SURVIVAL OF *STAPHYLOCOCCUS AUREUS* ON FOMITES

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ABSTRACT

The aim of this study was to evaluate duration of survival of Staphylococcus aureus on contaminated standardized fomites, such as sterilization paper (SP) and polyester previously sterilized in a steam autoclave, and to determine the potential inhibitory effects of the substrates (fabrics used to manufacture garments and special wrapping paper used in the dental setting) using the bacteriostasis test.

The test was performed on two types of sterile standardized samples (T1 and T2). Sterility of the samples was validated following the protocol in use at the Department of Microbiology, after which the samples were inoculated with 50µl of a calibrated suspension of Staphylococcus aureus (reference strain ATCC 25923) *in the exponential growth phase, in a final concentration of 10^7 cfu/ml and 10^6 cfu/ml). The samples were incubated at 27°C and survival and concentration of microorganisms attached to the surface of the substrates was determined at the following experimental time points: immediately post-contamination, and 3 hours, 24 hours, 3 days, and 7 days post-contamination. Recovery was determined and expressed as a percentage; the bacteriostasis test was performed and showed negative results. Our results suggest that the quantity of recovered microorganisms varies according to the type of substrate and that there is a relation between survival and incubation time of the inoculated substrate serving as an artificial niche.*

Key words: sterilization, infection control, survival of Staphylococcus aureus, fabrics, cross-infection.

VIABILIDAD DEL *STAPHYLOCOCCUS AUREUS* EN FOMITES

RESUMEN

El objetivo de este estudio fue evaluar la viabilidad del Staphylococcus aureus en el tiempo.Se contaminaron los siguientes fomites estandarizados: Papel de uso médico (T1) y poliester (T2) esterilizados en autoclave de vapor de agua, y comprobar los posibles efectos inhibitorios del soporte (telas usadas en indumentaria odontológica y papel de envoltorio) mediante protocolo de relación test de bacteriostasis.

Se evaluaron dos tipos de muestras estandarizadas y esterilizadas (T1 y T2) previa validación con controles biológicos según protocolo Cátedra de Microbiología. Se contaminaron con 50µl. de una suspensión microbiana calibrada de Staphylococcus aureus (cepa de referencia ATCC 25923) *en una concentración de 10^7 ufc/ml y 10^6 ufc/ml en fase de crecimiento exponencial. Dichos materiales se incubaron a 27ºC y se testearon la viabilidad y la concentración adheridas superficialmente en las mismas del mencionado microorganismo cumplidos los siguientes tiempos: recién contaminadas, 3 horas, 24 horas, 3 días y 7 días. Se calculó el porcentaje de recuperación del mismo y se realizó el test de bacteriostasis que resultó negativo.*

Los resultados sugirieron que la cantidad de microorganismos recuperada varia de acuerdo al tipo de soporte analizado y la viabilidad del microorganismo está en relación con el tiempo de incubación del soporte inoculado que actuaría de nicho artificial.

Palabras clave: esterilización, control de infección, viabilidad Staphylococcus aureus, telas, infección cruzada.

INTRODUCTION

The oral cavity is one of the most complex and heterogeneous ecosystems of the human body, since it is a reservoir for a number of microorganisms and a source of infection. Highest risk is associated with blood, saliva, and aerolization of potentially infective body fluids, which are very common in the dental setting[1]. *Staphylococcus aureus* is one of the gram positive *cocci* found in the oral microbiota. The hands are an essential element in the transmission of this infective agent, known to be a potential cause of cross-infection in the dental office, and to cause infectious endocarditis. In addition, some strains are multiresistant to antibiotics. *Staphylococcus aureus* has therefore become one of the nosocomial pathogens of great epidemiological interest[2].

The incidence of Infectious endocarditis (IE) is between 5 and 7 cases per 100,000 people, annually. Although it is a rare clinical entity, IE is associated with morbidity and substantial risk of mortality. IE, especially infection caused by *Staphylococcus aureus*, is one of the infections increasingly associated with oral health-care interventions[3].

