UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM
PÓS-GRADUAÇÃO EM ODONTOLOGIA

GUILHERME DE ALENCAR TEMÓTEO

ANÁLISE DA CONTAMINAÇÃO MICROBIANA DE DISPOSITIVOS ACRÍLICOS CONFECCIONADOS EM LABORATÓRIOS DE PRÓTESE DENTÁRIA

FORTALEZA
2014

GUILHERME DE ALENCAR TEMÓTEO

ANÁLISE DA CONTAMINAÇÃO MICROBIANA DE DISPOSITIVOS ACRÍLICOS CONFECCIONADOS EM LABORATÓRIOS DE PRÓTESE DENTÁRIA

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará como um dos requisitos para a obtenção do título de Mestre em Odontologia.

Área de Concentração: Clínica Odontológica

Orientadora: Profa. Dra. Karina de Matthes de Freitas Pontes

FORTALEZA

2014

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca de Ciências da Saúde

T278a Temóteo, Guilherme de Alencar.
Análise da contaminação microbiana de dispositivos acrílicos confeccionados em laboratórios de prótese dentária / Guilherme de Alencar Temóteo. – 2014.
51 f. : il. color., enc. ; 30 cm.

Dissertação (Mestrado) – Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Programa de Pós-Graduação em Odontologia, Mestrado em Odontologia, Fortaleza, 2014.
Área de Concentração: Clínica Odontológica.
Orientação: Profa. Dra. Karina de Matthes de Freitas Pontes.

1. Resinas Acrílicas. 2. Prótese Dentária. 3. Contaminação. 3. Desinfecção. 4. Esterilização. I. Título.

CDD 617.695

GUILHERME DE ALENCAR TEMÓTEO

ANÁLISE DA CONTAMINAÇÃO MICROBIANA DE DISPOSITIVOS ACRÍLICOS CONFECCIONADOS EM LABORATÓRIOS DE PRÓTESE DENTÁRIA

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará como um dos requisitos para a obtenção do título de Mestre em Odontologia.

Aprovada em 27/02/2014.

BANCA EXAMINADORA

_____

Profa Dra Karina Matthes de Freitas Pontes (Orientadora)

Faculdade de Farmácia, Odontologia e Enfermagem – UFC

_____

Prof. Dr Marcus Aurélio Rabelo Lima Verde

Faculdade de Farmácia, Odontologia e Enfermagem – UFC

_____

Prof. Dr. Eduardo Diogo Gurgel Filho

Curso de Odontologia - UNIFOR

AGRADECIMENTOS

A Deus por me iluminar com todo seu amor em todos os momentos, me fazendo ter dicernimento diante dos desafios da vida.

Aos meus queridos pais **Hermes e Celina** por me proporcionarem uma educação de qualidade e me ensinarem valores morais como honestidade e respeito. Sou eternamente grato por todo amor e dedicação.

À minha amada esposa **Carol** que sempre esteve ao meu lado em todos os desafios, me ajudando e incentivando.

AGRADECIMENTOS ESPECIAIS

Á Profª Drª **Karina Matthes de Freitas Pontes**, uma pessoa fantástica que une doçura e competência numa só pessoa. Chamou-me a atenção quando foi preciso, mas sempre me elogiou quando eu mereci. Serei sempre grato por todos os ensinamentos.

Ao **Carlos Eduardo Albuquerque** pela amizade e pela força para que eu fizesse a seleção do mestrado. À **Bruna Frota** por toda disponibilidade e boa vontade em ajudar nas pesquisas.

À **Bruninha Albuquerque** pela amizade e por ser meu braço direito durante toda a pesquisa! À **Camila Caracas** por não medir esforços para me ajudar ! À **Nádia Dantas** pelo apoio no inicio da pesquisa.

À **minha turma de mestrado** pela parceria durante essa caminhada . Em especial à **Ximena Trévia** por ter me ajudado durante vários momentos no mestrado.

À **Ramille Lima e David Queiroz** por me orientarem muitas vezes durante o período de realização dos experimentos microbiológicos.

Ao **Paulinho Goberlanio** por todo o apoio e dedicação na parte da estatística.

À toda **equipe de professores da Prótese Fixa**, além da **Malu**, **Gonzaga** e **Janaina Câncio** pelos bons momentos de convivência e aprendizado na clinica.

À coordenadora do Programa de Pós-graduação em Odontologia da UFC, Profª Drª **Lidiany Karla Azevedo Rodrigues**, pela atenção dada a todos á nossa turma do mestrado. À **Lúcia e Janaine** pela presteza em ajudar na parte burocrática do mestrado.

Aos **professores do Programa de Pós-graduação em Odontologia da UFC**, pelos ensinamentos.

Aos **professores titulares e suplentes** da banca de defesa, por sua disponibilidade.

“A persistência é o menor caminho do êxito.”

(Charles Chaplin)

RESUMO

A possível presença de microorganismos potencialmente patogênicos em próteses dentárias recém-chegadas dos laboratórios protéticos deve ser considerada. Este estudo avaliou o nível de contaminação bacteriana e fúngica de espécimes de resina acrílica confeccionados em 14 laboratórios de prótese dentária, inscritos no Conselho Regional de Odontologia do Ceará, na cidade de Fortaleza. Cada laboratório foi solicitado a confeccionar 10 espécimes de resina acrílica, a partir de modelos padronizados de silicona de adição estéreis, desconhecendo os objetivos da pesquisa. Os espécimes recebidos dos laboratórios foram colocados em tubos individuais contendo BHI caldo e incubados a 37°C por 48 horas e, em seguida, removidos, lavados, colocados em solução salina estéril e agitados para desprendimento microbiano. A suspensão obtida foi diluída em 1:100, 1:1000 e semeada em placas com Ágar Sangue, Sabouraud Dextrose Ágar e HICrome UTI Ágar®, para incubação por 48 horas a 37°C. Foi obtido o número de unidades formadoras de colônias (UFC) bacterianas e fúngicas viáveis, além da identificação e quantificação de algumas espécies de bactérias, comparando-se os laboratórios por meio dos testes de Kruskall-Wallis e Dunn ($\alpha=0.05$). Houve contaminação advinda de todos os laboratórios analizados, com uma contagem de UFC média de 101438 de bactérias e 71047 de fungos. *Pseudomonas spp* foi o microorganismo a mais prevalente identificado ($p<0,05$). Foi concluido que existe risco de contaminação por bactérias potencialmente patogênicas e fungos em dispositivos protéticos recém chegados dos laboratórios.

Palavras-chave: Resinas Acrílicas. Prótese Dentária. Contaminação. Desinfecção. Esterilização.

ABSTRACT

The possible presence of potentially pathogenic microorganisms in denture newly arrived from prosthetic laboratories should be considered. This study evaluated the level of bacterial and fungal contamination of specimens of acrylic resin made in 14 dental laboratories registered with the Regional Council of Dentistry of Ceará, Fortaleza. Each laboratory was asked to fabricate 10 specimens of acrylic resin, from standard models of sterile silicone addition, unaware of the research objectives. Specimens received from laboratories were placed in individual tubes containing BHI broth, incubated at 37°C for 48 hours and then removed, washed and placed in sterile saline and stirred for microbial detachment. The suspension obtained was diluted (1:100, 1:1000) and plated on blood agar plates, and Sabouraud Dextrose Agar and Agar HiCrome ICU by incubation for 48 hours at 37°C. The number of colony forming units (CFU) bacterial and fungal viable was obtained, besides the identification and quantification of some species of bacteria, comparing the laboratory by means of the Kruskal-Wallis and Dunn ($\alpha = 0.05$) tests. There was contamination originating from all laboratories analyzed, with a mean CFU counts of 101438 bacteria and 71047 fungi. *Pseudomonas spp* was the most prevalent microorganism identified ($p < 0.05$). It was concluded that there is a risk of contamination with potentially pathogenic bacteria and fungi in prosthetic devices newly arrived from dental laboratories.

Keywords: Acrylic Resin. Dental Prosthesis. Contamination. Disinfection. Sterilization.

SUMÁRIO

1 INTRODUÇÃO GERAL

Infecção cruzada entre consultório odontológico e laboratório de prótese dentária pode ocorrer quando procedimentos de biossegurança não são executados adequadamente, tanto por parte dos dentistas, quanto dos técnicos de laboratório. A desinfecção dos trabalhos protéticos é uma etapa importante para prevenção da contaminação entre pacientes, dentistas e técnicos de laboratório (Leung & Schonfeld , 1983; Kugel et al., 2000; Boas & Quirino, 2002).

Estudos têm sugerido que os laboratórios de prótese dentária são fontes importantes de contaminação cruzada. Os técnicos em laboratório devem estar cientes dos riscos potenciais de contaminação colocados pela presença de uma gama de patógenos oportunistas em trabalhos protéticos (Verran et al., 1996). Próteses, moldes, modelos ou outros objetos que mantiverem contato com a saliva ou sangue de pacientes podem servir como via indireta de transmissão de micro-organismos ao pessoal envolvido no processamento laboratorial de próteses dentárias, via contato direto ou pelos aerossóis produzidos durante os procedimentos de desgaste e polimento das próteses (Silva et al., 2010; Abichandani & Nadiger, 2013).

