Full Length Research Paper

Microbiological assessment of dentists' hands in clinical performance

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This study verified the presence of opportunist pathogenic bacteria in the hands of Surgeons Dentists. Biological materials were collected from the hands of 41 professionals, randomly selected. The professional washed his/her hands for around 30 s in a sterile plastic bag, filled with 250 mL of physiologic serum, in three distinct times: Timing 1 (T1), hands without wearing gloves, before attending to the patient; Timing 2 (T2), wearing gloves, right after the patient treatment, when the glove was contaminated with the patients' biological material; and Timing 3 (T3), collected after removing the gloves. The material obtained was inoculated in culture mediums such as: Brain Heart Infusion Agar (BHI), Manitol Salt Agar (MSA), Eosin Methylene Blue (EMB) and Bile Esculin Agar (BEA). There were identified *Staphylococcus aureus* and not aureus strains, besides Gram-negative bacteria, suggesting deficient hygiene of the hands. An antibiogram was made for all the Gram-positive and Gram-negative bacteria found. The result shows a high number of strains resistant to the most common used antibiotics. The microbiological count was higher in the T1 for all the culture mediums, suggesting that the act of washing the hands for 30 s decreases the microbiota resident in the hands.

Key words: Antimicrobial agents, hands washing, infection control, odontology, risk due to biological agents.

INTRODUCTION

Infection control has become one of the most discussed topics in odontology, being very important during the practice, so that professionals no longer question its importance. The knowledge about occupational hazards has been largely developed, but unfortunately it has been short to strengthen awareness and change behaviors, as policies of infection control are not being fully applied (Moraes, 2008).

According to Lehotsky et al. (2010) the failure in hand disinfection before surgical procedures is considered the major cause of nasocomiais infections worldwide,

contributing for the spread of multiresistant pathogens, besides playing an important role in the development of post operator complications. Lately, multiresistant bacterial strains are responsible for outbreaks all over the world and the therapeutic arsenal has become more and more scarce, increasing costs and time expending on treatment. The *Staphylococcus aureus* resistant to methicillin (MRSA) is responsible for important infections and hands are the most common way of transmission (Custódio et al., 2009).

According to Myers et al. (2008) Odontology profes-

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sionals must be bound to principles scientifically accepted and based on evidences of infection control, considering that hands hygiene is one of the most important processes to reduce the transmission of microorganisms between professional and patient.

Thus, the ethical responsibility of health professionals in reducing the risks of contamination is expected. According to Dejours (1995), the Health Area may be a health provider or a pathogenic producer. This study aimed to isolate and identify bacterial strains in the collected material extracted from hands of dentists during clinical procedures, being a helpful matter in the awareness about the possibility of transmission of pathogenic microorganisms through hands. It also intends to determinate the profile of sensibility of microorganisms to antibiotics, building up consciousness and a reasonable use of antimicrobials.

MATERIALS AND METHODS

The research was developed starting with a list of 120 names, provided by the Odontology Regional Council from Minas Gerais (CRO-MG), and intermediated by the Regional Office from Alfenas. Later, based on the names enrolled in the list, 41 participants were randomly selected and invited to participate in the survey.

Professionals were sought after in their workplaces and they were informed about the attribute of the study. The participation was linked to a consent form. On the research, dentists from both genders, specialists and general clinical dentist, who perform their activities in private and public offices, participated.

The study was developed starting with the collection of biological material on the active hand of the dentist. On each sample an sterile bag measuring 15x32 centimeters containing 250 mL of sterile physiologic serum was used, where the professional washed their hands for about 30 s, over three different times: Timing 1 (T1), bare hands before seeing the patient; Timing 2 (T2), gloved hands right after the patient treatment, when the glove was contaminated with the patients' biological material; and Timing 3 (T3), collected after glove removal looking forward to verify microbiota present within the glove.

Expecting to obtain standardization during the material collection, sterile Descarpack® latex gloves were provided to all participants (sample) in the research. The material collection did not interfere with the professional routine, in other words, the dentist was told to act just like his/her would normally do, which means that the dentist had full choice to wash hands before starting treatments and wear sterile gloves in the manner they judged more suitable.

Right after the three collecting timings in the physiologic serum, the obtained material was sent to the Microbiology Laboratory at the Universidade Federal de Alfenas, together with the glove used by the professional. The glove was filled with methylene blue and observed after 24 h in order to verify the presence of perforations.

