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GENETIC RELATEDNESS AMONG CLINICAL STRAINS OF *STENOTROPHOMONAS MALTOPHILIA* IN TERTIARY CARE HOSPITAL SETTINGS IN SÃO PAULO STATE, BRAZIL

Margarete Teresa Gottardo Almeida[1]*; Fernando Gôngora Rubio[1]; Doroti Oliveira Garcia[5]; Érika Cristina Pavarino-Bertelli[3]; Andrea Regina Baptista Rossit[1]; Silvia Yano Bando[2]; Suzane Silbert[4]; Eny Maria Goloni-Bertollo[3]; Márcia Maria Costa Nunes Soares[6] Marina Baquerizo Martinez[2]

[1]Departamento de Doenças Dermatológicas, Infecciosas e Parasitárias, Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brasil; [2]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brasil; [3]Departamento de Biologia Molecular, Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brasil; [4]Laboratório Especial de Microbiologia Clínica, Universidade Federal de São Paulo, São Paulo, SP, Brasil; [5]Seção de Bacteriologia, Instituto Adolfo Lutz, SãoPaulo, SP, Brasil; [6]Faculdade de Medicina de São José do Rio Preto, São José do Rio Preto, SP, Brasil

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ABSTRACT

Stenotrophomonas maltophilia is a Gram-negative bacillus, which is becoming widely recognized as an important nosocomial pathogen. The main objective of this study was to evaluate the genetic relatedness, by random amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) of 86 clinical isolates of *S. maltophilia* (colonization 22, infection 64) obtained from 79 hospitalized patients, from different geographic regions of São Paulo State. The genotypic analysis performed by RAPD and PFGE was used in 24 isolates for genetic identity confirmation. The results were congruent between the two methods but it was not possible to link genetic profiles with the studied variables, clinical state and geographic area, probably due to the great variability among the strains. The analyses by PFGE confirmed identity in 5 pairs of microorganisms and RAPD, in this study, showed to be a useful tool for investigation of diversity leading the identification of 85 genetic profiles. The genetic diversity shown may be due to re-infection by different strains or co-infection by multiple strains which suggests multiple entry sources of the bacterium in the hospital setting or of acquisition by patient. In this setting, colonization, infection and re-infection occur with unknown frequency, raising the need for the establishment of specific control measures.

Key words: *Stenotrophomonas maltophilia*, RAPD, PFGE

INTRODUCTION

Stenotrophomonas maltophilia is a non-fermenting Gram-negative bacillus that suffered several changes in its taxonomic name over the time (20) and is becoming widely recognized as an important nosocomial pathogen in immunosuppressed patients and, less frequently, in immunocompetent patients. This microorganism has been isolated from a great variety of microenvironments and geographical regions and occupies distinct ecological niches such as water, vegetables and soil, besides other sources in the hospital setting, including tap water, sinks, respirators, suction catheters (30), arterial pressure monitors, dialysis equipment (8), ice machines (6) and disinfectants (17). *S. maltophilia* is considered an opportunist agent, with hospital colonization and infection as its most common manifestation (4). Several outbreaks of infection have been described (6) including a few reports of infections originating outside the hospital setting (13,22). The risk factors associated with these infections include prolonged hospitalization, chemoprophylaxis, heart surgery (6,10) and

*Corresponding Author. Mailing address: Av. Brigadeiro Faria Lima, 5416 cep 15090-000 São José do Rio Preto, SP - Brasil. Tel.: (17) 3201-5741 ou (17) 3201-5734. E-mail: margarete@famerp.br

burns (28). Besides, in infectious processes there are reports of the production of several extracellular enzymes, including DNase, RNase, fibrinolysin, lipases, hyaluronidases, proteases and elastases, associated as virulence factors originating described to other microorganisms (6,10).

Considering the widespread environmental distribution of this bacterium, the evaluation of its dissemination pattern is difficult. Its distribution has been reported as cross-transmission by contaminated equipment (1) or environmental sources (34).

Due to the limitations of conventional typing methods, molecular assay techniques were adapted as additional or even definitive methods for tracing nosocomial pathogens (29). Typing enables the analysis of chromosomal DNA restriction patterns, as well as the characterization of the extra-chromosomal DNA portion (19).

Among the molecular techniques applied to epidemiological studies, one of the commonly utilized are randomly amplified polymorphic DNA (RAPD) investigations (2,11,31), which enables a very fast result and pulsed-field gel electrophoresis (PFGE) (1,7).

