

CHAPTER V

RESULTS

Selected demographic characteristics of the patients

Table 4 outlined the selected demographic characteristics of the patients who admitted to the RCU and the TICU at Siriraj Hospital during the 7 month of the study. The total number of patients who were admitted in both units were shown along with the number of patients who had either *P. aeruginosa* positive or negative in their throat. There was no different in the mean age of both groups of patients which were between 30-40 years. The average length of hospital stay before the patients were admitted to the RCU was 7 days which was longer than that the TICU patients which was 3 days. Among the 12 patients who had *P. aeruginosa* colonization in their throat, only one patient (8.33%) was admitted to RCU from home while the other 11 patients (91.66%) were all transferred from the other units. Among the 15 *P. aeruginosa* positive TICU patients, 7 patients (46.66%) were admitted from home while almost the same number of 8 patients (53.33%) were transferred from other units. In the RCU, the average length of the hospital stay before *P. aeruginosa* throat colonization was detected in all 12 patients was 10 days while the average length of RCU stay in the 4 patients who had acquired *P. aeruginosa* from the ward was also 10 days. Similar results were observed in the TICU, since all the 15 patients who were colonized with *P. aeruginosa* had stayed in the hospital for average of 11 days and the 8 patients who acquired the organism from the units had been admitted into TICU for about 12 days. All most all except one of the 12 RCU patients (91.67%) who carried *P. aeruginosa* in their throat had previous history of antimicrobial agents administration and were receiving antimicrobial agents at the time of specimen collection while only 11 out of

the 15 ICU trauma patients with *P. aeruginosa* colonization (73.3%) had previously received antibiotics before the organisms were detected and all of the patients were receiving antibiotics at the time of throat swab collection. Eight of the patients (66.7%) were colonized with *P. aeruginosa* upon the admission to RCU while the other 4 patients (33.3%) did acquired colonization in this unit. Much lower number of patients in TICU had been colonized with *P. aeruginosa* at the time of admission since there was only 7 out of 15 patients (46.7%) while 8 patients (53.3%) acquired colonization during their stay in the unit. There was only one RCU patient (8.3%) who developed lower respiratory tract infection after *P. aeruginosa* colonization. In contrast as high as 20% of the TICU patients with *P. aeruginosa* colonization (3 out of 15 patients) developed infection as shown in Table 5.

Additional results on the prevalence of *P. aeruginosa* isolated from patients and environment in the RCU and the TICU were also shown in Table 6 and Table 7, accordingly. In the RCU, 12 out of 82 patients (14.63%) carried *P. aeruginosa* in their throat. Among the 82 patients, only 32 patients were on respirator. These seemed to be no different on the prevalence of *P. aeruginosa* between the patients who were on and not on the respirators. Twenty-five out of 32 RCU patients had wound from tracheostomy and only 3 of them (12%) carried *P. aeruginosa* in their wounds. Only 1 out of the 20 patients (5%) who were on respirator had *P. aeruginosa* in the water from respirator. There were 5 sinks in the RCU where the sink swabs were collected for 24 times from each sink during the 7 months. Eventhough, each individual sink was not contaminated with *P. aeruginosa* at all the times of specimen collection but all 5 sinks (100%) were shown to be *P. aeruginosa* reservoirs once in awhile as shown in Table 8. There was a slightly lower number of patients with *P. aeruginosa* colonization in the TICU eventhough the number of total patients were higher since only 15 out of the 147 patients (10.20%) carried this organism in their throat and only 1 out of 22 patients with tracheostomy carried it in his wound. Surprisingly, among all 147 patients who were on respirator, none of them had *P. aeruginosa* in the water from their respirators.

All of the 7 sinks in ICU trauma (100%) were shown to be *P. aeruginosa* reservoirs once in awhile during the specimen collection time as also shown in Table 8.

Typing of Isolates

All of the 135 *P. aeruginosa* isolates obtained from the previous study were typed by PFGE. All most all except 5 isolates were discriminated into 72 pulsotypes. Figure 1-3 showed the photographs of the illustrative gels from PFGE typing. All lanes included a reasonable number of DNA bands for isolate comparison and were indicatives of clearly different banding pattern among the isolates compared. In Figure 1, identical banding patterns were evident in lane 2 and lane 9. Lane 6 was λ DNA ladder, with sizes of marker bands in kilobase (kb). The summary of total 72 pulsotypes of *P. aeruginosa* isolated from patients and environment in the RCU and the TICU together with those from sputum of infected patients from other ICU and other wards was shown in Figure 4 in the form of Diagrammatic pictures.

Table 9 summarized the results of molecular typing of all isolates according to the patients unit by PFGE. There seemed to be no prevalent pulsotype found from the isolates from the RCU, the TICU as well as from the sputum of infected patients. However, the results showed that there were specific types of *P. aeruginosa* in each unit such that types 7, 9, 10, 12, 13, 14, 15, 27, 28, 30, 33, and 72 were found only in the throats and wounds of patients in RCU while type 1, 4, 11, 17, 18, 19, 21, 26, 34, 47, and 71 were found in patients in the TICU. The sinks in the RCU were contaminated with *P. aeruginosa* type 2, 10, 28, 29, 31, and 38. There was only one isolate found in the RCU sink that was in the same pulsotype as one isolate from the patient's throat (pulsotype 10). *P. aeruginosa* with pulsotype 2 was shown to be persisted in one certain sink in RCU for as long as 6 weeks. Only *P. aeruginosa* pulsotype 7 was isolated from both throat and wound of one patient in the RCU who later got infection with this type of *P. aeruginosa*. Thus, in TICU, there were 3 patients

who carried *P. aeruginosa* pulsotype 11, 20 and 71, accordingly in their throat and got infection with *P. aeruginosa* in the same pulsotype as the colonized organism after transferring to the other ward. Among the 8 RCU patients who had *P. aeruginosa* in their wounds, 2 patients had been colonized with the same types of organism (type 7 and 72) in their throats while the only one patient in ICU trauma who had wound positive also had been colonized with the same type 4.

The molecular typing of *P. aeruginosa* isolated from sputum of the patients in 2 different groups of wards: the ICUs and the other wards had demonstrated that only the isolates that were obtained from the patients, who had been colonized with *P. aeruginosa* in their throat, were in the same types. The rest of the patients in both groups of wards had *P. aeruginosa* which were not genetically related to the others. For the ICUs groups, the most prevalent type of *P. aeruginosa* was shown to be type 42 which comprised 6 isolates from 4 different patients while there was no prevalent type found in the other ward group.

Susceptibility patterns of *P. aeruginosa*

Susceptibility test of 135 *P. aeruginosa* isolates against 15 antimicrobial agents was also performed. The intermediate susceptible isolates were also included in the resistant group. The susceptibility pattern was summarized as shown in Table 10. There were 30 different patterns (antibiograms) according to the number of the antimicrobial agents that the isolates were susceptible to which varied from 0 to 9. There were only 3 isolates obtaining from one throat colonized patient in the TICU and one infected patient that were resisted to all 15 antimicrobial agents. Fifty-nine isolates (43.7%) were susceptible to 8 antimicrobial agents which were piperacillin, ceftazidime, sulbactam/cefoperazone, imipenem, gentamicin, amikacin, netilmycin, and ciprofloxacin. Table 11 showed the distribution of the susceptibility patterns in all patients' units that were included in this study. The most prevalent pattern was pattern

26, which consisted of 18 isolates from the RCU, 14 isolates from the TICU, 15 isolates from sputum in the other ICU and 12 isolates from the other wards. The comparative susceptibility of *P. aeruginosa* isolated from different patients' unit was shown in Table 12. The isolates from sputum of the patients in the other ICU had the greatest proportion of the resistant strains. For *P. aeruginosa* isolates from the colonized patients, more than 70% of them were inhibited by piperacillin, ceftazidime, imipenem, gentamicin, amikacin, netilmycin and ciprofloxacin.

All the isolates from the RCU were less susceptible to sulbactam/cefoperazone (71.42%) than all the isolates from the TICU. *P. aeruginosa* isolates from sink in the RCU were slightly more susceptible to most of the antimicrobial agents included piperacillin, sulbactam/cefoperazone, imipenem, gentamicin, amikacin, netilmycin, and ciprofloxacin than those from colonized patients.

The isolates from colonized patients in the TICU were less susceptible to those from colonized patients in the RCU. Though more than 80% of the isolates were still susceptible to piperacillin, ceftazidime, gentamicin, amikacin, netilmycin, and ciprofloxacin and more than 70% were susceptible to sulbactam/cefoperazone and imipenem, but all of them were resist to sulfamethoxazole/trimetoprim. Isolates from sinks in the TICU were more resistant to various antimicrobial agents particularly piperacillin and gentamicin than those from the sinks in the RCU.

On the other hand, isolates from sputum from infected patients in the two patients' unit were much more resistant to most of the agents tested except imipenem, netilmycin, and ciprofloxacin. Not more than 70% of the isolates were susceptible to piperacillin, sulbactam-cefoperazone, and ceftriaxone. These isolates were very resistant to ceftriaxone and sulfamethoxazole/trimetoprim.

All of the isolates from all sources were resistant to ampicillin, ampicillin/sulbactam, cefazolin, cefotaxime, ceftriaxone (except for 5.5% of sputum isolates) and tetracycline.

Comparison of pulsotyping and antibiogram typing of *P. aeruginosa*

The comparison of pulsotypes and antibiograms from all *P. aeruginosa* isolates was shown in Table 13. It was clearly demonstrated that there was no correlation between pulsotype and antibiogram. Various pulsotypes expressed same antibiogram such that the pulsotypes 2, 7, 8, 9, 11, 12, 16, 17, 18, 19, 21, 23, 25, 27, 29, 37, 38, 42, 46, 63-68, 70, and 71 were all in the antibiogram type 26.

Correlation between the prior antimicrobial administration and the occurrence of *P. aeruginosa*.

The antimicrobial administration 7 days prior to the positive *P. aeruginosa* in the RCU and the TICU patients were shown in Table 14 and Table 15. Among the 12 patients with *P. aeruginosa* colonization in the RCU, 7 patients (58.3%) were on antimicrobial agents for at least 7 days before the first colonization was detected. The antimicrobial agents included ceftazidime (2 patients), amikacin (1 patient), penicillin G (1 patient), amoxicillin+penG (1 patient), and amikacin+ceftazidime (1 patient). The rest 5 patients did not received any agents or on the drug administration for less than 3 days.

In the TICU, the antimicrobial administrations in all 15 colonized patients were different. Four patients (26.7%) were on antimicrobial administration for longer than 5 days and were given the combined treatment with 2 or more agents including one patient on amikacin + ceftazidime, one patient on amikacin+vancomycin, one patient on amikacin + ceftazidime + clindamicin, and one patient on ceftriaxone + gentamicin + penicillin G. There were three patients who had been on single drug administration. These included 2 patients on amoxy+clavulanic and one patient on ceftazidime. More than half of the patients (8 patients) had not been on antimicrobial administration for longer than 2 days prior to *P. aeruginosa* detection.

The antimicrobial administration in the other ICU wards and the other wards were different from both the RCU and the TICU because the purpose of the antimicrobial administration was for the treatment of the lower respiratory tract infections while those in the first two units were for the other purposes (Table 16 and 17). Ten out of the 29 patients (34.5%) in the other ICUs did not receive antimicrobial treatment or received less than 3 days therapy during the 7 days prior to the first *P. aeruginosa* isolation from sputum. The rest of patients (65.5%) were on various antimicrobial therapy which mostly were the β -lactam groups. About 52.4% of the infected patients (10 out of 21) from the other wards had received the therapy for longer than 3 days prior to the first *P. aeruginosa* isolation from sputum. The antimicrobial agents used in these wards were similar to that in the ICUs which were the β -lactams.

Time course of *P. aeruginosa* colonization and infection in patients

After the pulsotypes of all isolates had been defined, the time course of *P. aeruginosa* colonization and infection in the RCU and the TICU patients were demonstrated as in Figure 5 and 6. This part of the study provided the information on the occurrence of *P. aeruginosa* colonization and infection in both units which were shown by the time of first occurrence and the pulsotypes of each isolate in the individual patient.

Figure 7 and Figure 8 showed the time course of *P. aeruginosa* positive sputum from the patients in the other ICU and in the other wards who were admitted in such wards for a long period of time so that their sputum were collected more than once. There were nine patients in other ICU whose sputum were positive for *P. aeruginosa*. Among the nine patients, 5 of them (55.6%) were infected with their own specific pulsotypes which were pulsotype 22, 39, 40, 45, and 46. The other four patients were infected with more than one *P. aeruginosa* strain such that the pulsotype of the

organism from the first isolation was different from the second isolation, which was about 10 days apart.



