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Microbiological analysis of sterile and nonsterile gloves before and during root canal treatment procedures

Análise microbiológica de luvas estéreis e não estéreis antes e durante procedimentos de tratamento endodôntico

Análisis microbiológico de guantes estériles y no estériles antes y durante los procedimientos de tratamiento de conducto

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Abstract

Objective: To evaluate the microbiological contamination of sterile (SG) and non-sterile (NSG) gloves before and during different stages of endodontic treatment. Methodology: Five brands (n = 10 per brand) of NSG (Supermax, UniGloves, Lemgruber, Nugard and Embramac) and four brands (n = 10 per brand) of SG (Sensitex, Sanro, Mucambo, Madeitex) were analyzed. Microbiological collections were performed at 3 different time-points: immediately after opening the packages (S1); after performing rubber dam placement (S2); and at the end of root canal treatment (S3). In the NSG group, to assess possible contamination by exposure to the clinical environment, samples were also collected within 24 hours (S4) and 7 days (S5) after opening the packages. After smearing the gloves with sterile swabs, the samples were immediately placed in test tubes containing specific BHI culture medium. These samples were incubated for 24 and 48h and the turbidity of the medium was evaluated. For turbid samples, bacterial identification was performed through culture and biochemical tests. Results: At S1, only the NSG UniGloves and Nugard brands showed contamination, while no contamination was observed in the SG. After S2 and S3 all brands showed contamination. In the NSG assessments, 24h after opening the packaging (S4) only the UniGloves brand showed no contamination, but this was not observed after 7 days (S5). The most observed species were *Staphylococcus aureus, Staphylococcus ssp, Staphylococcus intermedius, Neisseria ssp, Escherichia coli* and

Pseudomonas aeroginosa. Conclusion: The NSG can be contaminated after opening the packaging and their exposure to the dental office environment can make it contaminated. SG are sterile but can also become contaminated during procedures. In the trans-operative period of root canal treatment, both NSG and SG can be contaminated by Gram-positive and Gram-negative bacteria.
Keywords: Biological contamination; Endodontics; Gloves, Protective; Gloves, Surgical; Root canal therapy.

Resumo
Objetivo: Avaliar a contaminação microbiológica de luvas estéreis (LE) e não estéreis (LNE) antes e durante diferentes etapas do tratamento endodôntico. Metodologia: Foram analisadas cinco marcas (n = 10 por marca) de LNE (Supermax, UniGloves, Lemgruber, Nugard e Embramac) e quatro marcas (n = 10 por marca) de LE (Sensitex, Sanro, Mucambo, Madeitex). As coletas microbiológicas foram realizadas em 3 momentos distintos: imediatamente após a abertura das embalagens (S1); após a aplicação do dique de borracha (S2); e no final do tratamento endodôntico (S3). No grupo LNE, para avaliar possível contaminação por exposição ao ambiente clínico, as amostras também foram coletadas em até 24 horas (S4) e 7 dias (S5) após a abertura das embalagens. Após esfregar as luvas com swabs estéreis, as amostras foram imediatamente colocadas em tubos de ensaio contendo meio de cultura BHI específico. Essas amostras foram incubadas por 24 e 48h e a turbidez do meio foi avaliada. Para amostras turvas, a identificação bacteriana foi realizada por meio de cultura e testes bioquímicos. Resultados: Em S1, apenas as LNE das marcas UniGloves e Nugard apresentaram contaminação, enquanto em LE não foi observada contaminação. Após S2 e S3 todas as marcas apresentaram contaminação. Nas avaliações de LNE 24h após a abertura da embalagem (S4), apenas a marca UniGloves não apresentou contaminação, mas isso não foi observado após 7 dias (S5). As espécies mais observadas foram *Staphylococcus aureus*, *Staphylococcus ssp*, *Staphylococcus intermedius*, *Neisseria ssp*, *Escherichia coli* e *Pseudomonas aeroginosa*. Conclusão: As LNE podem ser contaminadas após a abertura da embalagem e sua exposição ao ambiente do consultório odontológico pode contaminá-las. LE são estéreis, mas também podem se contaminar durante os procedimentos. No período transoperatório do tratamento endodôntico, tanto o LNE quanto o LE podem ser contaminadas por bactérias Gram-positivas e Gram-negativas.
Palavras-chave: Contaminação biológica; Endodontia; Luvas cirúrgicas; Luvas protetoras; Tratamento do canal radicular.