The current study aimed at typing *S. maltophilia* isolates collected from clinical samples, using genotypic methods and correlating their molecular profiles with the geographical origins of the strains, the anatomic site and clinical state (colonization and infection).

MATERIALS AND METHODS

Bacterial Isolates

A total of 86 strains of *S. maltophilia* were isolated from 79 patients, 7 of these with two samples, hospitalized in four Health Institutions, located in São Paulo city, Instituto de Cardiologia Dante Pazzanese (IDPC – 48 isolates), Hospital das Clínicas da Faculdade de Medicina-USP (HC-USP - 23 isolates), Hospital Universitário da USP (HU-USP - 2 isolates) and São José do Rio Preto, São Paulo State, the Clinical and Surgical Intensive Care Units (ICU) of Hospital de Base (HB – 13 isolates), from March 1994 to June 2000, classified according to the clinical state as colonization (N=28) or infection (N=9). The sources of the *S. maltophilia* isolated in this study were classified as infection in 74.4% and colonization in 25.6% of the cases. All of these were recovered from a wide- range of clinical sites including colonization of the oropharynx and catheters (Table 1). Serial collections were made in Hospital de Base at two-day intervals to identify if the intra-hospital environment was the source of multiresistant strain transmission. Simultaneously, comparisons were made with the *S. maltophilia* strain ATCC 13637. All the strains were identified by the classical biochemical test as described by Gilardi (12) and maintained in the laboratory of microbiology under the same nutritional and physical conditions and analyzed after an average time of six years.

Table 1. Distribution of *S. maltophilia* strains accordingly to different Institutions of origin, cities and clinical specimens

City	Origin	Clinical Specimens	*S. maltophilia* Isolates Identification (Total)
SP	HC-FM	Catheter	10, 15 (2)
SP	IDPC	Catheter	50, 53, 55, 58, 62, 68 (6)
SP	HC-FM	Sputum	3, 6, 11, 13, 27, 29 (6)
SP	IDPC	Blood	28, 45 (2)
SP	HC-FM	Cerebrospinal fluid	8 (1)
SJRP	HB	Cerebrospinal fluid	86(1)
SP	HC-FM	Oropharynx	30, 25 (2)
SJRP	HB	Oropharynx	74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85 (12)
SP	HC-FM	Surgical wound secretion	16, 17, 22, 23 (4)
SP	IDPC	Surgical wound secretion	61, 64, 66, 67, 70, 71, 73 (7)
SP	IDPC	Skin Ulcer	44, 46 (2)
SP	IDPC	Tracheal secretion	1, 2, 12, 14, 26, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 47, 48, 49, 51, 52, 54, 56, 57, 59, 60, 63, 65 (29)
SP	HC-FM	Tracheal secretion	4, 18, 19, 21, 24 (5)
SP HU-USP	Tracheal secretion	33, 5 (2)	
SP	HC-FM	Urine	7, 9, 20 (3)
SP	IDPC	Urine	69, 72 (2)

SP - São Paulo; SJRP - São José do Rio Preto; HC-FM - Hospital das Clínicas Faculdade de Medicina da USP; IDPC - Instituto de Cardiologia Dante Pazzanese; HB - Hospital de Base; HU-USP - Hospital Universitário-USP.

Random Amplified Polymorphic DNA Typing

Standardization of RAPD was achieved using two amplification primers, primer I (5'-CTGGCGGCTG-3') and primer II (5'-TCACGATGCA-3'), according to the procedures described by Yao *et al.* (35) and Chatelut *et al.* (2) respectively, with alterations in relation to the extraction of the DNA, that was achieved using the Easy-DNA ™kit (Invitrogen). In the reaction with primer I a final volume of 50 µL was used comprised of 100 ng of genomic DNA, 20 mM Tris-HCl, [pH 8.4], 50mM KCl, 4 mM $MgCl_2$, 200 µM of each dNTP (*Amersham Pharmacia Biotech Inc*), l.25 U of *Taq* polymerase (GIBCO BRL) and 1 µM of the primer I. The amplification conditions included 35 nitial cycles of 2 minutes at 94ºC, 30 seconds at 94ºC, 30 seconds at 35ºC, 1 minute at 72ºC and end extension at 72ºC for 4 minutes. In the reaction with primer II, a final volume of 50 µL was used containing 100 ng of genomic DNA, 20mM Tris-HCl, [pH 8.4], 50 mM KCl, 2.5 mM of $MgCl_2$, 200 µM of each dNTP (*Amersham Pharmacia Biotech Inc*), 0.8 µM of primer II and 2 U of *Taq* polymerase (GIBCO BRL), with initial cycle of 5 minutes at 95ºC, 1 minute at 36ºC and 2 minutes at 72ºC and after 42 cycles of 40 seconds at 94ºC, 40 seconds at 36ºC and 80 seconds at 72ºC. These reactions were performed using a thermocycler (Eppendorf Master Cycler Personal). The protocols of RAPD were conducted for all clinical isolates twice, in order to check if they were reproductive, including the analysis with the ATCC 13637 strain.

