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Evaluation of the Spreading of Isolated Bacterias from Dental Consulting-Room using RAPD Technique

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SUMMARY. The aim of this study was to investigate the spreading of isolates recovered in dental office environment using Random Amplified Polymorphic DNA technique (RAPD). The bacteria isolated and identificated from the surfaces of different dental consulting-room and staff were obtained in two moments (47 isolates total). For *Klebsiella* sp, *Staphylococcus aureus* and *Escherichia coli* isolates, groups with high coefficient of similarity (Sab) were obtained. *Proteus vulgaris* and *Hafnia alvei* showed lower Sab, showing however greater relationships between individual species. It was possible to determine that the majority of the isolates of individual species were closely related.

INTRODUCTION

The cross-infection prevention in dental office have been a great challenge for healthcare professionals and researchers; in the professional exercise there is a constant contact with tissues from the mouth, saliva, microbiota oral and, most of the time, with patient's blood [1,2]. It's already known that group of benches surfaces, air, dental materials, instruments and water can be vehicles for cross-contamination for some microorganisms [3]. Diverse methods of disinfection and sterilization are available but there are many possible sources of contamination in the dental offices that can disseminate in different places [3]. Techniques of molecular typing can represent an additional discriminatory potential, especially when it makes possible to differ from isolates of the same strains. The molecular typing systems can be used for outbreaks inquiries, confirmation and delineation of the standards transmission of one or more strains, as well as tests of hypothesis about the origin and source of transmission of this microorganism and its encounter in the reservoirs [4].

The objective of the present work was to analyze the relationships of the genomic profile of isolated bacterial samples from dental surgery clinics using molecular typing by Random Amplified Polymorphic DNA (RAPD) technique as a molecular tool for the identification of possible origins of contamination and dissemination in dental offices.

MATERIALS AND METHODS

Bacterial strains

Samples collection, isolation and identification of bacteria from the surfaces of several sites of different dental offices [5] and staff (masks, gloves and eyeglasses) occurred in two moments, one in April 2006 (collection I) and other in june 2006 (collection II), in the Clinic of Surgery and Buco-Maxilo-Facial Traumatology of the University of Ribeirão Preto, São Paulo, Brazil. The samples collected by conventional procedures using swabs, were submitted to growth in BHI broth (Merck, Darmstad, Germany) or directly plated in 5% sheep blood agar, Mac Conkey agar and mannitol salt agar that was incubated in aerobic conditions for 24 h at 37 °C to allow differentiation of microor-

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ganisms. The samples were obtained before and after surgical procedures.

All isolates were identified according to Probac kit (Probac do Brasil, São Paulo, SP, Brazil), maintained as frozen stocks at -70 °C in the presence of 15% glycerol and cultured in Brain Heart Infusion medium (Biobrás, Montes Claros, MG, Brazil). Forty six isolates were selected (Table 1) due to their capacity of growth and recovery after 24 h in 37 °C in BHI: 12 *Staphylococcus aureus* isolates, 13 *Klebsiella oxytoca* isolates, 7 *Klebsiella pneumoniae* isolates, 5 *Hafnia alvei* isolates 5 *Proteus vulgaris* isolates and 4 *Escherichia coli* isolates. In order to make a comparison we use two ATCC strains: *S. aureus* 25923 and *E. coli* 25922. The bacterial strains were subcultured in BHI for 24 h at 37 °C, and 10^8 bacterias were again inoculated in the same conditions and spinning 20.800 rpm for 30 min in a centrifuge (Eppendorf Centrifuge 5417 R, Eppendorf AG Inc. Hamburg, Germany) for pellet formation to DNA extraction.

RAPD analysis

Genomic DNA was carried through using the GFX Genomic Blood DNA Purification Kit (Amersham Pharmacia Biosciences Inc., Buckinghamshire, U.K.) with some changes, adapted to enzymatic buffers for cell lyses: for Gram positive bacterias the Lysozyme buffer (0,1 M NaCl; 10 mM TRIS pH 8.0; 1 mM EDTA; 5% Triton X-100 (USM™ Cleveland, Ohio, USA) and Lysozyme (Pharmacia Biotech. Piscataway, NJ, USA) 20 mg/mL in 10 mM Tris-HCl pH 8.0) and for Gram negative bacterias the Proteinase K buffer (12 mM TRIS-HCl pH 8.0; 6 mM EDTA; 0.6% SDS (Gibco BRL Grand Island, NY, USA); Proteinase K (Pharmacia Biotech. Piscataway, NJ, USA) 100 mg/mL in 10 mM, TRIS-HCl p H8.0).