A ADA (*American Dental Association*) preconiza que os materiais, impressões e próteses intraorais devem ser limpas e desinfetadas antes de serem manipuladas, ajustadas, ou enviadas para um laboratório de prótese dentária (Bhat et al., 2007). Em alguns países, recomendações relativas à desinfecção de itens enviados para laboratórios já existem há vários anos, no entanto, essas recomendações normalmente são escassamente respeitadas e muito negligenciadas (Wakefield,1980; Verran et al., 1996; Sofou et al., 2002). De acordo com o *Centers for Disease Control* (Atlanta, Georgia, EUA), sangue e saliva devem ser minuciosamente limpos do material que foi usado na cavidade oral; também devem ser limpos e desinfetados antes de serem manipulados em laboratório de prótese dentária e antes que eles sejam colocados na cavidade oral de um paciente (Powell, 1990) .

O procedimento padrão de enxaguamento de moldes com água corrente imediatamente após sua remoção da cavidade oral, elimina uma

contaminação grosseira, juntamente com a maioria de saliva e sangue. No entanto, nem todos os micro-organismos são removidos, e eles podem ser uma fonte de infecção (Merchant et al., 1984). Estudo com moldes entregues a um grande laboratório dental na Suécia revelou que cerca de metade das clínicas relatou seguir algum tipo de rotina de desinfecção e, no entanto, 72% das impressões apresentavam crescimento de bactérias (Sofou et al., 2002).

A formação de biofilmes na cavidade oral pode acontecer não só em dentes, mas também em próteses dentárias, com a adesão de micro-organismos patogênicos (Nikawa et al*.,* 1998). Diferentes espécies de agentes patogênicos orais e não orais estão associados com a placa da dentadura, incluindo *Candida spp*., *Staphylococcus spp., Streptococcus spp., Lactobacillus spp., Pseudomonas spp., Enterobacter spp. e Actinomyces spp* (Glass et al., 2001*).* A presença desta microflora tem sido implicada em patologias locais e sistêmicas, tais como cárie, doença periodontal, inflamação da mucosa, infecções do trato urinário, conjuntivite, pneumonia, meningite, abcessos, endocardite e septicemia (Zarb & Mackay, 1980).

Agostinho et al*.* (2004) encontraram um alto índice de contaminação bacteriana e fúngica nas próteses totais provenientes de seus usuários e concluíram que, em virtude disto, se procedimentos adequados de desinfecção não fossem implantados também nos laboratórios quando fossem recebidas próteses para ajustes, consertos ou polimento, os micro-organismos poderiam contaminar os pacientes de outros consultórios, gerando uma infecção cruzada.

Em laboratórios de prótese dentária, tornos usados para polimento e acabamento de próteses têm sido descritos como uma das maiores fontes de contaminação. Witt & Hart (1990) publicaram que todas as amostras analisadas de discos de feltro embebidos com pedra-pomes e água estavam contaminadas com micro-organismos do tipo aeróbio bacilo Gram-positivo, incluindo *B. cereus*, *B. brevis*, *B. licheniformis* e com os membros do grupo *coli*.

Segundo Kahn et al. (1982), vírus, fungos e bactérias patogênicas podem ser facilmente transmitidos de paciente para paciente através do simples ato de polir uma dentadura. Levando em consideração que portadores de próteses dentárias normalmente são pessoas idosas, que podem ter a imunidade comprometida, doenças epidêmicas relativamente comuns, como a

gripe, podem causar-lhes uma morbidade mais significativa. Nestes pacientes podem estar presentes também problemas com higiene oral, periodontite, doenças sistêmicas, como pneumonia por aspiração, doenças cardiovasculares e diabetes. Dentre estes, a pneumonia por aspiração é um das principais causas de morte em idosos (Abaci et al., 2010). Desta maneira, o cuidado com a infecção cruzada deve ser redobrado em pacientes imunocomprometidos ou que tenham alguma outra condição sistêmica, como diabetes ou cardiopatias, por exemplo. O paciente diabético apresenta muitas alterações fisiológicas que diminuem a capacidade imunológica e a resposta inflamatória, aumentando a suceptibilidade às infecções (Sousa et al., 2003).

Os laboratórios de prótese dentária, de modo geral, não têm contato direto com os pacientes e, desta forma acreditam que não estão expostos a material biológico (Silva et al., 2010). Um estudo relatou que 39,5% de técnicos nunca usavam luvas ao trabalhar (Merchant et al., 1984). Talvez a realidade hoje não seja diferente. São escassos na literatura artigos atuais sobre a problemática da biossegurança em laboratórios de prótese dentária e no manejo de dispositivos protéticos no consultório odontológico.

2 PROPOSIÇÃO

2.1 Objetivo Geral:

O objetivo deste estudo observacional, descritivo e transversal foi avaliar o nível de contaminação bacteriana e fúngica, em superfície de resina acrílica, de espécimes confeccionados em diferentes laboratórios de prótese dentária da cidade de Fortaleza-CE.

2.2 Objetivos Específicos:

Os objetivos específicos desta dissertação foram:

- fazer um levantamento da prevalência média de contaminação por bactérias e fungos a ser transmitida via laboratório de prótese dentária para a clínica odontológica.
- comparar os laboratórios selecionados quanto ao nível de contaminação bacteriana e fúngica dos espécimes por eles produzidos.
- identificar alguns gêneros e/ou espécies bacterianas com potencial patogênico presentes nos espécimes de resina acrílica advindos dos laboratórios de prótese dentária, apontando sua prevalência.

3 CAPÍTULO

Esta dissertação baseia-se no Artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de mestrado e teses de doutorado e permite a inserção de artigos científicos de autoria e co-autoria do candidato. Assim sendo, essa dissertação é composta por um capítulo, contendo um artigo submetido à publicação ou em fase de redação, conforme descrito na sequência:

Capítulo 1 – artigo para publicação

"Analysis of microbial contamination of device acrylic manufactured in dental laboratories"

Pontes KMF, Temóteo GA, Garcia BA, Silva PGB, Sousa CCV

Este artigo será submetido à publicação no periódico **“The International Journal of Prosthodontics “.**

Analysis of microbial contamination of device acrylic manufactured in dental laboratories

Guilherme de Alencar Temóteo [a]

Bruna Albuquerque Garcia [a]

Paulo Goberlânio de Barros Silva [a]

Camila Caracas Vieira de Sousa [a]

Karina Matthes de Freitas Pontes [b]

DDS, graduate student, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará. – a

PhD, adjunct professor, Faculty of Pharmacy, Dentistry and Nursing, Federal University of Ceará. - b

Corresponding author:

Karina Matthes de Freitas Pontes

Rua Monsenhor Furtado S/N, Rodolfo Teófilo, CEP: 60430-350, Fortaleza, CE, Brazil.

E-mail: kamatthes@yahoo.com.br

Abstract

Purpose: This study evaluated the level of both bacterial and fungal contamination in acrylic resin specimens produced by different dental laboratories. ***Materials and Methods:*** A total of fourteen laboratories registered in the Regional Council of Dentistry of Ceará, in Fortaleza, were each requested to make 10 acrylic resin specimens based on sterile addition silicon models. Neither the laboratories did not know the aims of the research nor were their identifications informed to the experiment operator. The specimens brought from the laboratories were placed in individual tubes containing BHI broth and then incubated at 37ºC for 48 hours. Afterwards they were removed, washed, placed in sterile saline solution and then agitator to microbial release. The obtained microbial suspension was diluted 1:100, 1:1000 and plated in dishes containing blood agar, Sabouraud Dextrose agar and HICrome UTI agar® for incubation at 37°C for 48 hours. The number of viable bacterial and fungal colony forming units (CFU) was obtained, besides the identification and quantification of some bacterial species. The analysis was carried out by means of Kruskall-Wallis and Dunn tests (α=0.05). ***Results:*** Contamination was found in 14 laboratories. There was an average of 101438 CFU of viable bacteria and 71047 viable fungi; however, two laboratories stood-out by presenting more than 200000 CFU of bacteria and fungi ($p<0.05$). *Pseudomonas spp, Enterococcus spp*, *Staphylococcus aureus*, *Klebsiella*, *Staphylococcus saprophyticus* and *Escherichia coli* were identified, being the first one the most prevalent microorganism. ***Conclusion*****:** There is a risk of contamination with potentially pathogenic bacteria and fungi in prosthetic devices newly arrived from laboratories.

Descriptors: acrylic resin, dental prosthesis, contamination, disinfection, sterilization.

