PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA* MUCOID AND NON-MUCOID TYPE

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Abtract. *Pseudomonas aeruginosa* is a leading cause of nosocomial infections. One thousand two hundred and twenty strains of mucoid and non-mucoid types of *P. aeruginosa* isolated from different patients were examined at Siriraj Hospital from January 2001-October 2003. The prevalences of *P. aeruginosa* mucoid type and non-mucoid type were 3.6 % and 96.4%, respectively. Susceptibility testing was performed by Kirby-Bauer disk diffusion method as recommended by NCCLS. The isolates with mucoid phenotypes were more susceptible than the non-mucoid isolates. The antimicrobial susceptibility pattern of both types should provide guidelines for the selection of appropriate drugs for treatment.

INTRODUCTION

Pseudomonas aeruginosa is an important pathogen causing severe and life threatening infections in immunocompromised hosts, such as patients suffering from respiratory disease, chemotherapy cancer patients, and children and young adults with cystic fibrosis. Moreover, it is a leading cause of nosocomial infections and is associated with a high mortality rate. One reason for this high mortality is its notable resistance to many currently available antibiotics. Yet, comparative analyses of the emergence of resistance associated with different classes of antipseudomonal drugs are lacking, even through knowledge about the relative risks of resistance with different antibiotics could be useful in helping to guide theraputic choices (Carmell *et al*, 1999). In cystic fibrosis patients, colonization of the respiratory tract with *P. aeruginosa* occurs, mucoid variants of the original strain emerge and predominate (Ogle *et al*, 1987). The mucoid strain of *P. aeruginosa* leads to chronic pulmonary infection and a poor prognosis for the patient. The mucoid type is the result of an overproduction of the exopolysaccharide alginate. (Govan and Harris, 1986). The antimicrobial susceptibility pattern of mucoid and non-mucoid phenotypes is different. The reason for this difference in antibiotic susceptibility is not clear (Ciofu *et al*,

2001). In this study, we analyzed data for *P. aeruginosa,* mucoid type and non-mucoid type, isolated from patients admitted to Siriraj Hospital from January 2001-October 2003.

MATERIALS AND METHODS

Bacterial isolates and identification procedure

One thousand two hundred and twenty *P. aeruginosa* strains were collected from different patients who were admitted to Siriraj Hospital between January 2001 and October 2003. The isolates were obtained from different clinical specimens, including bronchial wash, bronchoalveolar lavage, throat swab, nasal swab, and sputa. Sputa were gram-stained and only sputums showing <25 squamous epitherial cells per low-power field were acceptable for culture on blood agar, chocolate agar and MacConkey agar. Bacterial pathogens were isolated and identified by the conventional biochemical tests (Murray *et al*, 1999) *ie*, gas from nitrate, growth at 42ºC, no growth at 4ºC, gelatin liquefaction test, pigment production, lactose O/F, maltose O/F, arginine dihydrolase activity and ornithine decarboxylase. For each isolate, the laboratory completed a case record by giving details of the patient's age, sex, site of infection, together with susceptibility testing results.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was performed as recommended by the National Com-

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mittee for Clinical Laboratory Standards (NCCLS, 2002). The Muller-Hinton plates were incubated overnight at 35ºC for 18 hours, after which the diameter of each inhibition zone was measured.

RESULTS

For the 1,220 strains of *P.aeruginosa* isolated, the sources of the specimens are shown in Table 1. They were mostly isolated from sputa followed by bronchial washings. Forty-four strains were mucoid types (3.6%) whereas 1,176 strains were non-mucoid types (96.4%) (Fig 1). For the mucoid type, the number of patients based on different age groups is shown in Table 2. The

Table 1
Source of clinical specimens from patients of Siriraj Hospital.

Isolation sites	No. of mucoid	%	No. of non-mucoid	%
Bronchoalveolar lavage	1	2.3	10	0.8
Bronchial wash	3	6.8	77	6.5
Nasal swab	-	-	5	0.4
ET tube	-	-	2	0.2
Throat swab	-	-	5	0.4
Sputa	40	90.9	1,077	91.6
Total	44	100	1,176	100

Table 2
The number of patients (mucoid type) in different age groups.

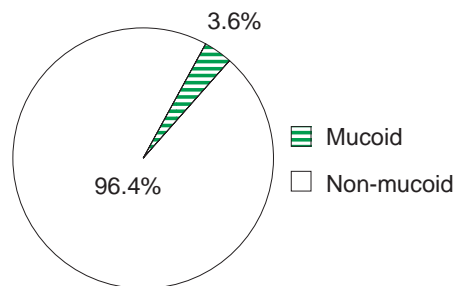| Age groups (years)	Number of patients (mucoid strains)	%
< 1	-	-
1-9	-	-
10-19	-	-
20-29	3	6.8
30-39	2	4.5
40-49	1	2.3
50-59	3	6.8
60-69	7	15.9
≥ 70	13	29.5
Data not available	15	34.1
Total	44	100

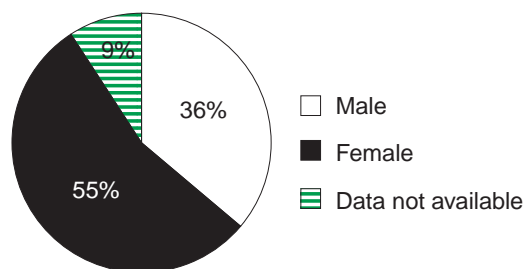Fig 1–The percentage of *P. aeruginosa* mucoid and non-mucoid types.