Quantification of the genomic DNA was analyzed estimating the intensity of the bands compared to the DNA of phage λ (Promega Inc. Madison, WI, USA), by means of 1% agarose gel electrophoresis. For RAPD analysis, preliminary assays were carried out to test 25 primers synthesized by Operon Technologies (Huntsville, CA, USA) and Amersham Pharmacia Biosciences Inc. (Little Chalfont, Buckinghamshire, U.K.). Under extensive analysis, the primer Operon 18 (5'-CAGCACCCAC-3') was selected for analysis with the isolation of *Klebsiella pneumoniae* and *Escherichia coli,* the primer Operon 21 (5'-TGCCGAGCTG-3') to *Klebsiella oxytoca,* the primer RAPD 7 (5'-GTGGATGCGA-3') to *Staphylococcus aureus,* the primer ERIC 1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') to *Proteus vulgaris* and primer UNIVERSAL (5'-TCACGATGCA-3') to *Hafnia alvei*. The PCR reactions were prepared individually for each group of isolates in a total volume of 30 µL per tube, containing 3 µL buffer 10x (Phoneutria Biotecnologia e Serviços Inc. Belo Horizonte, MG, Brazil) and dNTPs (25 mM) (Amersham Pharmacia Biosciences Inc., Buckinghamshire, U.K.). For isolates of *Escherichia coli* and *Hafnia alvei* the reactions contained 2.4 µL of $MgCl_2$ (25 mM) (Phoneutria Biotecnologia e Serviços Inc.) and to isolates of *Klebsiella oxytoca, Proteus vulgaris, Staphylococcus aureus* and *Klebsiella pneumoniae* 3.0 µL. Reactions of isolates of *Escherichia coli* contained 3 µL of primer (10 ng/µL), isolates of *Hafnia alvei, Klebsiella oxytoca* and *Klebsiella pneumoniae* 5 µL and isolates of *Proteus vulgaris* and *Staphylococcus aureus* 2 µL.

For *Taq* DNA polymerase (5 U/µL) (Phoneutria Biotecnologia e Serviços Inc.), the reactions for isolates of *Staphylococcus aureus* and *Escherichia coli* contained 0.3 µL of enzyme, to *Hafnia alvei* 0.4 µL, to *Klebsiella oxytoca* and *Klebsiella pneumoniae* 0.6 µL and to *Proteus vulgaris* 0.3 µL of enzyme. DNA (5 ng/µL) genomic for isolates of *Klebsiella oxytoca* and *Klebsiella pneumoniae* were added 3.0 µL per reaction, to *Staphylococcus aureus* 2.0 µL, to *Escherichia coli, Hafnia alvei* and *Proteus vulgaris* 1.0 µL. Controls for detection of false positive had been carried through parallel in all the reactions. Components of the mixture of PCR reaction had been tested without the presence of isolated DNA of the bacterial ones. The absence of bands after the amplification was interpreted as contamination absence. The amplification was done in thermocycler (model PTC-100 Programmable Thermal Controller, MJ Researcher Inc. Watertown, MA, USA) in the following conditions: 2 cycles of 2 min at 94 °C, 1 min at 40 °C and 2 min 72 °C followed by 33 cycles of 10 sec at 94 °C, 20 sec at 40 °C and 2 min at 72 °C, with a final extension at 72 °C for 5 min. Amplification products were resolved by electrophoresis on 2,0% agarose gel (Gibco/Invitrogen Amarillo, TX, USA) in TBE buffer 1x (Tris 90mM, boric acid 90mM, EDTA 2mM pH 8,0).

The amplification products were stained with ethidium bromide 0.5 µg/mL and visualized under violet light and then photographed (Image Master®VDS, Pharmacia Biotech. Piscataway, NJ, USA). A 110 pb Ladder (Amersham Pharmacia Biosciences Inc. Little Chalfont, Buckinghamshire, U.K.) was included in each PCR run.