Introduction

Cross-contamination among patients, dentists and laboratory technicians, when biosafety care is neglected, is real. In the dental environment, there is a possibility of exposure to a wide variety of pathogenic microorganisms in the blood and saliva, such as hepatitis B virus (HBV) and hepatitis C (HCV), HIV, *Pseudomonas, Acinetobacter, Diphteroids, Lactobacillus, Staphylococci, Streptococci, Mycobacterium* and other microorganisms that colonize the oral cavity and respiratory tract. These microorganisms can be transmitted through direct or indirect contact[1]. Prosthesis, impressions, models or other objects that had contact with the saliva or blood of patients can serve as an indirect route of transmission of microorganisms to the staff involved in laboratory processing of the prosthesis by contact or by aerosols produced during abrasion and polishing of prothesis[2].

During dental treatment, dentures are often transported from one place to another, and the lack of adequate disinfection is harmful to the dental office staff, patients and also to laboratory technicians[3]. If the rules of asepsis and antisepsis are well established in dental clinics, although not always strictly followed, the same might not be said for laboratories[4].

Studies have shown that microorganisms are transmitted from impressions to the plaster models[5] and from dentures to the pumice present in lathers, which remain viable impregnated in the felt cones or in the wet denim wheels[6]. If the polishing material in laboratory is not sterilized or disposable, it can perpetuate this contamination back to the office through other prosthetic materials that perhaps are polished. Therefore, in laboratories, pumice used for polishing dentures was identified as the major source of contamination and potential source of infection transmission[7].

Concurrently with the increase in the proportion of the eldrely within each population, the number of people with impaired immunity due to senile systemic health problems is increasing[8], thus they are more susceptible to the risks of contamination.

Biosafety care in dental offices is well-established[4]. However, carelessness in the handling of impressions, models and prosthetic devices that are tested by the patient can still be seen, as after testing they return to the lab

without going through processes of disinfection or sterilization. In the laboratories, neglecting biosecurity is even greater, since technicians generally have no direct contact with the patient; therefore they believe they are not exposed to materials biologic materials[2]. As such, a survey on the degree of contamination of materials coming from prosthetic laboratories is of great importance.

The purpose of this study was to conduct a microbiological evaluation of standard specimens produced by different dental laboratories located in a Brazilian capital. The amount of bacteria and viable yeast present in the surface as well as the identification of some microorganisms were evaluated. The null hypothesis tested was that there would be no contamination of bacteria and viable yeasts in the specimens.

Materials and Methods

This is an observational, descriptive, cross-sectional, double-blinded trial.

- Eligibility criteria of dental laboratories

Based on a data collection carried out in the Regional Council of Dentistry of Ceará, it was found that there are 32 registered dental laboratories in the city of Fortaleza. A number of 14 laboratories that met the proposed inclusion criteria were selected.

Inclusion criteria for the laboratories were: being registered in the Regional Council of Dentistry of Ceará, in Fortaleza, and usually working with acrylic resin.

There was exclusion of those ones, that have not worked with acrylic resin, laboratories whose address and phone number were incorrect, since it was not possible to make contact with them, and also the laboratories that did not accept the proposed work order due to the deadline stipulated by the researchers.

The order of approaching the laboratories was determined by draw performed by one of the researchers, called B. The lot was unknown by the

main researcher, named A, who performed the microbiological procedures blindly.

The laboratories received no information on the research aims, only a work order containing a requirement on the trademark of acrylic resin, finishing system and silicone models to be reproduced in acrylic, in order to standardize specimens in the study.

- Sample size

Based on the results obtained from an initial sample in a pilot test, the calculation of the statistical power was carried out by means of the BioEstat 5.0 software (Institute of Sustainable Development of Mamirauá, Manaus, AM, Brazil). It was verified that the number of required specimens from each dental laboratory for a minimum of 80% power with significance level of $\alpha = 0.05$ would be n equal to 10.

- Production of specimen models

The models delivered to the laboratories were made of addition silicon (Adsil, Vigodent, São Paulo, Brazil) measuring 1.0 cm in diameter and 2 mm thick. In this preparation, stainless steel arrays were used (fig. 1), where the material was inserted according to the manufacturer's instructions, being removed after setting. Then, the silicon models were wrapped in surgical paper and autoclaved (Cristófoli-Campo Mourão, Paraná, Brazil) at 121°C for 30 min.

The sterile silicon models, in the amount of 10, and the work order requesting the making of the acrylic resin specimens were delivered in each of the 14 laboratories. They were flasked, using a number 6 flask, (Jon, São Paulo, Brazil) and then reprinted by pressing the indicated acrylic resin.

- Work order to dental laboratories

A number of ten specimens were ordered from each laboratory (n = 10). The acrylic resin selected to make them was the autopolymerizing Clássico (Artigos Odontológicos Clássico Ltda. São Paulo, Brazil), in medium pink color.

The finishing and polishing were also performed by the laboratories, indicating the use of sandpaper numbers 220, 400 and 600. This procedure was followed by mechanical polishing using a polishing machine with felt cone and denim wheel embedded in pumice and Spain white.

The newly prepared specimens were packaged according to the preference of each laboratory and then taken from the dental laboratory to the laboratory of Microbiology of the Post. Graduate Program in Dentistry of the Federal University of Ceará. The specimens were transported in coded hermetically sealed sterile plastic box. Microbiological procedures were conducted after the fabrication and delivery of specimens. The logistics were taken by arrangements between researcher B and each laboratory, in which the dates for delivering the silicon models and for picking up ready acrylic specimens were scheduled.

- Microbiological procedures

After bringing the specimens from the laboratories, researcher B delivered them to researcher A, who was unaware of their origin. The shipping box received from each laboratory was only opened in the laminar flow for removal of specimens.

The specimens were placed individually in sterile Eppendorf tubes containing Brain Heart Infusion broth (BHI, Acumedia, Michigan, USA). After that, they were incubated for 48 hours at 37°C in bacteriological incubator.

After this period, the Eppendorf tubes were opened in laminar flow and the specimens were placed individually into other tubes containing sterile saline solution 0.9%, after washing to remove BHI excess. Each tube was placed in vortex agitator (Vertex QL-901) to microbial release, for one minute. The obtained microbial suspension went through a process of dilution 1:100, 1:1000 and the last two dilutions were 50 uL plated in Petri dishes containing blood agar culture media (Eximlab LTDA, São Paulo, Brazil), Sabouraud Dextrose agar (Eximlab LTDA, São Paulo, Brazil) and HiCrome UTI agar® (Himedia, New York, USA). The plates were incubated for 48 hours at 37°C for counting the colony forming units (CFU) afterwards.

The blood agar was used to allow the growth of colonies of viable bacteria, whereas Sabouraud agar permits the growth of viable yeasts. The HiCrome UTI agar® culture medium is a chromogenic selective medium which allows identification of specific colonies, for differences in their colors: *Escherichia coli, Enterococcus spp, Proteus, Pseudomonas spp, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella, Citrobacter*.

- Outcome analysis method

The data from the CFU counts in the petri dishes were exposed as mean ± standard error of means and submitted to Shapiro-Wilk test for normality, to assess the pattern of sample distribution. After analysis of normality, the elements were assessed using the Kruskal-Wallis test followed by Dunn's post-test (nonparametric data).

It was used confidence level of 95% ($\alpha = 0.05$) in all analyzes and GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, Ca, USA) for all assessments.

Results

Contamination was found in all laboratories. The results of the CFU counts regarding viable bacteria on plates with medium culture blood agar from the 14 laboratories are shown in figure 2. The laboratory eight had the highest count with an average of 430786 CFU, whereas the laboratory 12 was the one that had the lowest level with a mean of 102.3 CFU.

The results of the CFU counts regarding viable fungi on the plates with the medium culture Sabourad Dextrose Agar coming from the 14 laboratories are shown in figure 3. Laboratory eight was the one which got the highest level, with an average of 285667 CFU, whereas the laboratory 10 had the lowest score, showing a mean of 6890 CFU.

The bacteria found and identified by the culture medium HiCrome UTI® Agar were *Enterococcus, Pseudomonas spp, Staphylococcus aureus, Klebsiella, Escherichia coli* and *Staphylococcus saprophyticus* are represented according to a general differential count in figure 4. *Pseudomonas spp* is the

most common bacteria in all laboratories where it was identified found, as *Escherichia coli* was the one with appeared in a fewer quantity.

Figures 5 to 10 show the comparison of each bacterium found in the differential medium culture HICrome ITU® agar regarding each studied laboratory. *Pseudomonas spp* (fig. 5) was more present in the laboratory eight (120500 CFU) and less present in laboratory nine (840 CFU). *Enterococcus spp* (fig. 6) was more present in the laboratory eight (43540 CFU) and absent in the laboratory one. *Staphylococcus aureus* (fig. 7) was more present in the laboratory seven (76740 CFU) and absent in laboratories 1, 2, 8, 10, 11 and 12. *Staphylococcus saprophyticus* (fig. 8) was present mostly in the laboratory seven (41350 CFU) and absent in laboratories 1, 4, 5, 9, 10 and 12. The genus *Klebsiella* (fig. 9) was more present in the laboratory 11 (16290 CFU) and absent in laboratories two and three. *Escherichia coli* (fig.10) was more present in the laboratory one (46790 CFU) and absent in laboratories 2, 3 and 10.