Resumen
Objetivo: Evaluar la contaminación microbiológica de guantes estériles (GE) y no estériles (GNE) antes y durante las diferentes etapas del tratamiento endodóntico. Metodología: Se analizaron cinco marcas (*n* = 10 por marca) de NSG (Supermax, UniGloves, Lemgruber, Nugard y Embramac) y cuatro marcas (*n* = 10 por marca) de GE (Sensitex, Sanro, Mucambo, Madeitex). Las recolecciones microbiológicas se realizaron en 3 puntos de tiempo diferentes: inmediatamente después de abrir los paquetes (S1); después de realizar la colocación del dique de goma (S2); y al final del tratamiento de conducto (S3). En el grupo GNE, para evaluar la posible contaminación por exposición al ambiente clínico, también se recolectaron muestras dentro de las 24 horas (S4) y 7 días (S5) después de abrir los paquetes. Después de untar los guantes con hisopos estériles, las muestras se colocaron inmediatamente en tubos de ensayo que contenían medio de cultivo específico para BHI. Estas muestras se incubaron durante 24 y 48 horas y se evaluó la turbidez del medio. Para las muestras turbias, la identificación bacteriana se realizó mediante cultivo y pruebas bioquímicas. Resultados: En S1, solo las GNE de las marcas UniGloves y Nugard mostraron contaminación, mientras que en el GE no se observó contaminación. Después de S2 y S3 todas las marcas mostraron contaminación. En las evaluaciones de GNE, 24h después de abrir el empaque (S4), solo la marca UniGloves no mostró contaminación, pero esto no se observó después de 7 días (S5). Las especies más observadas fueron *Staphylococcus aureus*, *Staphylococcus ssp*, *Staphylococcus intermedius*, *Neisseria ssp*, *Escherichia coli* y *Pseudomonas aeroginosa*. Conclusión: Las GNE pueden contaminarse después de abrir el empaque y su exposición al entorno del consultorio dental puede contaminarlo. GE son estériles pero también pueden contaminarse durante los procedimientos. En el período transoperatorio del tratamiento de conductos, tanto GNE como GE pueden estar contaminados por bacterias Gram-positivas y Gram-negativas.
Palabras clave: Contaminación biológica; Endodoncia; Guantes quirúrgicos; Guantes protectores; Tratamiento del conducto radicular.

1. Introduction

Personal protective equipment (PPE) is considered any device for individual use and its function is to prevent risks that may threaten the safety and health of human beings. The need to use PPE by healthcare professionals is recommended by the Center for Disease Control (CDC) and the American Dental Association (ADA). It provides for standards and universal precautions infection control to reduce the risk of contamination (Zaheer et al., in press). In dentistry, two types of gloves are routinely recommended: sterile gloves (SG) and non-sterile gloves (NSG), whereas SE are usually indicated only for surgical

procedures (Centers for Disease Control and Prevention, 2016).

The coronavirus disease 2019 (COVID-19) outbreak increased the concern of dental care professionals regarding the use of PPE and barriers at the dental office, but the use of SG was not indicated (Bahador et al., 2021; Gund et al., 2022; Martinho & Griffin, 2021). The CDC recommended the use of gloves extended to cover wrist of isolation gown and to change gloves when torn or heavily contaminated (Centers for Disease Control and Prevention, 2020). Moreover, the type of PPE used should be modified according to the precautions required, such as standard and contact, droplet or airborne infection isolation precautions (Ather et al., 2020). The level of aerosols generated during dental treatment modifies according to the dental care procedure, even within the same area such as Endodontics (Bahador et al., 2021). Despite that, currently the procedures of endodontic practice still mainly performed with NSG.

The failure of root canal treatment is associated with the presence of persistent pathogenic microorganisms from the primary infection or from the breakdown of the aseptic chain during endodontic procedures (Gomes et al., 2021; Siqueira & Rôças, 2009). It is known that multiple surfaces acquire bacterial contamination during treatment at high frequencies and loads (Zahran et al., 2022). NSG can be contaminated and impair asepsis control during treatment. Such a situation can happen due to the glove's exposure to the clinical environment, in transport and in the form of storage in the professional office (Hall et al., 2014; Moran & Heuertz, 2017).