All the amplification had been reproducible therefore all the bands had been considered belonging products to the amplification profiles of the isolates.

Dendrograms were generated by the program NTSYS – Numerical Taxonomy and Multivariante Analysis System, version 2.1 [6] and it was used to determine the genetic relationship of such isolates. The similarity coefficients were calculated using the Sorensen-Dice coefficient and grouped by the Unweighted-Pair Group Method with Arithmetic mean clustering method (UPGMA). For the present study the interpretation of these results was: similarity coefficient 1.0 means isolates designated genetically indistinguishable. Similarity coefficient 0.99 to 0.80 means isolates closely related who is highly similar but wasn't identical and could be considered the same strain. Similarity coefficient 0.79 to 0.50 means isolates possibly related. Similarity coefficient lower than 0.50 means that isolates are unrelated [7,8].

RESULTS

Frequency of bacterial isolates

In our study was isolated several strains including *Streptococcus* sp, *Enterobacter* sp, *Citrobacter* sp, *Pseudomonas* sp, and the species represented in Table 1. The choice for these species is due to the significance in cross infection, mainly in dental office holding a great number of dental units, the number of isolates and to the great resistance capacity to survive in the environment.

In a total of 46 isolates, 50% (23 isolates) were obtained from a second collection (Table 1), and out of those, about 47.8% (11 isolates) were derived from related places to the lavatory basin (Pp, near wash basin; Pd, distant from wash basin), in this collection bacterial isolates were not found in the lavatory basin (Pi, in wash basin) or reflector (R), however a sample of the tap (T) was isolated. In some points of the collection we could observe an agreement in relation to the number of isolates, fact that

Isolates	Collection Area	Collection	Identification	Surgical procedure	
				Before	After
H. alvei	Mask	I / II	M29 / M4	X	
Total 5	Lavatory Basin	I	Pi11, Pi17		X
	Far from the Lavatory Basin	II	Pd18	X	
P. vulgaris	Far from Lavatory Basin	II	Pd36, Pd1	X	X
Total 5	Mask	I	M39		X
	Near the Lavatory Basin	I	Pp42		X
	Gloves	I	L251		X
K. pneumoniae	Lavatory Basin	I	Pi8		X
Total 7	Mask	I	M13		X
	Gloves	II	L14	X	
	Hand Skin	I / II	E49, E124 / E34	X	X
	Eyeglass	II	O48		X
K. oxytoca	Far from the Lavatory Basin	I / II	Pd43	X	
Total 13	Hand Skin	I	E3, E31 / E34, E35	X	X
	Reflector	I	R21		X
	Eyeglass	I	O33	X	
	Gloves	II	L40, L41	X	X
	Tap	II	T44	X	
	Near the Lavatory Basin	II	Pp45, Pp106	X	X
	Lavatory Basin	I	Pi199	X	
E. coli	Far from Lavatory Basin	I / II	Pd2 / Pd20, Pd32, Pd37	X	X
Total 4					
S. aureus	Far from the Lavatory Basin	I / II	Pd2, Pd9 / Pd12, Pd18	X	X
Total 12	Near the Lavatory Basin	I / II	Pp15 / Pp7		X
	Lavatory Basin	I	Pi17, Pi11, Pi8		X
	Gloves	II	L14	X	
	Hand skin	II	E16	X	
	Mask	II	M6	X	

Table 1. List of isolates related with area, period of the collection and surgical procedures. **Pi**: isolates from lavatory basin; **Pp**: isolates near the lavatory basin; **Pd**: isolates far from lavatory basin; **L**: isolates from gloves; **O**: isolates from eyeglasses; **T**: isolates from tap; **E**: isolates from skin of the hands of the surgeon-dentist; **R**: isolates from the reflector; **M**: isolates from mask.

was observed in isolates of the skin of the hands of the dentists (E) and eyeglasses (O) that maintained the same number of isolates. For isolates deriving from the mask (M), the results were basically the same in relation to the two carried through collections. Of all 12 isolates of *Staphylococcus aureus,* 75% derived from places related with the lavatory basin; 30. 7% of the isolates of *Klebsiella oxytoca* were collected from the hand skin of the dentist surgeon and 3 isolates of *Klebsiella pneumoniae* also were isolated from hand skin. For the isolates of *Hafnia alvei* and *Proteus vulgaris,* 60% of the isolates derived from places related with the lavatory basin and all isolates of *Escherichia coli* were isolated far from of the lavatory basin.

