International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Special Issue-Number2-April, 2016, pp102-110
http://www.bipublication.com

Research Article

Genotyping of *Pseudomonas aeruginosa* strains isolated from burn patients by RAPD-PCR molecular technique with primers 287

Z. Mahmmudi[1], A. Emami*[2] and AA. Gorzin[3]

[1]M.Sc. in Biology, Kazeroun Branch, Islamic Azad University, Kazeroon, Iran
[2]Department of Microbiology, Shiraz Burn Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran
[3]Assistant Professor of Bacteriology and Virology, School of Medicine,
Shiraz University of Medical Sciences, Shiraz, Iran
*Corresponding Author:Amir Emami,

ABSTRACT

Background and objectives: One of the most important opportunistic infectious agents is *P. aeruginosa*, especially in patients with weakened immune systems such as burn patients. For the treatment of resistant isolates to penicillin and aminoglycoside, carbapenem such as imipenem (IMP) and meropenem (MEM) are important antibiotics. Because of the importance of finding the source of contamination and the transfer patterns of resistance in the control of hospital infections, this research was conducted to assess the antibiotic susceptibility and genetic patterns of isolates of *P. aeruginosa* obtained from patients referring to Qutb al-Din Shirazi hospital.

Materials and methods: 200 samples were taken the patients. 50 cases were identified using standard biochemical tests and were examined by applying the Kirby-Bauer method to find the patterns of sensitivity and their genetic relationship was revealed through RAPD-PCR technique with the help of primer 287.

Results: RAPD-PCR technique is a genotyping tool with high discriminatory power in epidemiological studies and bacterial polymorphism. According to findings antibiotic resistance in isolates of *P. aeruginosa* is increasing along with various patterns in different wards of hospital. The children's ward had just common genetic patterns with other wards of the hospital due to contamination in other sectors.

Conclusion: Therefore, given to results serious measures must be done to find the infection sources through physicians and staff during work at hospital in order to control and prevent the transmission of the bacteria.

Keywords: *P. aeruginosa*, Burn Wounds, Drug sensitivity, Molecular Genotyping

INTRODUCTION

P. aeruginosa (Family Pseudomonadaceae) is one of the most common opportunistic pathogen that shows the inherent resistance against many antibiotics so that is a major problem in treatment. These bacteria can cause serious infections in burn patients due to damages in the first line of defense, i.e. skin; and they are main factor of deaths in about 77% of these patients in the past 25 years. *P. aeruginosa* genetic patterns using PCR-based molecular methods, which are less affected by environmental factors compared to phenotypic methods, play crucial roles in tracking the routes of pathogen transmission (Akopyanz *et al.*, 1992). Considering the researches, higher discriminatory power and reproducibility can be found in molecular techniques rather than phenotypic tests and these progresses are due to their ability to distinguish small genomic and

molecular stability, compared to phenotypic profiles of the same species. Advanced molecular typing methods such as RAPD - PCR (Random Amplified Polymorphic DNA- PCR) are efficient, complete and accurate to identify the bacterial genetic patterns (Hancock *et al*., 1983). This technique is one of the fastest typing methods, which can be done easily and it has been a lot of attention to it in recent years because of the simplicity, sensitivity, reproducibility, relatively low cost, high speed and differentiate between isolates (Hoogkamp-Korstanje *et al*., 1995). RAPD-PCR reaction that provides a quick method for DNA fingerprinting is the same as conventional PCR reaction; with the difference is that in this technique is used only a few short nucleotide primer (10 nucleotides containing 50% guanine and cytosine). If the double-stranded template primer binding sites are at a convenient distance and have the proper orientation relative to each other, DNA polymerase is able to follow the distance between the two primers connected. About 50 to 100 different DNA fragments can be obtained by careful optimization. The differences in the sequences of DNA at the primer binding sites create various genomic patterns of microorganisms. Bands observed are a reflection of the whole building of DNA molecule used as a template (Lightfoot *et al*., 1993). Given that *P. aeruginosa* bacteria are major agents in hospital acquired infections especially in burn patients, so knowing how to release bacterial strains have particular epidemiological importance in order to find the infection sources, assess the propagation of pathogenic isolates and quick control (Høiby *et al*., 1996).Because of the transfer patterns of resistance in the control of hospital infections, this research was conducted to evaluate the antibiotic susceptibility and genetic patterns of isolates of *P. aeruginosa* obtained from patients referring to Qutb al-Din Shirazi hospital through RAPD-PCR technique with the help of primer 287.

