Genotype and Antibiotic Susceptibility Patterns of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* Isolated from Cystic Fibrosis Patients

Hasan Nazik*, Betigül Öngen, Zayreuran and Melek Şalçioğlu

Department of Microbiology and Clinical Microbiology, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey

(Received July 20, 2006. Accepted December 25, 2006)

SUMMARY: The purpose of this study was to type the *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolates recovered from cystic fibrosis (CF) patients by random amplified polymorphic DNA (RAPD)-PCR and to determine the antibiotic susceptibility of these strains. *P. aeruginosa* (*n* = 49), and *S. maltophilia* (*n* = 11) isolates which had been recovered from 16 and 8 patients, respectively, during a 1-year period were investigated. Three primers were used for RAPD-PCR typing. Antibiotic susceptibility testing of all isolates was performed by the disc diffusion method. RAPD-PCR analysis revealed 21 (*P. aeruginosa*) and 9 (*S. maltophilia*) different genotypes. According to the antimicrobial susceptibility results, the *P. aeruginosa* and *S. maltophilia* strains were cumulated into 24 and 11 groups, respectively. The CF patients were colonized or infected with *P. aeruginosa* strains of single or sometimes multiple genotypes which remained stable over several months. Our results also revealed that cross-colonization might be possible among the patients who are followed up at the same center. Piperacillin-tazobactam for *P. aeruginosa* and trimethoprim-sulfamethoxazole for *S. maltophilia* were found to be the most active antibiotics according to our results.

INTRODUCTION

Respiratory tract colonization and infection in cystic fibrosis (CF) patients by bacterial pathogens are commonly associated with deterioration and lung function decline, and ultimately lead to pulmonary failure and death (1). Many bacterial species are associated with CF respiratory tract infection, including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Burkholderia cepacia* (2). Among these pathogens, chronic pulmonary infection with *P. aeruginosa* is a major cause of the morbidity and mortality in these patients (3). Once *P. aeruginosa* has colonized the patient’s lung, it is rarely possible to eradicate this pathogen by antimicrobial chemotherapy (2). *P. aeruginosa* infection occurs in over 80% of patients with CF and is the leading cause of progressive loss of lung function and early death (4).

*S. maltophilia* has been increasingly recognized as a cause of respiratory tract infection or colonization in CF patients, and its prevalence has increased in recent years (5-7). This increase has been associated with the extensive use of antipseudomonal antibiotics (8).

*P. aeruginosa* and *S. maltophilia* are highly resistant to various antimicrobial agents and understanding the role of both organisms in CF lung infections could have significant treatment implications (5,9).

The aim of this study was to determine the antibiotic susceptibility and random amplified polymorphic DNA (RAPD)-PCR genotypes of these strains, and to reveal whether CF patients are colonized with the same strain, if the respiratory tract specimens contain single or multiple genotypes, and if cross-colonization occurs between patients.

MATERIALS AND METHODS

Patients and isolates: In the present study, we investigated 49 *P. aeruginosa* and 11 *S. maltophilia* strains isolated from 16 and 8 CF patients (a total of 22 patients), respectively, who were followed during a 1-year period (2003) at the Istanbul Medical Faculty, Turkey. The strains were isolated from sputa and deep throat swab specimens. Of these samples, 22.5 and 77.5% were received from patients while they were in and outpatients, respectively. A total of 60 strains (49 *P. aeruginosa* from 31 samples: 10 throat swab and 21 sputum, 16 patients; and 11 *S. maltophilia* from 9 samples: 6 throat swab and 3 sputum, 8 patients) were investigated. Both species were isolated from 2 patients (Patients J and P: 9%). More than one sample was received from 8 (36%) patients. Simultaneous isolates of different colony morphotypes or antibiotic resistance patterns in one sample were taken as different strains.

Identification of isolates: All strains were identified by standard methods as *P. aeruginosa* and *S. maltophilia* (10). After identification, they were preserved at −70°C in trypticase soy broth supplemented with 15% glycerol.

Antimicrobial susceptibility testing: The antibiotic susceptibility of the strains was tested against various antibiotics using the disc diffusion method. The antibiotic discs (Oxoid, Hampshire, England) were purchased and used as directed by the manufacturer. The following antibiotics were tested: aztreonam (30 μg), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), ceftazidime (30 μg), cefoperazone-sulbactam (75/30 μg), cefepime (30 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), tobramycin (10 μg), netilmicin (30 μg), amikacin (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), and trimethoprim-sulfamethoxazole (1.25/25 μg) (for *S. maltophilia*).