Microbiological assessments in SG and NSG are necessary to improve the control of biosafety during clinical care, maintaining the aseptic chain and preventing cross-infections (Ferreira et al., 2011; Hall et al., 2014; Luckey et al., 2006; Moran & Heuertz, 2017). Due to the lack of literature regarding this issue, the aim of the present study was to evaluate the presence of contamination in different brands of SG and NSG, as well as the possibility of these gloves being contaminated during the different stages of a root canal treatment.

2. Methodology

2.1 Groups distribution and Samples collection

This study was approved by the Local Research Ethics Committee (CAAE: 11617512.0.0000.5220).

The NSG brands were Supermax Brasil Importadora S/A (Curitiba, PR, Brazil); UniGloves (Curitiba, PR, Brazil); Lemgruber (Rio de Janeiro, RJ, Brazil); Nugard (Blumenau, SC, Brazil); Embramac (Campinas, SP, Brazil). The SG brands were: Sensitex (Mucambo S.A.; São Paulo, SP, Brazil); Sanro (São Roque, SP, Brazil); Dial (Mucambo S.A; São Paulo, SP, Brazil); Madeitex (São José dos Campos, SP, Brazil).

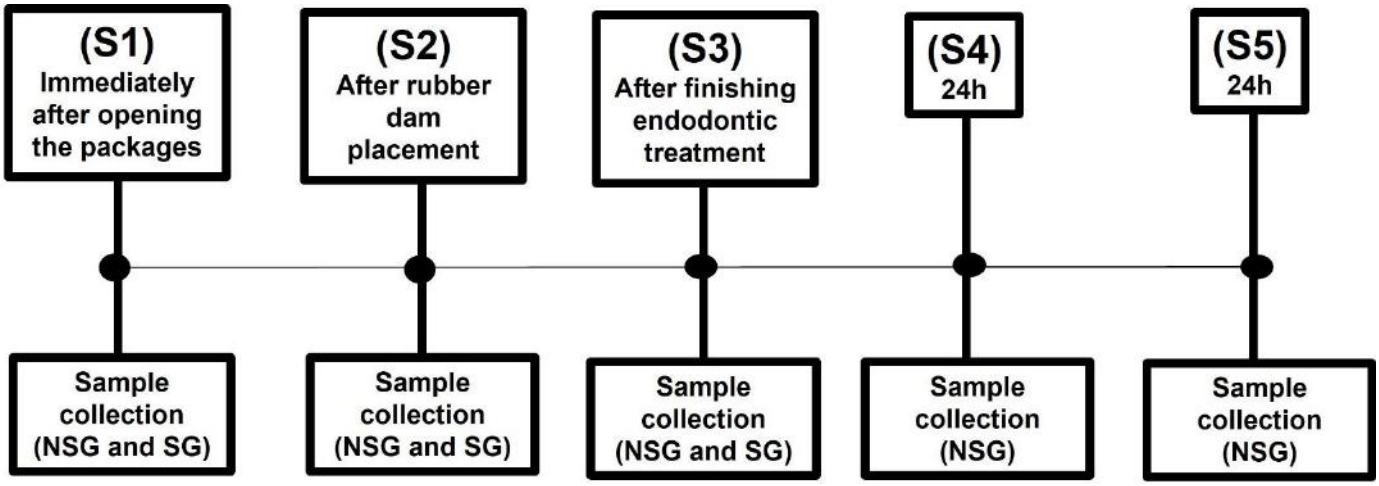
To assess the sterility of the gloves, microbiological collections with sterile swabs were performed. For each tested brand, 10 gloves were evaluated, and all collections were performed in triplicate. In addition, a positive control group and 2 negative control groups (sterility of the culture medium and the clinical environment) were performed.

Test tubes containing 10mL of BHI (Brain Heart Infusion) broth were autoclaved and kept in a bacteriological oven at 37ºC for 48 hours in order to confirm sterility. All gloves evaluated in this study were removed from previously sealed packages with sterile clinical forceps. The protocol described by the World Health Organization were used to put on the gloves (World Health Organization, 2009). At all stages of the collection, sterile swabs were rubbed on the back, palm and fingers on the hands covered with gloves and immediately transferred to the test tubes containing BHI. During the collection procedures, a nearby flame was kept, to minimize possible external contamination.