RAPD analysis for samples of Klebsiella oxytoca

All the isolates were grouped in two distinctive groups in the dendrogram analysis (Fig. 1) with average S_{ab} 0.86. The analysis showed 10 profiles in two main groups, where group I grouped the majority of isolates, 5 isolates were grouped with S_{ab} 1.0 deriving from skin, reflector, eyeglass and gloves. The remaining isolates in group I, small S_{ab} variation was obtained, varying from 0.91-0.94 being considered highly related.

RAPD analysis for samples of Klebsiella pneumoniae

For *Klebsiella pneumoniae* all the isolates were obtained in one group with average S_{ab} 0.79 (Fig. 2). The analysis showed 4 profiles with 2 identical isolates (S_{ab} 1.0), deriving from masks and gloves and an isolate from skin highly related. Two isolates from lavatory basin and skin showed highly similarity coefficients.

RAPD analysis for samples of Staphylococcus aureus

The RAPD analysis generated dendrogram with 9 profiles (Fig. 3). Analyzing all isolates, including a ATCC strain of *S. aureus,* an average S_{ab} of 0.78 of the total collection was shown. The profile IV contained 3 isolates with S_{ab} 1.0. The isolates of profile III, V and VI were highly relatedness with similarity coefficient varying from 0.84 to 0.94.

RAPD analysis for samples of Escherichia coli

The average S_{ab} to *E. coli* was 0.79 showing 2 identical isolates (S_{ab} 1.0), only one isolate had a low similarity degree in relation to others samples (Fig. 4).

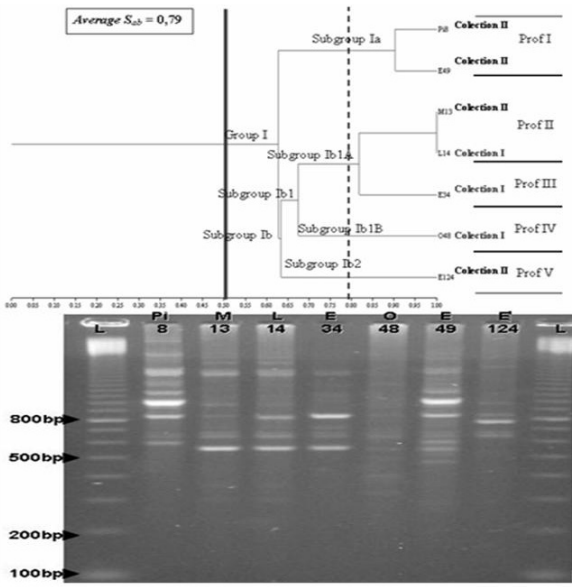

Figure 1. Gel electrophoresis and dendrogram of the RAPD profiles obtained from *Klebsiella oxytoca* isolates with primer Operon 21. **L***: Ladder 100pb; **Pi**: isolates from lavatory basin; **Pp**: isolates near the lavatory basin; **Pd**: isolates far from lavatory basin; **L**: isolates from gloves; **O**: isolates from eyeglasses; **T**: isolates from tap; **E**: isolates from skin of the hands of the surgeon-dentist; **R**: isolates from the reflector. **Prof**: Profile. The hatched line represents the S_{ab} average.