Discussion

As time passes, concern about cross-contamination between dental clinics and dental laboratories has been increasing[9]. During dental treatment, prosthesis are often transported from one place to another, and the lack of adequate disinfection is harmful to the dental office staff, patients and also to laboratory technicians[3].

Although there are implemented standards related to aseptic and antiseptic materials for the practice in dental clinic, Nevile & Zarb (2007) showed that over 60% of impressions that came from dental offices in the laboratories were contaminated with *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella oxytoca*[10]. Verran et al. (1996) say that prosthesis that were checked or adapted in the patients' mouths and then returned to adjustments in the laboratory can also transfer microorganisms. Thus, if the laboratory technicians are not careful enough to make a preventive disinfection of that material, they can run the risk of infecting themselves and their working materials[11].

According to Tatarciuc et al. (2010) there is a lack of established well-documented disinfecting protocols in dental laboratories[4]. Moreover, although the technician is aware of the possibility of contracting any disease, it seems

that the lack of direct contact with the patient makes such professional not to fear contamination and consequently not being protect adequately[12]. One study reported that 39.5% of laboratory technicians never wear gloves when working[13].

The literature says that the major source of contamination in dental laboratories is mainly present in the polishing lathes[4,8,14]. Junior (1974) adds that both pumice and polishing lathes used can be contaminated by microorganisms present on prosthesis coming from dental offices, by microorganisms present on hands, nose and mouth of the technicians, by aerosols and particles in the air of the laboratory or by tap water[15]. According to Orsi et al. (2011), the teeth of immunocompromised patients may be regarded as a greater source of contamination in dental laboratories when compared to normosystemic patients, as there is a higher possibility of presence of pathogenic microorganisms[8].

In this study, the acrylic resin was chosen because it is used in several works of prosthesis that are in constant flux between dental offices and laboratories, for example, total prosthesis, removable partial dentures, myorelaxing plates, surgical guides and provisional crowns.
It was found that all laboratories participating in this study had some type of contamination. The presence of fungi and some bacteria type *Enterococcus spp, Pseudomonas spp, Staphylococcus aureus, Escherichia coli, Klebsiella and Staphylococcus saprophyticus* was observed.

In relation to microorganisms present in blood agar culture, laboratory eight was the one which had the highest count of viable bacteria, whereas laboratory 12 had the lowest one. In relation to the amount fungi present in culture medium Sabourad agar, the laboratory eight received the highest score again, whereas the laboratory 10 was the one showing the lowest count, statistically similar to laboratories two and nine. Taking into consideration all the laboratories, the genus *Pseudomonas spp* was the most present in all laboratories where it was identified, as *Escherichia coli* was the species that appeared in smaller quantities. The study by Firoozeh et al. (2013) evaluated pumice samples from 24 laboratory and found both oral and non-oral microorganisms in the following proportions: *Staphylococcus aureus* (15.4%), *Streptococcus viridance* (10.8%), *Bacillus cereus* (18.7%), *Pseudomonas*

aeruginosa (12.8%), *Diphtheroids* (7.3%), *Enterobacter cloace* (4.3%), *Escherichia coli* (13.1%), *Klebsiella pneumonia* (5.4%), and *Acinetobacter spp*. (12.2%). Among the isolated fungi it was included Candida *albicans* (36.7%), other yeasts (17.3%), *Fusarium spp*. (13.8%), *Aspergillus spp*. (22.4 %) and *Penicillium spp*. (9.8%)[15].

Kahn et al. (1982), in their study on contamination of removable prosthesis when polished on lathes with denim wheel and pumice, found the following microorganisms: *Streptococcus, Lactobacillus, Neisseria and Diphtheroids*; besides @-hemolytic *Streptococcus* (group B), *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*[16]. In a study by Wakefield (1990), it was shown that nine out of ten sterile prosthesis sent to the laboratory for polishing were sent back contaminated with Gram negative bacilli, such as *Pseudomonas*, *Acinetobacter*, *Escherichia coli* and *Moraxella*[17].

Depending on the situation, some microorganisms can cause mild to severe inconvenience to patients. A cold for an immunocompromised patient, for instance, can bring great complications[16]. The coexistence of *Pseudomonas spp*., which was the most prevalent genus in this study, and *C. albicans* in elderly people, is a potential indicator of high risk for pneumonia and endocarditis[18]. Moreover, *Candida* may be present in denture stomatitis, since it can develop in both hard and soft tissues[19]. When *Enterococcus faecalis* enters the bloodstream inadvertently it can cause endocarditis as well as urinary tract and pelvic infections[20]. *S. aureus* is related to a number of oral infections such as osteomyelitis and stomatitis[19]. Gram-negative bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella*, when they enter the bloodstream of patients, particularly those who are weak, can cause a strong infection[21].

Acinetobacter, *Pseudomonas* and *Moraxella* microorganisms, which are not part of the normal oral flora, can cause serious illness if they are passed to patients by means of prosthesis polished with contaminated material or to laboratory technician, by exposure to contaminated aerosol[22]. Vojdani & Zibaei (2006) say that the prosthesis contaminated by potentially pathogenic microorganisms, such as gram negative bacilli, can cause serious illness when they enter the area of the oropharynx, increasing the incidence of pneumonia[7].

In case of installation of immediate prosthesis, after tooth extraction, one should pay special attention to this prosthesis disinfection due to the

greater possibility of contamination on the surgical wound[23]. Prosthesis are considered as semi critical items and must be subjected to strict sterilization or disinfection. However, because acrylic resins are heat-sensitive materials, in order to be undamaged, the use of chemical disinfectants is necessary[3]. Merchant (1997) suggest the use of sodium hypochlorite and glutaraldehyde[24].

Despite the instructions, there were small differences in the quality of the polishing of acrylic resin specimens from one laboratory to another. According to the literature, the surface roughness increases the adhesion of microorganisms and biofilm formation[25]. A denture showing poor surface smoothness or containing an old porous tissue conditioner can increase infection substantially[16]. In a study carried out by Kuroki (2010), antibacterial substances were placed in the composition of self-polymerizable acrylic resins.Their early results were encouraging, decreasing the amount of *Streptococcus ssp*[26]. According to Orsi et al. (2010), several procedures are recommended in order to reduce the risk of cross-infection, such as sterilization of polishing brushes and drills, replacement of pumice, or addition of disinfectants for polishing materials. However, many of these procedures might not be necessary if all devices were efficiently disinfected before being placed in the oral cavity[8].

Staff should also be encouraged to use personal protective equipment (PPE) more often not to contaminate the prosthetic work or not to be contaminated. In addition to that is worth remembering the importance of investigating the presence of viruses. When the dehydrated HIV virus is rapidly inactivated; however, the hepatitis B virus (HBV) can survive in 42% humidity for seven days[11]. In another study conducted in 1986, 22 out of 155 (14.2%) examined laboratory technicians had a positive sorologic test for hepatitis[27].

The results of this study also show the need for the introduction in laboratory of a good practical guide to biosafety and strict legislation regarding this work for dental prosthesis in order to reduce the risk of cross-contamination. In future studies, it is suggested to evaluate a greater number of laboratories in other cities and/or regions in Brazil, also comparing with the reality of other countries. A microbiological evaluation of actual prosthetic works, which come to different dental clinics in different districts and cities,

would also be important in order to strengthen or soften the findings of this study.

Conclusion

It was found bacterial and fungal contamination in all laboratories, especially in two of them. The genus *Pseudomonas* was the most prevalent, whereas *E. coli* was the least prevalent among the identified species.

References

1. Al-Saadi AK. Bacterial cross-contamination between clinic & dental laboratory during polishing procedure of complete denture. Mustansiria Dental Journal 2011;8(3):288-292.

2. Silva MCV, Cartaxo JUQ, Arioli Filho JN, Batista AUL. Evaluation of the Biosecurity Measures Adopted in Dental Prosthesis Laboratories of the City of João Pessoa, PB, Brazil. Pesq Bras Odontoped Clin Integr 2010;10(1):101-106.

3. Chassot AL, Poisl MI, Samuel SM. *In vivo* and *in vitro* evaluation of the efficacy of a peracetic acid-based disinfectant for decontamination of acrylic resins. Brazilian Dental Journal 2006;17(2):117-121.

4. Tatarciuc M, Zamfirache IC, Stefan M, Vitalariu A, Diaconu D. Microbiologic study regarding the risk of cross infection in the technical laboratory. Sectiunea Genetica si Biologie Moleculara, 2010;XI:53-58.

5. Leung RL, Schonfeld SE. Gypsum casts as a potential source of microbial cross-contamination. J Prosthet Dent 1983;49(2):210-211.