The first sample collection (S1) was performed immediately after the package was opened and before the SG and NSG were paved (Figure 1). After rubber dam placement, a new sample collection was performed (S2). Next, a new pair of gloves was used to complete the root canal treatment. At the end of the treatment, the third sample collection was performed (S3). The NSG packages remained open in a clinical environment for 24 hours (S4) and 7 days (S5), so the remaining samples

collections could be performed at these time-points.

Figure 1. Flowchart of study design showing the moment of sample collections during endodontic treatment procedures. *NSG: non-sterile gloves; SG: sterile gloves.



Source: Authors (2022).

After the sample's collections, the swabs were inserted in tubes containing BHI-broth, incubated in a bacteriological oven at 37ºC and kept in an anaerobic environment. The reading of the turbidity of the culture medium was performed using the visual method after 24 and 48h of incubation and were compared with the control groups. The turbidity of the culture medium showed microbial growth, characterizing contamination of the evaluated samples. Therefore, gloves were considered contaminated when the culture medium was cloudy and not contaminated in the absence of turbidity.

Contaminated samples were identified microbiologically by the traditional culture method and by phenotypic identification through biochemical tests as previously described (Gomes et al., 2006, 2008).

Inside a laminar flow cabinet, the test tubes were mechanically shaken for 1 minute to facilitate the dispersion of microorganisms. Then, serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were performed using BHI and 50 µL of each dilution were inoculated into pre-reduced plates containing: Fastidious Anaerobe Agar (FAA-LabM, Bury, UK), plus 5% defibrinated sheep blood supplemented by hemin (5 mg / L), and vitamin K1 (1 mg / L). These plates were incubated at 37°C in an atmosphere of 10% H_2, 10% CO_2, 80% N_2 for up to 14 days, to allow the detection of strict anaerobic microorganisms; and Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) blood agar and incubated aerobically at 37°C for 48h to allow the growth of aerobic and/or facultative microorganisms.

After incubation, each plate was examined using a stereoscopic magnifying glass in a 3-fold magnification. Colony-forming units were counted (CFU/mL) and the different types of colonies were subcultured on FAA plates (to obtain pure cultures of the species). The colonies were selected for their initial identification through their macroscopic characteristics on the agar, which is facilitated by the addition of blood. The morphological recognition of the colonies was made by: size, color, shape, elevation, edge, surface, texture, consistency, opacity, effect on the agar and effect on the blood (none, partial, complete hemolysis). Pure colonies were then identified by Gram stain, gas requirements, and catalase test (Gomes et al., 2006, 2008).

To determine the gaseous requirement, each colony obtained anaerobically was inoculated into two FAA plates. One plate was incubated for 2 days aerobically and the other for the same time anaerobically. The plates were compared to determine whether the isolates were optional or mandatory anaerobes. The species that grew only in anaerobiosis, were incubated in a CO_2 oven and if no growth was found, they were considered strict anaerobic. Bacterial morphology was

confirmed after each incubation by Gram stain.

Then, standardized and appropriate biochemical tests for identification (BioMérieux SA, Marcyl'Etoile, France) were used, Rapid ID 32A (for Gram-negative and Gram-positive rods, mandatory anaerobes); API Staph (for staphylococci and micrococci, Gram-positive, catalase-positive coconuts); API Strep (for streptococci, gram-positive, catalase-negative cocci); API 20E (for enterobacteria, Gram-negative enteric bacilli, catalase-positive, oxidase-negative); API NH (for *Eikenella*, *Haemophilus*, *Neisseria* and *Actinobacillus*, gram-negative and optional coconuts and bacilli, oxidasepositiva); AUX API (to identify *Candida* species).

2.2 Statistical analysis

Qualitative data were processed and analyzed using Excel software (Microsoft, Redmond, WA, USA).

