

## Infection risks related to aesthetic procedures: microbial profile and professional perception about infection prevention measures

*Riscos de infecção relacionados a procedimentos estéticos: perfil microbiano e percepção profissional sobre medidas de prevenção de infecção*

*Riesgos de infección relacionados con los procedimientos estéticos: perfil microbiano y percepción profesional sobre las medidas de prevención de infecciones*

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### ABSTRACT

**Background and Objectives:** This study aimed to identify the presence of microorganisms in the aesthetic environment and assess professionals' knowledge about relevant infection prevention measures, considering the importance of the issue and the lack of study in the area. **Methods:** A total of 100 clinics that perform minimally invasive aesthetic procedures in Porto Alegre (RS), Brazil, were visited. Procedures such as botulin-toxin, dermal fillers, collagen biostimulators, thread lift, chemical peels and laser hair removal were considered. A questionnaire about infection prevention measures were answered by 50 professionals. Also, 100 samples were collected from the environment for bacterial identification and antimicrobial susceptibility testing. **Results:** There was an infection prevention protocol in 40% of clinics, in which 95% of respondents had complete college education. Periodic professional training regarding infection control measures were performed in 72% of clinics. An autoclave was used for sterilization of materials and instruments in 66% of clinics. From the samples collected, 85% showed bacterial growth by microbiological methods. Coagulase-negative *Staphylococci* was the most prevalent genera found, and 16% of them were resistant to both cefoxitin, erythromycin, and clindamycin. Four isolates were positive for *mecA* by PCR. **Conclusion:** The presence of well-trained professionals is critical in aesthetic clinics so that biosafety and infection prevention measures are taken.

**Keywords:** Beauty and Aesthetics Centers; Infection Control; Delivery of Health Care; Environmental Microbiology.

### RESUMO

**Justificativa e Objetivos:** Este estudo teve como objetivo identificar a presença de microrganismos no ambiente estético e avaliar o conhecimento dos profissionais sobre medidas relevantes de prevenção de infecções, considerando a importância do tema e a falta de estudos nesta área. **Métodos:** Foram visitadas 100 clínicas que

realizam procedimentos estéticos minimamente invasivos em Porto Alegre (RS), Brasil. Foram considerados procedimentos injetáveis como aplicação de toxina botulínica, preenchedores faciais, microagulhamento, bioestimuladores de colágeno, fios de sustentação, *peelings* químicos e depilação a laser. Um questionário sobre medidas de prevenção de infecção foi respondido por 50 profissionais. Além disso, 100 amostras foram coletadas do ambiente para identificação bacteriana e teste de sensibilidade aos antimicrobianos. **Resultados:** Existia protocolo de prevenção de infecção em 40% dos ambulatorios, no qual 95% dos profissionais entrevistados possuíam ensino superior completo. Treinamento profissional periódico sobre medidas de controle de infecção foi realizado em 72% dos ambulatorios. Autoclave foi utilizada para esterilização de materiais e instrumentais em 66% das clínicas. Das amostras coletadas, 85% apresentaram crescimento bacteriano nas culturas microbiológicas. *Staphylococci* coagulase-negativo foi o gênero mais prevalente encontrado; e 16% deles eram resistentes à cefoxitina, eritromicina e clindamicina. Quatro isolados foram positivos para *mecA* por PCR. **Conclusão:** A presença de profissionais devidamente treinados é fundamental nas clínicas de estética, para que medidas de biossegurança e prevenção de infecções sejam tomadas.

**Descritores:** Centros de Embelezamento e Estética; Controle de Infecções; Assistência à saúde; Microbiologia Ambiental.

## RESUMEN

**Justificación y Objetivos:** Este estudio tuvo como objetivo identificar la presencia de microorganismos en el entorno estético y evaluar el conocimiento de los profesionales sobre las medidas de prevención de infecciones relevantes, considerando la importancia del tema y la falta de estudios en esta área. **Métodos:** Se visitaron 100 clínicas que realizan procedimientos estéticos mínimamente invasivos en Porto Alegre (RS), Brasil. Se consideraron procedimientos invasivos, como la aplicación de toxina botulínica, rellenos faciales, microagujas, bioestimuladores de colágeno, hilos de soporte, *peelings* químicos y depilación láser. Un cuestionario sobre medidas de prevención de infecciones fue respondido por 50 profesionales. Además, se recolectaron 100 muestras del medio ambiente para la identificación bacteriana y las pruebas de susceptibilidad a los antimicrobianos. **Resultados:** Existía un protocolo de prevención de infecciones en el 40% de las clínicas, en el que el 95% de los profesionales encuestados tenía educación universitaria completa. En el 72% de las clínicas se realizó capacitación profesional periódica sobre medidas de control de infecciones. Se utilizó un autoclave para la esterilización de materiales e instrumentos en el 66% de las clínicas. De las muestras recolectadas, el 85% mostró crecimiento bacteriano por métodos de cultivo microbiológicos. El *Staphylococci* coagulasa negativo fue el género más prevalente encontrado, y el 16% de ellos eran resistentes tanto a cefoxitina, eritromicina y clindamicina. Cuatro aislamientos fueron positivos para *mecA* por PCR. **Conclusión:** La presencia de profesionales debidamente capacitados es fundamental en las clínicas de estética, para la toma medidas de bioseguridad y prevención de infecciones.