6. Williams N. The persistence of contaminated bacteria in dental laboratory pumice. J Dent Res 1985;64:258.

7. Vojdani M, Zibaei M. Frequency of bacteria and fungi isolated from pumice in dental laboratories. J Res Health Sci 2006;6:33-38.

8. Orsi IA, Junior AG , Villabona CA, Fernandes FACN, Ito IY. Evaluation of the efficacy of chemical disinfectants for disinfection of heat-polymerized acrylic resin. Gerodontology 2011;28:253-257.

9. Bellissimo-Rodrigues WT, Bellissimo-Rodrigues F, Machado AA. Infection control practices among a cohort of Brazilian dentists. International Dental Journal 2009;59:53-81.

10. Neville D, Zarb M. Bacterial atmospheric contamination during routine dental activity. Malta Medical Journal, 2007;20(4):14-18.

11. Verran J, Kossar S, McCord JF. Microbiological study of selected risk areas in dental technology laboratories. J Dent 1996;24:77-80.

12. Glass RT, Bullard JW, Hadley CS, Mix EW, Conrad RS. Partial spectrum of microorganisms found in dentures and possible disease implications. J Am Osteopath Assoc 2001;101:92-94.

13. Merchant VA, McNeight MK, Ciborowski CJ, Molinari JA. Preliminary investigation of a method for disinfection of dental impressions. J Prosthet Dent 1984;52(6):877-879.

14. Firoozeh F, Zibaei M, Zendedel A, Rashidipour H, Kamran A. Microbial contamination of pumice used in dental laboratories. Healthcare in Low-resource Settings 2013;1(5):18-21.

15. Junior JWK. Cross-contamination via the prosthodontic laboratory. J. Prosthetic Dentistry 1974;32(4):412-419.

16. Kahn RC, Lancaster MV, Junior WK. The microbiologic cross-contamination of dental protstheses. The Journal of Prosthetic Dentistry 1982;47(5):556-559.

17. Wakefield CW. Laboratory contamination of dental prothesis. J Prosthet Dent 1990;44:143-146.

18. Abaci O, Haliki-Uztan A, Ozturk B, Toksavul S, Ulusoy M, Boyacioglu H. Determining Candida spp. incidence in denture wearers. Mycopathologia 2010;169:365-372.

19. Coco BJ, Bagg J, Cross LJ, Jose A, Cross J, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. OralMicrobiology and Immunology 2008;23:377-383.

20. Tankson JD, Thaxton JP, Vizzier-thaxton Y. Pulmonary hypertension syndrome in Broilers caused by *Enteroccoccus faecalis*. Infect Immun 2001;69:6318-6322.

21. Schuoter GS. Microbiology of the orofacial region in Topazian, oral and maxillofacial infection. 4th ed. Philadelphia, PA: WB Saunders; 2002.

22. Agostinho AM, Miyoshi PR, Gnoatto N, Aparanhos HF, Figueiredo LC, Salvador SL. Cross-contamination in the dental laboratory thought the polishing procedure of complete dentures. Braz Dent J 2004;15(2):138-143.

23. Autio KL, Rosen S, Reynolds NJ, Bright JS. Studies on cross-contamination in the dental clinic. J Am Dent Assoc 1980;100-358.

24. Merchant VA. An update on infection control in the dental laboratory. QDT 1997;2:157-169.

25. Yildirim MS, Hasanreisoglu U, Hasirci N, Sultan N. Adher-ence of *Candida albicans* to glow-discharge modified acrylic denture base polymers. Journal of Oral Rehabilitation 2005;32:518-525.

26. Kuroki K, Hayashi T, Sato K, Asai T, Okano M, Kominami Y, Takahashi Y, Kawai T. Effect of self-cured acrylic resin added with an inorganic antibacterial agent on *Streptococcus mutans.* Dental Materials Journal 2010;29(3):277-285.
27. Bôas MV, Quirino MRS. Controle da infecção cruzada: Laboratório versus consultório odontolgico. Rev. biociênc 2002;8(1):103-108.

Figure1. Matrix with addition silicon sent to laboratories, for making the acrylic resin specimens.

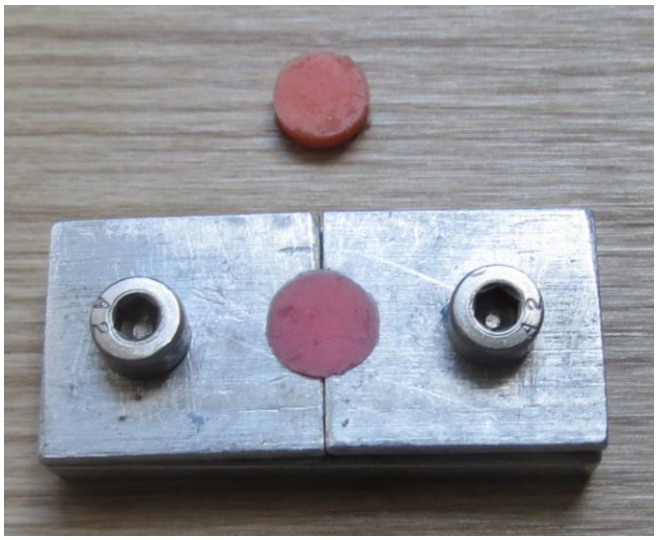
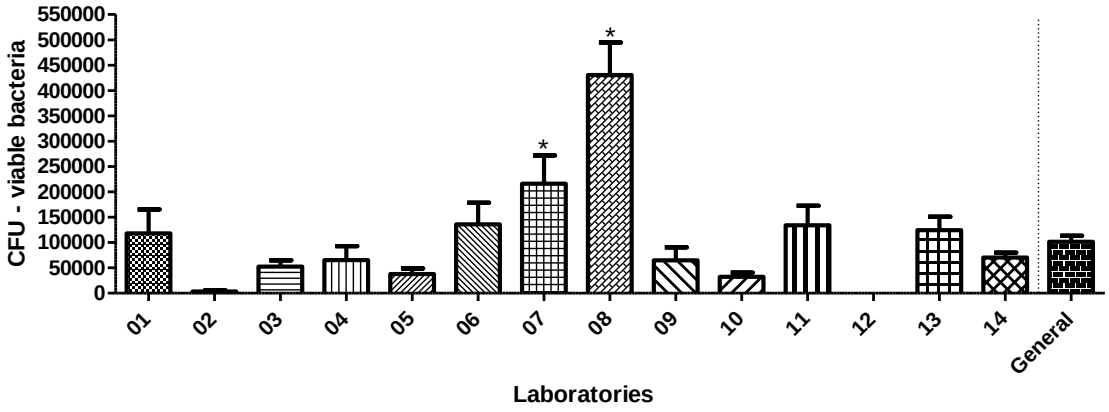
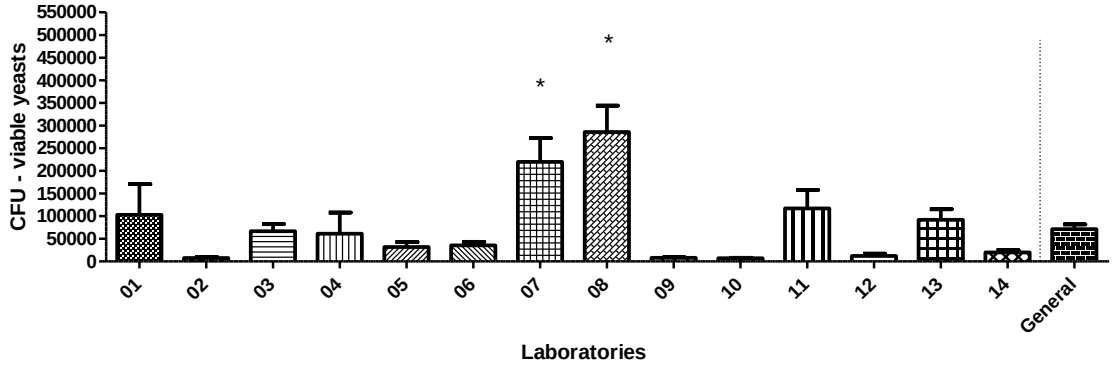


Figure 2. Counting colony forming units (CFU) of viable bacteria in different laboratories.