The antimicrobial susceptibility test was carried out
through the disc diffusion method according to the CLSI recommendations (11).

**Genotypic analysis by RAPD-PCR:** Isolation of bacterial genomic DNA: The whole-cell DNA of the bacteria was extracted as described previously. The DNA was stored at −20°C until the RAPD-PCR applied (12).

RAPD-PCR analysis: RAPD-PCR analysis was performed as described by Williams et al. (13,14) with some modifications. The PCR mixture was comprised of buffer (10 mM KCl, 10 mM (NH4)2SO4, 20 mM Tris HCl [pH 8.75], 0.1% Triton X-100, and 0.1 mg/ml BSA, 2 mM MgSO4), the four deoxynucleotide triphosphates (BioBasic, Markham, Ontario, Canada) at a concentration of 400 μM each, 150 pmol of primer, about 1 μg of DNA, and 2 U of Taq DNA polymerase (BioBasic), and the total volume was 50 μl.

The primers used in the study were 272 (5′-AGCGGGCCA A-3′), ERIC2 (5′-ATGTAAGCTCCT GGGGATTCA C-3′), and M13 (5′-GAGGGTGGCGGTTC3′).

Primer 272 was primarily used to type *P. aeruginosa*, primer ERIC2 was primarily used to type the *S. maltophilia* isolates, and primers ERIC2 and M13 were used for confirmation, respectively.

Each of the 40 subsequent cycles consisted of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min (the first cycle denaturation was at 94°C for 5 min, and the last cycle extension was at 72°C for 10 min) (Techne Thermal Cycler; Techne Inc., Burlington, N.J., USA). The RAPD-PCR products were separated by electrophoresis in 1.5% agarose gel, then stained with ethidium bromide and photographed (Techne Thermal Cycler; Techne Inc., Burlington, N.J., USA). A 1,353-bp DNA ladder (Φ X 174) was used as a molecular size standard. The fingerprints were compared visually, and the patterns were considered different when they differed by at least one amplification band, regardless of band intensity.

**RESULTS**

Tables 1 and 2 show an analysis of the strains according to the genotypes and antibiotic susceptibilities. Table 3 shows the correlation between RAPD and the antibiotic susceptibility pattern.

RAPD-PCR analysis revealed 21 (*P. aeruginosa*) and 9 (*S. maltophilia*) different genotypes. The *P. aeruginosa* and *S. maltophilia* strains were cumulated into 24 and 11 groups, respectively, according to the antimicrobial susceptibility results. We received more than one sample from 6 (37.5%) of the 16 patients, (Patients A, B, C, D, G and K) during the study period. Among these patients, Patient B was colonized with a single genotype and Patients D and K were colonized with a constant genotype and cocolonized with other genotypes simultaneously. Patients A, C, and G demonstrated replacement of one *P. aeruginosa* genotype with another.

Three patients 18.8% (Patients A, D, K) were colonized with more than one *P. aeruginosa* genotype in the same sample. Many isolates identical in genotype displayed variability in antibiotic susceptibility pattern (Table 3).

Five *P. aeruginosa* genotypes were shared among the patients. The most common one was genotype 5 (Patients A, C, D, J, M), followed by 9 (Patients B, F, I), 11 (Patients D, L, N), 7 (Patients A, P), and 8 (Patients A, G). Six patients (37.5%) (Patients F, H, J, L, M, N) had only nonmucoid and 4 patients (25%) (Patients E, I, O, P) had only mucoid *P. aeruginosa* isolates. Four (25%) of the patients (Patients B, D, G, K) harbored mucoid and nonmucoid isolates in the same sample.

Patient R had two samples which yielded a different genotype of *S. maltophilia*. Two patients with *S. maltophilia* (Patients P and T) harbored two different genotypes in the same sample, and they shared one of them (genotype III).

Genotype II was also shared between two patients (S and T) (Table 2).