**Descriptores:** Centros de Belleza y Estética; Control de Infecciones; Atención a la Salud; Microbiología Ambiental.

## INTRODUCTION

The number of aesthetic treatments has increased worldwide in recent years, mainly associate to minimally invasive aesthetic procedures.<sup>1</sup> Daily, thousands of people visit clinics seeking beauty treatments that bring rejuvenation and health<sup>2-4</sup>. In this scenario, in 2020, Brazil had the third-largest market for aesthetics and cosmetics in the world, staying behind the United States and China.<sup>2</sup>

According to Wang et.al. (2021), consumers' preference for minimally invasive aesthetic procedures have been increasing in the United States, even during the COVID pandemic.<sup>3</sup> In Brazil, this scenario is repeated; the search for minimally invasive aesthetic procedures and treatments have increased in 2020 and 2021, even during a pandemic and economic crisis.<sup>2</sup>

Even during non-invasive and minimally invasive aesthetic procedures, as botulin-toxin, dermal fillers, collagen biostimulators, thread lift, chemical peels, and laser hair removal, professionals handle body areas inhabited by microorganisms from both resident and transitory microbiota.<sup>5,6</sup> The skin is the habitat of millions of bacteria, fungi, and viruses that play an essential role

in our immune system and in the protection against invading pathogens.<sup>7</sup> These microbial communities interact competitively or synergistically for mutual benefits, driven by host or environmental factors. The resident skin microbiota is composed by *Staphylococcus spp.*, *Corynebacterium spp.*, and *Propionibacterium spp.* Species like *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella spp.*, *Candida spp.*, and even some respiratory viruses, can transiently colonize the skin and can be transmitted to a susceptible host by professional hands or the environment.<sup>7,8</sup>

When the skin barrier is broken, or when the proportion between commensals and pathogens is disturbed, diseases can occur, locally in the skin or even systemically.<sup>8,9</sup> Therefore, procedures that cause any skin injuries, including botulinum toxin and dermal filler injections, may increase the risk for infections if prevention protocols are not adopted.<sup>5</sup>

The environment is considered an important mediator in transmitting microorganisms, and the understanding of these transmission mechanisms can provide major opportunities for public health interventions.<sup>8,9</sup>

Many microorganisms have the capacity of surviving in the environment in a dormant state and can act as opportunistic pathogens under appropriate conditions<sup>10</sup>. For instance, bacteria of the genera *Bacillus* and *Clostridium* are capable of forming spores, which exhibit minimal metabolic activity and remains viable for a long time in the environment.<sup>10</sup>

According to the Report on Complaints of Health Associated Services, published annually by Brazilian National Health Regulatory Agency (ANVISA - *Agência Nacional de Vigilância Sanitária*), since 2016, the services that add up to the most complaints are aesthetic and beauty salon reaching 56.3% in 2020.<sup>11</sup>

Nowadays, many professionals with heterogeneous educational backgrounds are performing minimally invasive aesthetic procedures, looking for financial growth in this sector.<sup>2,5</sup> According to the same report from 2020, 18% of the complaints about aesthetic and beauty salons involve professional qualification, including complaints about the performance in some procedures by unqualified professionals.<sup>11</sup>

Considering the lack of information about infection prevalence associated with minimally invasive aesthetic procedures and the increasing number of complaints, this study aimed to identify the presence of microorganisms in the aesthetic environment and assess professionals' knowledge about relevant infection prevention measures.

## METHODS

### Service selection

We selected 100 aesthetic clinics located in Porto Alegre (RS), according to the commercial activity recorded from ANVISA in 2017. We visited the clinics from October 2018 to May 2019. Injection of dermal fillers (hyaluronic acid, calcium hydroxylapatite, and polylactic acid) and botulin toxin type A (BoNTA), chemical peel, laser hair removal, micro needling, and thread lift were included in the study and considered minimally invasive aesthetic procedures. Clinics that did not perform these procedures were excluded from the study. This study was approved by the *Universidade Federal do Rio Grande do Sul* Ethics Committee, under Protocol 2.909.825 and CAAE (*Certificado de Apresentação para Apreciação Ética* - Certificate of Presentation for Ethical Consideration) 92731018.8.0000.5347.

### Questionnaire about infection prevention measures

The information about infection prevention measures was obtained from a questionnaire with 18 objective questions (Table 1). The questionnaire was answered by professionals who declared themselves responsible for the establishment after signing the Informed Consent Form.

### Microbiological samples

We collected samples from tables, stretchers, equipment, products being used (such as eyebrow pigment inks and topical anesthetics), and autoclaved materials, like

tweezers and scissors. The collections were made aseptically, using sterile Stuart transport swabs (ABSORVE®), moistened in sterile buffered peptone water. After the samples were properly identified, the swabs were transported within two hours to the laboratory. The transportation occurred in appropriated boxes, at room temperature.