MATERIALS AND METHODS

Clinical samples

Burn Research Center, Shiraz University of Medical Sciences, was chosen because of the large number of burn patients. This research was a cross-experimental study and the period between March 2013 and March 2014 was selected for collecting samples from patients admitted to Qutb al-Din Shirazi hospital. Of 200 clinical samples including wound exudates, urine and blood isolated from patients, 50 strains were confirmed using standard biochemical tests and media such as SIM, MHA, TSI, citrate, oxidation and fermentation (OF), catalase, and pigment production on Mueller Hinton agar (Himedia, India). The samples were kept in the freezer for the next stage.

Genomic DNA extraction: Genomic DNAs of bacteria were extracted according to manufacturer instructions (Kit of Cinna Gene-Iran) as follows: bacteria were delivered using a sterile needle to 100 µl sterile phosphate buffered saline in microtubes 1.5 and 400 µl lysis buffers was added to each of the tubes and mixed by shaker. 300 µl precipitation buffers was poured and gently mixed. The entire contents of microtubes were transferred to special filter pipes and centrifuged at 12000-13000 rpm for a minute. Then, 400 µl washing buffer (1) was added to filter pipes and centrifuged for one minute at 12000-13000 rpm again. Next 400 µl washing buffer (2) was transferred to filter pipes and centrifuged for one minute at 12000-13000 rpm again and this action was repeated once more. In the next phase, filter pipes was centrifuged without adding any substance at the same speed and dry filters were removed and placed in sterile microtubes 1.5, then 50 µl of dilution buffer, which had been already subjected inside thermoblock at 65 °C for 3-5 min, was added to the filter pipes and centrifuged at the same speed. Finally, filters were discarded and the tube with bacterial DNA was stored in the

freezer at -20 ° C in order to do next stage of experiments(Liu *et al*., 1993).

Quantitative and qualitative control of extracted DNA: On the one hand, the concentration of extracted DNA; on the other hand, the ratio of possible contamination of DNA prepared with a mixture of sugar or protein were evaluated by spectrophotometry through measurement the light absorption of DNA at a wavelengths of 230, 260 and 280 nm (A_{230}, A_{260} & A_{280}). Quality of extracted DNA was considered favorable. All DNA samples with optimum quality were stored at -20 °C in the freezer(Ogle *et al*., 1987).

Genomic amplification reaction: Primer mentioned was prepared from Bioneer Inc. Germany. After receiving the lyophilized primers, stock and working solutions were prepared for daily use from the primers in microgram and molarity in terms of concentration provided by the company, including molecular weight, OD_{260} and kept at -20 °C (Mahenthiralingam *et al*., 1994,1995). Genomic amplification reaction was performed with 50 ml original mixes containing 5 µl template DNA, 2.5 µl buffer PCR 10X, 1 µl $MgCl_2$, 1.2 µl dNTPs mix, 0.4 µl Taq DNA polymerase, primer 287 (10 Pmo) and 36.4 µl Distilled Water, also a positive control was developed that included a components of genome replication reaction with DNAs of *P. aeruginosa* ATCC27853 as a representative of *P. aeruginosa* complex (Saiman. *et al*., 1990). Table 1 presents the conditions and program for 35 cycles used for genomic amplification reaction.