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Table 4 Demographics characteristics of the patients admitted to the RCU and the TICU at Siriraj Hospital during 7 months of the study

	RCU			TICU		
	Total patients	<i>P. aeruginosa</i> positive	<i>P. aeruginosa</i> negative	Total patients	<i>P. aeruginosa</i> positive	<i>P. aeruginosa</i> negative
Number of patients	82	12	70	147	15	132
Mean age	42 ^a	38	37 ^a	30	35	32
Average Length of hospital stay before admission to the RCU or the TICU	10	7	8 ^a	ND	3	ND
Number of admission from home (%)	25 ^a	1(8.33)	6 ^a	ND	7(46.66)	ND
Number of transfers from other units (%)	25 ^a	11(91.66)	14 ^a	ND	8(53.33)	ND
Number receiving antibiotics (%)	ND	11(91.66)	ND	ND	15(100)	ND
Number previously receiving antibiotics (%)	ND	11(91.66)	ND	ND	11(73.33)	ND
Average Length of hospital stay before <i>P. aeruginosa</i> positive	ND	10 ^b	ND	ND	11	ND
Average Length of the RCU or the TICU stay before <i>P. aeruginosa</i> positive	ND	10 ^c	ND	ND	12 ^d	ND

ND = No complete data available, a = out of 32 patients, b = out of 12 patients, c = out of 4 patients who acquired *P. aeruginosa* in the RCU,

d = out of 8 patients who acquired *P. aeruginosa* in the TICU, RCU = Respiratory Care Unit, TICU = Traumatic Intensive Care Unit

Table 5 Percentage of patients who were admitted to the RCU and the TICU during 7 months of the study who had throat colonization with *P. aeruginosa*

	RCU (n=12)	TICU (n=15)
Colonized on admission (%)	8 (66%)	7 (46.7%)
Acquired colonization (%)	4 (33%)	8 (53.3%)
Infection after colonized (%)	1 (8.3%)	3 (20%)

RCU = Respiratory Care Unit, TICU = Traumatic Intensive Care Unit



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Table 6 Prevalence of *P. aeruginosa* isolated from patients and environment in RCU

Source	No. of patients/samples	No. of positive (%)	Other bacteria (%)
Throat	82	12(14.63)	70(85.36)
1. on respirator	32	5(15.6)	27(84.4)
2. not on respirator	50	7(14.0)	43(86.0)
Wound	25	3(12.0)	20(80.0)
Water from respirator*	20	1(5.0)	19(95.0)
Sinks	5	5(100)	5(100)

* only from 20 patients who were on respirator were included

Table 7 Prevalence of *P. aeruginosa* isolated from patients and environment in ICU

Trauma

Source	No. of patients	No. of positive (%)	Other bacteria (%)
Throat	147	15(10.20)	131(89.12)
1. on respirator	147	15(10.20)	131(89.12)
2. not on respirator	0	0	0
Wound	22	1(4.54)	21(95.45)
Water from respirator	147	0(0)	147(100)
Sinks	7	7(100)	7(100)

Table 8 Prevalence of *P. aeruginosa* from the sinks

Sink	No. of specimen collection	No. of positive	Percent positive
RCU RSa	24	6	25.0
RSb	24	2	8.3
RSc	24	1	4.2
RSd	24	1	4.2
RSe	24	1	4.2
TICU ISa	24	1	4.2
ISb	24	2	8.3
ISc	24	1	4.2
ISd	24	4	16.7
ISe	24	5	20.8
ISf	24	1	4.2
ISg	24	2	8.3

RSa = sink a in the RCU, ISa = sink a in the TICU

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Table 9 Pulsotypes of *P. aeruginosa* isolated from different sources in the RCU, the TICU, the other ICU, and the other wards

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa(1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
1	-	-	-	-	2	-	-	-	-	-
2	-	-	-	6	-	-	-	-	-	-
3	-	-	-	-	-	-	-	2	-	-
4	-	-	-	-	2*	1	-	3	-	-
4A	-	-	-	-	-	-	-	-	-	1
5	-	-	-	-	-	-	-	2	2	-
6	-	-	-	-	-	-	-	2	-	-
7	2*	1	-	-	-	-	-	-	-	1 [♠]
8	-	-	-	-	-	-	-	1	2	-
9	2	-	-	-	-	-	-	-	-	-
10	1	-	-	1	-	-	-	-	-	-
11	-	-	-	-	1	-	-	-	-	1 [♠]
12	1	-	-	-	-	-	-	-	-	-

T = Throat W = Wound Wa = Water from respirator S = Sink * = One of the patient carried same type *P. aeruginosa* in both throat and wound [♠] = *P. aeruginosa* isolated from sputum of patient who had positive this organism in throat, () = Total isolates

Table 9 (continue)

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa(1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
13	1	-	-	-	-	-	-	-	-	-
14	-	1	-	-	-	-	-	-	-	-
15	1	-	-	-	-	-	-	-	-	-
16	1	-	-	-	-	-	-	-	-	1
17	-	-	-	-	1	-	-	-	-	-
18	-	-	-	-	1	-	-	-	-	-
19	-	-	-	-	1	-	-	-	-	-
20	-	-	-	-	1	-	-	-	-	1 ^φ
21	-	-	-	-	1	-	-	-	-	-
22	-	-	-	-	1	-	-	-	3	-
23	-	-	-	-	-	-	-	1	1	-
24	-	-	-	-	-	-	-	1	-	-
25	-	-	-	-	-	-	-	1	-	-
26	-	-	-	-	1	-	-	-	-	-

T = Throat W = Wound Wa = Water from respirator S = Sink ϕ = *P. aeruginosa* isolated from sputum of patient who had positive this organism in throat, () = Total isolates

Table 9 (continue)

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa(1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
27	2	-	-	-	-	-	-	-	-	-
28	-	-	-	1	-	-	-	-	-	-
29	-	-	-	1	-	-	-	-	1	-
30	1	-	-	-	-	-	-	-	-	-
31	-	-	-	1	-	-	-	-	-	-
32	-	-	-	-	-	-	-	1	1	-
33	1	-	1	-	-	-	-	-	-	-
34	-	-	-	-	1	-	-	-	-	-
35	-	-	-	-	-	-	-	1	-	-
36	-	-	-	-	-	-	-	1	-	-
37	-	-	-	-	-	-	-	-	2	-
38	-	-	-	1	-	-	-	-	-	-
39	-	-	-	-	-	-	-	-	2	-

T = Throat W = Wound Wa = Water from respirator S = Sink () = Total isolates

Table 9 (continue)

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa(1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
40	-	-	-	-	-	-	-	-	2	-
41	-	-	-	-	-	-	-	-	2	1
42	-	-	-	-	-	-	-	-	2	-
42A1	-	-	-	-	-	-	-	-	1	-
42A2	-	-	-	-	-	-	-	-	1	-
42A3	-	-	-	-	-	-	-	-	1	1
43	-	-	-	-	-	-	-	-	4	-
44	-	-	-	-	-	-	-	-	1	-
45	-	-	-	-	-	-	-	-	1	2
46	-	-	-	-	-	-	-	-	3	-
47	-	-	-	-	-	-	-	-	-	-
47A	-	-	-	-	-	-	-	-	1	-
48	-	-	-	-	-	-	-	-	1	1
49	-	-	-	-	-	-	-	-	1	1

T = Throat W = Wound Wa = Water from respirator S = Sink () = Total isolates

Table 9 (continue)

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa (1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
50	-	-	-	-	-	-	-	-	-	1
51	-	-	-	-	-	-	-	-	1	-
52	-	-	-	-	-	-	-	-	-	1
53	-	-	-	-	-	-	-	-	-	1
54	-	-	-	-	-	-	-	-	1	-
55	-	-	-	-	-	-	-	-	1	-
56	-	-	-	-	-	-	-	-	1	-
57	-	-	-	-	-	-	-	-	1	-
58	-	-	-	-	-	-	-	-	-	1
59	-	-	-	-	-	-	-	-	-	1
60	-	-	-	-	-	-	-	-	-	1
61	-	-	-	-	-	-	-	-	-	1
62	-	-	-	-	-	-	-	-	-	1
63	-	-	-	-	-	-	-	-	-	1

T = Throat W = Wound Wa = Water from respirator S = Sink () = Total isolates

Table 9 (continue)

Pulsotype	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa(1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
64	-	-	-	-	-	-	-	-	-	1
65	-	-	-	-	-	-	-	-	-	1
66	-	-	-	-	-	-	-	-	-	1
67	-	-	-	-	-	-	-	-	1	1
68	-	-	-	-	-	-	-	-	1	-
69	-	-	-	-	-	-	-	-	1	-
70	-	-	-	-	-	-	-	-	1	-
71	-	-	-	-	1	-	-	-	-	1 [†]
72	1	1	-	-	-	-	-	-	-	-
Untype	-	-	-	-	1	-	-	-	-	4

T = Throat W = Wound Wa = Water from respirator S = Sink ϕ = *P. aeruginosa* isolated from sputum of patient who had positive this organism in throat, () = Total isolates

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Table 10 Susceptibility patterns of *P. aeruginosa* to the 15 antimicrobial agents

Profile	Susceptibility antimicrobial agents	No. of susceptibility	Isolates
1		0	CIT1, 120A, 120B
2	IPM	1	CRT2B, CRW2, RSb2
3	AN	1	CIT13
4	NET	1	95A, 95B
5	PIP CAZ	2	131B
6	CAZ AN CIP	3	65
7	PIP 75 IPM	3	ISe1, ISg2
8	GM AN NET CIP	4	180
9	PIP CRO CAZ 75 CIP	5	91
10	PIP CAZ 75 IPM GM	5	131A
11	PIP CAZ 75 IPM CIP	5	CRT3, 87A
12	CAZ IPM GM AN NET	5	CRT5, 112
13	CAZ IPM AN NET CIP	5	ISc2

PIP = Piperacillin CRO = Ceftriaxone CAZ = Ceftazidime 75 = Sulbactam/cefoperazone IPM = Imipenem GM = Gentamicin

CI = TICU, CR = RCU, T = Throat; T1 = throat of patient no. 1, ...; W = wound; Wa = water from respirator; ISa1 = swab from sink a in TICU: first collection; RSa1 = swab from sink a in RCU: first collection; 1, 2, 3, ... = sputum from patients no. 1, 2, 3, ... and so on; 1A = first collection of sputum from patient no. 1; T1A = throat swab from patient no. 1; A = first collection B = second collection

Table 10 (continue)

Profile	Susceptibility antimicrobial agents								No. of susceptibility	Isolates
14				IPM	GM	AN	NET	CIP	5	78, 88D, 118A, 118B, 118C, 127, 133, 202B, 202C, RSc1
15		CAZ	75			AN	NET	CIP	5	183B
16	PIP	CAZ	75	IPM		AN		CIP	6	73B
17		CAZ	75	IPM		AN	NET	CIP	6	ISa1, ISc1, ISd3, Isf1, CII6
18	PIP	CAZ		IPM	GM	AN	NET		6	121, 168
19	PIP	CAZ		IPM		AN	NET	CIP	6	205
20	PIP	CAZ			GM	AN	NET	CIP	6	CIT8A, CIT8B, CIW8
21	PIP	CAZ	75	IPM	GM	AN	NET		7	CRT8, CIT6, 157
22	PIP	CAZ	75	IPM	GM	AN		CIP	7	CRT9, CIT5
23	PIP	CAZ	75	IPM	GM		NET	CIP	7	80, 93, 104, 114, 200
24	PIP	CAZ	75		GM	AN	NET	CIP	7	106, ISb1, ISb2, ISe5
25	PIP	CAZ		IPM	GM	AN	NET	CIP	7	88B, 88C, 92, 111, 113, 115, 122, 123, 146C, 156, CRT6, CRW9, ISg1

PIP = Piperacillin CRO = Ceftriaxone CAZ = Ceftazidime 75 = Sulbactam/cefoperazone IPM = Imipenem GM = Gentamicin

CI = TICU, CR = RCU, T = Throat; T1 = throat of patient no. 1, ...; W = wound; Wa = water from respirator; ISa1 = swab from sink a in TICU: first collection; RSc1 = swab from sink a in RCU: first collection; 1,2,3,... = sputum from patients no. 1,2,3,... and so on; IA = first collection of sputum from patient no. 1; T1A = throat swab from patient no. 1; A = first collection B = second collection; CII = sputum from infected patient with *P.aeruginosa* in TICU; CII1 = sputum from patient no. 1 CRI = sputum from infected patient in RCU