The samples were incubated in brain heart infusion broth (KASVI®) at 37°C for 24 hours. Afterwards, 1µl of the broth was incubated in three different culture media: blood agar, mannitol salt agar, and MacConkey agar (KASVI®). The plates were incubated for 24 hours at 37°C. After isolation, bacterial identification was performed using MALDI-TOF (Bruker®). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) is one of the most rapid and powerful method currently available for microbiologic identification. The technique is based on ionization to detect and precisely identify the microorganism by its ribosomal proteins.<sup>12</sup>

### Antimicrobial susceptibility profile

Disk diffusion test was performed according to BrCAST (Brazilian Committee on Antimicrobial Susceptibility Testing) protocols. Antibiotic disks (Amikacin, Amoxicillin + clavulanate, Ampicillin, Cephalexin, Cefoxitin, Ceftriaxone, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Imipenem, Levofloxacin, Linezolid, Meropenem, Sulfamethoxazole-trimethoprim, Oxoid® Tetracycline and Tigecycline) were placed on the surface of the inoculated plates. After incubation at 37°C for 18 to 24 hours, the inhibition zone diameters were measured. We also perform the D-test, to detect inducible or constitutive resistance to clindamycin. To perform the D-test, two additional pairs of erythromycin and clindamycin disks were placed to provide distances of 15 and 20 mm between the disks. Any significant ingrowth in a zone up to the edge of the disks was considered constitutive resistance. Inducible resistance was identified when there was any flattening or blunting of the shape of the clindamycin zone; in these cases, the isolates should be identified as clindamycin resistant. The inhibition zones were carefully examined using incident light using a simple lamp against a dark background. Control strains included *S. aureus* ATCC 25923 and *E.coli* ATCC 25922.

### Polymerase chain reaction for *mecA* gene

Cefoxitin-resistant isolates by disk diffusion test were selected to search *mecA* gene by in house polymerase chain reaction (PCR). The *mecA* gene is known for predict methicillin resistance. DNA extraction was performed by thermal lysis: about 5-10 colonies were suspended in 700µL of TE (Tris-EDTA) buffer (Sigma-Aldrich) and heated at 80°C for 20 min, and after that, samples were immediately frozen for 20 min at -20°C. Afterwards, samples were centrifuged, and the supernatant was submitted for quantification and purity analysis by spectrophotometry (NanoDrop, Kasvi®). In-house PCR was performed with the primers and methods described by Lawung et al. (2014)<sup>13</sup>. For the *mecA* amplification, a 25µL reaction mixture was used, containing 12.5 µL of Master

Mix (Quatro G<sup>®</sup>), 0.3125µL (0.125µM) of *mecA* forward and reverse primers, and 6.875µL of ultrapure water. The reaction was performed in a Thermal Cycler under the following conditions: initial denaturation at 95°C for 5 min, 35 cycles at 95°C for 1 min, 57°C for 1 min, and 72 °C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were subjected to electrophoresis on a 1.5% agarose gel to visualize the DNA fragments.

### Statistical analysis

The database was assembled using Excel 2013, and the analyses were performed with SPSS 18.0 (IBM, 2018). Qualitative variables were presented as frequencies and descriptive analysis of the independent variables. Bivariate analyses, using Pearson's chi-square test, were conducted to verify the associations between the dependent

variable (presence of an infection prevention protocol), categorized in a dichotomous way (yes/no) and the independent variables. The prevalence ratios and their 95% confidence intervals (95% CI) were estimated using Poisson regression with robust variances.

## RESULTS

### Questionnaire about infection prevention

From 100 clinics visited, fifty signed the consent form and answered the questionnaire. Of the professionals interviewed, 33 (66.0%) had complete college education in the healthcare or beauty sector, and 17 (34.0%) had technical instruction in the beauty sector. Participants' educational qualifications are shown in figure 1.

Among the participants, 20 (40.0%) declared that

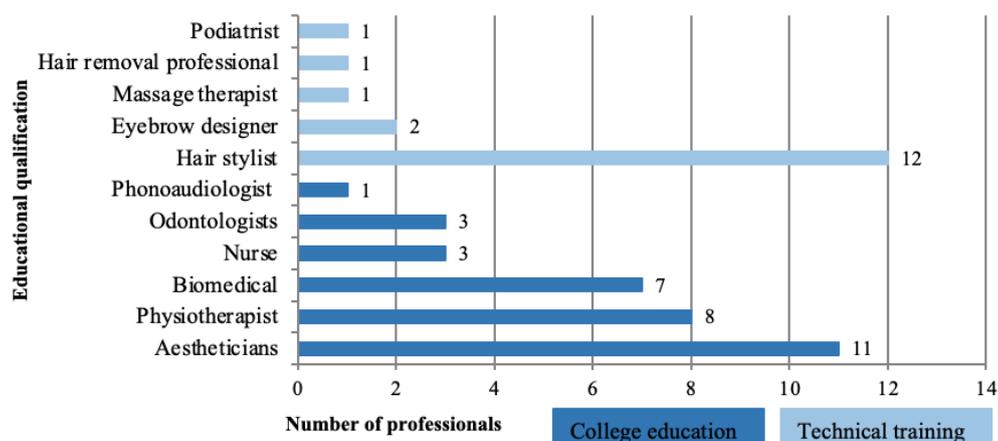


Figure 1. Educational qualification profile of interviewed professionals.