Overall, samples were collected 123 from the hands of 41 professionals. At the laboratory, 100 microliters (µL) of physiologic serum withdrew from hand washing was inoculated in the culture media: Brain Heart Infusion Agar (BHI - Himedia), Manitol Salt Agar (MSA), Eosin Methylene Blue (EMB) and Bile Esculin Agar (BEA), for each one of the timing (T1, T2, T3), in concentrations: undiluted and 1/10, in each one of the three times, and each sample for each one of the four culture medium, totaling 984 samples.

The culture media were incubated at 37°C (98.6°F) in a bacteriological incubator for 48 h. The colonies quantification was made using a colony counter, and the results were obtained in CFU/mL (Colony-Forming Unit per Milliliters).

Strains which were grown in MSA underwent the Gram Method, Catalase Test, DNase and Coagulase Test. Strains which were

grown in BEA underwent the Gram Method and Catalase Test. Finally, the ones grown in EMB Agar underwent the Gram Method and were identified using BacTray® Kit, for biochemical identification of Gram Negative bacilli with Negative or Positive Oxidase.

The antibiogram using the Agar Disc Diffusion Technique (also known as Agar Diffusion Method or Kirby- Bauer Test), according to CLSI (CLSI document, 2009), using antibiotics discs made by DME® Sensidisc. There were made the following antibiotics for Gram positive bacteria: Amoxicillin/Clavulanic Ac. (AMC 30 - 20/10 µg), Azithromycin (AZI 15 - 15 µg), Ciprofloxacin (CIP 05 - 5µg), Clindamycin (CLI 02 - 2 µg), Doxycycline (DOX 30 - 30 µg), Norfloxacin (NOR 10 - 10 µg), Oxacillin (OXA 01 - 1 µg), Vancomycin (VAN 30 - 30 µg), Linezolid (LNZ 30 - 30 µg), Table 4. For the Gram negative were used: Cephalotin (30 µg), Sulfazotrin (25µg), Tobramycin (10µg), Chloramphenicol (30µg), Gentamicin (10µg), Doxycycline (30µg), Ciprofloxacin (05µg), Azithromycin (15µg), Norfloxacin (10µg), Table 6.

The analysis of variance was used to evaluate the factors significance and the Scott-Knott test was applied at 5% level of significance to get the average difference.

This research was carried out after the Committee of Ethics in Research had authorized the realization of the project on Protocol No 216/10 in November, 25th, 2010.

RESULTS

The timing 1 (T1) which represents the first collection of the material from professional hands, always presented the highest number of Colony-Forming Unit (CFU/ml), when compared to the others timing (T2 and T3) from the research (Table 2). T2 presented the lowest count of CFU/ml when compared to T1 and T3, with statistical significance for the media used, according to Scott-Knott Test (Table 1).

41 pairs of Descarpack® sterile gloves were used, which did not show any damage during the collection, except one glove that had a perforation on the middle finger. The glove that presented the perforation was used in a long duration surgical procedure.

The material inoculation in the culture medium (BHI) was used to count the total bacteria, in each one of the three timing used in this research, totaling 246 samples.

The results show that the T1 relative to the time when the professional gets ready to start the clinical procedure, in other words, before wearing gloves, presented the highest count of total bacteria, reaching up to more than double of the CFU/ml count in the three media used.

The colonies count in the MSA was used to isolate the Gram positive cocci in the samples, specifically *Staphylococcus* sp. The colonies counting in this media followed the trend observed in BHI. In the same way, there was a reduction from T1 to T3, indicating that the act of washing hands in physiologic serum removes part of the microorganisms, thereby, reducing the number of CFU/ml (Table 3).

In T2, 27 samples had zero count. So, the unfolding of the significance for all the variable T presented a statistical three timing used in this research.

The results indicate the presence of 26 samples of *S. aureus* and 44 *Coagulase-negative Staphylococci* (CoNS), from analysis of the 246 samples distributed over the

Table 1. Colonies' counting average in the culture media BHI, MSA and EMB in CFU/ml.

Culture media	T1 (CFU/mL)	T2 (CFU/mL)	T3 (CFU/mL)
BHI	2624,27 ^a	701,83 ^c	1074,32 ^b
MAS	1837,93 ^a	48,66 ^c	807,93 ^b
BEM	89,27 ^a	0 ^a	20,49 ^a

T1= Bare hands before seeing the patient; T2= Gloved Hands, right after the patient treatment, when the glove was contaminated with the patients' biological material; T3= collected after glove removal. BHI= Brain Heart Infusion Agar; MSA= Manitol Salt Agar; EMB= Eosin Methylene Blue
 Note 1: it was not possible to calculate the statistics for the BEA medium, because only one was collected in T1; of all the 246 made showed positive result. The values are means. The means followed by the same lower case letter in the line do not have difference between then by Scott-Knott test, at level 5% of significance.