Table 10 (continue)

Profile	Susceptibility antimicrobial agents									No. of susceptibility	Isolates	
26	PIP		CAZ	75	IPM	GM	AN	NET	CIP	8	73A, 73C, 87B, 87C, 101, 110, 117, 119, 130, 132, 134, 136, 145, 146A, 146B, 147, 178, 183A, 198, 202A, 203A, 203B, 206, 212B, CRT1, CRT2A, CRT4, CRW _a 4, CRT7A, CRT7B, CRW7, CRI7, CRT10, CRT11, CRT12, CIT2, CII2, CIT3, CIT4, CIT7, CIT9, CII10, CIT11, CIT12, CIT14, CIT15, CII10, CRI7, R _{Sa} 1, R _{Sa} 2, R _{Sa} 3, R _{Sa} 4, R _{Sa} 5, R _{Sa} 6, R _{Sb} 1, R _{Se} 1, I _{Sd} 1, I _{Sd} 2, I _{Sd} 4, I _{Se} 3, I _{Se} 4	
27	PIP	CRO	CAZ	75	IPM		AN	NET	CIP	8	88A	
28	PIP		CAZ		IPM	GM	AN	NET	CIP	SXT	8	R _{Sd} 1
29	PIP	CRO	CAZ	75	IPM	GM	AN	NET	CIP		9	108, CIT10
30	PIP		CAZ	75	IPM	GM	AN	NET	CIP	SXT	9	181

PIP = Piperacillin CRO = Ceftriaxone CAZ = Cefazidime 75 = Sulbactam/cefoperazone IPM = Imipenem GM = Gentamicin

CI = TICU, CR = RCU, T = Throat; T1 = throat of patient no. 1, ...; W = wound; W_a = water from respirator; I_{Sa}1 = swab from sink a in TICU: first collection; R_{Sa}1 = swab from sink a in RCU: first collection; 1,2,3,... = sputum from patients no. 1,2,3,... and so on; 1A = first collection of sputum from patient no. 1; T1A = throat swab from patient no. 1; A = first collection B = second collection; CII = sputum from infected patient with *P.aeruginosa* in TICU; CII1 = sputum from patient no. 1; CRI = sputum from infected patient in RCU

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Table 11 Susceptibility pattern (Antibiogram) of *P. aeruginosa* from different sources in the RCU, the TICU, the other ICU, and the other wards

Susceptibility pattern	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa (1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
1	-	-	-	-	1	-	-	-	2	-
2	1	1	-	1	-	-	-	-	-	-
3	-	-	-	-	1	-	-	-	-	-
4	-	-	-	-	-	-	-	-	2	-
5	-	-	-	-	-	-	-	-	-	1
6	-	-	-	-	-	-	-	-	-	1
7	-	-	-	-	-	-	-	2	-	-
8	-	-	-	-	-	-	-	-	1	-
9	-	-	-	-	-	-	-	-	-	1
10	-	-	-	-	-	-	-	-	-	1
11	1	-	-	-	-	-	-	-	1	-
12	1	-	-	-	-	-	-	-	1	-
13	-	-	-	-	-	-	-	1	-	-
14	-	-	-	1	-	-	-	-	8	1
15	-	-	-	-	-	-	-	-	1	-

T = Throat W = Wound Wa = Water from respirator S = Sink

Table 11 (continue)

Susceptibility pattern	No. of isolates from the RCU				No. of isolates from the TICU				No. of isolates from the other ICU	No. of isolates from the other ward
	T(14)	W(3)	Wa (1)	S(11)	T(16)	W(1)	Wa(0)	S(16)	Sputum (44)	Sputum (29)
16	-	-	-	-	-	-	-	-	1	-
17	-	-	-	-	-	-	-	4	-	1
18	-	-	-	-	-	-	-	-	1	1
19	-	-	-	-	-	-	-	-	1	-
20	-	-	-	-	2	1	-	-	-	-
21	1	-	-	-	1	-	-	-	-	1
22	-	-	-	-	1	-	-	-	-	-
23	-	-	-	-	-	-	-	-	3	2
24	-	-	-	-	-	-	-	3	-	1
25	1	-	-	-	-	-	-	1	6	4
26	8	1	1	8	9	-	-	5	14	11
27	-	-	-	-	-	-	-	-	1	-
28	-	-	-	1	-	-	-	-	-	-
29	-	-	-	-	1	-	-	-	-	1
30	-	-	-	-	-	-	-	-	1	-

T = Throat W = Wound Wa = Water from respirator S = Sink

Table 12 Susceptibility of *P. aeruginosa* isolated from the different sources and the different wards against 15 antimicrobial agents

Antimicrobial agents	Wards								
	RCU (29)*				TICU (33)*				Other ICU and other wards (73) *
	T (14)**	W(3)**	Wa(1)**	S(11)**	T(16)**	W(1)**	Wa(0)**	S(16) **	Sputum (73) **
Ampicillin	0	0	0	0	0	0	0	0	0
Piperacillin	11(78.57)	1	1(100)	9(81.82)	14(87.5)	1	0	11(68.75)	57(78.1)
Ampicillin/Sulbactam	0	0	0	0	0	0	0	0	0
Cefazolin	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	0
Ceftriaxone	0	0	0	0	0	0	0	0	4(5.5)
Ceftazidime	12(85.71)	1	1(100)	9(81.82)	13(81.25)	1	0	14(87.5)	59(80.8)
Sulbactam/cefoperzone	10(71.42)	1	1(100)	8(72.72)	12(75.0)	0	0	14(87.5)	43(58.9)

* no. of total isolates, ** no. of isolates from individual source, T = throat, W = wound, Wa = water from respirator, S = sink

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Table 12 (continue)

Antimicrobial agents	Wards								
	RCU (29)*				TICU (33)*				Other ICU and other wards (73) *
	T (14)**	W(3)**	Wa(1)**	S(11)**	T(16)**	W(1)**	Wa(0)**	S(16)**	Sputum (73) **
Imipenem	12(85.71)	3	1(100)	11(100)	12(75.0)	0	0	13(81.25)	63(86.3)
Gentamicin	12(85.71)	2	1(100)	10(90.90)	14(87.5)	1	0	9(56.25)	60(82.2)
Amikacin	12(85.71)	2	1(100)	10(90.90)	15(93.75)	1	0	14(87.5)	58(79.5)
Netilmycin	12(85.71)	2	1(100)	10(90.90)	13(81.25)	1	0	14(87.5)	66(90.4)
Tetracyclin	0	0	0	0	0	0	0	0	0
Ciprofloxacin	11(78.57)	2	1(100)	10(90.90)	13(81.25)	1	0	16(100)	62(84.9)
Sulfamethoxazole/trimetoprim	8(57.14)	1	1(100)	9(81.82)	0	0	0	0	1(1.4)

* no. of total isolates, ** no. of isolates from individual source, T = throat, W = wound, Wa = water from respirator, S = sink

Table 13 Comparative of pulsotypes to antibiotic susceptibility patterns of *P. aeruginosa* isolated from The different wards

Pulsotype	Antibiotic pattern	Isolate
1	1	CIT1
	3	CIT13
2	26	RSa1, RSa2, RSa3, RSa4, RSa5, RSa6
3	17	ISa1, ISf1
4	20	CIT8A
	24	ISb1, ISb2, ISe5, CIW8
	27	CIT8B
4A	9	91
5	23	93
	26	ISe3, 117
6	7	ISe1, ISg2
7	26	CRT7A, CRW7, CRT7B, CRI7
8	26	ISd1, 183A, 203A
9	26	CRT11, CRT12
10	2	CRT2B
	28	RSd1
11	26	CIT2, CHI2
12	26	CRT10
13	21	CRT8
14	2	CRW2
15	11	CRT3
16	23	104
	26	CRT1
17	26	CIT12
18	26	CIT14
19	26	CIT7
20	21	CIT6

CI =TICU; CR = RCU; T = Throat (for example: T1=throat of patient no.1); W = wound; Wa = water from respirator; ISa1 = swab from sink a in ICU trauma, first collection; Rsa1 = swab from sink a in RCU; first collection; 1,2,3,... = sputum from patients no. 1,2,3,... ; 1A = sputum from patient no. 1; T1A = throat swab from patient no. 1; A=first collection , B = second collection; CHI = sputum from patient who positive *P. aeruginosa* in throat; CHI1 = sputum from patient no.1

Table 13 (continue)

Pulsotype	Antibiotic pattern	Isolate
20	17	CII6
21	26	CIT4
22	14	118A, 118B, 118C
	26	CIT9
23	26	ISd2
	30	181
24	17	ISc1
25	26	ISd4
26	22	CIT5
27	12	CRT5
	26	CRT2A
28	14	RSc1
29	26	RSb1, 198
30	25	CRT6
31	2	RSb2
32	13	ISc2
	15	183B
33	26	CRT4, CRWa4
34	26	CIT15
35	17	ISd3
36	25	ISg1
37	26	87B, 87C
38	26	RSc1
39	1	120A, 120B
40	4	95A, 95B
41	23	114
	25	113, 115
42	16	73B

CI = TICU; CR = RCU; T = Throat (for example: T1=throat of patient no.1); W = wound; Wa = water from respirator; ISa1 = swab from sink a in ICU trauma, first collection; Rsa1 = swab from sink a in RCU; first collection; 1,2,3,... = sputum from patients no. 1,2,3,... ; 1A = sputum from patient no. 1; T1A = throat swab from patient no. 1; A=first collection , B = second collection; CII = sputum from patient who positive *P. aeruginosa* in throat; CII1 = sputum from patient no.1

Table 13 (continue)

Pulsotype	Antibiotic pattern	Isolate
42	26	73C
42A1	26	73A
42A2	26	145
42A3	26	130, 212
43	11	87A
	14	88D
	25	88B, 88C
44	27	88A
45	5	131B
	10	131A
	14	133
46	14	202B, 202C
	26	202A
47	26	119, CIT11
	6	65
48	18	121
49	8	180
	14	78
50	21	157
51	25	123
52	25	122
53	14	127
54	19	205
55	23	80
56	29	108
57	23	200
58	25	111
59	12	112

CI =TICU; CR = RCU; T = Throat (for example: T1=throat of patient no.1); W = wound; Wa = water from respirator; ISa1 = swab from sink a in ICU trauma, first collection; Rsa1 = swab from sink a in RCU; first collection; 1,2,3,... = sputum from patients no. 1,2,3,... ; 1A = sputum from patient no. 1; T1A = throat swab from patient no. 1; A=first collection , B = second collection; CII = sputum from patient who positive *P. aeruginosa* in throat; CHI = sputum from patient no.1

Table 13 (continue)

Pulsotype	Antibiotic pattern	Isolate
60	18	168
61	24	106
62	25	92
63	26	206
64	26	136
65	26	132
66	26	101
67	26	178, 101
68	26	134
69	25	156
70	26	203B
71	26	CH10
	29	CIT10
72	22	CRT9, CRW9

CI = TICU; CR = RCU; T = Throat (for example: T1=throat of patient no.1); W = wound; Wa = water from respirator; ISa1 = swab from sink a in ICU trauma, first collection; Rsa1 = swab from sink a in RCU; first collection; 1,2,3,... = sputum from patients no. 1,2,3,... ; 1A = sputum from patient no. 1; T1A = throat swab from patient no. 1; A=first collection , B = second collection; CII = sputum from patient who positive *P. aeruginosa* in throat; CIII = sputum from patient no.1

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Table 14 Antimicrobial administration in patients in the RCU within 7 days prior to the *P. aeruginosa* detection

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
1	CRT1	← Ceftriaxone (R) →						
2	CRT2A	← Amikacin (S) →						
		← Cefazidime (S) →						
		← Imipenem (S) →						
	CRW2	← Amikacin (S) →						
		← Cefazidime (R) →						
		← Imipenem (S) →						
	CRT2B	← Amikacin (S) →						
3	CRT3	-						
4	CRT4	← Cefazidime (S) →						
	CRWa4	← Cefazidime (S) →						
5	CRT5						↔	
		Sulbactam/cefoperazone						
		← Cefazidime (S) →						
6	CRT6	← Amoxicillin (Amp R) →						
		← PGS (Amp R) →						
7	CRT7A	← Cloxacillin →						
	CRW7	← Cloxacillin →						
	CRT7B	← Cloxacillin →						
8	CRT8	← PGS (Amp R) →						
							← Cefotaxime (R) →	
9	CRT9	← Cefazidime (R) →						
	CRW9	← Cefazidime (R) →						
10	CRT10	← Amikacin (S) →						
		← Cefazidime (S) →						
11	CRT11	← Cloxacillin →						
12	CRT12						← Amp + Clavulanic →	
							↔	
		Ceftriaxone (R),						

CR = patient in RCU; T = throat; W = wound; Wa = water from respirator; a = first collection;

B = second collection; 1,2,3,... = patient no. 1,2,3,...