Table 1. Questionnaire responses and presence of infection prevention protocols in participant clinics.

Assessed questions	Does the service have an infection prevention protocol? N(%)			PR (95%CI) <sup>a</sup>	P value <sup>b</sup>
	Total 50 (100.0)	Yes 20 (40.00)	No 30 (60.00)		
Does the practitioner have complete graduation?	33 (66.00)	19 (95.00)	14 (46.67)	2,04 (1,37;3,02)	<0,001
Does the service actively search for post-procedure infections?	40 (80.00)	19 (95.00)	21 (70.00)	1,36 (1,05;1,75)	0,019
Does the service provide an infection prevention train-ing program?	36 (72.00)	19 (95.00)	17 (56.67)	1,68 (1,21;2,33)	0,002
Are there available information about infection risks in each procedure?	22 (44.00)	13 (65.00)	9 (30.00)	2,17 (1,15;4,09)	0,016
Are there cleaning routines written?	16 (32.00)	13 (65.00)	3 (10.00)	6,50 (2,12;19,93)	0,001
Are there asepsis routine written?	19 (39.00)	14 (70.00)	5 (16.67)	4,20 (1,80;9,83)	0,001
Is there hand hygiene technique written?	11 (22.00)	9 (45.00)	2 (6.67)	6,75 (1,63;28,03)	0,009
Are there hand hygiene supplies available?	47 (94.00)	20 (100.0)	27 (90.00)	1,11 (0,99;1,25)	0,083
Is there tracking for used products and substances?	18 (36.00)	11 (55.00)	7 (23.33)	2,36 (1,10;5,04)	0,027
Is there a clear movement of materials and people?	41 (82.00)	19 (95.00)	22 (73.33)	1,30 (1,02;1,64)	0,033
Does the cleaning of materials and instruments follow a unidirectional flow?	33 (66.00)	10 (50.00)	23 (76.67)	0,65 (0,40;1,05)	0,081
Is there a specific room for products and instruments cleaning?	20 (40.00)	7 (35.00)	13 (43.33)	0,81 (0,39;1,67)	0,563
Is there a contract with a specialized company for water tank cleaning?	48 (96.00)	20 (100.0)	28 (93.33)	1,07 (0,97;1,18)	0,157
Is there a contract with a specialized company for pest control?	49 (98.00)	20 (100.0)	29 (96.67)	1,03 (0,97;1,11)	0,317
Does the service use autoclave for sterilization?	33 (66.00)	10 (50.00)	23 (76.70)	0,81 (0,39;1,67)	0,563
Is autoclave quality control used Biological?	13 (39.40)	4 (40.00)	9 (39.10)	6,75 (1,63;28,03)	0,009
Is autoclave quality control used Chemical?	2 (6.10)	0 (0.00)	2 (8.70)	1,35 (1,63;28,03)	0,421
Is autoclave quality control used Physical?	18 (54.50)	6 (60.00)	12 (52.20)	2,36 (1,10;5,04)	0,027
Is the autoclave quality control performed:					
Weekly?	7 (21.20)	1 (10.00)	6 (26.10)	1,78 (1,53;28,03)	0,474
Monthly?	18 (54.50)	7 (70.00)	11 (47.80)	2,36 (1,10;5,04)	0,027
Half-yearly?	3 (9.10)	1 (10.00)	2 (8.70)	1,35 (1,63;28,03)	0,584
Daily?	2 (6.10)	0 (0.00)	2 (8.70)	1,35 (1,63;25,03)	0,977
Annual?	2 (6.10)	1 (10.00)	1 (4.30)	0,36 (1,87;27,03)	0,124
Unanswered	1 (3.00)	0 (0.00)	1 (4.30)	0,54 (1,27;28,03)	0,421

<sup>a</sup> PR (95% CI) – Prevalence Ratio (95% confidence interval). <sup>b</sup> p value < 0.005 value was considered statistically significant.