Number of cycles	Denaturing	Annealing	Elongation	Profile
1	95			temperature (°C)
	600			time (s)
35	95	44	72	temperature (°C)
	60	180	120	time (s)
1	72			temperature (°C)
	600			time (s)

Table 1- program, temperature, time and cycles for RAPD-PCR (primer 287)

RAPD-PCR products were prepared on agarose gel 1.5% for electrophoresis of 16srRNA. After heating and dissolving the gel and reaching to 60 °C at room temperature, it was poured into the electrophoresis cassette, which the comb had been placed correctly in it. After complete solidification of the gel, it was placed inside the tank and tris acetic acid buffer 1x was poured on it up to 1 ml above inside the tank.

45 µl of genomic amplification products were mixed with 5 µl of loading buffer. Then mixed with loading buffer was placed in wells of agarose gel 1.5% floating in the buffer. Electrophoresis was performed at a voltage of 70. Then, gels containing genomic amplification products were stained with ethidium bromide for 30 minutes. Stained gel was observed by the UV system and scanned with the help of Gel Doc device and the results were digitally recorded and printed. After the molecular detection of *P. aeruginosa*, based on amplification of 16srRNA, samples containing this gene fragment were identified by UV and confirmed as *P. aeruginosa* isolates (Sokol *et al*., 1994).

Statistical analysis: Data analyzing was performed using software SPSS version sixteenth, analysis of variance and T test ($p < 0.05$).

Cluster analysis: clustering the results of electrophoresis for PCR products was done by software Numerical Taxonomy and Multivariate Analysis System (NTSYSpc). For each primer was drawn a dendrogram as well as an overall dendrogram derived from all the bands amplified by the primers (Simpson *et al*., 1995).At last, genetic distance was calculated using matrix of Jaccard similarity coefficients and Unweighted Pair Group Method with Arithmetic Mean method (UPGMA).

RESULTS

Pseudomonas aeruginosa confirmed samples, differentiated by gender were obtained from 117 (58%) males and 83 (42%) females, overall including: 27% from the women's ward; 41% from the men's ward; 17% the children's ward

and 14% from the ICU. Of the total 200 samples, those that results of microbiological tests were positive for citrate, oxidase, catalase, indole, with pigment production and no sugar fermentation in TSI as well as growth was at a temperature of 42 °C have been identified as *P. aeruginosa* and stored in specific medium at -20 °C.

The results of Antibiogram

The patterns of sensitivity were assessed by applying disk diffusion test (Kirby-Bauer method) on Mueller-Hinton agar; antibiotics were: gentamicin (5 µg), amikacin (30 µg), piperacillin (100µg), erythromycin (30µg), ciprofloxacin (10 µg), cefotaxime (30 µg), meropenem (10 µg), imipenem (10 µg), ceftazidime (30 µg) and chloramphenicol (30 µg). Isolates of *Pseudomonas aeruginosa ATCC 27853* was used as a positive control.

The frequency of antibiotic resistance

Of the 11 antibiotics used, isolates resistance to a variety of antibiotics has been shown in Figure 1.

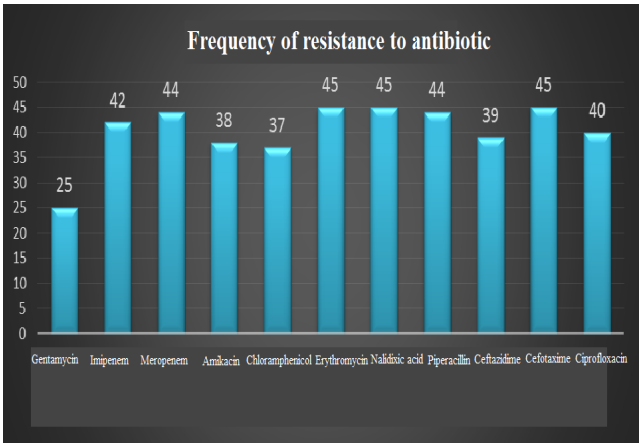

Fig 1- The antibiotic susceptibility of *Pseudomonas aeruginosa* isolates from the Iranian Burn patients using the disk diffusion method.Amikacin (AN), Ceftazidime(CAZ), **Chloramphenicol** (CK), **Erythromycin** (E), **Nalidixic acid (NA), Cefotaxime (CTX),**Ciprofloxacin (CP), Gentamycin (GN), Imipenem (IPM), Meropenem (MEM), Piperacillin (PIP),and Piperacillin (PIP) were used**.**

