EPIDEMIOLOGICAL STUDY OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SPECIMENS

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Abstract. The epidemiology of *Pseudomonas aeruginosa* infection was studied in Siriraj Hospital. During April 1989 - June 1990, *P. aeruginosa* 436 strains were isolated from clinical specimens of 260 patients, *ie* blood (19 strains), pus (192 strains), sputum (159 strains) and urine (66 strains). By using a combination of serogroups and pyocin types as epidemiological markers, it was found that there were 10 serogroups and 8 pyocin types which can be differentiated into 33 serogroup/pyocin types or patterns. The most common pattern was E 211111 (26.3%) followed by B 121614 (24.5%), G 373112 (13%) and L 888888 (7.1%), respectively.

INTRODUCTION

Among the common gram-negative bacilli that cause nosocomial infections, *Pseudomonas aeruginosa* is exceptional in its ability to infect various patients, particularly those with burns, traumatic wounds, immunosuppression (especially granulocytopenia), and cystic fibrosis (Aber and Mackel, 1981). Infections produced by *P. aeruginosa* have been increasing reported during the past few years. The organism is a major cause of morbidity and mortality among hospitalized compromised hosts (Morrisson and Wenzel, 1984). The source of human infections is usually a colonized or infected patient or a moist item in the hospital environment. Suppression of the normal flora by antimicrobial agents and depression of normal host defense mechanisms by immunosupressive and other agents have favored *P. aeruginosa* invasion which is highly resistant to antibiotics. Even though the mortality rate in patients with localized infection is not high, pseudomonal septicemia is often associated with a high mortality rate, exceeding 70% (Curtin *et al,* 1961; Flick and Cluft, 1976).

Our knowledge of the epidemiology and control of *Pseudomonas* infections depends on the availability of accurate typing procedures. Among the numerous typing schemes suggested (Aber and Mackel, 1981, Bergan and Midtredt, 1975; Bobo *et al,* 1973; Brokopp *et al,* 1977; Deighton *et al,* 1971; Edmons *et al,* 1972; Gilardy, 1985; Homma, 1976; Jones *et al,* 1974; Thomas *et al,* 1975) serotyping and pyocin typing have proved to be a rapid, practical, and reproducible method for *P. aeruginosa* isolates. We were interested in investigating epidemiology of infections due to *P. aeruginosa.*

MATERIALS AND METHODS

Bacterial strains

The set of 18 ALA (Alabama) indicator strains of *P. aeruginosa* was kindly provided by Dr CD Cox, the University of Iowa, USA. They were kept in the stock nutrient agar at room temperature. For clinical isolates, 436 strains of *P. aeruginosa* were isolated from pus, urine, sputum and blood of patients admitted to Siriraj Hospital during April 1989 - June 1990. Indentification of the isolates was done by using biochemical tests (Gilardy, 1985).

Serotyping

The slide agglutination method previously described by Homma (1976) was performed. The polyvalent antisera (I, II, III) and monospecific antisera (Denka Seiken Ltd, Tokyo, Japan) were used.

Pyocin typing

The methods previously described by Jones *et al* (1974) and Fyle *et al* (1984) were performed. Reporting the results of pyocin typing required simplification. A set of rules has been proposed in which numbers were assigned for inhibition patterns. The series of positive and negative reactions were converted to pyocin type as shown in Table 1 (Farmer and Herman, 1974).

Table 1

Conversion of pyocin reactions into simplified notation.

Three pyocin reactions	Notation
+ + +	1
+ + −	2
+ − +	3
− + +	4
+ − −	5
− + −	6
− − +	7
− − −	8

Note — For example, isolate No. 1 : + + +, + + −, + + +, − + −, + + +, − + +. These were converted to the simplified notation, isolate No. 1 : pyocin type 121614.

RESULTS

During April 1989 - June 1990, 436 *P. aeruginosa* strains were isolated from clinical specimens of 260 patients admitted to Siriraj Hospital which is the biggest hospital in Thailand as follows : blood (19 strains), pus (192 strains), sputum (159 strains) and urine (66 strains).

Serogroups

Three hundred and ninety-six strains (92.8%) were typable, ten serogroups were identified, whereas 37 strains (8.49%) were non-typable and the another 3 strains (0.69%) were autoagglutinated in normal saline solution. Table 2 shows the serogroup distribution.

Pyocin types

All isolates (436 strains) were typed into eight pyocin types. Table 3 shows that the most common type was 121614 (30.95%) followed by 211111 (30.25%) 888888 (17.43%) and 373112 (17.43%), respectively

Epidemiology of *P. aeruginosa* by a combination of serogrouping and pyocin typing

When the isolates were classified according to serogroup and pyocin type, it was found that strains of the same serogroup were apparently distinguished into one to four pyocin types. For example, 115 out of 122 strains in serogroup E fell into pyocin type 211111 whereas the other 7 strains were 888888. In addition, the strains belonging to the same pyocin type were also subdivided into different serogroups. *P. aeruginosa* pyocin type 211111 was distinguished into 4 serogroups (E, L, I and F). Another examples are

Table 2

Serogroup of *P. aeruginosa* isolated in Siriraj Hospital.

O serogroup	No. of *P. aeruginosa* strains	%
E	122	27.98
B	113	25.91
G	62	14.21
L	44	10.10
H	27	6.20
I	13	2.98
F	7	1.61
A	4	0.91
C	3	0.69
J	1	0.23
Nt*	37	8.49
Au*	3	0.69
Total	436	100.00

* Non-typable strain
** Autoagglutinable strain

Table 3

Pyocin types of *P. aeruginosa* isolated in Siriraj Hospital.