Table 15 Antimicrobial administration in patients in the TICU within 7 days prior to the *P. aeruginosa* detection

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
1	CIT1					← Teicoplanin →		
		←			Ceftazidime (R)		←	→
							←	→
								Netilmycin (R)
2	CIT2	← Cefoxitin →						
		←			Ceftazidime (S)			→
		←			Amikacin (S)			→
3	CIT3						←	→
								Cefazolin (R)
4	CIT4						←	→
								Ceftazidime(S)
5	CIT5						←	→
								PGS (Amp R)
							←	→
								Amikacin (S)
							←	→
								Ceftazidime (S)
6	CIT6	← Ceftriaxone (R) →				Amp + Clavulanic		→
7	CIT7						←	→
								Chloramphenical
8	CIT8A	←			Amp + Clavulanic			→
	CIT8B	←			Amp + Clavulanic			→
							←	→
								Ceftriaxone (R)
	CIW8	←			Amp + Clavulanic			→
							←	→
								Ceftriaxone (R)
9	CIT9	←			Amikacin (S)			→
		←			Ceftazidime (S)			→
		←			Clindamycin			→
10	CIT10						←	→
								Ceftriaxone (S)
11	CIT11						←	→
								Ceftriaxone (R)
12	CIT12			←		PGS (Amp R)		→
				←		Gentamicin (S)		→
				←		Ceftriaxone (R)		→
							←	→
								Ceftazidime (S)
13	CIT13	←			Amikacin (S)			→
		←			Vancomycin			→

CI = patient in the TICU; T = throat; W = wound; A = first collection; B = second collection;

1,2,3,... = patient no. 1,2,3,...

Table 15 (continue)

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
14	CIT14							↔ Cloxacillin
15	CIT15							↔ Ceftriaxone (R)

CI = patient in the TICU; T = throat; W = wound; A = first collection; B = second collection;

1,2,3,... = patient no. 1,2,3,...



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Table 16 Antimicrobial administration in patients in the other ICU within 7 days prior to the *P. aeruginosa* detection

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
1	73A						← Amp + Clavulanic →	
	73B						← Amp + Clavulanic →	
								← Cefotaxim (R) →
								← Vancomycin →
	73C	←			Cefotaxim (R)			→
		←			Vancomycin			→
2	78	-						
3	80	←			Ceftazidime (S)			→
		←			Cloxacillin			→
4	87A				Ceftazidime (S), Ceftriaxone (R), Amikacin (S)			
	87B						← Cefazolin (R) →	
	87C	←			Cefazolin (R)			→
5	88A	←			PGS (Amp R)			→
	88B	-						
	88C	-						
	88D	←			Ampicillin (R)			→
		←			Ceftazidime (R)			→
		←			Ciprofloxacin (S)			→
6	93					←	Gentamicin (S)	→
7	95A	←			Clindamycin			→
		←			Ceftazidime (R)			→
	95B	←			Ceftazidime (R)			→
8	108							
9	113	←			Cefotaxime (R)			→
10	115						← PGS (Amp R) →	
		←			Cefoxitin			→
11	117	←			Netilmycin (S)			→
					Cefotaxime (R)			
						←	Sulbactam + Ampicillin	→

1,2,3,... sputum from patient no. 1,2,3,... ; A = first collection; B = second collection; C = third collection; D = forth collection

Table 16 (continue)

Patients	isolates	Antimicrobial agents (susceptibility)							
		1	2	3	4	5	6	7	
12	118A			←		Cefotaxime (R)		→	↔
									Ceftazidime (R)
	118B	←	Cefotaxime (R)	→					
	118C	←			←	Ceftazidime (R)		→	↔
									Amikacin (S), Ciprofloxacin (S)
	13	119	←			Cefazolin (R)		→	↔
14	120A	←				Imipenem (R)		→	↔
									Ticoplanin
	120B	←				Imipenem (R)		→	↔
15	121	←							↔
									Sulbactam + Ampicillin, Netilmicin (S)
	123	←				Ceftazidime (S)		→	↔
16	123								Amikacin (S)
									↔
	17	133	-						
18	134	-							
19	145	-							
20	156	←				Cefazolin (R)		→	↔
									Ceftriaxone (R)
21	178	-							↔
22	181								Ceftazidime (S), Amikacin (S)
		←							↔
	183A	Cefazolin (R)	→						↔
23	183A								↔
									Sulbactam + Ampicillin
	183B								↔
24	198	-							↔
									Ceftazidime (S)
	200	-							↔
26	202A	-							↔
	202B	←							↔
	202C								↔
									Ceftazidime (R), Amikacin (S), Vancomycin

1,2,3,... sputum from patient no. 1,2,3,... ; A = first collection; B = second collection; C = third collection;

D = forth collection

Table 16 (continue)

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
27	203A	-						
	203B	-						
28	205	-						
29	212							

↔
Cefotaxime(R), Clindamycin

1,2,3,... sputum from patient no. 1,2,3,... ; A = first collection; B = second collection; C = third collection;
D = forth collection

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Table 17 Antimicrobial administration in patients in the other wards within 7 days prior to the *P. aeruginosa* detection

Patients	isolates	Antimicrobial agents (susceptibility)						
		1	2	3	4	5	6	7
1	65	-						
2	91	← Fosfomycin →						
3	92	← Cefotaxime (R) →						
4	101	← Amp + Clavulanic →					← Cefotaxime (R) →	
5	104						← Ampicillin (R) →	
6	106	← Ceftazidime (S) →						
7	110						← Fosfomycin →	
8	111							← Cefazolin (R) →
9	112							
10	114	← Ceftazidime (S) →						
11	122						← Gentamycin (S), Ceftazidime (S) →	← Clindamycin →
12	127	← Cefotaxime (R) →						← Ceftriaxone (R) →
13	130							
14	131A	← Amikacin (R) →					← Ceftazidime (S) →	
	131B						← Ceftazidime (S) →	
15	132							
16	136							
17	146A	← Amp + Clavulanic →						← Cefotaxime (R) →
	146B	← Cefotaxime (R) →						← Cefotaxime (R) →
	146C	← Ciprofloxacin (S) →						
18	147	← Amp + Clavulanic →						
19	157	← Cephalosporin →						← Ceftazidime (S) →
								← PGS →
20	168							
21	180	← Cefotaxime (R) →						
		← Cloxacillin →						

1,2,3,... sputum from patient no. 1,2,3,... ; A = first collection; B = second collection; C = third collection



Figure 1 Pulsed-field gel electrophoresis with a contour-clamped homogenous electric field (CHEF) of DNA of *P. aeruginosa*. DNA were digested with *SpeI*, and electrophoresis conditions consisted of pulsed times of 1s-40s for 23 h at 200v. Lane 1,10: DNA of *P. aeruginosa* isolated from sputum of infected patient in the other ward; lane 4: DNA of *P. aeruginosa* isolated from patient's throat who were colonization in the RCU; lane 3,7,8: DNA of *P. aeruginosa* isolated from sinks in the RCU; lane 6: DNA of *P. aeruginosa* isolated from sinks in the TICU; lane 2,9: DNA of reference strain; and lane 5: standard lambda ladder marker.

1 2 3 4 5 6 7 8 9 10



Figure 2 Pulsed-field gel electrophoresis with a contour-clamped homogeneous electric field (CHEF) of DNA of *P. aeruginosa*. DNAs were digested with *SpeI*, and electrophoresis conditions consisted of pulsed times of 1s-40s for 23 h at 200V. Lane 1: DNA of *P. aeruginosa* isolated from patient's throat who were colonization in the RCU; lane 3,4,6,7,: DNA of *P. aeruginosa* isolated form patient's throat who were colonization in the TICU; lane 10: DNA of *P. aeruginosa* isolated from sputum of infected patient in the other ward; lane 2,9: DNA of reference strain; and lane 5: standard lambda ladder marker.



Figure 3 Pulsed-field gel electrophoresis with a contour-clamped homogeneous electric field (CHEF) of DNA of *P. aeruginosa*. DNAs were digested with *SpeI*, and electrophoresis conditions consisted of pulsed times 1s-40s for 23 h at 200v. Lane 1,3,7: DNA of *P. aeruginosa* isolated from sinks in the TICU; lane 4,6,8,10: DNA of *P. aeruginosa* isolated from patient's throat who were colonization in the RCU; lane 2,9: DNA from reference strain; and lane 5: standard lambda ladder marker.

Figure 4 Diagrammatic pattern of 72 pulsotypes of *P. aeruginosa*

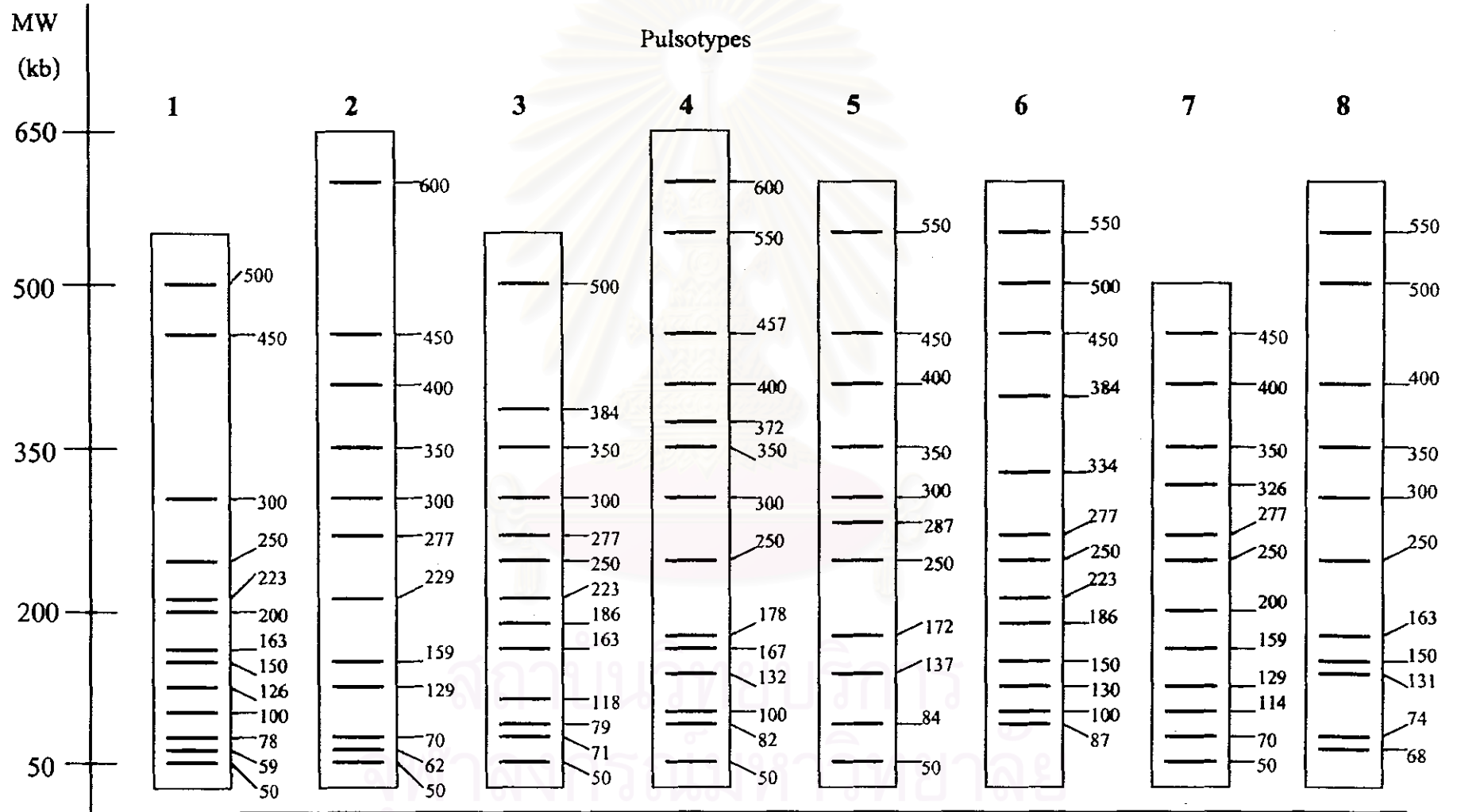


Figure 4 (continue)