The results of RAPD-PCR method for Primer 287

Genetic patterns of 50 *P. aeruginosa* isolates that were identified by phenotypic and molecular techniques were determined using RAPD-PCR with primer 287.

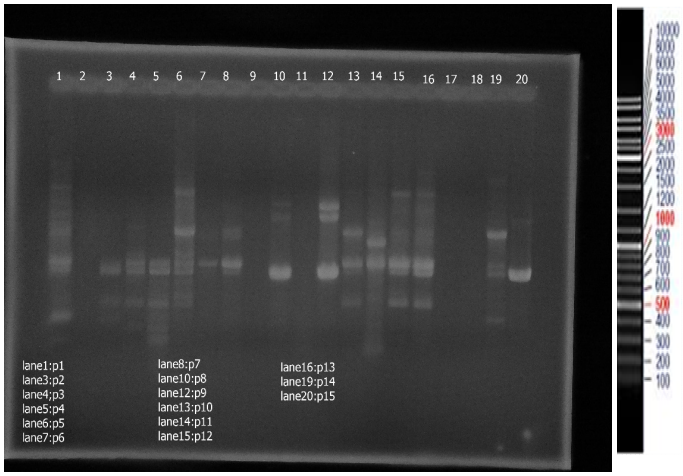

Fig 2- Gel electrophoresis: detection of *Pseudomonas aeruginosa* isolates with primers 287

As is clear from Figure 2, samples 1 to 20 are different strains of *P. aeruginosa* with primer 287 and fermentas gel has been shown along with ladder. The results of electrophoresis for isolates of *P. aeruginosa* with primer 287 in Excell software have been shown as 0 and 1 with the presence and absence of the bands (Table 2). And then the collected data in Excel software was transferred to program NTSYSpc and drawn dendrogram to determine the number of clusters for finding the relationship with each other. At last, genetic distance was calculated using matrix of Jaccard similarity coefficients.

Table 2- Number of genetic patterns and the bands range for *Pseudomonas aeruginosa* isolates with primers 287

Bands (bp)	1	2	3	4	5	6	7	8	9	10	11
300	1	0	1	0	0	0	0	0	0	1	0
400	0	0	0	0	0	0	0	0	0	0	0
500	1	1	0	0	0	0	1	0	0	1	0
600	0	0	0	0	0	0	1	0	0	1	0

700	0	1	0	0	0	0	0	0	0	0	0
800	0	1	0	0	1	1	0	0	0	0	0
900	1	1	1	0	1	0	1	0	1	0	0
1000	1	1	1	1	0	0	1	1	1	0	1
1100	0	0	0	0	0	0	0	0	0	1	0
1200	0	0	0	0	0	0	0	0	0	0	0
1300	0	0	0	0	0	0	0	0	1	0	0
1400	0	0	0	0	0	0	1	0	0	1	0
1500	0	1	0	0	1	1	0	0	0	0	0
1600	0	0	0	0	0	1	0	0	1	1	1
1700	0	1	0	0	0	0	0	0	0	0	1
1800	0	0	0	0	0	0	0	0	0	0	0
1900	0	0	0	0	0	0	0	0	0	0	0
2000	0	0	0	0	0	0	0	0	0	0	0
2100	0	1	0	0	0	0	0	0	0	0	0
2500	0	0	0	0	0	0	0	0	0	0	0
3000	0	0	0	0	0	0	0	1	0	0	0
No. of bands	3	8	3	1	3	3	4	2	4	5	3

According to Table 2, number 1 means the presence of bands and zero means the absence of bands in a variety of genetic patterns in isolates of *P. aeruginosa*.