Investigation of the amplified fragments was done by electrophoresis using agarose gel. Six µL of each of the amplification products with 2.5 µL of bromophenol blue stain (40% sucrose, 0.025% bromophenol blue), as well as a molecular Ladder (DNA Ladder 100 bp *Amersham Pharmacia Biotech Inc*) were run in 1.5% agarose gel. A 90-V magnetic field was applied for 3 hours, using a TAE buffer (Tris-acetate 4 mM, EDTA l mM, pH 8.5). The agarose gel was stained with ethidium bromide solution (10 mg/mL) and observed under an ultra-violet light transilluminator (Fisher Scientific DLT) followed by image recording using the Kodak lD vs. 3.5 system (EDAS-Electrophoresis Documentation and Analysis System) for image capture and analysis. The fragments were analyzed in respect to the presence (a light band) or absence (no band) in the gel.

Pulsed-Field Gel Electrophoresis Typing

Twenty-four isolates that showed similar genetic profiles obtained by RAPD with two separated primers were studied by macro restriction analysis of chromosomal DNA by PFGE. Genomic DNA inserts were digested at 37ºC overnight with 10U of *Spe* I restriction enzyme (New England Biolabs, Inc., Beverly, Mass). Electrophoresis was performed in a CHEF-DRIII apparatus (Bio-Rad, Richmond, Calif.) under the following conditions: 0.5 Tris-borate-EDTA, 1% agarose at 13ºC, and 200V (6 V/cm). The electrophoresis was run for 23 hours, and the switch interval ramped from 5 to 90s. Photographs of ethidium bromide-stained gels were examined macroscopically (26).

Phylogenetic Analysis

DNA bands, obtained using two primers, were scored as present (1) or absent (0) to construct a binary matrix in order to determine Jaccard (25) similarity coefficient of 86 isolates. Comparison among the individuals was performed to evaluate the relationships among them.

Data analysis was performed with the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System program version 1.7, Exeter Software, Setauked, N.Y) (18). Dendrogram was constructed from the Jaccard similarity coefficient data by the UPGMA (unweighted pair-group method using arithmetic) means clustering method.

RESULTS

RAPD Fingerprinting

The 86 clinical *S. maltophilia* isolates and the standard ATCC strain were submitted to analysis by RAPD with the two decameric primers. Analyses using the two primers resulted in 81 profiles with primer I and 83 with primer II. Thus, 111 fragments were identified with molecular weights ranging from 154 to 3591 bp. The greatest number of bands seen in one isolate was 31 (isolate 9) and the smallest number was 4 (isolate 37). Monomorphic bands were not observed. Genotypic analysis of isolates identified twenty-four bands which were exclusively from strains isolated from infections. The frequency of these bands varied from 1.56% (N=1) to approximately 23.5% (N=15). In contrast, only two isolates of colonization exhibited exclusive bands, one with 1777 bp and the other with 1922 bp (data not shown). Comparing the bands in respect to the geographical origin of isolates, it was possible to observe that isolates from São Paulo presented thirty-nine exclusive bands, with frequencies varying from 1.36% to 41.09% and the isolates from São José do Rio Preto, presented only three exclusive bands with frequencies of from 7.7% to 23%.

Analysis of the profiles using the two primers showed the existence of eighty-five distinct patterns which were used to construct the dendrogram presented in Figure 1. The genotypic similarity among the isolates led to the formation of seven main interdependent clusters, which presented a similarity coefficient of approximately 20%. Subsequent analysis of these clusters demonstrated the existence of fourteen subgroups with similarity rates from 25 to 50%. Isolates 29 and 30 (distinct patients), originating from an oropharynx secretion and spittle in HC (June 1995), exhibited a similarity coefficient of 59.65%. A similarity rate of 84.75% was found between isolates 28 and 47 coming from a blood culture and a tracheal secretion of patients hospitalized in the IDPC - in distinct periods (June 1995 and October 1996). Isolates 81 and 83, which were obtained from oropharynx secretions of two patients hospitalized in HB, in different periods (January 1997 and May 1998), were indistinct. All these clusters presenting main