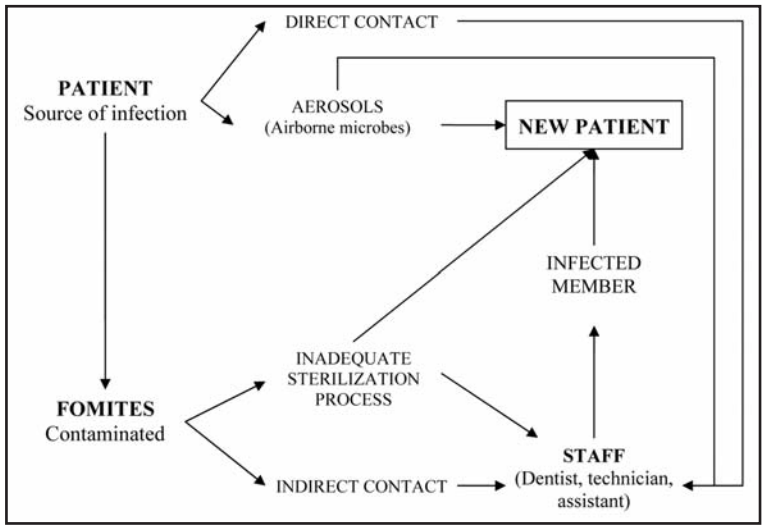

Fig. 1: Routes and ways of infection transmission in the dental office.

How can healthcare professionals be a source of infection? The aforementioned germs can be transmitted directly via wounds, body fluids, aerosols, or indirectly by dental impressions, instruments, removable prostheses, and so on. Body parts such as the nose and hands of the dentist, healthcare worker, patient, or technician, and inert elements including materials, garments, floors, and instruments, can serve as transmission vectors (Fig. 1)[4-6].

Survival of microorganisms on different fabrics used to manufacture garments used in the medical-dental setting, on dental and surgical appliances, and wrapping paper used for sterilization, has gained increasing concern among physicians and dentists, since evaluation of contamination with these microbes would reveal the importance of maintaining an aseptic chain of sterility in order to diminish the risk of cross-infection when providing health care[7-9].

A number of studies have examined microorganism survival, particularly *Staphylococcus aureus*, on inert materials[10-12].

The usefulness of asepsis and antisepsis, disinfection, and sterilization is beyond doubt, especially in the dental setting where the likelihood of cross-infection caused by highly pathogenous microorganisms is well documented.

Biosafety is the term used to refer to the principles, techniques, and practices applied to avoid unintentional exposure to pathogens and toxins, as well as their accidental release into the ennvironment.

OBJECTIVE

To evaluate duration of survival of *Stphylococcus aureus* inoculated on autoclaved medical and dental materials, and determine the possible inhibitory effect of the substrates using the bacteriostasis test. We sought to contribute to the body of knowledge on how to prevent the risk of cross-infection in the dental practice setting.

MATERIALS AND METHODS

The following standardized materials were used: sterilization paper (Group T1, n: 48) and polyester fabric (Group T2, n: 48) sterilized in a steam autoclave (Högner) at 134°C for 20 minutes, in keeping with the protocol in use at the Department of Microbiology of the School of Dentistry of the University of Buenos Aires.

In order to evaluate survival of *Staphylococcus aureus* inoculated on the medical/dental materials, the samples were contaminated with 50µl of a calibrated suspension of *Staphylococcus aureus* (reference strain ATCC 25923) in the exponential growth phase, in a final concentration of $3.1x10^7$ cfu/ml and $3.1x10^6$ cfu/ml. Total counts were obtained using the plate count method, following incubation in plate count agar at 35±2ºC, for 48±2 hours (Fig. 2).

