 86 different pulsotypes designated as type 1 to type 86 and 5 subtypes designated ; 3a, 8a, 9a, 9b, 10a accordingly. Almost all isolates were sporadic types because there were only 3 isolates at most that were in the same pulsotypes (pulsotype 1 and 2).

Table 4.1 Total number of *K. pneumoniae* isolates from various clinical specimens and from the feces of normal persons during August 2000 to January 2001

Month	Blood (9075)*	Sputum (4757)	Urine (8346)	Feces (100)
August	8	16	24	ND
September	26	18	21	18
October	10	22	28	7
November	16	34	27	11
December	7	26	37	ND
January	8	24	48	ND
Total	**75 (0.83%)**	**140 (2.94%)**	**185 (2.21%)**	**36 (36%)**

***( ) = Number of total specimens**

ND = Not done

Table 4.2.1 Number of suspicious extended spectrum β - lactamase (ESBL) producing *K. pneumoniae* * isolates from various clinical specimens based on NCCLS initial screen test

Specimen	No. of *Klebsiella pneumonaie* isolates (Total = 436)	Suspicious ESBL producing *Klebsiella pneumoniae* (%)
Blood	75 (17.2%)**	20 (26.7)***
Sputum	140 (32.1%)	53 (37.9)
Urine	185 (42.4 %)	50 (27.0)

* ESBL screening test using either one of three drugs ; ceftazidime, or cefotaxime or ceftriaxone

** Percentage from total 436 isolates

*** Percentage from the total *K. pneumoniae* isolated from each type of specimen

Table 4.2.2 Number of suspicious extended spectrum β - lactamase (ESBL) producing *K. pneumoniae* * isolates from feces of normal person based on NCCLS initial screen test

Specimen	No. of *Klebsiella pneumonaie* isolates (Total = 436)	Suspicious ESBL producing *Klebsiella pneumoniae* (%)
Feces	36 (36.0%)**	0

Table 4.3 Number of suspicious extended spectrum β - lactamase (ESBL) producing *K. pneumoniae* isolates from various clinical specimens in relation to the number of the antimicrobial agents tested

Specimen	Number of suspicious ESBL producing *Klebsiella pneumoniae*			
	One antimicrobial agent test	Two** antimicrobial agents test	All three*** Antimicrobial agents test	Total
Blood	4	2	14	20
Sputum	5	6	42	53
Urine	2	0	44	55
Total	**11**	**8**	**104**	**123**

* One agent : ceftazidime or cefotaxime or ceftriaxone

** Two agents : ceftazidime and cefotaxime

: ceftazidime and ceftriaxone

: cefotaxime and ceftriaxone

*** Three agents : ceftazidime and cefotaxime and ceftriaxone

Table 4.4 Number of extended spectrum β - lactamase (ESBL)* producing *K. pneumoniae* isolates from various clinical specimens determined by phenotypic confirmatory test as compared with the initial screen test

Specimen	Number of suspicious ESBL producing *K.pneumoniae*	Comparative number of ESBL producing isolated determined by 2 different tests		
		Initial screen by only one antimicrobial agent	Initial screen by two antimicrobial agetns	Initial screen by all three antimicrobial agents
Blood	20	0/4*	0/2	14/14
Sputum	53	0/5	0/6	42/42
Urine	50	0/2	0/0	44/48
Total	**123**	**0/11**	**0/8**	**100/104**

* = Number of ESBL producer determined by phenotypic confirmatory test / Number of positive test determine by initial screen test

Table 4.5 Number of extended spectrum β - lactamase producing *K. pneumoniae* isolates from various clinical specimens determined by E -test ESBL strips* and the initial screen test

Specimen	Number of suspicious ESBL producing *K.pneumoniae*	Comparative number of ESBL producing isolated determined by 2 different tests		
		Initial screen by only one antimicrobial agent	Initial screen by two antimicrobial agetns	Initial screen by all three antimicrobial agents
Blood	20	0/4**	0/2	14/14
Sputum	53	0/5	0/6	33/42
Urine	50	0/2	0/0	24/48
Total	**123**	**0/11**	**0/8**	**71/104**

* E –test ESBL strip = CTX/ CTL and CAZ/ CZL

** = Number of ESBL producer determined by E -test ESBL strips / Number of isolates determined by initial screen test

Table 4.6 Antimicrobial susceptibility of extended spectrum β – lactamase producing *K. pneumoniae* isolates from each clinical specimen against cephalosporin and imipenem detected by E -test