Table 2. Minimum and Maximum CFU/ml counting in the 4 culture media used.

Culture Media	T1 (CFU/ml)		T2(CFU/ml)		T3 (CFU/ml)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
BHI	0	Countless	0	9,400	0	6,010
MAS	0	6,000	0	920	0	5,320
BEM	0	1,530	0	0	0	830
BEA	0	Countless	0	0	0	0

T1= Bare Hands before seeing the patient; T2= Gloved hands right after the patient treatment, when the glove was contaminated with the patients' biological material; T3= collected after glove removal. BHI= Brain Heart Infusion Agar; MSA= Manitol Salt Agar; EMB= Eosin Methylene Blue; BEA= Bile Esculin Agar.

Table 3. Identification of the strains of *Staphylococcus aureus* and CNS - Coagulase negative *Staphylococcus*, obtained in T1, T2 and T3.

Timing	<i>Staphylococcus aureus</i>	CNS - Coagulase negative <i>Staphylococcus</i>
T1	13	19
T2	03	07
T3	10	18
Total	26	44

T1= Bare hand before seeing the patient; T2= Gloved hand right after the patient treatment, when the glove was contaminated with the patients' biological material; T3= collected after glove removal.

three timings established in this research, although, with a higher colonies concentration in T1, for both identifications (Table 3).

Both *S. aureus* and *Coagulase-negative Staphylococci* strains isolated and identified in this study underwent an antibiogram, being totally, sensitive to Amoxicillin/Clavulanic Acid and Norfloxacin. Also, they presented a high resistance to Azitromicin, Clindamicin and Vancomycin, according to Table 4.

The culture medium EMB is used to differentiate and isolate Gram negative bacilli (*Enterobacteriaceae* and others Gram negative bacilli). Following up the tendencies of the others culture media, T1 in EMB presented the highest concentration of CFU/mL, resulting to positive in six cases with minimum counting as 90 CFU/ml and the highest counting as 1,530 CFU/ml. In T3, results were

positive for two samples, and they were: 10 and 830 CFU/ml. The T2 was equal to zero and for 100% of the samples, indicating that those bacteria are not common in the oral cavity (Table 2). 246 samples were analyzed in the EMB culture medium, and for the three timings used in the research; there were no statistical significance in the results (Table 1).

The presence of *Escherichia coli* in 8 samples was verified, *Citrobacter freundii* in 7 samples, and one sample presented *Enterobacter cloacae*, totaling 16 samples with positive results for Gram negative bacteria, for a total of 86 samples.

After identification of the 16 samples positive for Gram negative bacteria, the antibiogram was done, and the strains were sensitive in 100% of the cases to Cephalotin, Gentamicin and Norfloxacin. It is important to point out the

Table 4. Susceptibility profile of 26 strains of *Staphylococcus aureus* and 44 strains of CNS - coagulase negative *Staphylococcus*, in response to 9 clinical drugs.

Antibiotic/Strain	AMC 30		AZI 15		CIP 05		CLI 02		DOX 30		NOR 10		OXA 01		VAN 30		LNZ 30	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
<i>S. aureus</i>	0	26	10	16	0	26	9	17	0	26	0	26	7	19	12	14	0	26
SCNS	0	44	26	18	4	40	26	18	14	30	0	44	13	31	25	19	1	43

AMC 30µg: Amoxicillin/Clavulanic Acid; AZI 15µg: Azitromycin; CIP 05µg: Ciprofloxacin; CLI 02µg: Clindamicin; DOX 30µg: Doxycycline; NOR 10µg: Norfloxacin; OXA 01µg: Oxacilin; VAN 30µg: Vancomycin; LNZ 30µg: Linezolid.

Table 5. Distribution of the Gram negative strains (*Enterobacteriaceae*) isolated in the culture media.

Gram negative Bacilli	N° (%)
<i>Escherichia coli</i>	08 (50.0)
<i>Citrobacter freundii</i>	07 (43.75)
<i>Enterobacter cloacae</i>	01(6.25)
Total	16 (100)

elevated number of strains resistant to Chloramphenicol, Sulfazotrin, Azithromycin, Ciprofloxacin (Table 6).