**Table 2.** Identified bacteria from the different collected samples in 43 establishments.

Clinic ID	Infection Prevention Protocol (Yes/No)	Table (N = 25)	Identified bacteria	Stretcher <sup>b</sup> (N = 25)	Identified bacteria	Equipment <sup>c</sup> (N = 19)	Identified bacteria	Product <sup>d</sup> (N = 20)	Identified bacteria	Autoclaved material <sup>e</sup> (N = 11)	Identified bacteria	Total collected samples per clinic N (%)
1	No	1	<i>E.coli</i>	1	<i>S. cohnii</i>	1	<i>S. saprophyticus</i>	1	<i>S. caprae</i>	NA <sup>f</sup>	... <sup>g</sup>	4 (4%)
2	Yes	1	<i>S. aureus</i>	NA	...	1	<i>S. epidermidis</i>	1	<i>S. haemolyticus</i>	NA	...	3 (3%)
3	Yes	1	<i>S. haemolyticus</i>	NA	...	1	<i>S. epidermidis</i>	1	NG <sup>h</sup>	1	NG	4 (4%)
4	No	1	<i>E.coli</i>	NA	...	1	<i>Acinetobacter spp.</i>	1	<i>S. caprae</i>	1	<i>Acinetobacter spp.</i>	4 (4%)
5	Yes	1	<i>S. warnerii</i>	1	<i>S. warnerii</i>	1	NG	NA	...	NA	...	3 (3%)
6	No	1	<i>E.coli</i>	1	<i>E.coli</i>	1	<i>S. aureus</i>	1	NG	NA	...	4 (4%)
7	Yes	1	<i>S. warnerii</i>	1	<i>S. warnerii</i>	NA	...	NA	...	1	NG	3 (3%)
8	Yes	1	<i>S. warnerii</i>	NA	...	NA	...	NA	...	NA	...	1 (1%)
11	No	NA	...	NA	...	NA	...	1	<i>S. aureus</i>	1	<i>Bacillus sp</i>	2 (2%)
12	No	1	<i>Bacillus sp.</i>	NA	...	1	<i>S. epidermidis</i>	NA	...	1	<i>S. epidermidis</i>	3 (3%)
13	No	NA	...	2	NG	NA	...	NA	...	NA	...	2 (2%)
14	No	1	<i>S. saprophyticus</i>	NA	...	1	NG	NA	...	NA	...	2 (2%)
15	Yes	NA	...	NA	...	1	<i>S. cohnii</i>	1	NG	1	NG	3 (3%)
16	Yes	NA	...	NA	...	1	<i>S. warnerii</i>	NA	...	1	NG	2 (2%)
18	Yes	1	<i>Bacillus sp.</i>	NA	...	1	<i>S. warnerii</i>	NA	...	NA	...	2 (2%)
19	No	1	<i>S. cohnii</i>	NA	...	NA	...	1	<i>S. cohnii</i>	NA	...	2 (2%)
20	No	1	<i>S. cohnii</i>	1	<i>S. hominis</i>	1	<i>S. cohnii</i>	1	<i>S. epidermidis</i>	NA	...	4 (4%)
21	No	1	<i>Bacillus sp.</i>	NA	...	NA	...	1	<i>S. epidermidis</i>	NA	...	2 (2%)
22	No	NA	...	1	<i>S. haemolyti-cus</i>	1	<i>S. warnerii</i>	NA	...	NA	...	2 (2%)
23	No	NA	...	1	<i>S. haemolyti-cus</i>	1	NG	NA	...	NA	...	2 (2%)
24	No	1	<i>Bacillus sp.</i>	NA	...	1	<i>E. coli</i>	NA	...	NA	...	2 (2%)
26	Yes	NA	...	1	<i>S. hominis</i>	NA	...	1	<i>S. epidermidis</i>	NA	...	2 (2%)
27	Yes	1	<i>S. aureus</i>	1	<i>Bacillus sp</i>	NA	...	1	NG	1	NG	4 (4%)
28	No	NA	...	1	<i>S. aureus</i>	NA	...	NA	...	NA	...	1 (1%)
29	No	1	<i>S. epidermidis</i>	1	<i>Bacillus sp</i>	NA	...	NA	...	NA	...	2 (2%)
					<i>S. haemolyti-cus</i>							
30	No	1	<i>S. epidermidis</i>	1	<i>S. hominis</i>	NA	...	NA	...	NA	...	2 (2%)
31	No	NA	...	1	<i>S. aureus</i>	NA	...	1	<i>S. saprophyticus</i>	NA	...	2 (2%)
32	No	NA	...	1	<i>S. aureus</i>	1	<i>S. aureus</i>	1	<i>S. saprophyticus</i>	NA	...	3 (3%)
33	No	NA	...	1	<i>S. aureus</i>	NA	...	NA	...	NA	...	1 (1%)
34	Yes	NA	...	1	<i>S. aureus</i>	NA	...	1	NG	NA	...	2 (2%)
35	No	NA	...	1	<i>S. haemolyti-cus</i>	1	<i>S. haemolyticus</i>	NA	...	1	<i>S. saprophyti-cus</i>	3 (3%)
37	Yes	1	<i>S. epidermidis</i>	NA	...	NA	...	NA	...	NA	...	1 (1%)
38	Yes	NA	...	NA	...	1	<i>S. caprae</i>	NA	...	1	NG	2 (2%)
39	Yes	1	<i>S. epidermidis</i>	NA	...	NA	...	NA	...	NA	...	1 (1%)
40	Yes	1	<i>S. warnerii</i>	NA	<i>S. epidermidis</i>	NA	...	NA	...	1	NG	3 (3%)
41	No	1	<i>S. epidermidis</i>	1	<i>S. epidermidis</i>	NA	...	1	<i>S. hominis</i>	NA	...	3 (3%)
43	No	NA	...	1	<i>S. caprae</i>	NA	...	1	<i>S. saprophyticus</i>	NA	...	2 (2%)
44	No	1	<i>S. cohnii</i>	1	<i>S. cohnii</i>	1	<i>S. hominis</i>	NA	...	NA	...	3 (3%)
45	No	1	<i>S. hominis</i>	1	<i>S. epidermidis</i>	NA	...	NA	...	NA	...	2 (2%)
47	No	NA	...	1	<i>S. hominis</i>	NA	...	1	<i>S. warnerii</i>	NA	...	2 (2%)
48	No	NA	...	NA	...	NA	...	1	<i>S. warnerii</i>	NA	...	1 (1%)
49	No	NA	...	1	<i>S. epidermidis</i>	NA	...	1	<i>S. warnerii</i>	NA	...	2 (2%)
50	No	1	<i>S. hominis</i>	NA	...	NA	...	NA	...	NA	...	1 (1%)
<b>Total samples with bacterial growth N (%)</b>		25 (100%)		25 (100%)		16 (84.2%)		15 (75.0%)		4 (36.0%)		