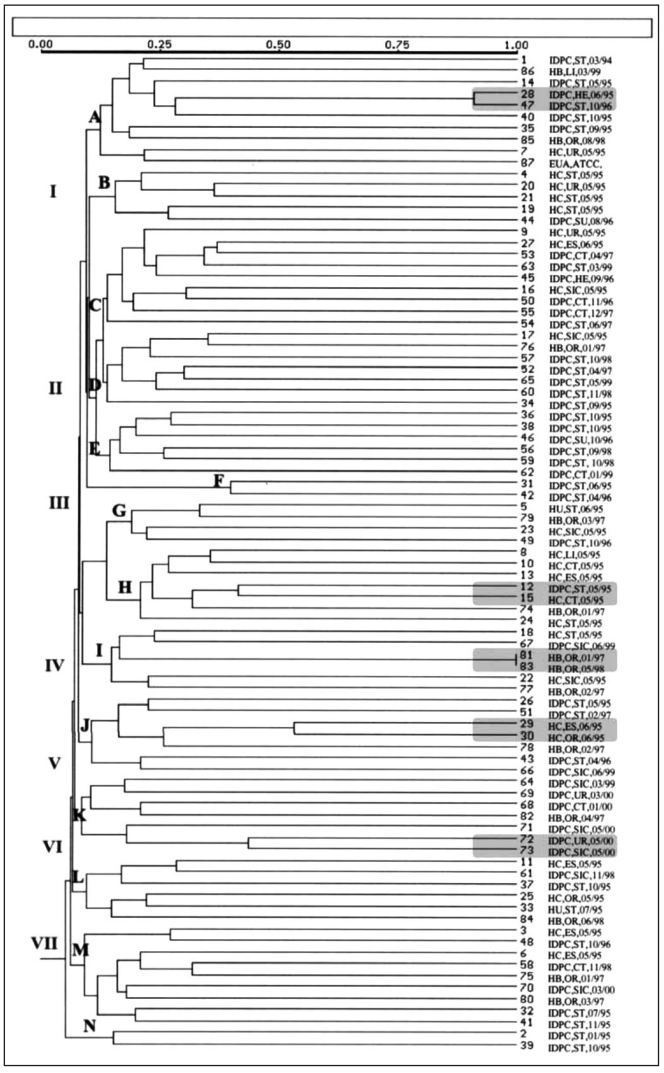

Figure 1. Dendrogram obtained by the UPGMA group analysis method for the Jaccard coefficient. I–VII: 7 groups; A-N: 14 subgroups; the number 1 to 86 represent samples; the last column represents Hospital, clinical sample and month and year of collection. In highlight clusters of main similarity.

genetic similarity are highlighted in Fig. 1, including some without any specific clonal formation.

Interpretation of the results of RAPD, obtained with two primers separately, demonstrated twelve pairs of isolates with the same genetic profile. However, when these results were analyzed together, only one pair of isolates continued to have an indistinguishable genomic pattern.

PFGE Typing

The twelve pairs of isolates identified as having genotypic similarities using the RAPD method were also typed using the

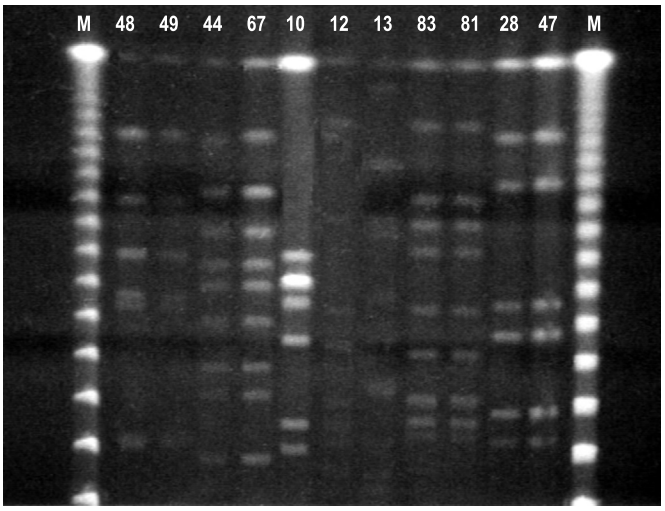

Figure 2. Genomic DNA macrorestriction profiles of *S. maltophilia* produced by PFGE after *Spe* 1 digestion. The four pairs 48-49; 44-67; 83-81; 28-47 show indistinct genetic profiles, and the isolates 10;12;13 show distinct genetic profiles. M: Lambda ladder marker (48,5Kb).