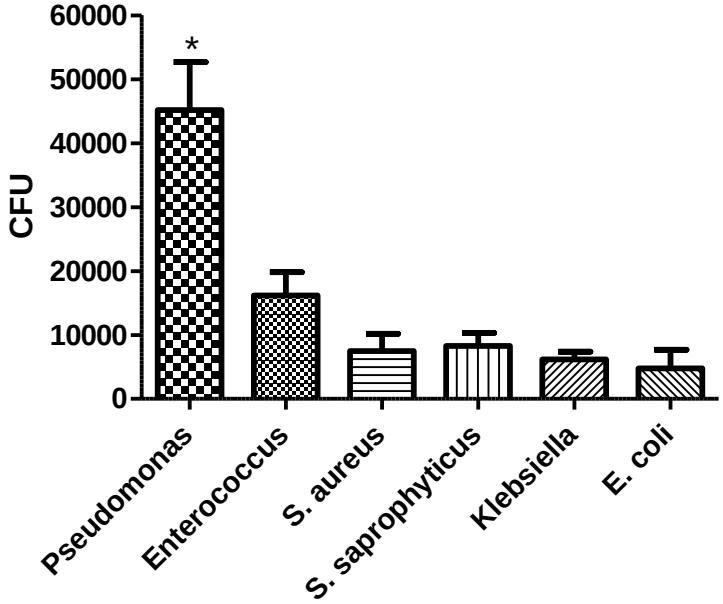
*p<0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM)

Figure 3 Counting colony forming units (CFU) of viable fungi in different laboratories.



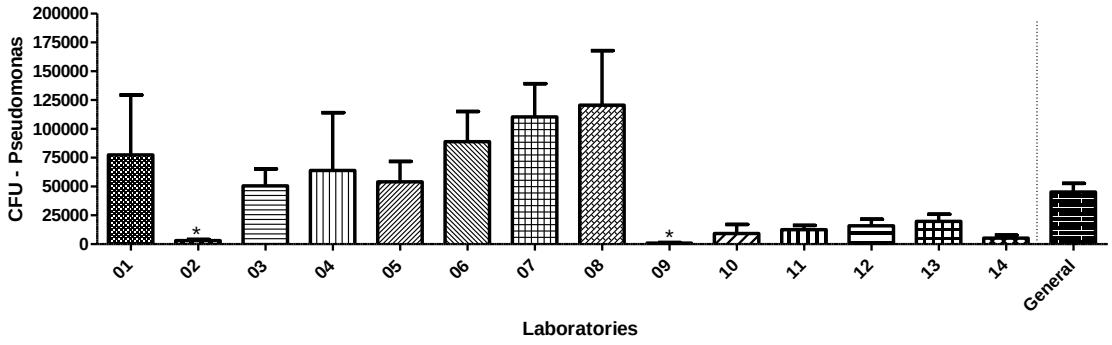
*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 4. Counting colony forming units (CFU) of bacterial species identified in specimens coming from laboratories.



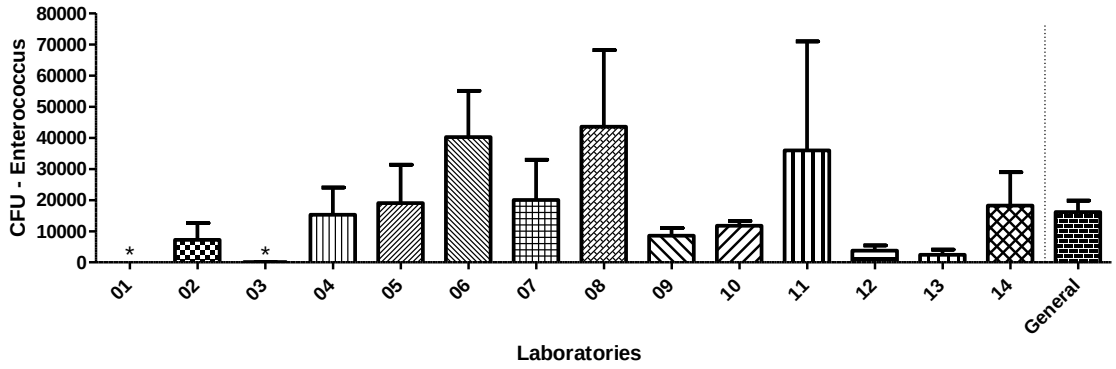
* p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 5. Counting CFU of *Pseudomonas spp* found in different laboratories.



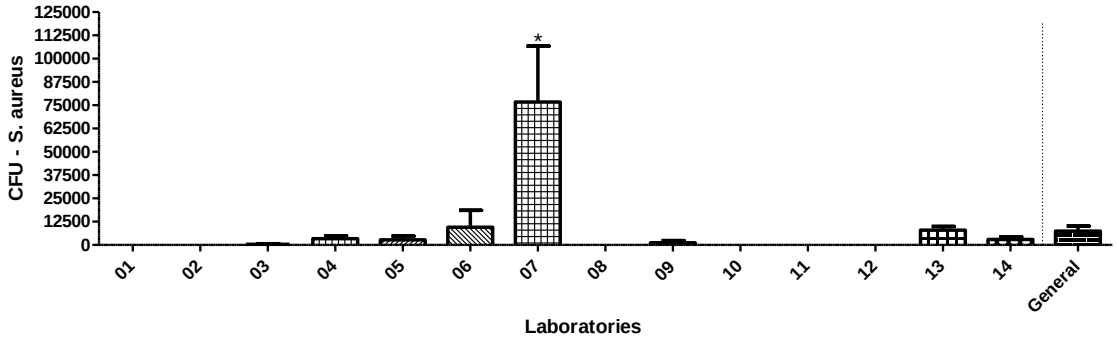
*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 6. Counting CFU of *Enterococcus spp* found in different laboratories.



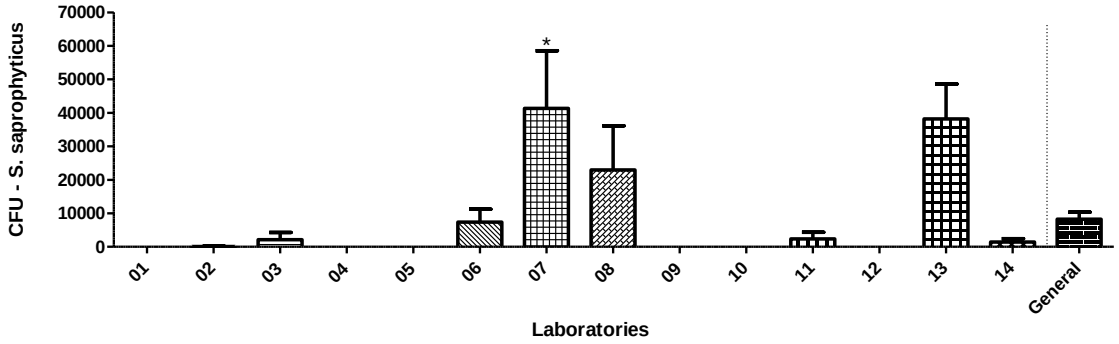
*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 7. Counting CFU of Staphylococcus aureus found in different laboratories.



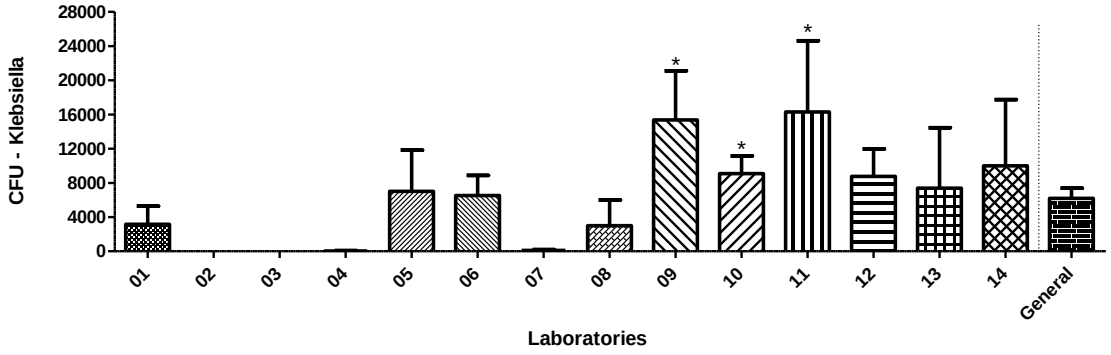
*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 8. Counting CFU *Staphylococcus saprophyticus* found in different laboratories.



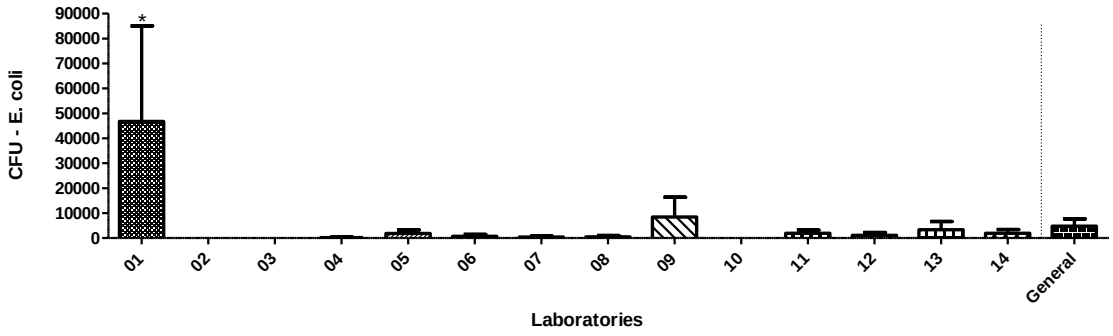
*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 9. Counting CFU of *Klebsiella spp* found in different laboratories.