Number of susceptible isolates (Percent)						
Specimen	**CAZ**	**CTX**	**CRO**	**CXM**	**FOX**	**IPM**
Blood (14)	1 (7.1)	14 (100)*	13 (50)	5 (35.7)	14 (100)	14 (100)
Sputum(42)	3 (7.1)	36 (66.7)	33 (78.6)	15 (35.7)	40 (95.2)	42 (100)
Urine(44)	1 (2.3)	37 (84.1)	33 (75.0)	10 (22.7)	36 (81.8)	44 (100)
Total (100)	**5**	**87**	**79**	**30**	**90**	**100**

* CAZ= ceftazidime, CTX = cefotaxime , CRO = ceftriaxone

CXM = cefuroxime, FOX = cefoxitin, IPM = imipenem

Table 4.7.1 Antimicrobial susceptibility of ESBL producing *Klebsiella pneumoniae* isolates from each clinical specimen against other groups of antimicrobial agents besides beta - lactams as detected by agar disk diffusion method

Number of susceptible isolates (Percent)					
Specimen	**GN**	**AK**	**TOB**	**CIP**	**SXT**
Blood (14)	10 (71.4)*	8 (57.1)	2 (14.3)	6 (42.9)	6 (42.9)
Sputum (42)	22 (52.3)	32 (76.2)	9 (21.4)	32 (76.2)	21 (50.0)
Urine (44)	18 (40.9)	31 (70.5)	5 (11.3)	29 (65.9)	11 (25.0)
Total (100)	**50**	**71**	**16**	**67**	**38**

*GN = gentamicin , AK = amikacin , TOB = tobramycin ,

CIP = ciprofloxacin , SXT = trimethoprim –sulfamethoxazole

Table 4.7.2 Antimicrobial susceptibility of non - ESBL producing *K. pneumoniae* isolates from feces of normal person against other groups of antimicrobial agents besides beta - lactams detected by agar disk diffusion method

Number of susceptible isolates (Percent)					
Specimen	**GN**	**AK**	**TOB**	**CIP**	**SXT**
Feces (36)	36 (100)	36 (100)	36 (100)	36 (100)	36 (100)

Table 4.8 Distribution of phenotype of extended spectrum β - lactamase (ESBL) producing *K. pneumoniae*

Suggestive	Urine	Sputum	Blood	Number of isolates
Broad type	16	13	3	32
Ceftazidimase	3	7	3	13
Marginal	0	0	0	0
Undetermined Type I	1	3	1	5
Undetermined Type II	24	19	10	50

Table 4.9 Pulsotypes of extended spectrum β - lactamase producing *K. pneumoniae* isolates from various clinical specimens

Pulsotypes and subtypes	Codes of isolates	Number of isolates
1	KB012, KB013, KB014	3
2	KS030, KS032, KS036	3
3	KB011, KB015	2
3a	KB019	1
4	KS023, KS024	2
5	KS050, KS051	2
6	KU011, KU012	2
7	KU013, KU015	2
8	KU001	1
8a	KU009	1
9	KU016	1
9a	KU027	1
9b	KU028	1
10	KU032	1
10a	KU033	1
11	KB002	1
12	KB003	1

Table 4.9 (cont.)

Pulsotypes and subtypes	Codes of isolates	Number of isolates
13	KB006	1
14	KB009	1
15	KB016	1
16	KB017	1
17	KB018	1
18	KB020	1
19	KS001	1
20	KS002	1
21	KS005	1
22	KS008	1
23	KS010	1
24	KS011	1
25	KS012	1
26	KS017	1
27	KS018	1
28	KS019	1
29	KS020	1
30	KS021	1
31	KS022	1

Table 4.9 (cont.)

Pulsotypes and subtypes	Codes of isolates	Number of isolates
32	KS026	1
33	KS028	1
34	KS029	1
35	KS031	1
36	KS033	1
37	KS034	1
38	KS035	1
39	KS037	1
40	KS038	1
41	KS039	1
42	KS040	1
43	KS041	1
44	KS042	1
45	KS043	1
46	KS044	1
47	KS045	1
48	KS046	1
49	KS047	1
50	KS048	1

Table 4.9 (cont.)