PFGE method. Fourteen different PFGE profiles were identified out of 24 studied strains, with 5 non-typable strains because DNA was digested before analysis. Nine strains displayed different PFGE profiles, while 4 pairs showed in figure 2(48-49; 44-67;83-81; 28-47) and one, 72-73 (data not shown), confirmed these similarities. Among the clonal isolates, two pairs came from the same hospital ward and three pairs from the same hospital but from different places within the hospital.

DISCUSSION

S. maltophilia is considered to be a low-virulence opportunistic microorganism; however, the infections caused by this bacterium are difficult to treat due to its intrinsic resistance to severalantimicrobial agents. Among the patients of this study, 57% were not from ICU and strain isolation occurred in other hospital wards. These data show that, although *S. maltophilia* is considered an important nosocomial pathogen within ICUs (23), it plays a significant role as an etiological agent of infections in other settings including no-ICU environments.

Analysis of anatomical sources of samples identified the respiratory tract as the most commonly affected structure (49%), a similar result to other published studies which reported frequencies of from 42% to 33% of *S. maltophilia* isolates found in the airways (5,16). Usually, this bacillus is not considered a significant cause of postoperative infections but, in the current study a significant number of samples originated from surgical wounds. The importance of this microorganism as an etiological agent of post surgical infections should not be ignored,

specifically in immunosuppressed patients or patients who are submitted to wide-ranging prophylactic therapy.

One of the current challenges in biology is to discover how to utilize molecular information in order to understand the phylogenetic relationships between microorganisms (24). In the last years, molecular epidemiological typing methods have substituted the phenotypic methods, since the exposure of microorganisms to different environments contributes to phenotypic instability (9). Among the techniques available, RAPD has been widely used for genetic diversity studies of genera and species (10).

PFGE is another methodology utilized in epidemiological investigation studies of microorganisms, which has a good discriminatory power (26).

To define the genetic variability utilizing the RAPD technique, 111 bands of amplification were considered, independent of their frequencies. In a previous study with eukaryotes, it was evident that the presence of exclusive bands can contribute as an additional resource to determine the geographical origin of the strain (21). The current study demonstrated that thirty-nine bands were exclusive in the isolates from Sao Paulo, whilst in Sao José do Rio Preto there were only three. The isolates were from distinct geographical areas and collection periods and, therefore, were probably exposed to different selective pressures. Moreover, the environment and the time of storage of the bacterial samples in the laboratory until molecular investigation is carried out, can allow slight alterations in the genome, resulting in variations in the molecular profile (26).

The results produced using the RAPD technique, confirmed the great genomic variability, as has been previously demonstrated (3). Only one clone, involving two samples, presented 100% equivalence. The other 84 samples presented identities which varied from 20 to 85% of similarity. Even observing the groups formed in general by samples from a single hospital as in the case of Group II (IDPC), a low potential of this species involved in outbreaks is observed. Neither the samples obtained from patients hospitalized in Hospital de Base, Sao José do Rio Preto nor the isolates originating in Sao Paulo collected from sites of colonization or infection, were classified in specific subgroups by the dendrogram as they were not grouped in specific clusters. This is probably owing to the pressure of continuous selection exerted by the microenvironment of the infection or by the external environment.

Several studies utilized PFGE for the molecular investigation of *S. maltophilia*, a technique that is considered the gold standard in bacterial epidemiological investigations (26,14). In the current study, a wide variety of genotypes of the *S. maltophilia* strains were detected therefore, from twenty-four samples analyzed, only five pairs of clinical isolates showed similar profiles by both PFGE and RAPD, this last, done with results of independent primers. Again, the investigation of consecutive isolates originating from the same patient showed that the restriction profiles found by PFGE were widely divergent. These data suggest the involvement of distinct strains causing infection or colonization in the assessed patients.

In 62 cases of hospital infection no epidemic strain was detected. Additionally, no monomorphic bands were found, reinforcing the idea of a wide genomic variability. This data suggests that the lack of detection of *S. maltophilia* outbreaks does not imply that it has a lower potential as a nosocomial pathogen.