<sup>a</sup>Samples were collected from auxiliary tables located in procedures rooms, used in minimally invasive aesthetic procedures. <sup>b</sup>Samples were collected from stretchers used in minimally invasive aesthetic procedures. <sup>c</sup>Samples were collected from surfaces of equipment such as diamond peeling tips. <sup>d</sup>Samples were collected from open products such as eyebrow pigments. <sup>e</sup>Samples were collected from autoclaved materials such as scissors and tweezers. <sup>f</sup>NA: no samples collected. <sup>g</sup>Not applicable. <sup>h</sup>NG: no bacterial growth.

there was an infection prevention protocol available in the clinic (Table 1), and 22 (44.0%) stated that they have access to information about infection risks for each procedure, together with specific operational protocols.

Infection prevention training for employees was provided in 36 (72.0%) of clinics. According to the responses, the occurrence of post-procedure infections was actively monitored in 40 (80.0%) clinics, by contacting the patients over the phone.

Concerning hand hygiene, 47 (94.0%) of professionals stated that supplies for hand hygiene were available. On the other hand, only 11 (22.0%) establishments had written protocols for hand hygiene available.

For the cleaning and aseptic routines of materials and instruments, 33 (66.0%) establishments declared to obey a unidirectional flow, not mixing clean materials with dirty ones. Also, 41 (82.0%) interviewed professionals declared that there are a clear movement of materials and people, avoiding recontamination of any kind. Opposite to that, only 20 (40.0%) establishments had proper rooms for cleaning, decontamination, and sterilization of materials and instruments.

Regarding biological contaminated waste, 32 (64.0%) establishments had contracts with specialized companies to provide this service. To sterilize the instruments, 33 (66.0%) establishments declared to use an autoclave, in which the majority, 18 (54.0%), perform the process monitoring with a physyc quality control, and just 13 (39.4%) use a biological control to it.

### Microbiological findings

The samples were collected in 43 clinics that signed a consent term. We collected 100 different samples, as follow: 25 from tables; 25 from stretchers; 19 from equipment surfaces; 20 from open products that were currently in use, as eyebrow pigmentation inks; and 11 from autoclaved materials, such as tweezers and scissors.

Of the 100 samples collected, 85 had bacterial growth by cultural methods (Table 2), in which 83.5% was identified coagulase-negative staphylococci (CNS) species, mostly *Staphylococcus epidermidis* (15/85; 21.2%). *Staphylococcus aureus* was found in 10/85 (1.7%) samples. Gram-negative bacteria, such as *Escherichia coli* and *Acinetobacter spp.*, were found in 7/85 (8.3%) of the samples as well as *Bacillus spp.* Moreover, 4/11 (36.4%) of the samples collected from autoclaved materials were contaminated.

Regarding the association between bacterial growth and the presence of infection prevention protocols, 75.3% (64/85) of contaminated samples were from services that did not have infection prevention protocols (Table 2). Here, we highlight the statistically significant association between contaminated open products and the absence of infection prevention protocols: 15/20 collected samples showed bacterial growth, in which 86.7% (13) of establishments did not have infection prevention protocols ( $p < 0.001$ ). Contamination in autoclaved materials among clinics without infection prevention protocols was also statistically significant ( $p < 0.001$ ).

Antimicrobial susceptibility tests were performed for 78 isolates, (7 *Bacillus sp.* isolates were excluded according to BrCAST protocol). The resistance profiles are shown in table 3.

The results showed that 22 (28.2%) of isolates showed resistance at least one antibiotic. Among the staphylococci, 19 (26.7%) showed resistance to cefoxitin, clindamycin, and erythromycin, and 16 (84.2%) of these isolates had a positive D-test. Among 21 isolates resistant to cefoxitin, 4 of them (19.1%) were positive for the *mecA* gene.