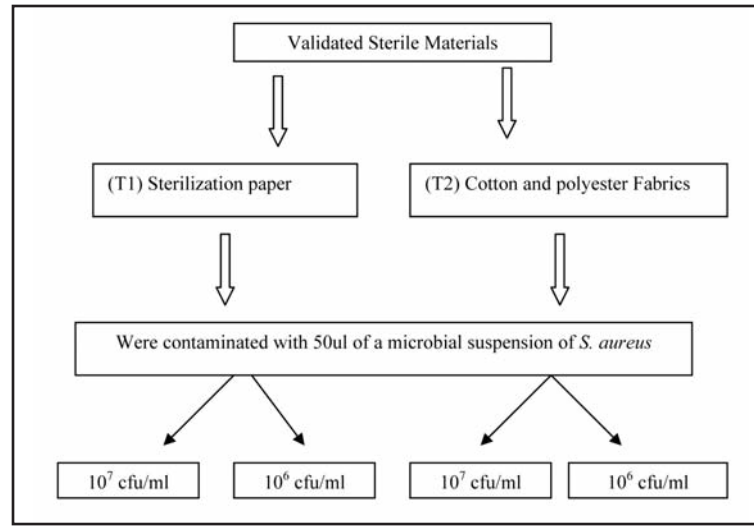

Fig. 2: Experimental Design to evaluate survival of Staphylococcus aureus inoculated onto materials commonly used in the medical and dental setting.

The inoculated samples were kept in different Petri dishes at 27±2ºC, 30 to 50% humidity, to be used at the corresponding time points: immediately post-contamination, 3 hours, 24 hours, 72 hours (3 days), and 168 hours (7 days) post-contamination (Fig. 3). Four samples of each of the materials contaminated with each of the inoculum's concentrations and a negative control (uninoculated sample) were processed at each experimental time. For this purpose, using sterile tweezers, the samples were placed in 10 ml of Brain-Heart–Infusion (BHI) broth and vortexed (Velp Scientifics) at 20 Hertz during 30 seconds. The samples were removed using sterile tweezers and placed onto Petri dishes containing BHI agar. The supernatants were processed to perform CFU counts on solid medium and thus evaluate the percentage of detached microorganisms in the liquid inside the processed tubes. The tubes and plates were incubated in an oven at 35±2°C for 48±2 hours. Turbidity of the tubes was assessed and gram staining was performed. Survival and concentration of microorganisms attached to the surface of T1 and T2 was tested (Table 1 and Table 2). Preparation of the inoculums the Bacteriostasis test: *Staphylococcus aureus* was incubated at 37°C for 18 to 24 hours. A microbial suspension was obtained and diluted in a solution of 0.85% NaCl, pH 7.2±0.2, to reach a concentration of 10^5 cfu/ml[13-15]. In order to perform the study, 0.1 ml of the microorganism were cultured on Petri dishes (20 mm in diameter) containing 15 to 18 ml of solid Brain-Heart Infusion (BHI) agar following the technique of surface dissemination. Using sterile tweezers, the sterile samples (T1 and T2) were then placed on the dishes. The samples were placed in the center of each inoculated plate pressing gently and avoiding the formation of bubbles. The plates were incubated at 37°C for 18-24 hours. Microbial growth and sterility were monitored on the inoculated and uninoculated plates respectively. Following incubation, the inhibition zone (zone surrounding the sample that is free of microbial growth) was measured in millimetres. The test is positive when the inhibition zone or halo is > 1 mm, indicating lack of microbial growth around the fomite.

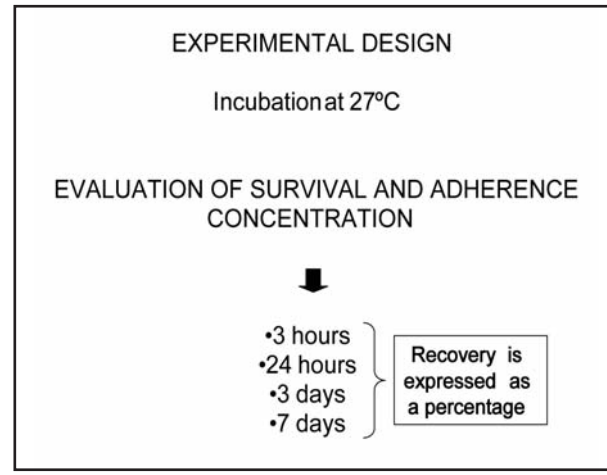

Fig. 3: Experimental design.