In two different studies the great genomic diversity of S. *maltophilia* was observed. According to Travassos *et al.* (27), 33 out of 39 clinical isolates presented different profiles and in the studies of Valdezate *et al.* (30), out of 112 cases, 99 presented differences. The results of these studies, together with the results of the present investigation, suggest that a multiple route of acquisition of this bacteria is more common than cross-transmission.

In the present study some patients with intermittent infections were characterized with different strains of *S. maltophilia*, corroborating the report by Krziwinski *et al.* (15). Furthermore, it was not possible to correlate a determined genotypic profile with the anatomic site, geographical area of origin (different hospitals) or with the clinical states (colonization or infection).

Even though the RAPD technique is considered less discriminatory when compared with PFGE (32), it presents some advantages and thus it may be a practical option for molecular typing in investigations of the genetical diversity of *S. maltophilia*. The analysis of the results obtained from RAPD, using two primers, proved to be more discriminatory than PFGE therefore, only one pair of *S. maltophilia* strains (81 and 83) was allocated in the same genetic profile, whilst with PFGE, five pairs were indistinct.

By utilizing the RAPD technique as a molecular tool of investigation, it was possible to show the high degree of variability and the presence of exclusive bands in the group of strains isolated from infected body sites when compared with those obtained from colonization ones. With the lack of detection of a genetical pattern among the different studied isolates in the different clinical or environment settings, it is possible to suggest that *S. maltophilia* can undergo high rates of mutation.

It is interesting to emphasize that the patients with identical isolates were not inside the hospital concomitantly (January 1997 and May 1998), but occupied the same physical area (ICU – Hospital de Base). This data indicate a common environmental source of transmission, as previously reported (33). It is probably that intermediary means of environmental dissemination are involved in the process of colonization between the source and the patient. Future characterization of these routes will permit effective action control against this microorganism in the ICU of Hospital de Base, São José do Rio Preto.

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RESUMO

Relação genética entre isolados clínicos de *Stenotrophomonas maltophilia* em hospitais terciários do Estado de São Paulo, Brasil

Stenotrophomonas maltophilia é um bacilo Gram-negativo, conhecido como importante patógeno nosocomial. O principal objetivo desse estudo foi avaliar a relação genética, através da análise randômica do polimorfismo de DNA (RAPD) e eletroforese em gel de campo pulsado (PFGE), de 86 isolados clínicos de *S. maltophilia* (22 de colonização, 64 de infecção) obtidos de 79 pacientes hospitalizados em diferentes regiões geográficas do estado de São Paulo. A análise genotípica foi realizada através da técnica RAPD e o PFGE foi usado em 24 isolados para confirmar a identidade genética dos mesmos. Os resultados foram coerentes entre os dois métodos, mas não foi possível correlacionar um perfil genético com as variáveis estudadas, estado clínico e área geográfica, provavelmente pela ampla variabilidade entre as linhagens. A análise por PFGE confirmou a identidade genética em 5 pares de microrganismos e o RAPD, neste estudo, mostrou ser uma ferramenta útil para investigação da diversidade, possibilitando identificar 85 perfis genéticos. A diversidade genética observada através do RAPD pode ser devido à re-infecção por diferentes linhagens ou co-infecção por linhagens distintas, sugerindo múltiplas fontes de entrada da bactéria no hospital ou de aquisição pelo paciente. Nesse ambiente, a colonização, infecção e re-infecção ocorrem com freqüência, o que leva à necessidade do estabelecimento de medidas de controle específicas.