Pulsotypes and subtypes	Codes of isolates	Number of isolates
51	KS049	1
52	KS052	1
53	KS053	1
54	KU002	1
55	KU004	1
56	KU006	1
57	KU007	1
58	KU008	1
59	KU010	1
60	KU014	1
61	KU017	1
62	KU018	1
63	KU019	1
64	KU020	1
65	KU021	1
66	KU023	1
67	KU024	1
68	KU029	1
69	KU030	1

Table 4.9 (cont.)

Pulsotypes and subtypes	Codes of isolates	Number of isolates
70	KU034	1
71	KU035	1
72	KU036	1
73	KU037	1
74	KU038	1
75	KU039	1
76	KU040	1
77	KU041	1
78	KU042	1
79	KU043	1
80	KU044	1
81	KU045	1
82	KU046	1
83	KU047	1
84	KU048	1
85	KU049	1
86	KU050	1

Figure 4.1 Comparison of PFGE of whole - cell DNA from ESBL producing *K. pneumoniae* isolates digested with *Spe* I. The pulse time was 10 to 20s at 6 v/cm and runtime 22 h. Lane 1 showed the lambda ladder (molecular marker). Lane 2-15 showed the PFGE patterns of isolates from blood. Lane 2: pulsotype 11, Lane 3: pulsotype 12, Lane 4: pulsotype 13, Lane 5: pulsotype -, Lane 6: pulsotype 3, Lane 7 - 9: pulsotype 1, Lane 10: pulsotype 3, Lane11: pulsotype 15, Lane 12: pulsotype 16, Lane 13: pulsotype 17, Lane 14: pulsotype 18, Lane 15: pulsotype 19

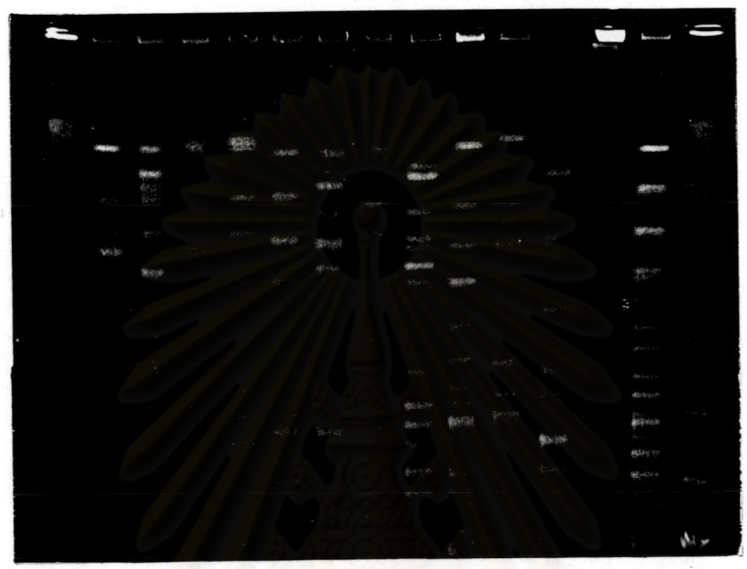

Figure 4.2 Comparison of PFGE of whole - cell DNA from ESBL producing *K.pneumoniae* isolates digested with *Spe* I. The pulse time was 10 to 20s at 6 v/cm and runtime 22h. Lane 1,15 showed the lambda ladder (molecular marker). Lane 2 - 14 showed the PFGE patterns of isolates from sputum : Lane 2: pulsotype 19, Lane 3: pulsotype 20, Lane 4: pulsotype 21, Lane 5: pulsotype 22, Lane 6: pulsotype 23, Lane 7: pulsotype 24, Lane 8: pulostype 25, Lane 9: pulsotype 26, Lane 10: pulsotype 27, Lane11: pulsotype 28, Lane 12: pulsotype 32, Lane 13: -, Lane 14: pulsotype 5

Figure 4.3 Comparison of PFGE of whole - cell DNA from ESBL producing *K.pneumoniae* isolates digested with *Spe* I. The pulse time was 10 to 20s at 6 v/cm and runtime 22h. Lane 1 showed the lambda ladder (molecular marker). Lane 2 -15 showed the PFGE patterns of isolates from urine : Lane 2: pulsotype 8, Lane 3: pulsotype 54, Lane 4: pulsotype 55, Lane 5: pulsotype 56, Lane 6: pulsotype 57, Lane 7: pulsotype 58, Lane 8: subtype 8a, Lane 9:pulsotype 59, Lane 10-11: pulsotype 6, Lane 12,14: pulsotype 7, Lane 13: pulsotype 60, Lane 15: pulsotype 61