RESULTS

Survival of *Staphylococcus aureus* on fabrics: *Staphylococcus aureus* was recovered from both the studied dilutions up to 7 days post-contamination, in agreement with reports in the literature. In the case of the 3.1 x 10^6 cfu/ml dilution and the 1.6 x 10^5 cfu inoculums, recovery from sterilization paper samples was four-times that observed in fabrics. Microorganism recovery decreased with time; the decrease peaked at 24 hours and was more marked in the sterilization paper samples. After this peak, recovery rate remained at 2% in both types of samples up to day 7 (Fig. 4 and 5, Table 1).

Table 1: Recovery of Staphylococcus aureus.

Material	Inoculum [Log_{10}ufc] (mean ± SD)	Count in [Log_{10}ufc] (mean ± SD) Time				
		Immediately post-contamination	3 hours	24 hours	3 days	7 days
Sterilization paper	6.20±0.22	5.30±0.32	5.18±0.21	3.88±0.28	*NG*	*NG*
Cotton and polyester fabric	6.20±0.22	4.38±0.24	4.06±0.11	3.71±0.18	3.18±0.12	*NG*
Sterilization paper	5.20±0.22	4.08±0.31	3.60±0.29	2.80±0.38	*NG*	*NG*
Cotton and polyester fabric	5.20±0.22	3.14±0.23	2.84±0.21	2.60±0.22	*NG*	*NG*

NG: No Growth

Table 2: Survival of Staphylococcus aureus on inoculated materials.

Time	INOCULUM			
	$1.6x10^6$ cfu		$1.6x10^5$ cfu	
	Sterilization paper	Cotton and polyester fabric	Sterilization paper	Cotton and polyester fabric
0 hours	growth	growth	growth	growth
3 hours	growth	growth	growth	growth
24 hours	growth	growth	growth	growth
3 days	growth	growth	growth	growth
7 days	growth	growth	growth	growth

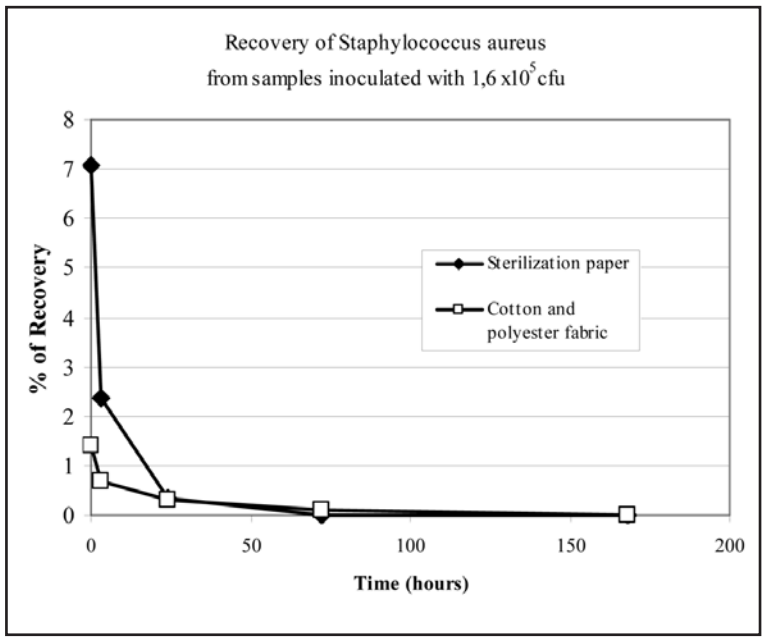

Fig. 4: Recovery of Staphylococcus aureus from samples inoculated with 1.6 x 10^5 cfu.