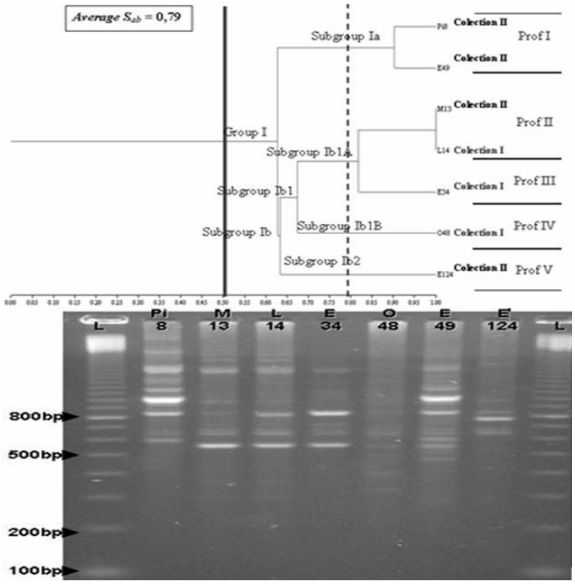

Figure 2. Gel electrophoresis and dendrogram of the RAPD profiles obtained from *Klebsiella pneumoniae* isolates with primer Operon 18. **L***: Ladder 100pb; **Pi**: isolates from lavatory basin; **L**: isolates from gloves; **O**: isolates from eyeglasses; **E**: isolates from skin of the hands of the surgeon-dentist; **M**: isolates from mask. **Prof**: Profile. The hatched line represents the S_{ab} average.

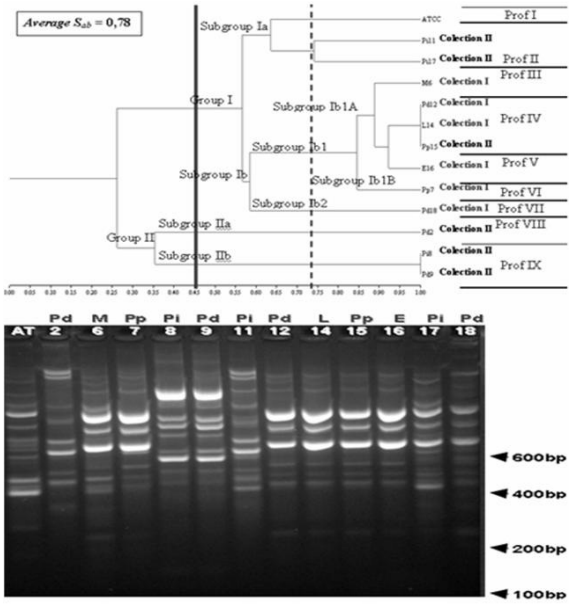

Figure 3. Gel electrophoresis and dendrogram of the RAPD profiles obtained from *Staphylococcus aureus* isolates with primer RAPD 7. AT: ATCC 25923 strain of *Staphylococcus aureus;* **Pi**: isolates from lavatory basin; **Pp**: isolates near the lavatory basin; **Pd**: isolates far from lavatory basin; **L**: isolates from gloves; **O**: isolates from eyeglasses; **E**: isolates from skin of the hands of the surgeon-dentist. **Prof**: Profile. The hatched line represents the S_{ab} average.

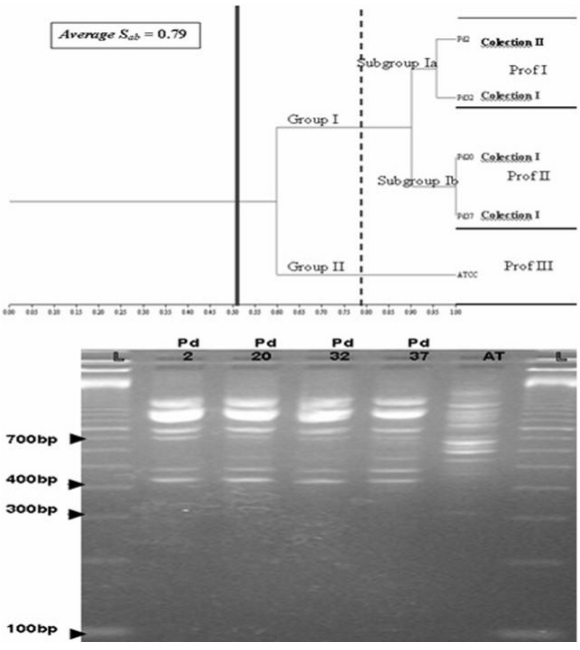

Figure 4. Gel electrophoresis and dendrogram of the RAPD profiles obtained from *Escherichia coli* isolates with primer Operon 18. **L***: Ladder 100pb; AT: ATCC 25922 strain of *Escherichia coli;* **Pd**: isolates far from lavatory basin. **Prof**: Profile. The hatched line represents the S_{ab} average.