*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

Figure 10. Counting CFU of *Escherichia coli* found in different laboratories.



*p <0.05, post-test of Kruskal-Wallis and Dunn (Mean ± EPM).

4 CONCLUSÕES GERAIS

Ao final deste trabalho e diante de suas limitações, foi possível concluir que:

- nenhum laboratório de prótese dentária avaliado ficou isento da presença de contaminação bacteriana em seus espécimes;
- todos os laboratórios avaliados também apresentaram contaminação dos espécimes por fungos;
- houve heterogeneidade entre os laboratórios quanto ao grau de contaminação, sendo que dois se destacaram por apresentarem contaminações bacteriana e fúngica mais significativas.
- o gênero *Pseudomonas* foi o mais prevalente, enquanto o *E. coli* foi o menos prevalente entre as espécies identificadas.

REFERÊNCIAS GERAIS

ABACI O, HALIKI-UZTAN A, OZTURK B, TOKSAVUL S, ULUSOY M, BOYACIOGLU H. Determining Candida spp. incidence in denture wearers. **Mycopathologia** 2010; 169: 365–372.

ABICHANDANI SJ, NADIGER R. Cross-contamination in dentistry: A comprehensive overview. **Chron Young Sci** 2013;4:51-8.

AGOSTINHO AM, MIYOSHI PR, GNOATTO N, APARANHOS HF, FIGUEIREDO LC, SALVADOR SL. Cross-contamination in the dental laboratory thought the polishing procedure of complete dentures. **Braz Dent J**. 2004;15(2):138-143.

AL-SAADI AK. Bacterial cross-contamination between clinic & dental laboratory during polishing procedure of complete denture. **Mustansiria Dental Journal** 2011;8:288-92.

AUTIO, K. L., ROSEN, S., REYNOLDS, N. J., AND BRIGHT, J. S.: Studies on cross-contamination in the dental clinic. **J Am Dent Assoc** 1980:100~358,.

BELLISSIMO-RODRIGUES WT, BELLISSIMO-RODRIGUES F, MACHADO AA. Infection control practices among a cohort of Brazilian dentists. **International Dental Journal**. 2009;59:53—8.

BURTON, W. E., AND MILLER, R. I,.: The Role of Aerobiology in Dentistry, **?U Prw. Fir\! Int. Symp. Aerobiol.,** Berkeley, 1963.

BHAT VS, SHETTY MS, SHENOY KK. Infection control in the prosthodontic laboratory. **The Journal of Indian Prosthdontic Society**. 2007; 7(2):62-65.

BÔAS MV, QUIRINO. Controle de infecção cruzada: laboratório de prótese versus consultório odontológico. **Rev. biociênc.,Taubaté**, 2002:8(1):103-108.

CHASSOT AL, POISL MI, SAMUEL SM. *In vivo* and *in vitro* evalu-ation of the efficacy of a peracetic acid-based disinfectantfor decontamination of acrylic resins. **Brazilian Dental Journal** 2006;17:117—21

COCO BJ, BAGG J, CROSS LJ, JOSE A, CROSS J, RAMAGE G. Mixed*Candida albicans* and *Candida glabrata* populations asso-ciated with the pathogenesis of denture stomatitis. **OralMicrobiology and Immunology** 2008;23:377—83.

DONALD L. MITCHELL, NADJMEH M. HARIRI,MANVILLE G. DUNCANSON, JR., NANCY L. JACOBSEN, RODERICK E. MCCALLUM, Quantitative study of bacterial colonization of dental casts **,J Prosthet Dent** 1997(5);78:518-21.

FIROOZEH F, ZIBAEI M, ZENDEDEL A, RASHIDIPOUR H, KAMRAN A. Microbial contamination of pumice used in dental laboratories. **Healthcare in Low-resource Settings** 2013; volume 1:e5. 18-21.

GLASS RT, BULLARD JW, HADLEY CS, MIX EW, CONRAD RS. Partial spectrum of microorganisms found in dentures and possible disease implications. **J Am Osteopath Assoc**. 2001;101:92–94.

JUNIOR JWK . Cross-contamination via the prosthodontic laboratory. **J. Prosthetic Dentistry**.1974 Out; 32(4): 412-19.

KAHN RC, LANCASTER MV,JUNIOR WK. The microbiologic cross-contamination of dental protstheses.**The Journal of Prosthetic Dentistry**.1982 May; 47(5):556-559.

KUGEL G, PERRY RD, FERRAR M, LALICATA P. Disinfection and communication practices: a survey of U.S. dental laboratories. **J Am Dent Assoc** 2000;131(6):786-92.

KUROKI K, HAYASHI T, SATO K, ASAI T, OKANO M, KOMINAMI Y, TAKAHASHI Y, KAWAI T. Effect of self-cured acrylic resin added with an

inorganic antibacterial agent on *Streptococcus mutans.* **Dental Materials Journal** 2010; 29(3): 277–285

LEUNG RL, SCHONFELD SE .Gypsum casts as a potential source of microbial cross-contamination. **J Prosthet Dent.** 1983 Feb;49(2):210-1.

MERCHANT VA, MCNEIGHT MK, CIBOROWSKI CJ, MOLINARI JA. Preliminary investigation of a method for disinfection of dental impressions. **J Prosthet Dent**. 1984 Dec;52(6):877-9.

NEVILLE DEBATTISTA, ZARB M., 2007- Bacterial atmospheric copntamination during routine dental activity. **Malta Medical Journal**, 20(4):14-18.

NIKAWA H, HAMADA T, YAMAMOTO T. Denture plaque-past and recent concerns. **J Dent**. 1998;26:299–304.

ORSI IA, JUNIOR AG , VILLABONA CA, FERNANDES FACN , ITO IY . Evaluation of the efficacy of chemical disinfectants for disinfection of heat-polymerised acrylic resin. **The Gerodontology Society and John Wiley & Sons A/S, Gerodontology** 2011; 28: 253–257.

POWELL GL, RUNNELLS RD, SAXON BA, WHISENANT BK. The presence and identification of organisms transmitted to dental laboratories. **J Prosthet Dent.** 1990 Aug;64(2):235-7.

SCHUOTER GS. Microbiology of the orofacial region in Topazian, **oral and maxillofacial infection**. 4th ed. Philadelphia, PA: WB Saunders; 2002.

SILVA MCV, CARTAXO JUQ, ARIOLI FILHO JN , BATISTA AUL. Evaluation of the Biosecurity Measures Adopted in Dental Prosthesis Laboratories of the City of João Pessoa, PB, Brazil. **Pesq Bras Odontoped Clin Integr**, 2010;10(1):101-106.

SOFOU A,LARSER T, FIEHN NE,OWELL B. Contamination level of alginate impressions arriving at a dental laboratory. **Clin Oral Investig** 2002;6:161-5.

SOUSA RR, CASTRO RD, MONTEIRO CH, SILVA SC, NUNES AB. O Paciente Odontológico Portador de Diabetes Mellitus: Uma Revisão da Literatura. **Pesq Bras Odontoped Clin Integr, João Pessoa**, 2003;3(2):71-77.

TANKSON JD, THAXTON JP, VIZZIER-THAXTON Y. Pulmonary hypertension syndrome in Broilers caused by Enteroccoccus faecalis. **Infect Immun** 2001; 69: 6318– 3322.

TATARCIUC M, ZAMFIRACHE IC, STEFAM M, VITALARIU A , DIACONU D. Microbiologic study regarding the risk of cross infection in the technical laboratory . Analele Stiintifice ale Universitatii Alexandru Ioan Cuza, **Sectiunea Genetica si Biologie Moleculara**, XI(4), pg.53-59.

VERRAN J, KOSSAR S, MCCORD JF. Microbiological study of selected risk areas in dental technology laboratories. **J Dent** 1996;24:77-80

VOJDANI M, ZIBAEI M. Frequency of bacteria and fungi isolated from pumice in dental laboratories. **J Res Health** Sci 2006;6:33-8.

WAKEFIELD CW. Laboratory contamination of dental prostheses. **J Prosthet Dent**. 1980 Aug;44(2):143-6.

WILLIAMS N. The persistence of contaminatedbacteria in dental laboratory pumice. **J Dent Res** 1985;64:258.

WITT S, HART P. Cross-infection hazards associated with the use of pumice in dental laboratories. **J Dent** 1990;18:281-3.

YILDIRIM MS, HASANREISOGLU U, HASIRCI N, SULTAN N. Adher-ence of *Candida albicans* to glow-discharge modified acrylicdenture base polymers. **Journal of Oral Rehabilitation** 2005;32:518—25.

ZARB GA, MACKAY HF. The partially edentulous patient. I. The biologic price of prosthodontic intervention. **Aust Dent J**. 1980;25:63–68.