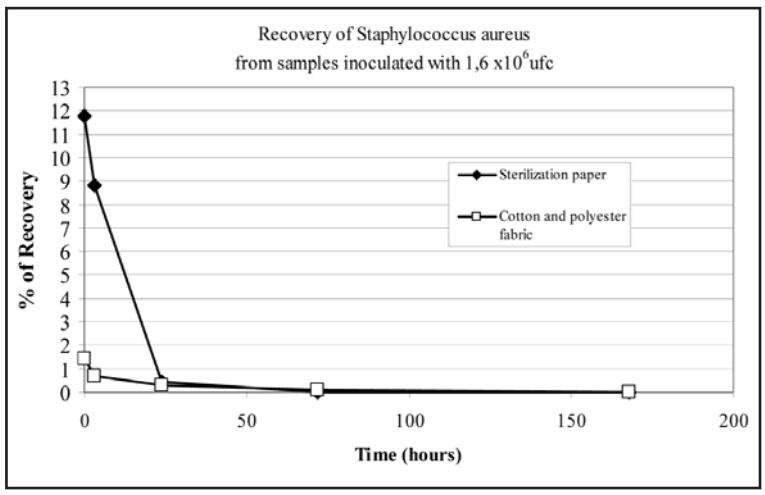

Fig. 5: Recovery of Staphylococcus aureus from samples inoculated with 1.6 x 10^6 cfu.

Higher recovery was observed in the 3.1 x 10^7 cfu/ml dilution and 1.6 x 10^6 cfu inoculums. The increase only reached statistical significance in the sterilization paper samples. The decrease in the recovery rate also peaked at 24 hours (Fig. 4 and 5, Table 1).

Our results suggest that the quantity of recovered microorganisms varied according to the type of substrate, and that there is association between microorganism survival and the incubation time of the inoculated substrates serving as an artificial niche. A larger amount of microorganisms were retained on the sterilization paper samples than on the cotton and polyester fabric samples (Table 1).

The experimental conditions employed in the study resembled the ambient temperature and relative humidity of a dental office, in order to mimic the effect of desiccation.

Very small droplets were inoculated on the fabrics in order to mimic the micro droplets produced by respiratory aerosol or due to spraying during dental treatments.

The bacteriostasis test was used to evaluate the potential inhibition of growth of *Staphylococcus aureus* (ATCC 25923) in the exponential growth phase on the standardized samples of the studied materials.

The results of the Bacteriostasis test showed that none of the materials exhibited an inhibition zone, allowing us to infer that the tested materials do not exert an antimicrobial effect (Table 2).

DISCUSSION

Survival of microorganisms is related with the quantity and quality of the inoculums, adherence, and the capacity of the microorganisms to interact with the substrate components; the teeth, mucosa, prostheses (fixed or removable), and orthodontic appliances can serve as substrates[15]. These substrates can act as an artificial niche favoring microbial growth and horizontal transmission.

What can be said about contamination of garments used by healthcare professionals and workers? Do garments act as fomites, favouring cross-infection?

Dissemination of micro organisms by fabrics has long been recognized as an important Public Health problem[16]. Fabrics made of cotton and widely used in the medical and dental setting, and are employed to manufacture laboratory coats and bandages, as well as bed-clothes and patient-gowns. Is it possible then, that healthcare professionals and workers facilitate the dissemination of microorganisms by their contaminated garments?

According to our experimental results, it would seem that the answer to the question stated above is "yes". In addition, a number of studies performed at our laboratory evaluating survival of other microorganisms, showed that Herpes simplex survives 2 days, different yeasts of the Candida species survive up to 15 days, and *Actinobacillus actinomycetemcomitans* survives up to 7 days[17].

According to our results it can be concluded that *Staphylococcus aureus* survives 7 days on the materials commonly used in the dental setting that were tested in this study.

Microorganism recovery dropped sharply at 20 hours, possibly due to the desiccation effect. After this time, the probability of cross-contamination would diminish. However, given that the microbes inside the fabric remain viable, the fabric, which acts as a protective niche, could still allow microorganism dissemination.

Our results suggest that:

a) The quantity of recovered microorganisms varies according to the type of substrate
b) Survival of the microorganism is associated with incubation time
c) The inoculated substrate acts as an artificial niche

The results on survival obtained in this study indicate that materials commonly used in the dental office serve as a reservoir for and vector of *Staphylococcus aureus*. It is well documented that different microorganisms survive in fabrics, wrapping paper, and appliances commonly used in the medical and dental setting[18]. It is therefore clearly evident that healthcare professionals and workers must comply with standards to control infection, one of the most important of which is sterilization of all non-disposable materials and instruments. It is important that the conditions under which dentists deliver oral-care do not pose a threat to the safety of their patients. Continuing education of healthcare workers and professionals is a key tool to successfully implement these objectives.

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