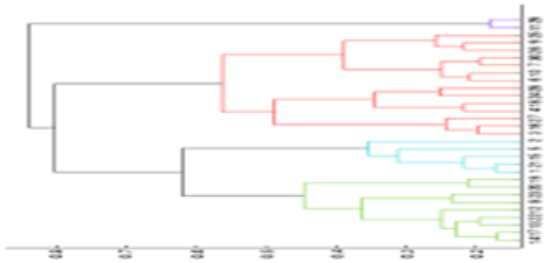

Fig 3- Dendrogram obtained from cluster analysis in isolates of *P. aeruginosa* using UPGMA through NTSYS-PC software with primers 287

According to the dendrogram, primer 287 has the ability to identify 11 genetic patterns in form of 12 band patterns (300-3000) among obtained isolates.

Table 3- Genetic patterns of *P. aeruginosa* strains based on primer 287

Genetic patterns	samples
1	17,20,24,6,18,30,50,52,92
2	25,16,31,46,49,51,23
3	13,15
4	27,65,64,57
5	2,7,91,33,1
6	29,32,14,99,28
7	61,67,29,9,34,58
8	82
9	11,22,19,21
10	4,48,54,38,56
11	47,53
11	50

As it can be seen in the Table 3, according to primer 287, genetic patterns 1 and 2, respectively, with 9 and 7 isolates had the highest number of *P. aeruginosa* isolates studied in this research.

Table 4- Distribution of genetic patterns obtained from *P. aeruginosa* isolates with primer 287

Genetic patterns	Number of samples	Percent
1	9	18
2	7	14
3	2	4
4	4	8
5	5	10
6	5	10
7	6	12
8	1	2
9	4	8
10	5	10
11	2	4
11	50	100

According to the table, the genetic patterns of 1 (18%) and 2 (14%) had the highest frequencies among the 11 genetic patterns of *P. aeruginosa* with primer 287**.**

Table 5- Frequency of genetic patterns of *P. aeruginosa* with primer 287 in different wards of hospital

Ward	
Men	1,2,4,5,6,7,9,10
Women	31,2,4,5,6,7,9,10,8,11
Pediatric	1,2,4,5,6,7,9,10
ICU	1,2,4,5,6,7,9,10

DISCUSSION AND CONCLUSION

Since *P. aeruginosa* is an important factor for nosocomial infections, particularly in burn patients, hence being aware of how to release bacterial strains have a special epidemiological importance to detect the infection sources, assess the propagation of pathogenic isolates and quick control, and because it seems certain genetic patterns of *P. aeruginosa* are imported in various infections, so we used the technique of RAPD - PCR to determine the diversity and genetic differences among patients. Of the total 200 samples collected from burn hospital of Qutb al-Din Shirazi in this study, 50 samples were identified as *P. aeruginosa* isolates. Kirby-Bauer disk diffusion method was used to assess antibiotic susceptibility of isolates. Of 11 antibiotics the highest sensitivity was achieved to gentamicin (25 samples, 50%), and the least sensitivities (90%) were related to nalidixic acid, erythromycin and cefotaxime. However, their sensitivity to the rest of the antibiotic disc was as follows: 16% to imipenem; 12% to meropenem; 24% to amikacin; 26% to chloramphenicol; 12% to piperacillin; 22% to ceftazidime and 20% to ciprofloxacin. A study was performed by Pier *et al*., in 1992 for six months on all patients hospitalized in the burn ward, so that 72% isolates were *P. aeruginosa* and the rest ones were *E. coli*, staphylococcus, candida and proteus, respectively (Pier *et al*., 1992). The findings of this research were not in line with ours and the ratio of infection was high, may be due to a lack of hygiene and inappropriate sterilization and other factors in his study. In another work performed by Struelens *et al* (2011), the greatest common causative organisms of burn wound infections were, respectively, staphylococcus, klebsiella and *P. aeruginosa*. The incidence of *P. aeruginosa* isolates in this study was 25% similar to present work and it was shown that, unlike previous years, the prevalence has been reduced (Struelens *et al*., 2011).