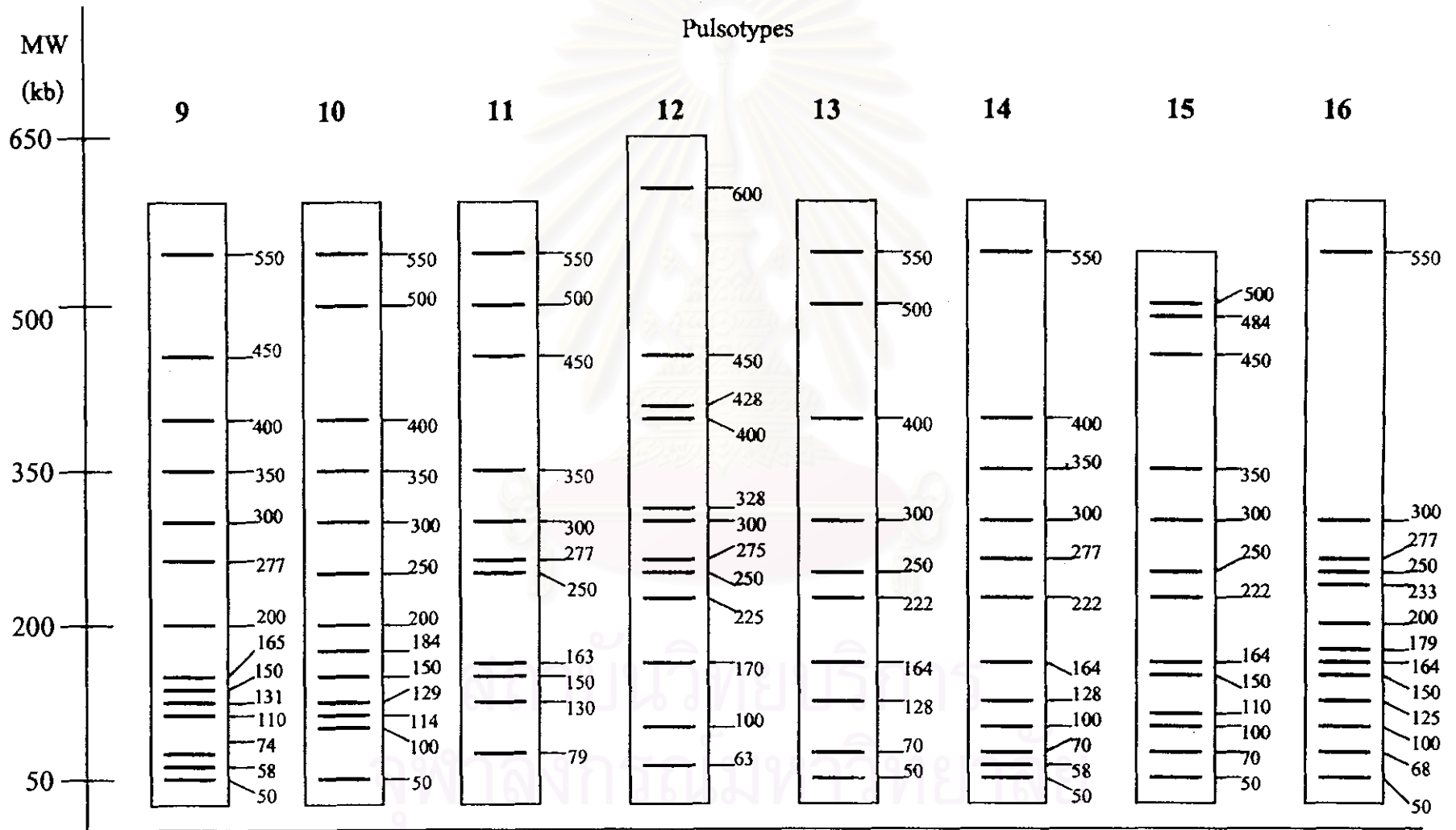


Figure 4 (continue)

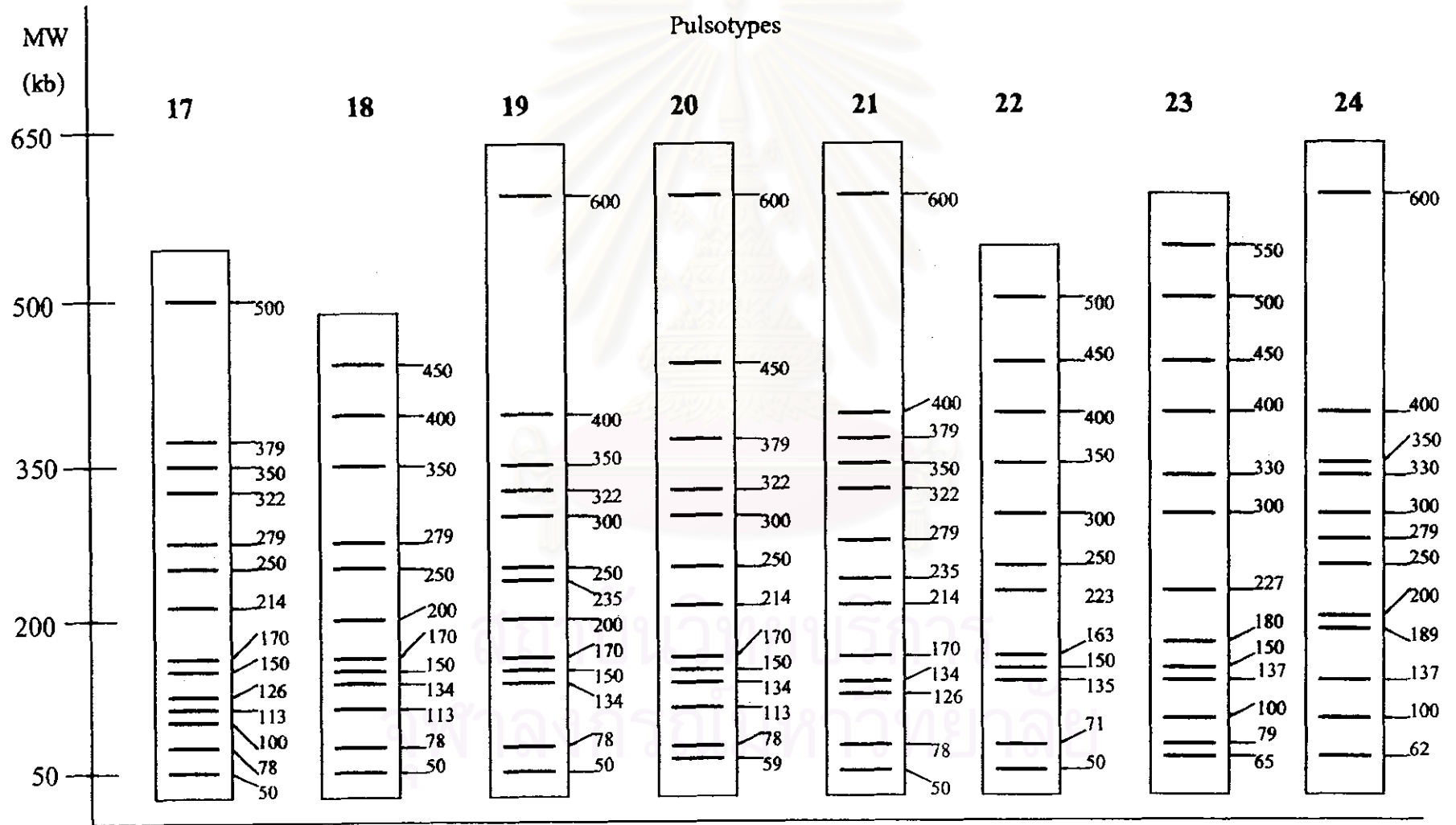


Figure 4 (continue)

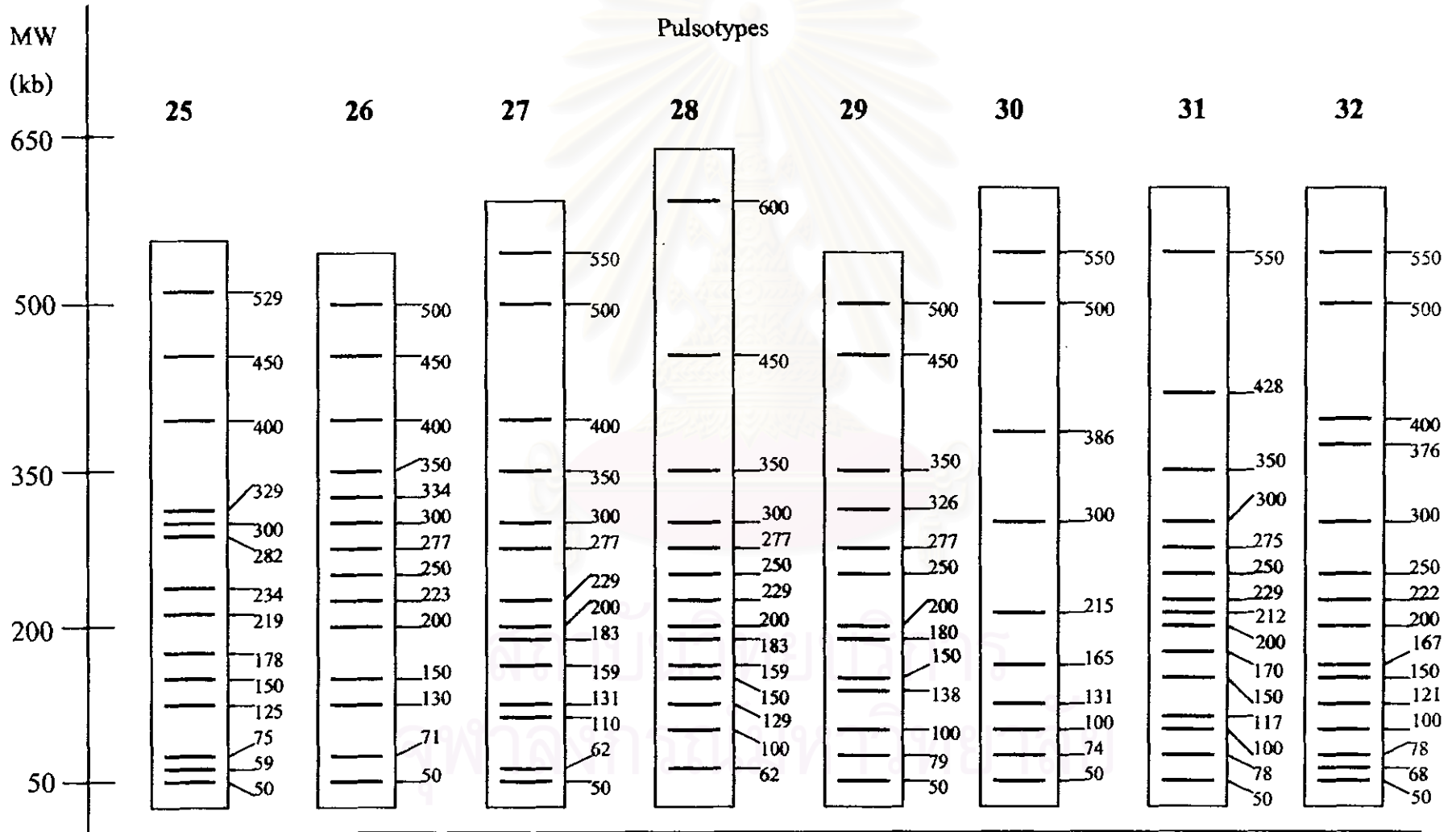


Figure 4 (continue)