3. Results

At S1, only the NSG UniGloves and Nugard brands showed contamination (Table 1), whereas no contamination was observed in the SG group (Table 2). After S2 and S3 all brands showed contamination. In the NSG assessments, 24h after opening the packaging (S4) only the UniGloves brand showed no contamination, but this was not observed after 7 days (S5). The most observed species were *Staphylococcus aureus*, *Staphylococcus ssp*, *Staphylococcus intermedius*, *Neisseria ssp*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1. Percentage of specimens of non-sterile gloves detected with microbiological contamination and bacteria identified during root canal treatment procedures:

				SUPERMAX		
Contamination	**Bacteria**	**S1**	**S2**	**S3**	**S4**	**S5**
Contaminated	*Staphylococcus aureus*	33.3%	33.3%	-	66.7%	33.3%
	Staphylococcus intermedius	33.3%	-	-	-	33.3%
	Staphylococcus ssp	-	66.7%	33.3%	33.3%	33.3%
	Neisseria ssp	-	-	66.7%	-	-
	Escherichia coli	-	-	33.3%	-	-
	Pseudomonas aeruginosa	33.3%	-	-	-	-
Non-Contaminated	-	33.3%	-	-	-	-
				UNIGLOVES		
Contamination	**Bacteria**	**S1**	**S2**	**S3**	**S4**	**S5**
Contaminated	*Staphylococcus aureus*	-	-	-	-	-
	Staphylococcus intermedius	-	-	-	-	-
	Staphylococcus ssp	-	33.3%	33.3%	-	66.7%
	Neisseria ssp	-	33.3%	66.7%	-	-
	Escherichia coli	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-
Non-Contaminated	-	100%	33.3%		100%	33.3%
				LEMGRUBER		
Contamination	**Bacteria**	**S1**	**S2**	**S3**	**S4**	**S5**
Contaminated	*Staphylococcus aureus*	33.3%	66.7%	33.3%	33.3%	33.3%
	Staphylococcus intermedius	33.3%	33.3%	66.7%	-	-
	Staphylococcus ssp	-	-	-	-	-
	Neisseria ssp	-	-	-	-	-
	Escherichia coli	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-
Non-Contaminated	-	33.3%	-	-	66.7%	66.7%
				NUGARD		
Contamination	**Bacteria**	**S1**	**S2**	**S3**	**S4**	**S5**
Contaminated	*Staphylococcus aureus*	-	-	66.7%	33.3%	66.7%
	Staphylococcus intermedius	-	33.3%	-	-	-
	Staphylococcus ssp	-	-	-	-	-
	Neisseria ssp	-	33.3%	33.3%	66.7%	-
	Escherichia coli	-	33.3%	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-
Non-Contaminated	-	100%	33.3%	-	-	33.3%
				EMBRAMAC		
Contamination	**Bacteria**	**S1**	**S2**	**S3**	**S4**	**S5**
Contaminated	*Staphylococcus aureus*	-	-	-	-	-
	Staphylococcus intermedius	33.3%	-	66.7%	-	66.7%
	Staphylococcus ssp	66.7%	100%	33.3%	33.3%	33.3%
	Neisseria ssp	-	-	-	-	-
	Escherichia coli	-	-	-	33.3%	-
	Pseudomonas aeruginosa	-	-	-	-	-
Non-Contaminated	-	-	-	-	66.7%	-

- : 0%. Time-points during root canal treatment: S1 = immediately after opening the packages; S2 = after performing rubber dam placement; S3 = at the end of root canal treatment; S4 = 24 hours after opening the gloves package; S5 = 7 days (S5) after opening the gloves packages. Source: Authors (2022).

Table 2. Percentage of specimens of sterile gloves detected with microbiological contamination and bacteria identified during root canal treatment procedures:

			SENSITEX	
Contamination	**Bacteria**	**S1**	**S2**	**S3**
Contaminated	*Staphylococcus aureus*	-	66.7%	100%
	Staphylococcus intermedius	-	-	-
	Staphylococcus ssp	-	-	-
	Neisseria ssp	-	-	-
	Escherichia coli	-	-	-
	Pseudomonas aeruginosa	-	-	-
Non-Contaminated	-	100%	33.3%	-
			SANRO	
Contamination	**Bacteria**	**S1**	**S2**	**S3**
Contaminated	*Staphylococcus aureus*	-	100%	66.7%
	Staphylococcus intermedius	-	-	-
	Staphylococcus ssp	-	-	-
	Neisseria ssp	-	-	33.3%
	Escherichia coli	-	-	-
	Pseudomonas aeruginosa	-	-	-
Non-Contaminated	-	100%	-	-
			DIAL	
Contamination	**Bacteria**	**S1**	**S2**	**S3**
Contaminated	*Staphylococcus aureus*	-	100%	33.3%
	Staphylococcus intermedius	-	-	-
	Staphylococcus ssp	-	-	-
	Neisseria ssp	-	-	-
	Escherichia coli	-	-	-
	Pseudomonas aeruginosa	-	-	-
Non-Contaminated	-	100%	-	66.7%
			MADEITEX	
Contamination	**Bacteria**	**S1**	**S2**	**S3**
Contaminated	*Staphylococcus aureus*	-	33.3%	-
	Staphylococcus intermedius	-	-	-
	Staphylococcus ssp	-	-	-
	Neisseria ssp	-	66.7%	100%
	Escherichia coli	-	-	-
	Pseudomonas aeruginosa	-	-	-
Non-Contaminated	-	100%	-	-

- : 0%. Time-points during root canal treatment: S1 =; S2 = after performing rubber dam placement; S3 = at the end of root canal treatment. Source: Authors (2022).

4. Discussion

Gloves used in root canal treatment are often neglected (Patel et al., 2022). The present study pointed out that gloves can serve as a potential means for breaking the aseptic chain, if no previous decontamination protocol is carried out. This potential was evidenced in the NSG group, as only two brands were not associated with contamination at S1. The absence of bacteria in all SG brands confirms their sterility guaranteed by the manufacturers.

The present study also pointed out that NSG that remained with the packages open for 24 hours and 7 days, may be subject to contamination present in the clinical environment. This fact was evident in the Uniglove brand group, which showed no contamination in 24 hours, but in 7 days it was contaminated. These groups were carried out precisely because this is a common clinical condition in offices, where packaging is exposed to the environment. The literature shows that dental procedures produce aerosols that may be contaminated; thus, presenting a high risk for the spread of microorganisms (Gomes et al., 2006; Gund et al., 2022; Meng et al., 2020).

In addition to the risk of contamination by aerosols, the risk of contamination of gloves by handling the packaging that remain open and continued to be handled was previously reported (Ferreira & Andrade, 2010). For these authors, the contamination found was due to their handling, transferring the resident microbiota in the operator's hands to the gloves that remained in the packaging. Such findings corroborate this work. However, divergent results were also reported, as no difference was observed in the contamination of gloves from newly opened packages to almost empty packages that were handled and were exposed to the clinical environment (Luckey et al., 2006). The presence of a large number of bacteria in the NEG group, corroborates with these findings, that showed a 10-fold increase in the number of cultivable colonies in newly placed gloves when compared to gloves after the installation of rubber dam.

The contamination found in the groups after rubber dam placement and at the end of root canal treatment can be considered by an inevitable factor, as in these stages there is contact with the patient's saliva (Luckey et al., 2006). Thus, even when SG are used, at the end of treatment these gloves might be contaminated. These findings demonstrate that the use of SG or change the gloves after the installation of rubber dam does not guarantee the maintenance of their asepsis during the entire care.

In the present study, the most prevalent microorganisms were Gram-positive species. However, three species of Gram-negative bacteria were identified, diverging from previous findings (Hall et al., 2014; Moran & Heuertz, 2017) that did not find any Gram-negative species. This divergence can be explained due to the evaluated environment, because in hospitals the protocols for decontamination of the environment are more rigorous than in a dental clinic. Such microorganisms are extremely resistant to harsh environments and are often associated with endodontic failure (Cardoso et al., 2016). Considering this, there is a need for further studies on the topic, as the literature is still very scarce when it comes to the impact of the use of gloves and their contamination in endodontic treatment.

5. Conclusion

The results of the present study demonstrated that NSG may be contaminated as soon as package is opened and their exposure to the dental office environment can also promote contamination. SG are sterile as indicated by the manufacturer but can also become contaminated during procedures. In the trans-operative of endodontic treatment, both NSG and SG can be contaminated by Gram-positive and Gram-negative bacteria.

Future studies on this subject including the coronavirus (COVID-19) and a broad range of microorganisms would add more relevant information to Endodontics' field. The potential of root canal cross infection from the contaminated gloves should also be more explored in further research.

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