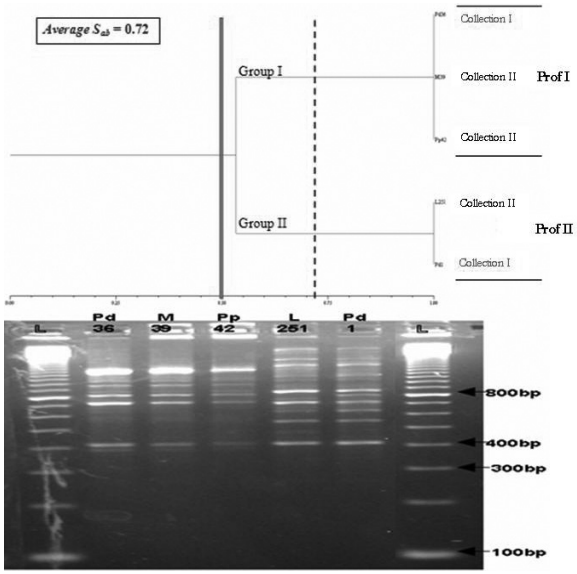

Figure 5. Gel electrophoresis and dendrogram of the RAPD profiles obtained from *Proteus vulgaris* isolates with primer ERIC 1. **L***: Ladder 100pb; **Pd**: isolates far from lavatory basin; **Pp**: isolates near the lavatory basin; **L**: isolates from gloves; **M**: isolates from mask. **Prof**: Profile. The hatched line represents the S_{ab} average.

RAPD analysis for samples of Proteus vulgaris

For *Proteus vulgaris* isolates, the average S_{ab} was 0.72, which grouped in two profiles containing 3 and 2 isolates with S_{ab} 1.0. The relation between the profiles was about 0.55 (Fig. 5).

RAPD analysis for samples of Hafnia alvei

All isolates of *Hafnia alvei* showed highly dissimilarity based on S_{ab} average, about 0.48.

DISCUSSION

The risks of infection are frequent in the odontological practice, due to drops, aerosols, contaminated instruments and or equipment which can transmit many kinds of infections. Professionals of healthcare area are exposed, and dentists, nurses and others can transmit infectious diseases to patients through the usage of dental instruments and contaminated hands. This transversal microbiological contamination is particularly dangerous when considering individual immunodeficiency [3].

The dissemination can be related to some particular aspects like survival in the environmental and possible sources or reservoir of microorganism. There are studies indicating that the survival of microorganisms in some surfaces is dependent of the type of microorganism stud-

ied and can vary from 0.2 % to 2 % of the initial inoculum [9]. *Porphyromonas gingivalis, S. mutans* and *Candida albicans,* after 24 h, were found retained on surfaces of dental brushes [10]. No periodontopathogen remained detectable at 8 hours, except for Fusobacterium nucleatum and the proportion of vital bacteria decreased in 48 hours from 50% to 30% [10].

In all isolates found in both collections of this present work, 54.34% were located in places related to the lavatory basin (in the lavatory basin, close and distant benches and taps) in agreement with Barbeau *et al.* [11], who considers some odontological environments as an aquatic ecosystem where the opportunist pathogen colonize synthetic surfaces successfully, being able to increase the concentration of this same pathogen in the water to potentially dangerous levels, therefore enabling this environment to an area that can propitiate dissemination. Similar results were verified in our study where the highest amount of isolated was found in the lavatory basin, but interestingly, in distant places from the lavatory basin were found higher amount of isolates than in close areas. The fact that a higher number of isolates was found in these areas probably is due to dissemination of aerosol generated during surgical procedures and/or deriving contamination of abiotics sources.

It is known that air, surfaces, dental materials and instruments, and water in dental units could be vehicles for cross contamination with several microorganisms; there is almost any information of contamination of dental surgery environment [3]. There are studies showing high level of contamination of water lines feeding dental units [12-14] which analyzing the quality of water in a dental unit showed the occurrence of microbial contamination probably produced by the presence of biofilm in tubules of water distribution. In our study we found *E. coli* far from lavatory basin probably due to aerosol that had spread the bacteria for the environment to the dental offices. We cannot affirm that isolated of *E. coli* had been proceeding from reservoirs of water of dental offices, but this bacterium was isolated of distant environments of the sinks of laundering, showing that the spreading occurred for the oral aerosol dissemination during the procedures of equipment washing launderings of clinical sources. These findings can possibly be confirmed by the absence of these isolated ones in the sink, were the laundering of the hands occurred. The use of these procedures of laundering of the hands with disinfecting products had reduced the number of bacteria in these places.

