

***Staphylococcus aureus*'s IDENTIFICATION IN SURGICAL FIELDS AT A DENTISTRY SCHOOL OF BAHIA - BRAZIL**

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ABSTRACT

In an Ambulatory environment, before, during and after surgical procedures is extremely important to control contamination, mainly where your instruments will be used, taking into consideration odontogenic infections' development risk. Those infections compose themselves of a mixed microbiota; however, recent studies demonstrated that *Staphylococcus aureus* is the most commonly found species and pointed out by the Sanitary Vigilance Agency (Agência de Vigilância Sanitária –ANVISA in Portuguese) as the etiological agent of a series of infections. This studies' objective was to evaluate whether there was contamination in the surgical tables of the students of the second and last year of the Dentistry course of the Independent College of the Northeast through the isolation and identification of *Staphylococcus aureus*. This research consists of an observational study, in vitro, characterized by a search in which was analyzed TNT usage in surgical fields during oral surgeries for the surgical table's protection. For such purpose, it was collected samples of two groups corresponding to dentistry course's second year and last year students and biochemical tests were used to identify *Staphylococcus aureus*. The prevalence of contamination by *Staphylococcus aureus* in surgical tables operated by students of the Dentistry course of the Independent College of the Northeast was quite high, corresponding to half of the fields evaluated. In none of the groups was the maintenance of the aseptic chain fully effective. Therefore, there is a need for greater care regarding the factors that interfere in the contamination of the fields either by the students or by the professionals involved.

KEYWORDS: Biosafety, bacteria, surgical fields.

1. INTRODUCTION

The perioperative contamination control is a mandatory measure in dental surgery as well as in the other specialties, considering that the operative field is considered contaminated due to the microbiota present in the oral cavity and the dental environment is considered a site of possible cross contamination. Thus, it is necessary to use protective barriers in order to minimize the risk of contamination of the surgical site

and the development of possible odontogenic infections¹.

It is known that the professional and the patient are exposed mainly to biological risks that are represented by bacteria, fungi, parasites, viruses, among others. These microorganisms can survive in places with varied physical conditions; their resistance, however, finds limitations in environments that are unfavorable to them, such as places where disinfection has been done with great discretion^{2,3}.

Odontogenic infections are diseases arising from dental and periodontal tissues and may originate from periapical lesions, pericoronites, direct trauma and post-surgical infections that can lead to septicemia. Although it has a multifactorial etiology, the presence of pathogenic microorganisms in the surgical environment tends to facilitate its installation by its polymicrobial nature^{4,5,6}.

These infections are composed of a mixed microbiota, however, recent studies have shown that the *Staphylococcus* is the most commonly found species^{6,7,8} and pointed out by Agência de Vigilância Sanitária (ANVISA) as the etiological agent of a series of infections⁹. Therefore, it is important to know that microorganisms present in both the oral cavity and the clinical environment can be transferred to the dental equipment that is exposed, and this transmission is through direct contact, blood and saliva dispersed by aerosols¹⁰.

For this reason, the importance of the protection not only of the professional, who is constantly exposed to the patient's contaminated fluids, that when subjected to surgical procedures has its protection barrier exposed, as well as of the operative environment, which must be done by sterile surgical fields in order to avoid contamination of tissues incised by microorganisms. In addition, it is essential that, in addition to being protected, surfaces and equipment should be disinfected, since non-compliance with biosafety standards can be synonymous with the emergence of infections¹¹.

The care of the environment includes the cleaning of floors and walls, control of the movement of the doors and the traffic of people, as well as the care with the ventilation system, considering that

microorganisms have the capacity to carry particles found in the air and in objects inanimate that makes up the surgical site and consequently function as a vehicle for infection^{12,13}.

In clinical practice it is common to use surgical fields made of non-woven fabric (TNT), which has the advantages of being sterilized and inexpensive. However, the fact that they are sterilized does not prevent the occurrence of contamination by microorganisms during the assembly of the workbench and the surgery, due to the breaking of the biosafety barriers by the team or contamination by the aerosol, which could compromise surgical success^{14,15}.