Palavras-chave: *Stenotrophomonas maltophilia*, RAPD, PFGE

REFERENCES

1. Alfieri, N.; Ramotar, K.; Armstrong, P.; Spornitz, M.E.; Ross, G.; Winnick, J.; Cook, D.R. (1999). Two consecutive outbreaks of *Stenotrophomonas maltophilia* (*Xanthomonas maltophilia*) in an intensive-care unit defined by restriction fragment-length polymorphism typing. *Infect. Control Hosp. Epidemiol*., 20, 553-556.
2. Chatelut, M.; Dournes, J.L.; Chabanon, G.; Marty, N. (1995). Epidemiological typing of *Stenotrophomonas (Xanthomonas) maltophilia* by PCR. *J. Clin. Microbiol.*, 33, 912-914.
3. Davin-Regli, A.; Bollet, C.; Auffray, J.P.; Saux, P.; De Micco, P. (1996). Use of random amplied polymorphic DNA for epidemiological typing of *S. maltophilia*. *J. Hosp. Infect*., 32, 39-50.
4. Del Toro, M.D.; Rodríguez-Baño, J.; Herrero, M.; Rivero, R.; Garcia-Ordoñez, M.A.; Corzo, J.; Pérez-Canoa, R. (2002). Clinical epidemiology of *Stenotrophomonas maltophilia* colonization and infection. *Medicin.*, 81, 228-239.
5. Del Toro, M.D.; Rodríguez-Baño, J.; Martínez-Martínez, L.; Pascual, A.; Pérez-Canoa, R.; Perea, E.J.; Muniain, M.A. (2006). Epidemiology, clinical features and prognosis of infections due to *Stenotrophomonas maltophilia. Enferm. Infecc. Microbiol. Cli.*, 24, 4-9.
6. Denton, M.; Kerr, K.G. (1998). Microbiological and Clinical Aspects of Infection Associated with *Stenotrophomonas maltophilia*. *Clin. Microbiol. Re.*, 11, 57-80.
7. Denton, M.; Todd, N.J.; Kerr, K.G.; Hawkey, P.M.; Littlewood, J.M. (1998). Molecular epidemiology of *Stenotrophomonas maltophilia* isolated from clinical specimens from patients with cystic fibrosis and associated environmental samples. *J. Clin. Microbiol.*, 36, 1953-1958.
8. Flaherty, J.P.; Garcia-Houchins, S.; Chudy, R.; Arnow, P.M. (1993). An outbreak of Gram- negative bacteremia traced to contaminated O-rings in reprocessed dialysers. *Ann. Intern. Med*., 119, 1072-1078.
9. Franzot, S.P.; Mukherjee, J.; Cherniak, R.; Chen, L.C.; Hamdan, J.S.; Casadevall, A. (1998). A microevolution of standard of *Cryptococcus neoformans* resulting in differences in virulence and other phenotypes. *Infect. Immu.*, 66, 89-97.
10. Garcia, D.O.; Timenetsky, J.; Martinez, M.B.; Francisco, W.; Sinto, S.I.; Yanaguita, R.M. (2002). Proteases (caseinase and elastase), hemolysins, adhesion and susceptibility to antimicrobials of *Stenotrophomonas maltophilia* isolates obtained from clinical specimens. *Braz. J. Microbiol.*, 33, 157-162.
11. Garcia de Viedma, D.; Marin, M.; Cercenado, E.; Alonso, R.; Rodrigues-Créixems, M.; Bouza, E. (1999). Evidence of nosocomial *Stenotrophomonas maltophilia* cross-infection in a neonatology unit analysed by three molecular typing methods. *Infect. Control. Hosp. Epidemiol.*, 20, 816-820.
12. Gilardi, G.L. (1991). *Pseudomonas* and related genera. In: Ballows A. (ed), *Manual of Clinical Microbiology*, 5 ed. American Society for Microbiology, Washington, 429-441.
13. Heath, T.; Currie, B. (1995). Nosocomial and community-acquired *Xanthomonas maltophilia* infection in tropical Australia. *J. Hosp. Infect*., 30, 309-313.
14. Khardori, H.; Elting, L.; Wong, E.; Schable, B.; Bodey, G. (1990). Nosocomial infections due to *S. maltophilia* in patients with cancer. *Rev. Infect. Dis*., 12, 997-1003.
15. Krzewinski, J.W.; Nguyen, C.D.; Foster, J.M.; Burns, J.L. (2001). Use of random amplified polymorphic DNA PCR to examine epidemiology of *S. maltophilia* and *Achromobacter (alcaligenes) xylosoxidans*, from patients with cystic fibrosis. *J. Clin. Microbiol*., 3597-3602.
16. Lai, C.H.; Chi, C.Y.; Chen, H.P.; Chen, T.L.; Lai, C.J.; Fung, C.P., Yu, K.M.; Wong, W.W.; Liu, C.Y. (2004). Clinical characteristics and prognostic factors of patients with *Stenotrophomonas maltophilia* bacteremia. *J. Microbiol. Immunol. Infect*., 37, 350-358.
17. Mukhopadhyay, C.; Bhargava, A.; Ayyágari, A. (2003). Novel nosocomial infections by *Stenotrophomonas maltophilia*: first reported case from Lucknow, North India. *J. Clin. Microbiol.*, 41, 3989-3990.
18. NTSYS-pc. (1992). Numerical Taxonomy and Multivariate Analyses System. Version 1.70. Exeter Software, NY.
19. Olive, D.M.; Bean, P. (1999). Principles and applications of methods for DNA-based typing of microbial organisms. *J. Clin. Microbiol.*, 137, 1661-1669.
20. Palleroni, N.J.; Bradbury, J.F. (1993). *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh, 1980) Swings *et al*. 1983. *Int. J. Syst. Bacteriol*., 43, 606-609.
21. Ruiz, L.C. (2001). Caracterização molecular de populações de *Haematobia irritans* (Mosca-do-chifre) pela técnica de RAPD-PCR. São José do Rio Preto, Brasil, (Tese. Instituto de Biociências, Letras e Ciências Exatas. UNESP).