Speert et al., (2010)in the USA conducted a study on the frequency of *P. aeruginosa* among 200 burn patients during 8 months; the results showed that the prevalence of the bacterium was 80%, more than our research (Speert *et al*., 2010). Fegan et al., (2009) in the Netherlands in a similar study demonstrated that the prevalence of bacteria involved in infections over a period of two years was as follows: *Staphylococcus aureus* (70%), *Pseudomonas aeruginosa* (15%), Klebsiella (9%), Candida (5 %) and Proteus (1%). The prevalence of *P. aeruginosa* isolates in this study was lower than ours, which represents the difference of bacteria involved in infections in various parts of the world (Fegan *et al*., 2009).

Grundmann, et al(1995) in Italy studied on 104 strains of *P. aeruginosa*; isolates resistance was evaluated by disk diffusion for 11 antibiotics and broth microdilution for 4 antibiotics. In this study, the highest rate of resistance was to antibiotics of nalidixic acid (86.54%), ceftriaxone (82.2%) and ofloxacin (81.78%). There was also the least resistance to the antibiotic of imipenem (40.19%), piperacillin (44.9%) and tetracycline (48.03%). MIC results showed the greatest sensitivity was related to tetracycline and the most resistant was to ceftriaxone. The results of this study were in line with findings of present research (Grundmann *et al*., 1955).

RAPD-PCR technique is a quick and easy method for detecting polymorphisms in isolates of *P. aeruginosa* and it has been a valuable tool for epidemiological study which the results reveal the difference among strains in the population under study.

In the present study, we attempted for the unification of PCR conditions and DNA concentrations were calculated from 8 to 10 ng

.Kersulyte et al (2005) found that genomic amplification with stored DNA is difficult to interpret and use of fresh DNA was advised (Kersulyte *et al.*, 2005). In this study, we tried to use as much as possible from the fresh DNA; because more than a month after extraction, the bands were not clearly observed due to drop in genetic markers. Investigation of RAPD-PCR technique for typing isolates of *P. aeruginosa* using two ten-nucleotide primers created specific profiles and different genetic patterns were obtained with these primers, reflecting the ability of primers to differentiate the isolates (Speert *et al.*, 1993, 1995). Ramphal et al., in 2006 in Taiwan with RAPD-PCR analysis over 64 strains of *P. aeruginosa* showed that in burn patients of Taipei City, the disease occurred mainly due to the transfer of isolates which had similar genetic fingerprinting results placed in a genetic pattern and only 4 isolates had a unique genetic pattern, which determines the probability of being infected with the new strain (Ramphal *et al.*, 2006).

A study was carried out by Ro¨mling et al., in 1999 in Russia on 41 patients with cystic fibrosis caused by *P. aeruginosa* using RAPD-PCR molecular technique to analyze the genetic patterns of isolates involved in the creation of the infection. It was found that 4 isolates had unique patterns and drug resistance revealed that these isolates compared to other isolates had higher drug resistance expressing probability of mutations as well as in total five different genetic patterns were obtained (Ro¨mling *et al.*, 1999).

The present study shows that RAPD-PCR markers are powerful and effective techniques in identifying and initial screening of samples, examining the differences among species and identify at the level of strain as well as compared with biochemical methods need to spend less time and lower cost.

To ensure the amplification power in RAPD-PCR technique several cases should be considered:

1. Samples should be evaluated by two different primers to confirm the differences among isolates;
2. All reactions should be repeated twice;
3. It is very important to standardize the PCR reaction mixture and unify the conditions for amplification;
4. Several dilution of DNA should be used for PCR reaction and the best dilution should be applied with gel analysis(Sambrook *et al.*, 1989).