Thus, the objective of this study was to evaluate if there was contamination of the surgical tables manipulated by the students of the second and last year of the Dentistry course of a Higher Education Institution of Bahia through the isolation and identification of *Staphylococcus aureus*.

2. MATERIAL AND METHODS

An in vitro observational study characterized by a study in which surgical fields of TNT used in oral surgeries for the protection of the surgical table were analyzed. We collected 40 samples from two groups corresponding to the students of the second and last year of the Dentistry course at the research site.

Initially, a pilot study was carried out with 5 samples from each group, which defined the biochemical tests that would be used from the microorganisms found in this study: Mannitol, Catalase and Gram stain.

Collection Procedure

Sterile swab collection was performed: the researcher placed sterile gloves before beginning collection. After the assembly of the table was finished, the swab (Global Swab) was passed in only 1 area of approximately 10 cm² making movements of rotation near the surface and movements from left to right and from top to bottom. The swab was then inserted into a test tube containing Brain Heart Infusion (BHI) (BBLTM) (Figure 1). The BHI was chosen because it is a nutrient broth that allows the growth of microorganisms.

The test tube was closed after inserting the inoculated swab, identified and immediately taken to Fainor's microbiology laboratory. The tubes containing the swab were incubated in a bacteriological oven at 37 ° C for 24 hours. If the result was negative (without turbidity), they were incubated for another 24 hours, always having a negative control: a test tube with BHI broth without inoculated swab.

The inoculated samples, which were turbid (Figure 2), that is, positive for the presence of microorganisms, were removed with a sterile ferrule (100 µl) and seeded with a Drigalski loop in a medium containing Mannitol Agar (Acumedia) (Figure 3) and placed in an oven at 37° C for 24 hours. Mannitol is a selective medium for this species of bacterium researched, leading to the

production of lactic acid, and thus, colonies are visualized with yellowish coloration.



Figure 1. Tube containing BHI without growth of microorganisms.

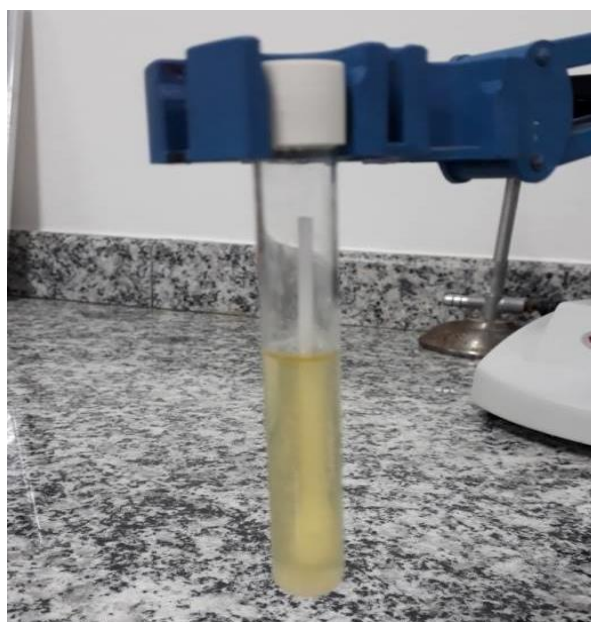


Figura 2. Tubo contendo BHI turvado indicando o crescimento de microrganismos.

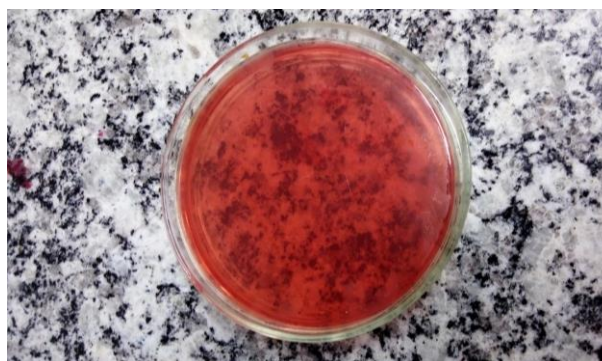


Figure 3. Mannitol Agar culture medium without sowing of bacteria.