Colonization of water systems with Enterobacteriaceae other than *E. coli* had been reported throughout several countries mainly in the United States and Canada. Drinking water distribution systems are colonized by saprophytic heterotrophic microorganisms (bacteria, fungi, yeasts, etc.) [11,15-18] that grow on biodegradable organic matter [19-22]. Potentially pathogenic microorganisms *(e.g., Legionella* spp.) and microorganisms of fecal origin *(e.g., Escherichia coli)* may also find favorable conditions and proliferate in these systems [23-27].

S. aureus also was the only isolated Gram-positive cocci in some places, with exception of eyeglasses, taps and reflectors, suggesting that the dissemination wasn't caused by the hands [28,29]. The persistence of determined bacterial species in different places of a dental clinical can characterize possible sources of contamination. The presence of *K. pneumoniae, K. oxytoca* and *S. aureus* suggest that the dissemination was through the hands therefore this study revealed that disinfection procedures must be revised by surgeon-dentist [30]. A high correlation between distinctives genetic profiles was observed in this work which was identified by RAPD analysis pointing the genus *Klebsiella* and *S. aureus* the ones with the highest values of similarity coefficient.

Several studies using RAPD as tool had obtained satisfactory results in the determination of closely related strains, demonstrating that polymorphic low intensity bands can be considered to differentiate the profiles. The speed, simplicity of this method could represent a suitable first screening approach for identification of distinct genetic lineages, besides; RAPD markers could reveal possible relationship between host origin, mutation and genetic variation among bacterial isolates. The RAPD assay demonstrated its fingerprinting and diagnostic potential and makes it a valuable addition to current molecular epidemiological tool in the investigation of infections [5,16,31-34].

Most studies of molecular epidemiology can discriminate bacterial strains from hospital environment, but the use in dental office this approach is relatively new. In Brazil few studies show dissemination in dental office through epidemiologic inquire in the environment. In our study most of the strains were considered closely related, suggesting that the surgical procedures can disseminate these species in the environment. The relatedness found between strains means that only one source of contamination

was happening in the area. The origin of the contamination source wasn't clear in this study but may be related to surgical sources.

The genetic relationship obtained through the isolates based on analysis of RAPD profiles, suggested that the most common way of dissemination for bacterial isolates were through the hands and surgical aerosols. A RAPD technique demonstrated an efficient and adjusted biotechnological tool in the understanding of the spreading of certain species in dental offices mainly the strains with great resistance and capacity to survive in these environments. This technique can be used in epidemiologics studies, in association with genetic analysis similarity helping in the identification of possible origins of spreading, contamination and infection [34-36].