22. Sakhnini, E.; Weissmann, A.; Oren, I. (2002). Fulminant *Stenotrophomonas maltophilia* soft tissue infection in immunocompromised patients: an outbreak transmitted tap water. *Am. J. Med. Sci*., 323, 269-272.
23. Sattler, C.A.; Mason, E.O.; Kaplan, S.L. (2000). Nonrespiratory *Stenotrophomonas maltophilia* infection at a children's hospital. *Clin. Infect. Dis*., 31, 1321-1330.
24. Selander, K.R.; Musser, J.M. (1990). Population genetics of bacterial pathogenesis. *In*: Iglewski, B.H. and Clark, V.L. (eds). *Molecular basis of bacterial pathogenesis.* Academic Press Inc. Orlando, Florida, USA.
25. Sneath, P.H.; Sokal, R.R. (1973). *Numerical Taxonomy*. W.H. Freeman & Co. San Francisco, Calif.
26. Tenover, F.C.; Arbeit, R.D.; Goering, R.V. (1997). How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for health epidemiologists. *Infect. Control. Hosp. Epidemiol*., 18, 426-439.
27. Travassos, L.H.; Pinheiro, M.N.; Coelho, F.S.; Sampaio, J.L.M.; Merquior, V.L.C.; Marques, E.A. (2004). Phenotypic properties, drug susceptibility and genetic relatedness of *Stenotrophomonas maltophilia* clinical strains from seven hospitals in Rio de Janeiro, Brazil. *J. Appl. Microbiol.*, 96, 1143-1150.
28. Tsai, W.P.; Chen, C.L.; Ko, W.C.; Pan, S.C. (2006). *Stenotrophomonas maltophilia* bacteremia in burn patients. *Burns.*, 32, 155-158.
29. Tyler, K.D.; Wang, G.; Tyler, S.D.; Johnson, W.M. (1997). Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. *J. Clin. Microbiol*., 339-346.
30. Valdezate, S.; Vindel, A.; Martín-Dávila, P.; Saz, B.S.D.; Baquero, F.; Cantón, R. (2004). High Genetic Diversity among *Stenotrophomonas maltophilia* Strains Despite Their Originating at a Single Hospital. *J. Clin. Microbiol.*, 42(2), 693-699.
31. VanCouwenberghe, C.J.; Cohen, S.H.; Tang, Y.J.; Gumerlock, P.H.; Silva, J.R. (1995). Genomic fingerprinting of epidemic and endemic strains of *Stenotrophomonas maltophilia* (formerly *Xanthomonas maltophilia*) by arbitrarily primed PCR. *J. Clin. Microbiol.*, 33, 1289-1291.
32. VanCouwenberghe, C.J.; Farver, T.B.; Cohen, S.H. (1997). Risk factors associated with isolation of *Stenotrophomonas maltophilia (Xanthomonas) maltophilia* in clinical specimens. *Infect. Control Hosp. Epidemiol*., 18, 316-321.
33. Verweij, P.E.; Meis, J. F.; Christimann, V.; Van Der Bor, M.; Melchers, W.J.; Hilderink, B.G.; Voss, A. (1998). Nosocomial outbreak of colonization and infection with *Stenotrophomonas maltophilia* in preterm infants associated with contaminated tap water. *Epidemiol. Infect.*, 120, 251-256.
34. Villarino, M.E.; Stevens, L.E.; Schable, B.; Mayers, G.; Miller, J.M.; Burke, J.P.; Jarvis, W.R. (1992). Risk factors for epidemic *Xanthomonas maltophilia* infection/colonization in intensive unit patients. *Infect. Control Hosp. Epidemiol*., 13, 201-206.
35. Yao, J.D.C.; Conly, J.M.; Krajden, M. (1995). Molecular Typing of *Stenotrophomonas (Xanthomonas) maltophilia* by DNA Macrorestriction Analysis and Randon Amplified Polymorphic DNA Analysis. *J. Clin. Microbiol*., 33, 2195-2198.