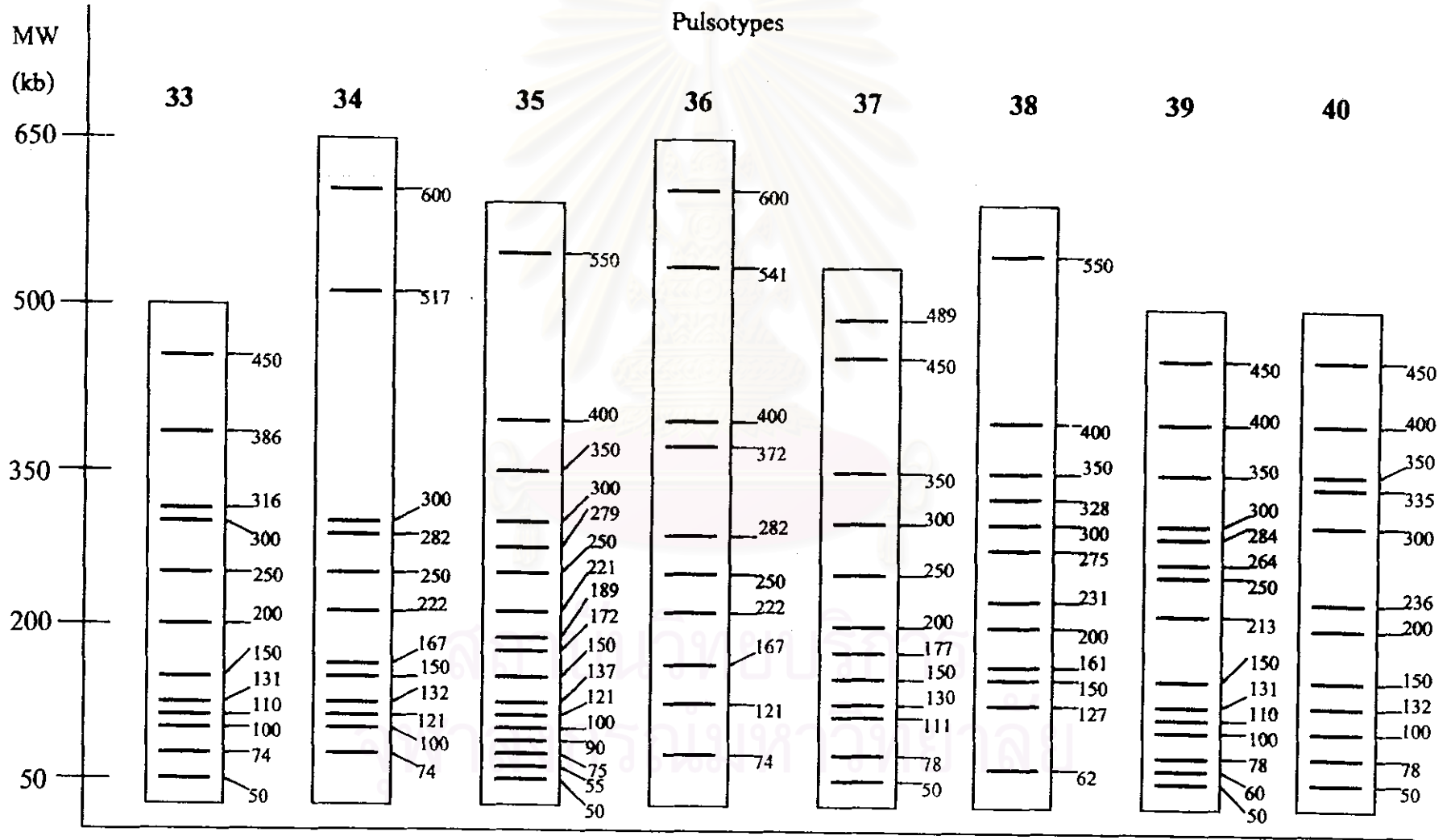


Figure 4 (continue)

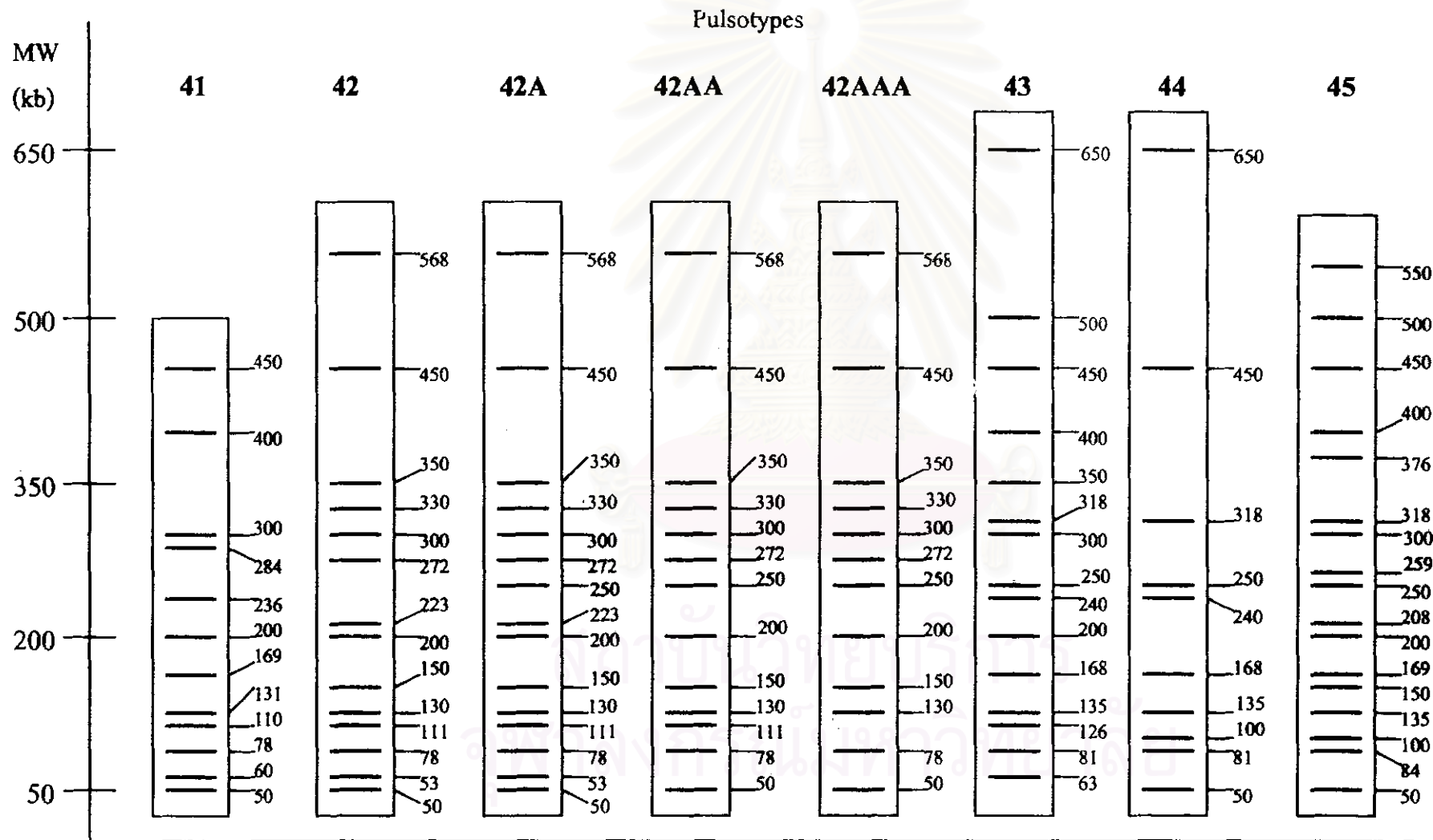


Figure 4 (continue)

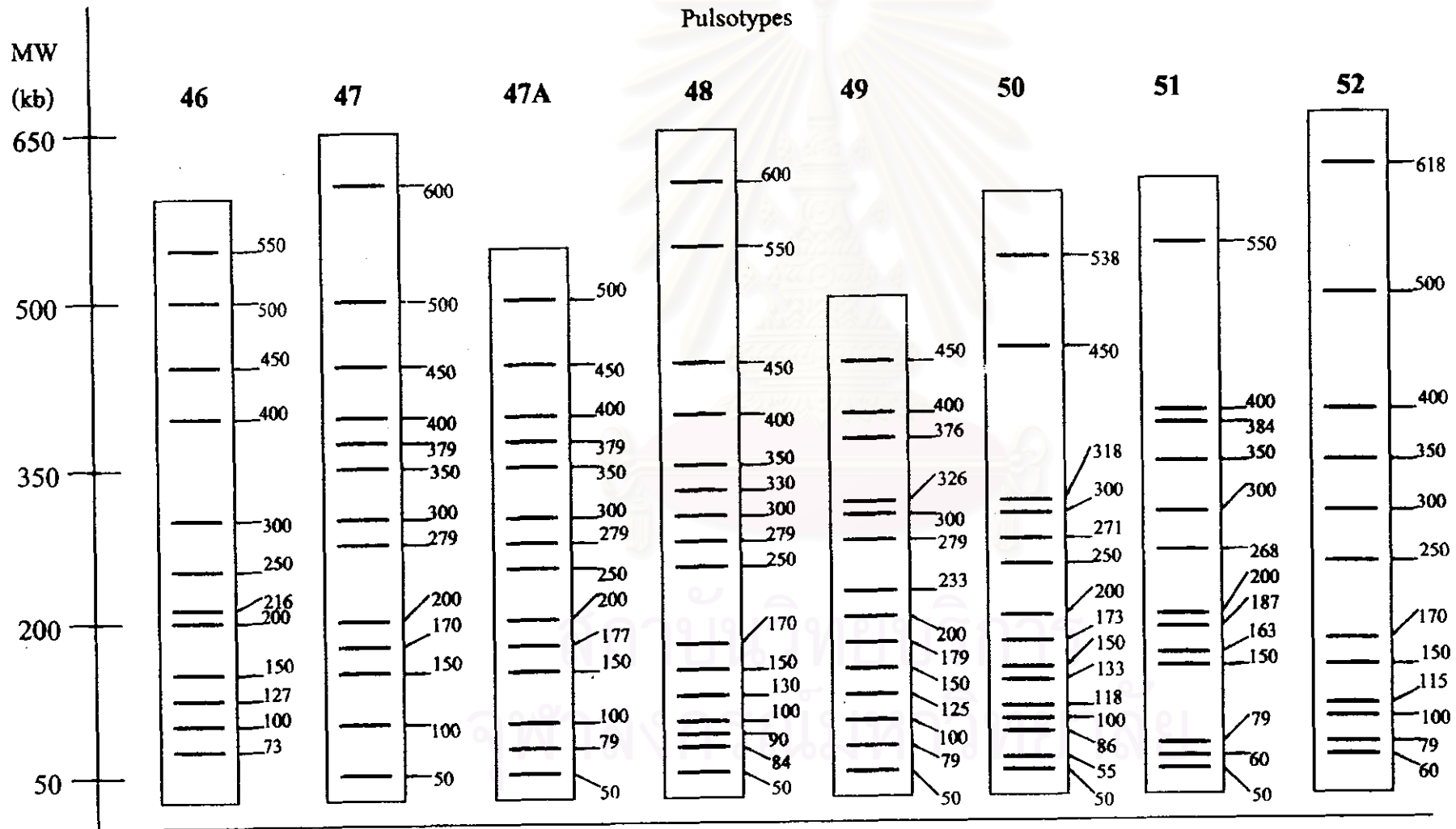


Figure 4 (continue)

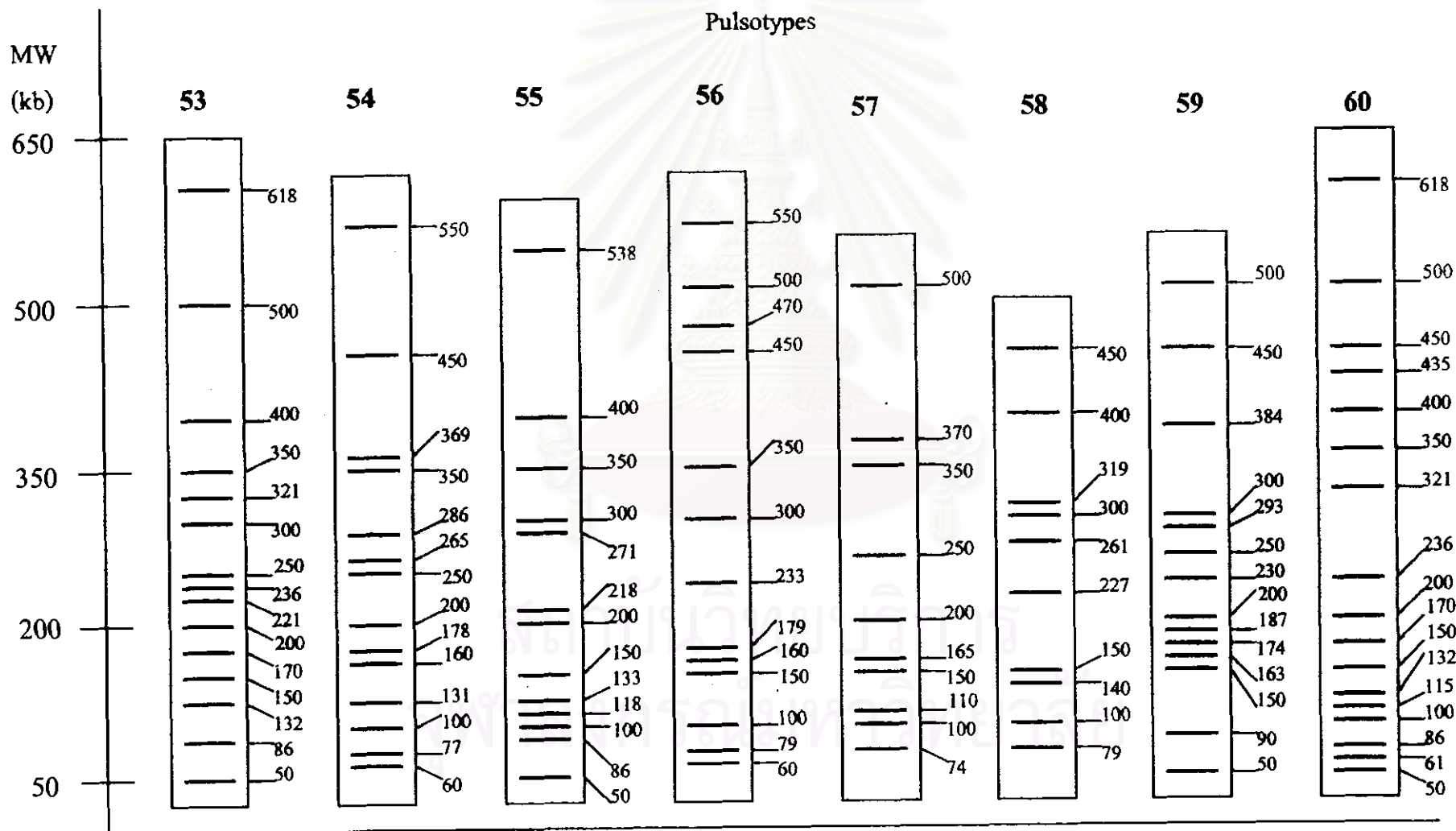


Figure 4 (continue)