Fig 2–The number of male and female in *P. aeruginosa* mucoid type.

patients ages ranged from 23 to 81 years old (Table 2). The highest percentage group was patients ≥ 70 years old (29.5%) followed by 60-69 years old (15.9%). Of these, the ratio of male:female was 1:1.5 (Fig 2).

Antimicrobial susceptibility test

The percentages of mucoid and non-mucoid strains susceptible and resistant to each drug are shown in Table 3. For the mucoid type, the sensitivities were: amikacin (86%), gentamicin (86%), netilmycin (97%), ciprofloxacin (90%), ofloxacin (87%), imipenem (95%), meropenem (93%), piperacillin/tazobactam (97%), cefepime (96%), cefpirome (86%), cefoperazone/sulbactam (95%), ceftazidime (91%) and ceftriaxone (58%). For the non-mucoid type, the sensitivities were: amikacin (70.5%), gentamicin (53.5%), netilmycin (82%), ciprofloxacin (70%), ofloxacin (60.5%), imipenem (69.5%), meropenem (68%) and piperacillin/ tazobactam (78%). Both types were resistant to ampicillin, ampicillin/sulbactam, amoxicillin/ clavulanate, co-trimoxazole, cefotaxime and cefoxitin.

Table 3
Susceptibility patterns of *P.aeruginosa* mucoid type and non-mucoid type.

Antibiotics	% Susceptible	
	Mucoid	Non-mucoid
Amikacin	86	70.5
Gentamicin	86	53.5
Netilmycin	97	82
Ceftazidime	91	66
Ciprofloxacin	90	70
Ofloxacin	87	60.5
Imipenem	95	69.5
Meropenem	93	68
Piperacillin /tazobactam	97	78
Cefepime	96	65
Cefpirome	86	57
Cefoperazone/sulbactam	95	65
Ceftriaxone	58	5.5
Ampicillin	8	0
Ampicillin/sulbactam	36	10
Amoxicillin/clavulanate	17	25
Co-trimoxazole	41	17.5
Cefotaxime	45	3
Cefazolin	0	6

DISCUSSION

P. aeruginosa infection is a serious cause of nosocomial infections. In this study, it was found that the incidence of *P. aeruginosa* mucoid type was very rare compared to non-mucoid type. From a previous study in Iran (Ahangarzadeh-Rezaee *et al*, 2002), out of 133 clinically isolated *P. aeruginosa* strains, 43 cases (32.3%) were identified as mucoid type and 90 cases (67.7%) were non-mucoid type. Mutation may induce mucoid variants, which synthesize large amounts of extracellular alginate, emerging within months of colonization. Thus, transition from early colonization to chronic infection may be associated with a change in *P. aeruginosa* phenotype from non-mucoid to mucoid colony formation (Mathee *et al*, 1999). Our study also investigates the relationship between age and the mucoid type. It is associated with patients in age groups ≥ 60 years old (45.5%).

Drug resistant *P. aeruginosa* may develop during therapy. Data from our study demonstrated that the mucoid isolates were generally more sus-ceptible to the drugs tested than non-mucoid isolates. These results are in accordance with previously published data from southwestern Germany. They found that non-mucoid strains were more resistant to antibiotics, and inversely correlated to alginate production (Schulin, 2002). Other *in vitro* investigations have noted similar results (Shawar *et al*, 1999). In addition, a study in Denmark found that mucoid strains of *P. aeruginosa* were more susceptible to antibiotics, and had lower β-lactamase activity than the corresponding non-mucoid strains. They proposed that the maintenance of antibiotic susceptibility of alginate-overproducing isolates might be explained by the co-existence in biofilm of non-mucoid resistant isolates that may play a protective role (Ciofu *et al*, 2001). A recent study in Iran reported that mucoid strains were significantly more resistant to amikacin, gentamicin, and tobramycin than non-mucoid strains (Ahangarzadeh-Rezaee *et al*, 2002). Of all the isolates in our study, netilmycin was the most effective antibiotic (89.5%), followed by amikacin (78.2%) and gentamicin (69.7%) in the aminoglycoside group. Of the β-lactam antibiotics, piperacillin/tazobactam (87.5%) was the most effective, followed by imipenem (82.2%), meropenem (80.5%), cefepime (80.5%), cefoperazone/sulbactam (80%), ceftazidime (78.5%) and cefpirome (71.5%). In fluoroquinolone group, ciprofloxacin (80%) was the most effective, followed by ofloxacin (73.7%). These data are in agreement with a previous study in Belgium which demonstrated that amikacin was the most effective (10.5% resistance) in aminoglycoside group and ciprofloxacin was most effective agent in the fluoroquinolone group. Levofloxacin and oflaxacin had only 27.5% and 37.5% sensitivities, respectively. In terms of percentages of susceptibility, both piperacillin/tazobactam and meropenem scored best (Eldere, 2003). Similar to a study in western Germany, meropenem, ceftazidime, and piperacillin were most effective in mucoid and non-mucoid strains (susceptibilities were 86.2, 84.2 and 84%, respectively). However, only 46.2 and 41.8% of strains were susceptible to ciprofloxacin and fosfomycin, respectively (Schulin, 2002). It was reported that the majority of meropenem-resistant *P. aeruginosa* were resistant to imipenem, but almost half the

imipenem resistant strains were susceptible to meropenem. Moreover, the strains resistant to meropenem were also resistant to ciprofloxacin and carbenicillin (Bonfiglio *et al*, 1998).

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