According to the results obtained in this study, with more than 95% antibiotic resistance to carbapenem in *P. aeruginosa* strains isolated from burn patients at Qutb al-Din Shirazi hospital, it seems the efficacy of the drug has been greatly dropped and we need new patterns of treatment. Detected reasons for resistance of *P. aeruginosa* isolates to carbapenem include: reduced the permeability of the outer membrane, increased expression of efflux pumps, changes in binding proteins and the presence of carbapenem hydrolyzing enzymes such as metallo beta lactamases. The results of this research and other studies suggest that antibiotic resistance patterns in *P. aeruginosa* are various in different parts of the world and even the resistances among species of pseudomonas are also different. Given to the reasons mentioned, the prevalence of resistance has often genetic origins and is due to add or remove the genetic fragments. This leads to changes in the pattern of chromosome sets in isolates of *P. aeruginosa*. As a result, precise examinations of drug-resistant infectious agents are required for proper treatment of bacterial infections and then prescribe medications. Understanding the state of sensitivity and resistance of these bacteria in hospitals is a fundamental measure in order to determine the therapeutic plan in early dealing and control the bacteria's resistance to antibiotics. Nowadays, there are several molecular techniques to identify the genetic patterns of *P. aeruginosa* isolates. Several researches throughout the world demonstrated that higher discriminatory power and reproducibility compared with phenotypic tests can be observed due to molecular techniques and this is owing to their ability in distinguish of small genomic differences and high molecular stability compared to the phenotypic profiles of

same strains. Advanced molecular typing methods such as Random Amplified Polymorphic DNA-PCR (RAPD - PCR) are efficient, complete and accurate to identify the bacterial genetic patterns. The results of the present experimental work indicated that there were high polymorphisms among strains of *P. aeruginosa* bacteria isolated from burn patients referred to Qutb al-Din Shirazi hospital, Iran; and we should mention that these reports were published for the first time concerning the genetic diversity related to burn patients in the study area. It is recommended that, identify and classify the genetic patterns of *P. aeruginosa* bacteria isolated from these patients play an important role in the finding the causes of colonization the isolates in patients in terms of virulence severity and drug resistance and provide beneficial guides for physicians in order to carry out preventative and treatment measures.

REFERENCES

1. Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg.1992. DNA diversity among clinical isolates of Helicobacter pyloridetected by PCR-based RAPD fingerprinting. Nucleic Acids Res.20:5137–5142.
2. Fegan, M., P. Francis, A. C. Hayward, and J. A. Fuerst.2009. Heterogeneity,persistence, and distribution of Pseudomonas aeruginosagenotypes in cysticfibrosis. J. Clin. Microbiol.29:2151–2157.
3. Grundmann, H., C. Schneider, D. Hartung, F. D. Daschner, and T. L. Pitt.1995. Discriminatory power of three DNA-based typing techniques for Pseudomonas aeruginosa. J. Clin. Microbiol. 33:528–534.
4. Hancock, R. E. W., L. M. Mutharia, L. Chan, R. P. Darveau, D. P. Speert,and G. B. Pier.1983.Pseudomonas aeruginosa isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. Infect. Immun. 42:170–177.
5. Høiby, N., and K. Rosendal.1996. Epidemiology of Pseudomonas aeruginosa infection in patients treated at a cystic fibrosis centre. Acta Pathol. Microbiol. Scand. Sect. B88:125–131.
6. Hoogkamp-Korstanje, J. A. A., J. F. G. M. Meis, J. Kissing, J. van der Laag,and W. J. G. Melchers.1995. Risk of cross-colonization and infection by Pseudomonas aeruginosain a holiday camp for cystic fibrosis patients. J. Clin.Microbiol.33:572–575.
7. Kersulyte, D., M. J. Struelens, A. Deplano, and D. E. Berg.20055. Comparison of arbitrarily primed PCR and macrorestriction (pulsed-field gel electrophoresis) typing of Pseudomonas aeruginosa strains from cystic fibrosis patients. J. Clin. Microbiol.33:2216–2219.
8. Lightfoot, J., and J. S. Lam.1993. Chromosomal mapping, expression and synthesis of lipopolysaccharide in Pseudomonas aeruginosa: a role for guanosine diphospho (GDP)-D-mannose. Mol. Microbiol.8:771–782.
9. Liu, S. L., A. Hessel, and K. E. Sanderson.1993. Genomic mapping with I-Ceu I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in Salmonella spp., Escherichia coli, and other bacteria. Proc. Natl.Acad. Sci. USA90:6874–6878.
10. Mahenthiralingam, E., M. E. Campbell, and D. P. Speert.1994. Nonmotility and phagocytic resistance of Pseudomonas aeruginosaisolates from chronically colonized patients with cystic fibrosis. Infect. Immun.62:596–605.
11. Mahenthiralingam, E., and D. P. Speert.1995. Non opsonic phagocytosis of Pseudomonas aeruginosaby macrophages and polymorphonuclear leukocytes requires the presence of the bacterial flagellum. Infect. Immun.63:4519–4523.