### Table 1. Typing and antibiotic susceptibility pattern results of *P. aeruginosa* strains

<table>
<thead>
<tr>
<th>Patient</th>
<th>Isolation date (2003)</th>
<th>Sample</th>
<th>ip/op</th>
<th>Phenotype</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14 January</td>
<td>sp</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>04 March</td>
<td>sp</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>23 June</td>
<td>sp</td>
<td>op</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>28 July</td>
<td>sp</td>
<td>op</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>20 October</td>
<td>op</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>19 November</td>
<td>op</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>01 December</td>
<td>op</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>20 January</td>
<td>op</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>25 June</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>21 January</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>13 August</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>10 November</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>05 February</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>16 October</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>24 October</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>14 March</td>
<td>sp</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>19 March</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>26 June</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>26 August</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>31 October</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>04 December</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>03 July</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>15 July</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>J</td>
<td>05 August</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>25 September</td>
<td>sp</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>25 September</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>06 October</td>
<td>ts</td>
<td>op</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>06 October</td>
<td>ts</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>O</td>
<td>23 December</td>
<td>sp</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P</td>
<td>23 July</td>
<td>sp</td>
<td>ip</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1): two strains.
2): three strains.

Antibiotic susceptibility patterns: 1, Resistant to piperacillin (PRL); 2, Susceptible to all antibiotics tested; 3, Resistant to ceftazidime-sulbactam (SCF), ceftazidime (CAZ), cefepime (FEP), PRL, aztreonam (ATM), gentamicin (CN), tobramycin (TOB), netilmicin (NET), amikacin (AK), ciprofloxacin (CIP), ofloxacin (OFX); 4, Resistant to SCF, CAZ, PRL, CN; 5, Resistant to SCF, PRL, ATM, CIP, OFX; 6, Resistant to meropenem (IPM), meropenem (MEM), SCF, FEP, PRL, piperacillin-tazobactam (TZP), ATM; 7, Resistant to IPM, PRL, MEM, SCF, CAZ, FEP, ATM, CIP, OFX; 8, Resistant to IPM, MEM, SCF, CAZ, FEP, PRL, ATM, CIP, OFX; 9, Resistant to CIP, OFX; 10, Resistant to SCF; 11, Resistant to CN, CIP, OFX; 12, Resistant to CIP, FEP, PRL, ATM, CN, TOB, NET, AK, CIP, OFX; 13, Resistant to ATM, MEM, OFX, PRL, ATM; 14, Resistant to FEP, ATM, CN, TOB, NET, AK, CIP, OFX; 15, Resistant to SCF, CAZ, ATM, PRL, TZP; 16, Resistant to FEP, CAZ, PRL, ATM, TZP, ATM; 17, Resistant to PRL, CIP, OFX; 20, Resistant to ATM, ATM; 21, Resistant to SCF, CAZ, FEP, PRL, ATM, CN, AK, NET, OFX; 22, Resistant to ATM, ATM; 23, Resistant to SCF, IPM, MEM, PRL, CAZ, PRL, ATM, CN, AK, NET, CIP, OFX.
Fig. 1 shows the antibiotic susceptibility results of the *P. aeruginosa* and *S. maltophilia* strains. For the *P. aeruginosa* isolates, amikacin (80% of strains), netilmicin (80%) and tobramycin (82%) were found to be more active than gentamicin (73%). The *P. aeruginosa* isolates were most susceptible to piperacillin-tazobactam (86%), followed by meropenem (84%), and imipenem (82%). Trimethoprim-sulfamethoxazole was found to be the most active antibiotic against the *S. maltophilia* strains (all were susceptible), followed by ofloxacin (82%), and ciprofloxacin (73%).

**DISCUSSION**

CF patients are susceptible to chronic respiratory tract infections by many microorganisms including *P. aeruginosa* and *S. maltophilia*. They suffer from repeated bacterial infections which cause an irreversible loss of lung function which determined morbidity and mortality (2,15).

*P. aeruginosa* strains which were isolated from chronically colonized CF patients had significantly different phenotypes than strains collected from other patients or from the environment (16,17).

Investigation of the epidemiology of these organisms may help us to better understand their role in CF lung disease (5,9). The typing of *P. aeruginosa* isolates of CF origin by conventional phenotypic methods has been found to be difficult due to the unique feature of these isolates (18). Also, our study demonstrated that there was not a good correlation between RAPD and the antibiotic susceptibility pattern (Table 3). The RAPD method is simple, highly reproducible, and able to differentiate unrelated strains and should be useful in clarifying the epidemiology of *P. aeruginosa* (19) and *S. maltophilia* in patients with CF (5).

We examined the RAPD fingerprints of the 49 *P. aeruginosa* and 11 *S. maltophilia* strains isolated from CF patients during a 1-year period.