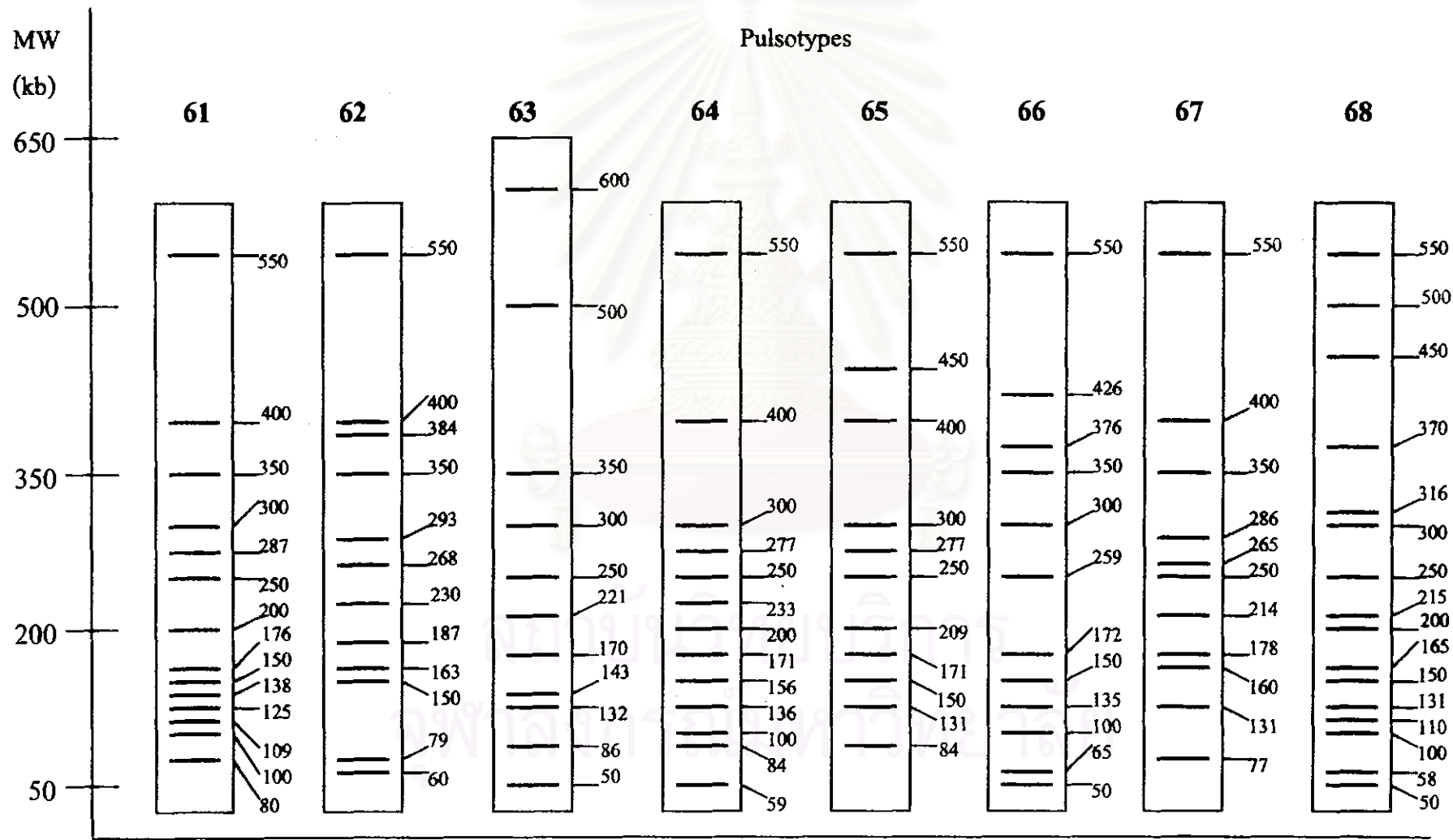
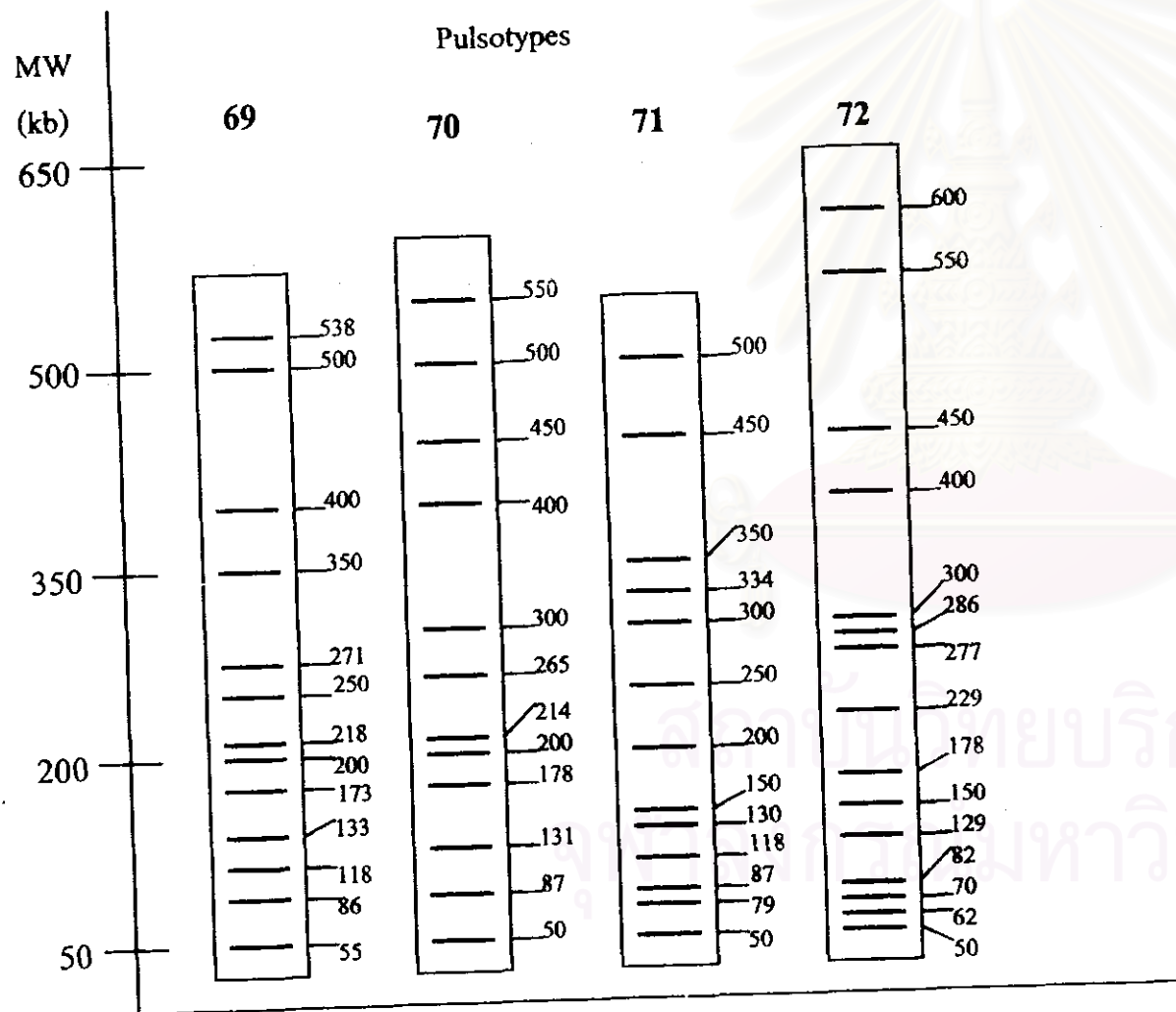


Figure 4 (continue)