The BEA is used to isolate and used as presumptive identification of *Enterococo faecalis*. This bacterium was found in only one sample in T1 (with formation of bacterial biofilm) for a total of 246 samples.

DISCUSSION

Professional's choice of washing or not of hands before wearing gloves, was determinant to the highest CFU/ml counting found in all culture media used. It was so because T1 had the highest count of CFU/ml, when compared to T2 and T3. And, besides, in some cases in T1, the formation of biofilm could be noticed. This topic was also studied by Agbor and Azodo (2010), when only 63.4% of the interviewed reported the practice of hand washing.

Poor hand hygiene (HH) among professionals on the health area has motivated a lot of researches. And, although this practice is a simple act, its interdependence with the behavior sciences makes them complex and it depends on a set of factors and attitudes, beliefs and knowledge (Pessoa-Silva et al., 2005). For researchers, although professionals valorized and recognize the importance of the HH, the inadequate habit results in the non adhesion and only the divulgation is not enough to change behavior (Larson et al., 2007).

For the culture media EMB and BEA, where T2 was equal to zero, no result in T3 was higher than in T1; relevant fact considered is that Gram negative bacteria (EMB culture) and Enterococcus (BEA culture) are not usually found in the oral cavity, being found, most times in places poorly sanitized. The Gram negative bacilli are normally found in the environment and make part of the intestinal flora of humans and other animals, and may be

associated to diarrhea and pyogenic infections (Custódio et al., 2009).

In this study, in the MSA culture were found values that range from zero to 6,000 CFU/ml. Also isolated in the MSA, in T1 was the presence of 13 *S. aureus* and 19 other *Staphylococcus*; not *aureus*, from a total of 26 and 44 colonies, respectively (Table 3); totaling 86 samples in the MSA. In the same way Silva et al. (2003) found a high rate of contamination for *Staphylococcus* and a lower number of Gram negative bacilli, in a study that verified the microbiological contamination on surfaces, including the operator hands' and the places touched by him.

S. aureus is responsible for several types of infection in human body, and hands are the main way of transmission. In this way, MRSA can be transmitted from one patient to another if hands hygiene is neglected. Lately, MRSA has become an important topic, but it is not more aggressive than *Staphylococcus not aureus*, and the actual challenge is a shortage of antibiotics that can combat the bacterium and not its virulence (Johnston and Bryce, 2009). Due to the increasingly frequent and unnecessary use of antibiotics, infections for MRSA have become more frequent. The infections caused by *S. aureus* are usually treated with penicillin derivatives such as Oxacilin, Cefazolin and Cefalotin; all of them were used in this study.

The *S. aureus* strains were sensitive, in all cases to Amoxicilin and Clavulanic acid, Ciprofloxacin, Doxycycline, Norfloxacin and Linezolid, according to Table 4, and were resistant to Azitromycin, Clindamicin, Oxacilin and Vancomycin. The occurrence of bacteria resistance to antibiotics is a critical point, affecting sharply morbidity rate and treatment costs (Oliveira et al., 2010). MRSA rates clinical isolated from *S. aureus* vary from less than 1% in Norway and Sweden, from 5% to 10% in Canada, 25% to 50% in USA, reaching up to more than

Table 6. Sensitivity profile of the 16 strains of Enterobacteriaceae in response to 9 clinical drugs.

Antibiotic (µg)	Sensitive strain	Resistant strain
Cefalotin (30)	16	0
Sulfazotrin (25)	8	8
Tobramycin (10)	13	3
Chloraphenicol (30)	5	11
Gentamicin (10)	16	0
Doxycycline (30)	14	2
Ciprofloxacin (05)	10	6
Azitromicin (15)	5	11
Norfloxacin (10)	16	0

50% in Hong Kong and Singapore (Michael and Martin, 2010). MRSA are not only resistant to all the regular antibiotics, but also to a combination of them.

In this study 44 strains of *Staphylococcus* coagulase negative were found, for a total of 70 *Staphylococcus* sp. Until the last couple of years, the *Staphylococcus* coagulase negative, were seen as reduced risk in causing infections, due to presence in the skin of microbiota. However, the Negative Coagulase *Staphylococcus* begin to be identified as a pathogenic agent, being considered the main cause of bacteremia in the USA; caused by the increase in the incidence of infections (Grundman et al., 2006). The highest concentration of this pathogen was collected on the professionals' hands in T1 and T3, according to Table 3. Due to similar finding, warning on how easy it is to transport the bacterium from one place to another, completing the circle of microbial infection should be done.