### DISCUSSION

Although there are sanitary recommendations that establish technical standards for the operation of establishments that perform aesthetic procedures without

**Table 3.** Resistance profile of 78 bacteria isolates collected from 43 establishments.

Antimicrobial	Resistance Profile n (%) <sup>a</sup>								
	<i>Acinetobacter sp</i> (n=2)	<i>E. coli</i> (n=5)	<i>S. aureus</i> (n=10)	<i>S. caprae</i> (n=5)	<i>S. cohnii</i> (n=8)	<i>S. epidermidis</i> (n=15)	<i>S. haemolyticus</i> (n=7)	<i>S. saprophyticus</i> (n=6)	<i>S. warneri</i> (n=12)
Amikacin	0	0	0	0	0	0	0	0	0
Amoxicillin + clavulanate	NA <sup>b</sup>	1 (20.0)	NA	NA	NA	NA	NA	NA	NA
Ampicillin	NA	0	NA	NA	NA	NA	NA	NA	NA
Cephalexin	NA	0	NA	NA	NA	NA	NA	NA	NA
Cefoxitin	NA	0	5 (50.0) <sup>c</sup>	0	2 (25.0) <sup>c</sup>	6 (13.3) <sup>c</sup>	2 (28.5) <sup>c</sup>	2 (16.6)	3 (8.3)
Ceftriaxone	NA	0	NA	NA	NA	NA	NA	NA	NA
Ciprofloxacin	0	0	0	0	0	0	0	0	0
Clindamycin	NA	NA	4 (40.0) <sup>d</sup>	0	2 (25.0) <sup>e</sup>	6 (40.0) <sup>e</sup>	2 (28.5) <sup>e</sup>	2 (16.6) <sup>f</sup>	3 (25.0) <sup>f</sup>
Erythromycin	NA	NA	4 (40.0)	0	2 (25.0)	6 (40.0)	2 (28.5)	2 (16.6)	3 (25.0)
Gentamicin	0	0	0	0	0	0	0	0	0
Imipenem	0	0	NA	NA	NA	NA	NA	NA	NA
Levofloxacin	0	0	0	0	0	0	0	0	0
Linezolid	NA	NA	0	0	0	0	0	0	0
Meropenem	0	0	NA	NA	NA	NA	NA	NA	NA
Sulfamethoxazol-trimetropim	0	0	0	0	0	0	0	0	0
Tetracycline	NA	NA	0	0	0	0	0	0	0
Tigecycline	NA	0	0	0	0	0	0	0	0

<sup>a</sup> Resistance profile was interpreted according to BrCAST – Brazilian Committee on Antimicrobial Susceptibility Testing. <sup>b</sup> NA: antibiotic is not standardized for testing according to BrCast. <sup>c</sup> One isolate with positive *mecA*. <sup>d</sup> Three isolates with D test positive. <sup>e</sup> All isolates with D-test positive. <sup>f</sup> One isolate with D-test positive.

medical responsibility, problems related to infection prevention measures in these services are frequent.<sup>5,15</sup> Even though these establishments receive thousands of patients daily, there are few records of aesthetic procedure-related infections, not due to the lack of these events, but probably due to the absence of well-conducted national and international specific epidemiological studies.<sup>11</sup>

In Brazil, there are more than three million professionals working in beauty and aesthetics, with heterogeneous educational and professional backgrounds, and with different knowledge and perceptions about infection prevention measures.<sup>2</sup> Our study showed that infection prevention protocols were available at 20 (40.0%) of the clinics, and, in 19 of them (95%), professionals had higher educational degrees. This result suggests that the presence of professionals with higher levels of education can be associated with increased compliance with infection prevention practices, in accordance to Garbaccio and Oliveira (2013).<sup>5</sup>

Besides that, many studies also consider continuing education programs for professionals as very effective methods for infection prevention.<sup>2,6,9</sup> In our study, 36 (72%) clinics declared they periodically provide infection prevention trainings for employees. As suggested by Garbaccio and Oliveira (2013), clinics that provide staff training are less associated with infection related to aesthetic procedures.<sup>5</sup>

Furthermore, protocols for hand washing technique were available at only 11 (22.0%) clinics. Curiously, hand washing supplies, such as paper towels and soap, were present in 47 (94.0%) clinics. Also, in accordance to Garbaccio & Oliveira (2013), it seems that most beauty and aesthetics professionals consider hand washing as just a hygiene measure, not as an infection prevention method. Since microorganisms are transmitted mainly through the hands, the adoption of correct hygiene practices is essential and should be routine in professional practice.<sup>5,9,14,16</sup>

As seen by a study conducted by Graveto et al. (2017), staff training of nurses about hand hygiene provided satisfactory results in daily routines in infection prevention measures, including the presence of written protocols about infection prevention<sup>16</sup>. In our study, the percentage of clinics with written hand hygiene protocols were higher in the ones that had written protocols for infection prevention measures (45%).