The inoculated samples, which were turbid (Figure 2), that is, positive for the presence of microorganisms, were removed with a sterile ferrule (100 µl) and seeded with a Drigalski loop in a medium containing Mannitol Agar (Acumedia) (Figure 3) and placed in an oven at 37° C for 24 hours. Mannitol is a selective medium for this species of bacterium researched, leading to the production of lactic acid, and thus, colonies are visualized with yellowish coloration.

Microorganism's Identification

The growth of colonies producing yellowish staining in Mannitol is indicative of *Staphylococcus aureus*, leading to the next step of biochemical tests to identify the species. The tests used were catalase (Figure 4) and staining with Gram (Figure 5).

Catalase is produced in abundance by this microorganism and when in the presence of hydrogen peroxide it releases oxygen, producing bubbles. In contrast, the Gram test allows the classification of the bacteria as being positive or negative, in addition to being used for structural analysis of the colonies. *Staphylococcus aureus* are gram positive cocci that form rounded colonies that when attached are similar to bunches of grapes.



Figure 4. Catalase test showing positive result.

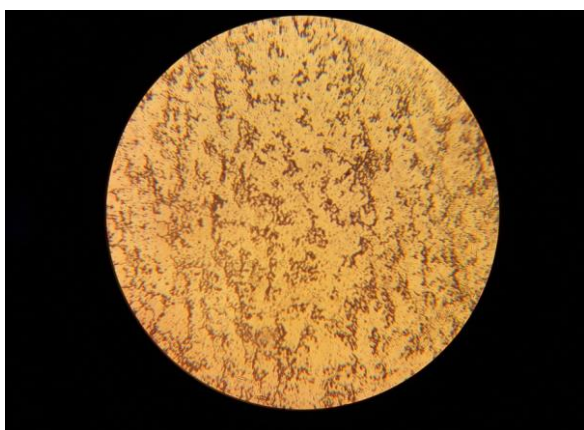


Figure 5. Gram stain showing results for Gram positive.

Statistical analysis

The comparisons of frequencies between the groups (2nd year students vs. 5th year students) were performed using the chi-square test with continuity correction. The level of significance was 5% ($\alpha = 0.05$). The data were tabulated and analyzed in IBM

SPSS Statistics for Windows (IBM SPSS, 21.0, 2012, Armonk, NY: IBM Corp.).

3. RESULTS

The results were expressed as absolute and relative frequencies.

As shown in Figure 6, of the 40 surgical fields evaluated, half presented growth of colonies producing yellowish staining in Mannitol (Figure 7). *Staphylococcus aureus* was confirmed by the Gram and Catalase tests.

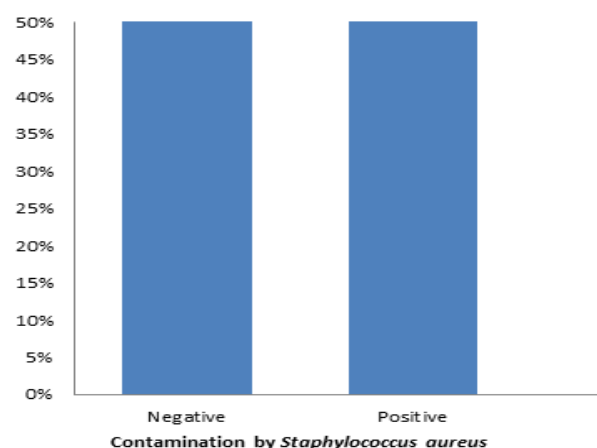


Figure 6. Contamination by *Staphylococcus aureus* in surgical tables of students of the second and last years of the Dentistry course of the research site.

The prevalence of contamination by *Staphylococcus aureus* in surgical tables of dental students, according to the year of the course, are shown in Table 1.