The antibiogram for Coagulase Negative *Staphylococcus* was found in this study; 29.5% of strains were resistant to Oxacilin, 9% to Ciprofloxacin and 56.8% to Vancomycin. What makes the *Staphylococcus* a pathogen with the highest resistant rate to antibiotics is shown in Table 4.

The World Health Organization (2007) has made reference to the excessive using of antibiotics worldwide, and shows that the resistance to them is one of the three biggest threats to human health. Odontology overuses antibiotics, and most of the time with no justification. This alert must be considered, because this study found 2.27% of strains resistant to Linezolid, 56.8% to Vancomycin, 29.5% to Oxacilin, 31.8% to Doxycycline, 59% to Clindamycin, 9% to Ciprofloxacin and 59% to Azitromicin. Only the Amoxicilin associated to the Clavulanic acid and the Norfloxacin, was shown to be effective in 100% to the cases in this study (Table 4).

Nowadays, the main concern is also applied to the specific treatments for infections caused by Gram negative bacteria and multidrug resistant to *E. coli*, just like the ones that were isolated in this study, and are indicated in Table 5. These bacteria can survive for around 48 h after being deposited on surfaces (Rodrigues et al., 2008).

The presence of those bacteria in T1 and T3, in other words on dentists' hands indicate fecal contamination.

This situation is unacceptable in clinical offices. This Gram negative bacilli, was also found and it is the main cause of infections in the urinary tract and neonatal meningitis, causing 80% of mortality. It can also cause infections in wounded skin, peritonitis and septicemia (Fraser and Cunha, 2012)

Strains of *Citrobacter freundii* (Gran Negative) identified in this study, (Table 5), usually can be found in human and other animals feces. Fraser and Cunha (2012) had already isolated strains in clinical samples of urine, throat swabs, expectorations, blood and wound swabs, with characteristics of opportunist pathogen. The presence of this bacterium on the hands and in the clinical offices environment is critical, and not only increases the risk of infection, but also chances of one be infected by a resistant bacterium. Besides epidemiological vigilance, this issue also needs prioritization of health programs, effective health education, and aiming a reasonable use of antibiotics (Oliveira et al., 2010).

The strains of *E. cloacae*, also isolated in this study, are enterotoxigenic, and showed resistance to antibiotics, according to Table 6. *Enterobacter* can infect any surgical wounds, and these infections are clinically indistinguishable from infections caused by others bacteria (Fraser and Cunha, 2012).

The antibiogram for Gram negative bacilli was made (Table 6). Most of the strains were resistant to most of the antibiotics tested, with exception to Cefalotin, Gentamicin and Norfloxacin.

Among the samples analyzed in T1, a strain of *E. faecalis* was identified, commensal of human digestive tract, causing over 90% of the enterococci human infections (Johnston and Bryce, 2009). The strain isolated was resistant to Clindamycin, Cefalotin, Chloraphenicol and Oxacilin, with no inhibition, and with Norfloxacin, there was the formation of a ring of 14 mm. Although, it was sensitive to the others antibiotics used, including Vancomycin. A multinational study (including countries such as South Africa, Egypt, Saudi Arabia and Lebanon) on nosocomial pathogens has found a similar result, because none of the *Enterococcus* found in the study were resistant to Vancomycin. However, 7% of the *Enterococcus* isolated in Germany and 16.7% of the ones

isolated in Switzerland and Greece were resistant to Vancomycin. Other studies show that environments frequented by patients with MRSA and *Enterococcus* resistant to Vancomycin is often contaminated by MRSA and *Enterococcus*, just like the same contamination found on hands, coats and equipments used by service providers (Johnston and Bryce, 2009).

The scene is very critical, because in the late years the pharmaceutical industry did not invest on new antibiotics. According to Piddock (2011), this has occurred due to merging pharmaceutical companies, small profit rates, high costs and regulatory barriers. Besides all these barriers, when the new drug has been finally approved, it is will not be effective in a long term, for the bacterium will soon develop resistance mechanism.

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Conclusion

This study found the presence of Gram negative and Gram positive bacteria, based on the collection of biological material on hands of Surgeons Dentists, during clinical procedures, showing deficiency in hand hygiene procedures. The CFU/ml count from hands of professionals before starting the procedure (Timing 1) was the highest for all the culture media used, suggesting that hand washing was neglected or was made improperly. The antibiogram showed the existence of strains resistant to most used antibiotics in the office. Thus, this press the recommendable need for effective programs in orienting professionals to a reasonable use of antibiotics.

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