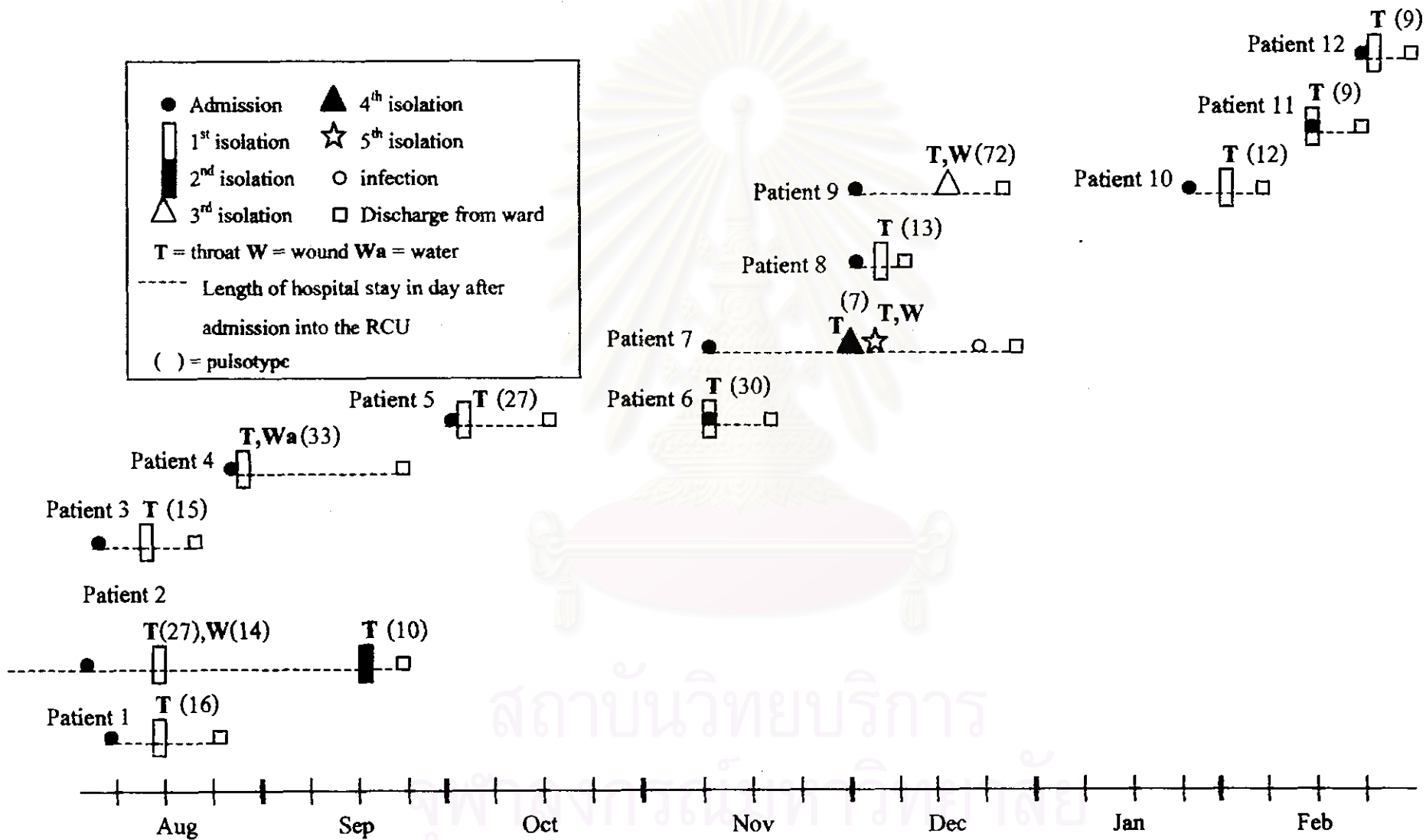


Figure 5 Time course of *P. aeruginosa* from patients in the RCU

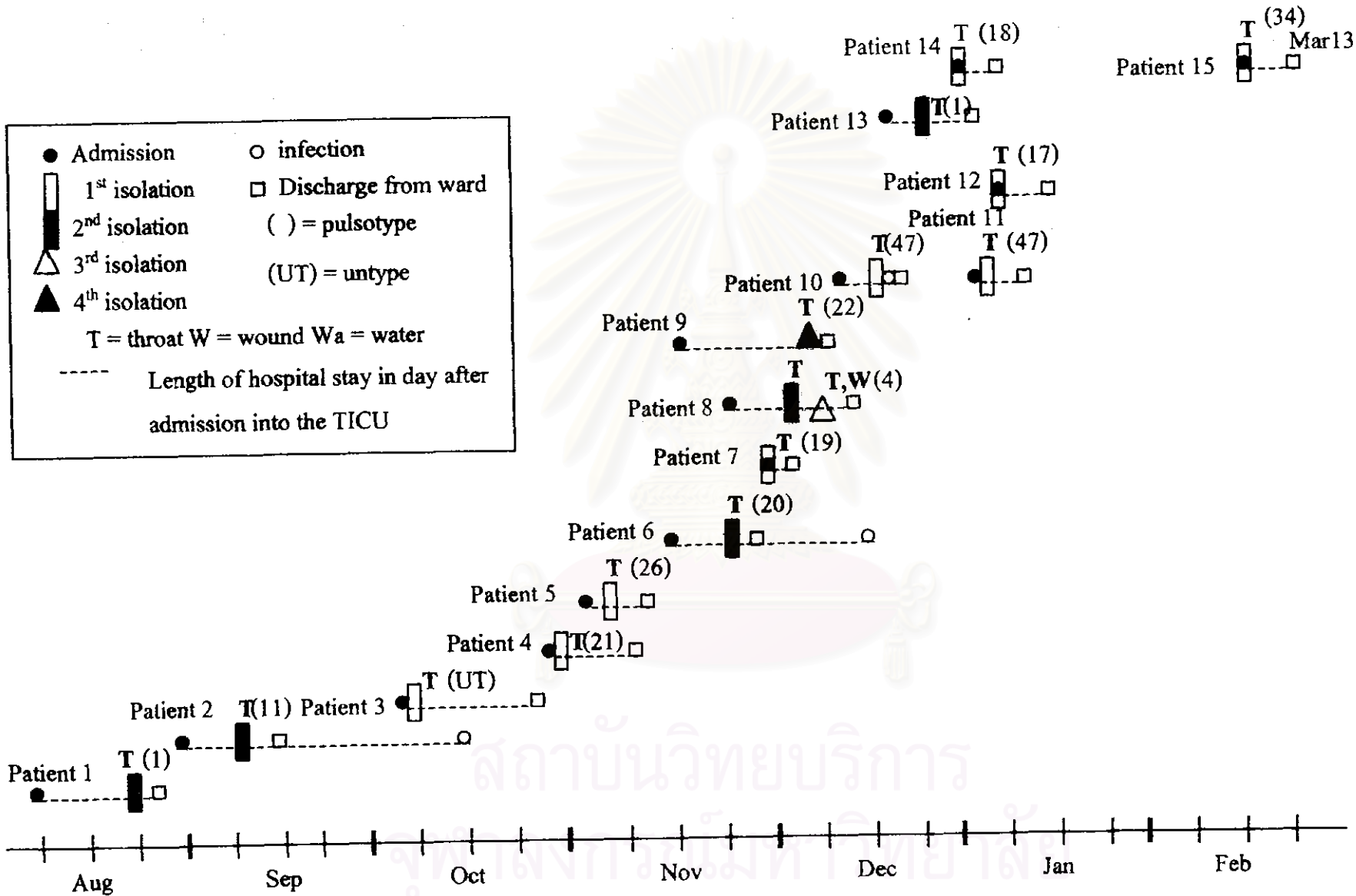


Figure 6 Time course of *P. aeruginosa* from the patients in the TICU

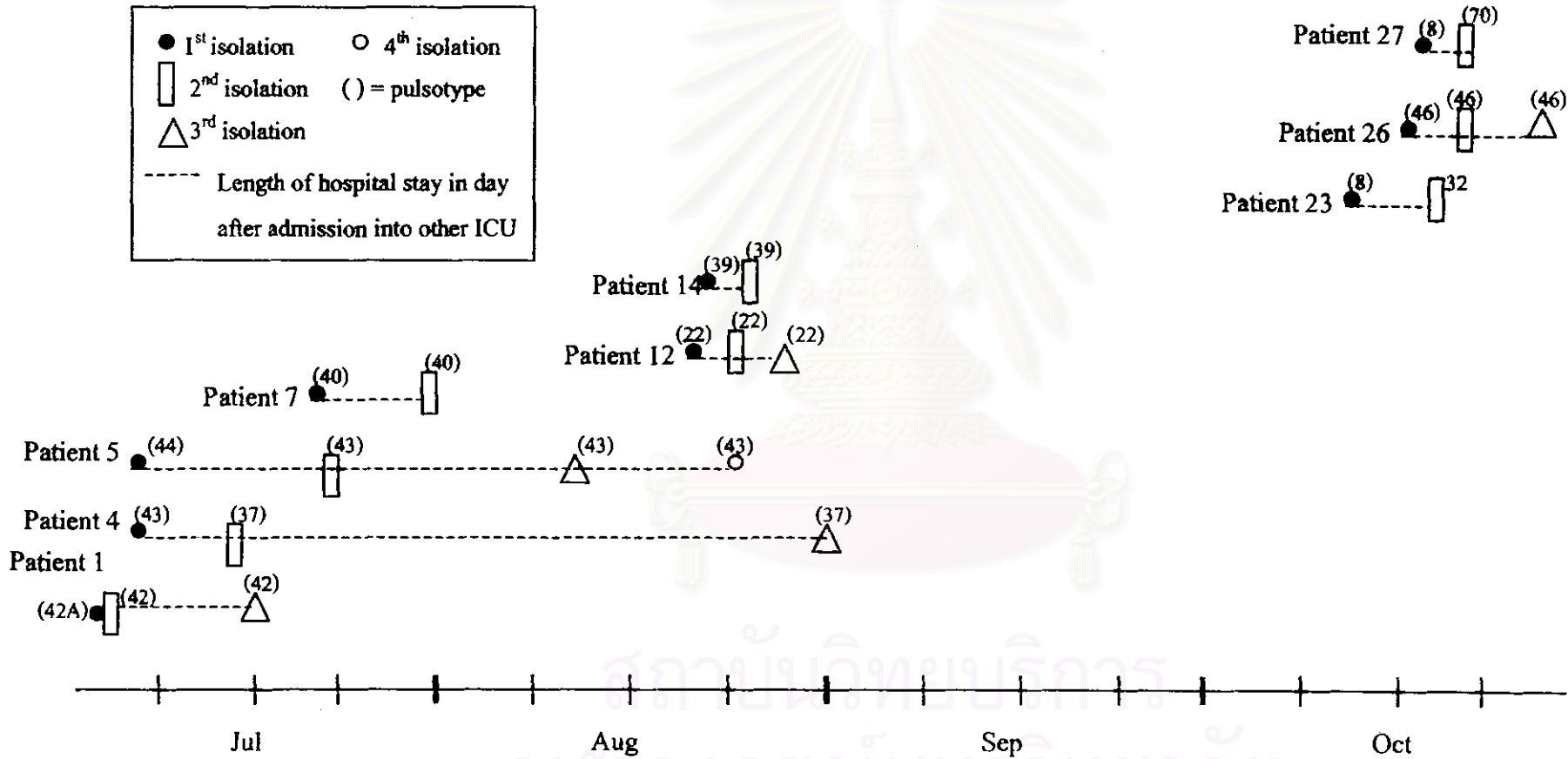


Figure 7 Time course of *P. aeruginosa* positive sputum from the patient in the other ICU

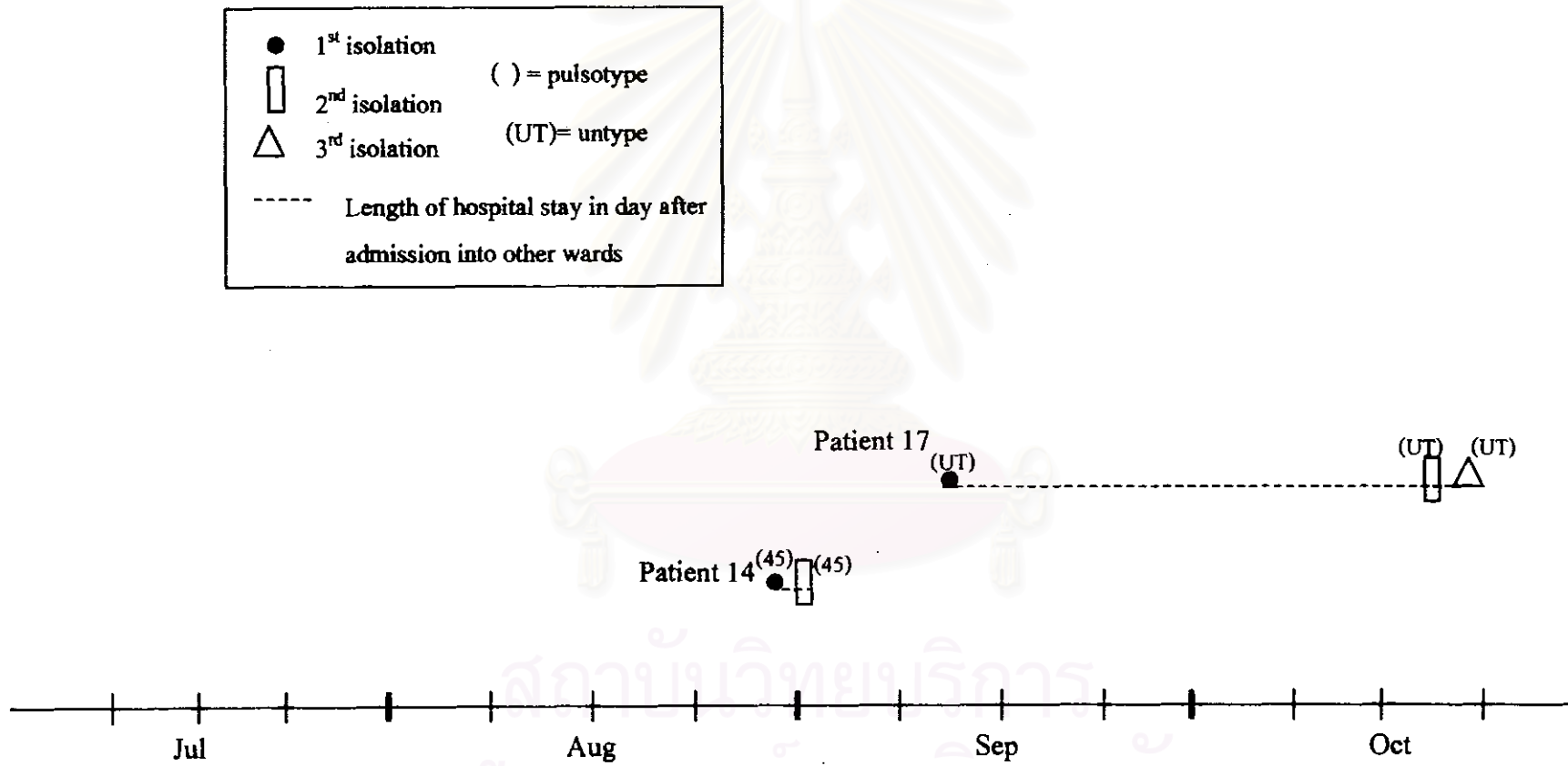


Figure 8 Time course of *P. aeruginosa* positive sputum from the patients in the other wards