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REFERENCES

1. Samaranayake, L. (1993) *Int. Dent. J.* **43**: 578-84.
2. Miller, C.H. (1996) *Dent. Clin. North Am.* **40**: 437-56.
3. Monarca, S., M. Grotollo, D. Renzi, C. Paganelli, P. Sapelli, I. Zerbinni & G. Nardi (2000) *Occup. Environ. Med.* **57**: 721-6.
4. Santos, L.R. (2004) *Rev. Fac. Zootec. Vet. Agr.* **10**: 72-86.
5. Khivsara, A., T.V. Sushma & B. Dhanashree (2006) *Curr. Sci.* **90**: 910-2.
6. Rohlf, J.F. (2000) NTSYS – pc: Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Software. Setauket. NY.
7. Tenover, F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing & B. Swaminathan (1995) *J. Clin. Microbiol.* **33**: 2233-9.
8. Soll, D.R. (2000) *J. Clin. Microbiol.* **13**: 332-70.
9. Quirynen, M., M. de Soete, M. Pauwels, K. Goossens, W. Teughels, J. van Elderevan & D. van Steenberghe (2001) *J. Clin. Periodontol.* **28**: 1106-14.
10. Bunetel, L., S. Tricot-Doleux, G. Agnani & M. Bonnaure-Mallet (2000) *Oral Microbiol. Immunol.* **15**: 313-6.
11. Barbeau, J., R. Taguay, E. Faucher, C. Avezard, L. Trudel, L. Cöte & A.P. Prévost (1996) *Appl. Environ. Microbiol.* **62**: 3954-9.
12. Meiller, T.F., J.I. Kelley, A.A. Baqui & L.G. De Paola (2000) *J. Clin. Dent.* **11**: 11-5.
13. Gross, A., M.J. Devine & D.E. Cutright (1976) *J. Periodontal.* **47**: 670-3.
14. Linger, J.B., J.A. Molinari, W.C. Forbes, C.F. Frathing & W.J. Winget. (2001) *J. Am. Dent. Assoc.* **132**: 1287-91.
15. Amblard, C., G. Bourdier, J.F. Carrias, N. Maurin & C. Quiblier (1996) *Water Res.* **30**: 613-24.
16. Block, J.C., K. Haudidier, J.L. Paquin, J. Miazga & Y. Lévi (1993) *Biofouling*. **6**: 333-43.
17. LeChevallier, M.W., T.M. Babcock & R.G. Lee (1987) *Appl. Environ. Microbiol.* **53**: 2714-24.
18. Servais, P., G. Billen, P. Laurent, Y. Lévi & G. Randon (1992) *Sci. Eau*. **5**: 69-89.
19. LeChevallier, M.W., W. Schultz & R.G. Lee (1991) *Appl. Environ. Microbiol.* **57**: 857-62.
20. Mathieu, L., J.L. Paquin, J.C. Block, G. Randon & J. Maillard (1992) *Sci Eau*. **5** : 91-112.
21. Servais, P., P. Laurent & G. Randon (1995) *J. Water SRT-Aqua.* **44**: 10-17.
22. Steinert, M., M. Ott, P.C. Lück, E. Tannick & J. Hacker (1994) *FEMS Microbiol. Ecol.* **15**: 299-308.
23. Camper, A.K. (1994) "Coliform regrowth and biofilm accumulation in drinking water systems: a review", en *"Biofouling and biocorrosion in industrial water systems"* (G.G. Geesey, Z. H. Levandowski, C.Flemming, eds.), Lewis Publishers Inc., Chelsea, MI, USA, págs. 91-105.
24. Fass, S., M.L. Dincher, D.J. Reasoner, D. Gatel & D.J.C. Block (1996) *Water Res.* 30: 2215-21.
25. LeChevallier, M.W. (1990) *J. Am. Water Works Assoc.* **82**: 74-86.
26. Morin, P., A. Camper, W. Jones, D. Gatel & J.C. Goldman (1996) *Appl. Environ. Microbiol.* **62**: 4428-32.
27. Verity, P.G. (1988) *Bull. Mar. Sci.* **43**: 772-82.
28. Murdoch, F.E., R.L. Sammons & I.L.C. Chaplle (2004) *Oral Dis.* **10**: 155-62.
29. Bernardo, W.L.C., M.F.G. Boriollo, R.B. Gonçalves & J.F. Hofling (2005) *Rev. Inst. Med. Trop.* **47**: 19-24.
30. Toroglu, M.S., M.C. Haytac & F. Koksal (2001) *Angle Orthod.* **71**: 299-306.
31. Saulnier, P., C. Bourneix, G. Prévost & A. Andremont (1993) *J. Clin. Microbiol.* **31**: 982-5.
32. Eisen, D., E.G. Russell, M. Tymms, E.J. Roper, M.L. Grayson & J. Turnidge (1995) *J. Clin. Microbiol.* **33**: 713-17.
33. Brisse, S. & J. Verhoef (2001) *Int. J. Syst. Evol. Microbiol.* **51**: 915-24.
34. Onasanya, A., H.D. Mignouna & G. Thottappilly (2003) *Afric. J. Biotech.* **2**: 246-250
35. Barnini, S., C. Dodi & M. Campa (2004) *Lett. Appl. Microbiol.* **39**: 274-7.
36. Romalde, J.L., D. Castro, B. Magariños, L. Lopes-Cortes & J.J. Borrego (2002) *Syst. Appl. Microbiol.* **25**: 544-50.