Table 1. Contamination by *Staphylococcus aureus* in surgical tables of students of the Dentistry course of the research site of the Faculdade Independente do Nordeste, according to the year of the course.

Group	Contamination by <i>Staphylococcus aureus</i>		p-value
	Negative	Positive	
2ºseries	8 (40,0%)	12 (60,0%)	0,343
5º series	12 (60,0%)	8 (40,0%)	

4. DISCUSSION

The use of biosafety standards aims to protect staff and patients in a clinical setting. These standards include, among others, the control of physical and chemical risks and biological risks, including methods of sterilization and disinfection of equipment and use of individual protection barriers¹⁶.

The development of this research is important for the clinical environment, considering the need to start surgery with a surgical table free of microorganisms. However, from the data, it was observed that at the beginning of the surgery, when the collection was performed, the presence of *Staphylococcus aureus* was confirmed in 50% of the tables, a result that was not expected, since the used fields were sterilized.

Although the proportion of contaminated surgical

fields was slightly higher among second-year students, the chi-square test indicated no significant difference between the groups compared ($p > 0.05$).

According to Chauveaux (2015)¹², *Staphylococcus aureus* are frequently found in human skin and, in this region, are not pathogenic. However, these can be transported by particles dispersed in the air and can reach the surgical table and the instruments that will be used, thus approaching the surgical site and consequently the bloodstream. In this place, as quoted by Evangelista Oliveira (2015)¹⁷, acquire great potential for the development of odontogenic infections. As shown in the study, the collection site, that is, the surgical table is subject to contamination as well as the instruments that are there.

Kumari *et al.* (2018)⁶, show that odontogenic infections are mostly *Staphylococcus aureus*, and their installation should be avoided because it is a serious condition that can involve facial spaces and present symptoms such as edema, mouth floor pain, fever, dysphagia, odynophagia, sialosis, trismus, toothache and fetid breathing. In addition, changes in phonation, respiratory distress and cyanosis can be observed that reflect the signs of airway compromise¹⁸. From the study, through the biochemical tests it was possible to conclude that this microorganism can be found at the tables.

However, odontogenic infections depend on a number of factors to be established as seen in Gizaw, Gebrehiwot, Yenew (2016)¹⁹. These infections may range from mild to very severe and of an emergency nature, and the factors that will determine such condition are: virulence of the microorganism involved, amount of pathogen within the tissues, the anatomy of the affected region, and the systemic condition of the patients. Therefore, the presence of microorganisms in the surgical tables is not indicative that the patient will develop an infectious²⁰.

The environment of the school clinic in which the research was carried out presents conditions for the transmission of *Staphylococcus aureus* to the surgical table, such as: no air renewal due to the air conditioning system, constant traffic of people including employees, students and patients; which prevents the control of the aseptic chain, besides the constant use of aerosols, which according to Moreira, Gouvêia, Pávoa (2010)¹⁰ facilitates the dispersion of the microorganisms in the air.

Therefore, from the results, it is possible to affirm that the sterilization of the kits containing the surgical fields is not enough to have a procedure free of microorganisms in view of the various factors that interfere in the contamination of the same. In addition, the care to maintain an aseptic chain should be based on the teachers, the students and also the employees who are involved in the daily routine of the clinic, demonstrating the need and importance of having this theme reinforced in all semesters.

5. CONCLUSION

Based on the results it is possible to conclude that:

1. A) The prevalence of *Staphylococcus aureus* contamination in surgical tables at the study site was quite high, corresponding to half of the evaluated fields;
2. B) There was no statistically significant difference in the prevalence of *Staphylococcus aureus* contamination in surgical tables manipulated by students of the 2nd and 5th graders in Dentistry.
3. C) In none of the groups was the maintenance of the aseptic chain fully effective. Therefore, there is a need for greater care regarding the factors that interfere with the contamination of the fields either by the students or by the professionals involved.
- D) The presented results may suggest that the disciplines related to microbiology and clinical practices are directed to the training and evidence of the importance of the prevention of microbiological contamination.

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