.

.

APÊNDICE A – Tabelas de resultados.

Tabela 1 Quantidade média de UFC de bactérias no meio de cultura Agar Sangue.

Contagem no Ágar Sangue															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	117910	3440	52467	65320	37590	135811	216333	430786	64750	32400	134125	102,3	124356	70289	101438
Erro-padrão	47291	2430	12461	27511	11310	42682	55534	64022	25560	8316	38579	21,02	26651	9560	11979

Tabela 2 Quantidade média de UFC de fungos no meio de cultura Agar Sabourad.

Contagem no Saboraud															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	102870	7713	66700	61160	32080	35740	219970	285667	8067	6890	116889	12000	91867	20160	71047
Erro-padrão	67865	2345	15845	46749	10592	7699	52540	58111	1137	1099	41035	5377	23545	4638	10617

Tabela 3 Quantidade média de UFC de Enterococcus no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® (Enterococcus)															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	0	7210	70	15280	19060	40240	20060	43540	8580	11770	35990	3780	2400	18230	16158
Erro-padrão	0	5417	42,3	8745	12281	14882	12917	24601	2481	1535	34974	1667	1678	10721	3669

Tabela 4 Quantidade média de UFC de Pseudomonas no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® (Pseudomonas)															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	77430	3030	50530	63880	54130	88970	110470	120500	840	9310	12510	15980	19800	5230	45186
Erro-padrão	51979	1130	14697	50232	17685	26183	28820	47336	634,4	7760	3788	5625	6182	2594	7502

Tabela 5 Quantidade média de UFC de S. Aureus no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® (S. aureus)															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	0	0	330	3400	2810	9500	76740	0	1210	0	0	0	7920	2960	7491
Erro padrão	0	0	330	1554	1879	9064	29946	0	1103	0	0	0	2069	1334	2703

Tabela 6 Quantidade média de UFC de S. Saprophyticus no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® **(S. saprophyticus)**															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	0	90	2130	0	0	7370	41350	22950	0	0	2370	0	38190	1400	8275
Erro-padrão	0	90	2130	0	0	3881	17169	13211	0	0	1993	0	10456	1035	2057

Tabela 7 Quantidade média de UFC de Klebsiella no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® **(Kleibsiella)**															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	3160	0	0	40	7020	6510	100	3000	15370	9090	16290	8760	7390	9990	6194
Erro-padrão	2118	0	0	30,55	4826	2365	100	3000	5734	2076	8332	3217	7063	7748	1202

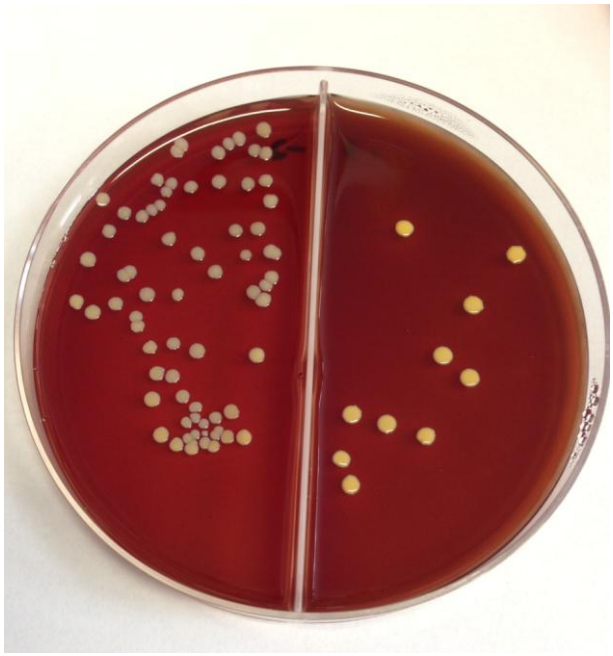
Tabela 8 Quantidade média de UFC de E. Coli no meio de cultura HICrome UTI Ágar®.

Contagem no HICrome UTI Ágar® **(E. coli)**															
	LAB 1	LAB 2	LAB 3	LAB 4	LAB 5	LAB 6	LAB 7	LAB 8	LAB 9	LAB 10	LAB 11	LAB 12	LAB 13	LAB 14	Geral
Média	46790	0	0	200	1840	730	400	500	8450	0	1940	1110	3300	1910	4798
Erro-padrão	38315	0	0	200	1443	730	400	500	7963	0	1284	1110	3300	1519	2860

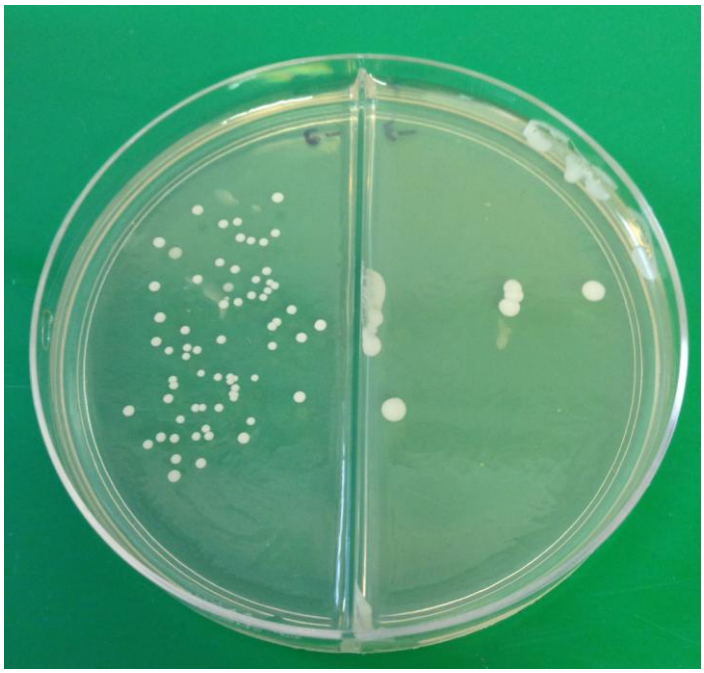
Tabela 9 Prevalência de quantidades de UFC de bactérias especificas.

Geral						
	Enterococcus	Pseudomonas	S. aureus	S. saprophyticus	Kleibsiela	E. coli
Média	16158	45186	7491	8275	6194	4798
Erro-padrão	3669	7502	2703	2057	1202	2860

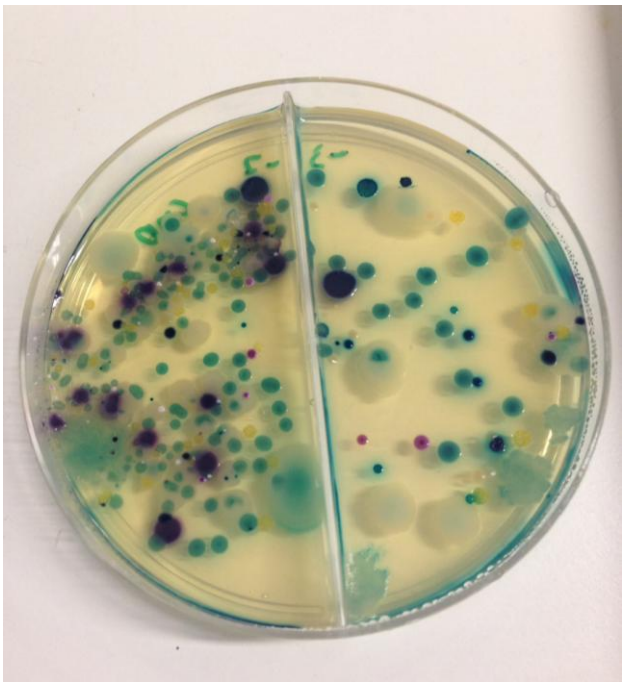
APÊNDICE B- Exemplo do crescimento bacteriano no meio de cultura Ágar Sangue nas diluições 1:100 (Lado esquerdo) e 1:1000 (Lado direito).



APÊNDICE C - Exemplo do crescimento fúngico no meio de cultura Ágar Sabourad nas diluições 1:100 (Lado esquerdo) e 1:1000 (lado direito).



APÊNDICE D - Exemplo do crescimento bacteriano especifico no meio de cultura nas HICrome UTI Ágar® nas diluições 1:100 (Lado esquerdo) e 1:1000 (lado direito).



ANEXO

Identificação dos microorganismos através do meio de cultura HICrome UTI Ágar®:

Cor Típica da Colônia	**Microorganismo pré-identificado**
Vermelho *	*Escherichia coli*
Azul Turquesa	*Enterococcus spp*
Azul Metálico	*Klebsiella ssp, Enterobacter spp, Citrobacter spp*
Halo marrom *	*Proteus spp*
Creme, translúcida	*Pseudomonas spp*
Dourada, opaca, pequena	*Staphylococcus aureus*
Rosa opaca, pequena	*Staphylococcus saprophyticus*