Most isolated microorganisms in our study are normal members of the skin microbiome<sup>7</sup>, but skin injury that occurs during certain minimally invasive procedures can represent a gateway for invasion of microorganisms, which can result in colonization of pathogenic organisms or infectious diseases.<sup>17</sup> The high level of contamination in supposedly sterile materials (four contaminated samples, out of 11 collected samples) demonstrates the inadequacy in the use or functioning of the autoclave devices and represent a risk for infection.<sup>18</sup>

Of the clinics that performed sterilization of materials with an autoclave, 18 (54.5%) used physical control as the main measure of verification of the sterilization process. Physical control is not the most appropriate,

since it varies in reading by subjectivity in the interpretation of results.<sup>6,9</sup> The most effective method for autoclave quality control is biological, which uses biological indicators to simulate microbial death and should be performed weekly.<sup>9</sup> Only 13 (39.4%) clinics performed this type of quality control. The lack in autoclave maintenance and also the use of inadequate types of quality control can lead to major health concerns, considering that these inadequately autoclaved objects will probably be used during cosmetic procedures.<sup>9,14</sup>

One important bacterium that we found that acts as an opportunistic pathogen is *Staphylococcus aureus*.<sup>18</sup> Currently, methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious public health concern and is among the pathogens of greatest clinical importance, according to the World Health Organization.<sup>19</sup> In our study, 5/10 *Staphylococcus aureus* isolates demonstrated resistance to cefoxitin, and one isolate was positive for *mecA*. Resistance to cefoxitin is reported to be highly accurate to predict methicillin resistance, and detection of the *mecA* gene remains the most reliable method for identifying these isolates.<sup>19-21</sup> Four *S. aureus* isolates demonstrated resistance to both clindamycin and erythromycin, with a positive D-test. The test positivity indicates the inducible resistance to these antibiotics, which is frequently mediated by the *ermA* and *ermC* genes.<sup>20,21</sup>

Despite the isolation in different environments, the real prevalence of MRSA transmission in aesthetic services is not known. A study carried out in the Netherlands with eleven people, including professionals, clients, and family members who developed abscesses after waxing, revealed positives samples in all of them for the same strain of MRSA. Contamination was mainly caused by wax reuse and the fact that the devices used in this procedure were not properly sanitized.<sup>18</sup>

Coagulase-negative staphylococci (CNS) is also one of the main opportunistic pathogens in the hospital and community environments, and is able to colonize different parts of the skin and soft tissues.<sup>21-24</sup> CNS were isolated in 61 (61.0%) samples in our study, in which 16 (26.2%) isolates showed resistance to cefoxitin. Among the cefoxitin resistant isolates, three (18.7%) were positive for *mecA*. Inducible resistance to MLS<sub>B</sub> (Macrolide, Lincosamide and Streptogramin-B) antibiotics were found in 12 CNS isolates, and constitutive resistance to MLS<sub>B</sub> was identified in three isolates of the same group.

It is known that CNS can act as a reservoir of genetic elements that lead to resistance to beta-lactams and other classes of antibiotics, and they can pass these elements on to more virulent bacteria, such as *S. aureus*.<sup>21,22</sup> Resistance levels among coagulase-negative staphylococci are increasing dramatically.<sup>25</sup> Currently, less than 10% of the clinical isolates of *S. epidermidis* and *S. haemolyticus* are sensitive to penicillin,<sup>13</sup> representing a major public health concern, especially when related to cosmetic procedures, because there is a known risk for infection if infection prevention measures are not taken.<sup>5,24</sup>

Also, our study showed that the association between bacterial growth and the presence of infection pre-

vention protocols were statistically significant ( $p < 0.001$ ), highlighting the importance of infection prevention protocols in these establishments. For instance, in autoclaved materials, all samples that showed bacterial growth were collected from clinics that did not have infection prevention protocols. Similarly, bacterial growth in open products was 86.7% higher in clinics that did not have infection prevention protocols. These results help us understand the importance of the existence and following infection prevention protocols, in order to prevent any infection associated to minimally invasive aesthetic procedures, since open products, as well as autoclaved materials and instruments, could be a source to spread bacteria and cause infections, as happened in the Netherlands in 2009.<sup>18</sup>

An important limitation of our study was the acceptance of study participation in only 50% of the clinics visited. Non-acceptance may have been due to the fear that the research would have a supervisory character, and maybe these clinics could have problems related to infection prevention measures.

In conclusion, the presence of opportunistic pathogens in the environment associated with a lack of infection prevention measures may represent a risk of infections for patients. Surfaces, products, and autoclaved materials can be contaminated even with multidrug-resistant microorganisms, such as MRSA, exposing patients to risk of infection. This fact highlights the importance of disinfection and aseptic protocols, as well as hand hygiene, to avoid contamination, especially of multidrug-resistant bacteria. The study also leads us to the conclusion that the presence of higher educated professionals is critical in aesthetic clinics so that biosafety and infection prevention measures are taken.

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**Daniela Signori, Andreza Francisco Martins** and **Taís Fernanda da Silva Anelo** made substantial contributions to the study's conception and design, data collection, analysis and interpretation, drafting and critical revision of important intellectual content;

**William Machado de Souza, Malena Rostirola Miri, Lilian Berger de Oliveira, Jéssica Daiane Cardozo** and **Gabriela Santos da Rosa** made substantial contributions to the data collection, analysis, and interpretation;

**Andreza Francisco Martins** made substantial contribution to analysis and approval of the final version for publication.

All authors have approved the final version to be published and are responsible for all aspects of the work including ensuring its accuracy and integrity.