12. Ogle, J. W., J. M. Janda, D. E. Woods, and M. L. Vasil.1987. Characterization of and use of a DNA probe as an epidemiological marker for Pseudomonas aeruginosa. J. Infect. Dis. 155:119 126.
13. Pier, G. B., G. Meluleni, and E. Neuger.1992. A murine model of chronic mucosal colonization byPseudomonas aeruginosa. Infect. Immun. 60:4768–4776.
14. Ramphal, R., L. Koo, K. S. Ishimoto, P. A. Totten, J. C. Lara, and S. Lory.2006. Adhesion of Pseudomonas aeruginosapilin-deficient mutants to mucin.Infect. Immun.59:1307–1311.
15. Ro¨mling, U., B. Fiedler, J. Boßhammer, D. Gorthues, J. Greipel, H. von derHardt, and B. Tu¨mmler.1999. Epidemiology of chronic Pseudomonas aeruginosa infections in cystic fibrosis. J. Infect. Dis. 170:1611–1621.
16. Saiman, L., K. Ishimoto, S. Lory, and A. Prince.1990. The effect of piliation and exoproduct expression on the adherence of Pseudomonas aeruginosato respiratory epithelial monolayers. J. Infect. Dis.161:541–548.
17. Sambrook, J., E. F. Fritsch, and T. Maniatis.1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
18. Simpson, D. A., E. Mahenthiralingam, and D. P. Speert.1995. Characterization of nonmotile Pseudomonas aeruginosaisolated from cystic fibrosis patients, abstr. 238, p. 246.InProgram and Abstracts of the Ninth Annual North American Cystic Fibrosis Conference. Pediatric Pulmonology, Suppl.12. Wiley-Liss, New York.
19. Sokol, P. A., M. Z. Luan, D. G. Storey, and P. Thirukkumaran.1994. Genetic rearrangement associated with in vivo mucoid conversion of Pseudomonas aeruginosa PAO is due to insertion elements. J. Bacteriol.176:553–562.
20. Speert, D. P.1993.Pseudomonas aeruginosa-phagocytic cell interactions, p.163–178.InM. Campio (ed.),Pseudomonas aeruginosa as an opportunistic pathogen. Plenum Press, New York.
21. Speert, D. P., and M. E. Campbell.1995. Hospital epidemiology of Pseudomonas aeruginosa from patients with cystic fibrosis. J. Hosp. Infect.9:11–21.
22. Speert, D. P., M. E. Campbell, S. W. Farmer, K. Vopel, A. M. Joffe, and W.Paranchych.2010. Use of a pilin gene probe to study molecular epidemiology ofPseudomonas aeruginosa. J. Clin. Microbiol. 27:2589–2593.
23. Struelens, M. J., V. Schwam, A. Deplano, and D. Baran.2011. Genome macro restriction analysis of diversity and variability of Pseudomonas aeruginosastrains infecting cystic fibrosis patients. J. Clin. Microbiol. 31:2320–2326.