The genotyping of sequentially isolated *P. aeruginosa* strains demonstrated that each of these patients were colonized or infected with strains of single or multiple genotypes which remained stable over several months. Patients A, D and K were colonized with two or more *P. aeruginosa* genotypes, and three different patterns in the same sample were recovered from Patient K. This means that different...
genotypes may be recovered simultaneously beside the consistent genotypes in some patients. Our study revealed that most of the *P. aeruginosa* isolates with dissimilar colony morphology or antibiotic susceptibility isolated from these CF patients were of the same genotype; however, colonization or infection with only one genotype is not a rule (Fig. 2). These results were also found in other previous studies (20, 21).

To date, several epidemiologic studies have addressed cross-colonization with *P. aeruginosa* in CF patients, but the reported results are contradictory (20,22,23). It has been shown that the transmission of strains between patients is possible, especially in CF camps (22,23). The results of our study demonstrated that there is a risk of cross-colonization between CF patients followed-up at the same CF center. It was observed that genotype 5 was the most commonly shared *P. aeruginosa* genotype in this study population. This genotype was recovered from 5 patients. It was first isolated from Patient C in January and last isolated from Patient M in October. These findings are also similar to Sener et al.’s results from Turkey (20). Their patients were followed up at the same date and in the same CF center. Cross-colonization between patients may have occurred because the patients were together in a small, crowded room or because the strains came from a common source. In our study, the patients could have gotten the strains from the same source because patients with the same genotype had no contact with each other. The most common genotypes were isolated from Patients A, D and J while they were outpatients.

Although a limited number of *S. maltophilia* strains were included in this study, our findings showed that some patients can carry more than one genotype in the same sample (Patients P and T), and this is very similar with *P. aeruginosa* colonization/infection in CF patients. The epidemiology of cross-colonization of *S. maltophilia* between CF patients has not been fully clarified. In our study, two genotypes were shared in two patients. These findings were also supported by Krzewinski et al. and Valdezate et al. (5,24).

Antibiotic therapy reduces the morbidity of CF lung disease. Although the treatment of lung infection in CF patients is based on the patient’s age, the colonizing organisms, and the severity of the patient’s pulmonary exacerbation, choosing antibiotics according to the resistance pattern of the strains is highly recommended (1). The result of susceptibility testing of *P. aeruginosa* isolates indicated that tobramycin (82%), amikacin (80%), and netilmicin (80%) are more active than gentamicin (73%). The *P. aeruginosa* isolates are most susceptible to piperacillin-tazobactam (86%) followed by meropenem and imipenem (84 and 82%, respectively). These results are similar to Yagci et al. (23) suggesting that carbapenems are the most active components (93.5% susceptibility), followed by piperacillin (80.5%). In that study, amikacin was the most active aminoglycoside (91.4%) compared to gentamicin (71.7%) and tobramycin (71.7%). Manno et al. (25) recently demonstrated that ceftazidime is the most active agent (86%), followed by piperacillin-tazobactam, aztreonam and imipenem, (81.7, 80.3 and 80%, respectively). They reported that tobramycin was the most active aminoglycoside (76.5%) compared to amikacin (69.5%) and netilmicin (56.5%).

Trimethoprim-sulfamethoxazole is found to be the most active antibiotic against the *S. maltophilia* strains (all strains were susceptible), followed by ofloxacin and ciprofloxacin, (82 and 73%, respectively). Koseoglu et al. (26) suggested similar results with strains isolated from non-CF patients. According to their results, trimethoprim-sulfamethoxazole was the most susceptible agent (95%), followed by ciprofloxacin (80%).

This retrospective study demonstrated that CF patients who were colonized or infected with *P. aeruginosa* strains of single or sometimes more consistent genotypes with different phenotypes remained stable over several months. However, patients could be colonized with more than one genotype simultaneously. Although further studies are needed, this was similar for *S. maltophilia*. Our results also revealed that cross-colonization might be possible among patients who are followed up at the same center. Although piperacillin-tazobactam for *P. aeruginosa* and trimethoprim-sulfamethoxazole for *S. maltophilia* are the most active antibiotic, therapy should be chosen according to the antibiotic susceptibility tests.

**ACKNOWLEDGMENTS**

This study was partly presented as a oral presentation at the 21th ANKEM Klinikleri ve Tip Bilimleri Kongresi (ANKEM Congress of Clinics and Medical Sciences), June 4-8, 2006, Antalya, Turkey.

The present work was supported by the Research Fund of Istanbul University (Project Number T-483/25062004).

**REFERENCES**