Pyocin Type	No. of *P. aeruginosa* strains	%
121614	135	30.95
211111	132	30.25
373112	76	17.43
888888	76	17.43
341112	9	2.10
221131	4	0.92
141111	2	0.46
111111	2	0.46
Total	436	100.00

Table 4

Epidemiology of *P. aeruginosa* by a combination of serogrouping and pyocin typing.

Sero-group	No.	Pyocin type							
		121614	211111	373112	888888	341112	221131	141111	111111
E	122	-	115	-	7	-	-	-	-
B	133	107	-	6	-	-	-	-	-
G	62	1	-	57	4	-	-	-	-
L	44	8	2	2	31	-	-	-	1
H	27	15	-	2	-	9	-	-	1
I	13	-	1	1	11	-	-	-	-
F	7	-	3	-	-	-	4	-	-
A	4	-	-	-	4	-	-	-	-
C	3	1	-	1	-	-	-	1	-
J	1	-	-	-	1	-	-	-	-
Nt**	37	2	9	7	18	-	-	1	-
Au**	3	1	2	-	-	-	-	-	-
Total	436	135	132	76	76	9	4	2	2

* Non-typable strain
** Autoagglutinable strain

shown in Table 4. Therefore, all 436 *P. aeruginosa* strains could be differentiated into 33 "serogroup/pyocin type" or "pattern". The results are illustrated in Fig 1. The most common pattern was E 211111 with the percentage of 26.3% followed by B 121614 (24.5%), G 373112 (13%) and L 888888 (7.1%), respectively. In the other groups (65 strains), there were 24 patterns which the number of strains in each pattern was less than 8 isolates.

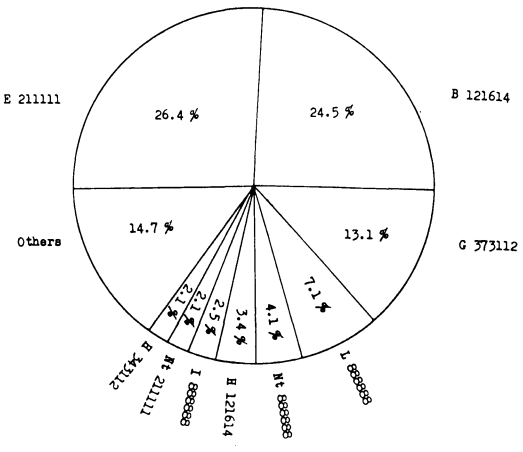

Fig 1—Percentage of 33 different serogroup/pyocin type (pattern) of *P. aeruginosa* in Siriraj Hospital.
Other : *P. aeruginosa* which belonged to the pattern that had the number of isolates ≤ 8.

DISCUSSION

It has been reported that among data on site-specific infections, *P. aeruginosa* was the leading pathogen identified in nosocomial lower respiratory tract infections (9.7%), and it was the second leading pathogen identified in nosocomial urinary tract infections (16.4%) as well as in burn/wound infections (19.7%) (Morrisson *et al,* 1984).

In Thailand, according to data of the study by the Committee of Infectious Disease Control at Ramathibodi Hospital, it was shown that *P. aeruginosa* was the most common isolate from patients with nosocomial lower respiratory tract infections (34%) and was the second leading pathogen (14%) in patients with nosocomial septicemia. For nosocomial surgical wound infections, *P. aeruginosa* was the third leading pathogen (22%) and for nosocomial urinary tract infection, it was the fourth leading pathogen (12%) (Lolekha *et al,* 1981).

There are at least three factors which confer advantages for survival of *P. aeruginosa* in the hospital environment. Firstly, it can survive in environment with simple nutrient requirements, due to its ability to metabolize a variety of organic chemical substrates. Secondly, it is widely distributed in nature. Thirdly, it is highly resistant to many antibiotics. To monitor successfully the epidemiology of this organism, it is necessary to positively establish the relationship between all isolates. Epidemiological markers, including the determination of general physiological properties (biotyping), bacteriophage typing, pyocin typing, antibiotic susceptibility, genome fingerprinting and serotyping are used. Some of them have been useful, but others have given misleading results (Thomas *et al,* 1975).

In this study, serotyping and pyocin typing were chosen to type *P. aeruginosa.* Since there were no previous reports about *serogroups* and pyocin tyes of *P. aeruginosa* published in Thailand, comparison of the results should be performed with those from other countries. For serotyping, 10 serogroups were found in Siriraj Hospital. In this study, serogroup E was predominant (27.98%) which was also found in Greece 14% (Legakis *et al,* 1982), Italy 23.4% (Piano *et al,* 1986). USA 28% Mayo *et al,* 1986), India 40% (Agarwal *et al,* 1983), and Taiwan 28% (Ding and Wu, 1988). The reason for this observation could not be explained, but it may be related to virulence factors and antibiotic resistance of this serogroup (Morrison and Wenzel, 1984).

The results of pyocin typing could not be compared with other reports because of the use of different sets of *P. aeruginosa* indicator strains, *eg* The studies of Darrell and Wahba (1964), Jones *et al* (1974). Pyocin types from their studies were different, furthermore, comparative study between their pyocin-type systems has never been carried out. In this study, with the use of ALA indicator strains, 8 pyocin types were found in Siriraj Hospital and 121614 type was predominant (30.95%).

Epidemiological study of *P. aeruginosa* infection using serotyping and pyocin typing showed that strains belonging to one serogroup differed in the distribution of pyocin typing or in the other hand, the same pyocin types were also diffenent in serogroup. Combining these two markers, greater numbers of epidemiological strains of *P. aeruginosa* were apparently obtained. In this study, the most common pattern was E211111. This information is not only useful in epidemiology but also helpful in research for development of *P. aeruginosa* vaccines.

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