Original Article

Does storage of silicone tubes prior to packaging prevent sterilization?

Armazenar tubos de silicone antes do empacotamento impede a esterilização?

¿Almacenar tubos de silicona antes del empaque impide la esterilización?

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Abstract
Objective: To determine the microbial load of silicone tubes, immediately after cleaning, and at different storage intervals.
Methods: Experimental study that analyzed silicone tubes from surgical patient care, conducted after approval by the Ethics Committee (protocol no. 1,277,077), from September to November of 2015, with tubes from the Central Processing Department (CPD) of a large general hospital in the West Central region of Brazil. The tubes were segmented (end 1 and 2, and the middle) and were then segmented again, according to established time intervals (zero, 12, and 24 hours). The fragments were filled with sterile water, sealed, and exposed to five minutes of sonication. The water was filtered via 0.45µm Millipore, and the membranes were incubated at 35°C for 24 hours, on nutrient agar. The membranes were removed and placed in test tubes containing 1mL of saline, which were mixed for five minutes, and subjected to a calibrated loop technique.
Results: An increase in microbial load was identified, in the order of a logarithmic magnitude every 12 hours ($p<0.05$), in the cleaning and storage conditions provided by the institution, in the experimental and positive control groups, and no difference was identified when comparing the middle and ends of the silicone tubes ($p> 0.05$) at periods zero, 12, and 24 hours.
Conclusion: Depending on the initial microbial load, an increase in the order of magnitude can result in sterilization failure, which corroborates the need to not maintain healthcare products in the storage place while awaiting processing.

Resumo
Objetivo: Determinar a carga microbiana de tubos de silicone imediatamente após a limpeza e em diferentes intervalos de armazenamento.
Métodos: Estudo experimental que analisou tubos de silicone oriundos da assistência ao paciente cirúrgico. Foi conduzido após aprovação do Comitê de Ética (protocolo nº 1.277.077), no período de setembro a novembro de 2015, com tubos oriundos do Centro de Material e Esterilização (CME) de um hospital geral de grande porte da região Centro-Oeste do Brasil. Os tubos foram segmentados: extremidade 01, 02 e meio e novamente segmentados, conforme intervalos de tempo preestabelecidos em zero, 12 e 24 horas. Os fragmentos foram preenchidos com água estéril, vedados e submetidos a cinco minutos de sonicação. A água foi filtrada em *Millipore 0,45 µm* e as membranas incubadas a 35ºC por 24 horas em ágar nutriente. As membranas foram removidas e dispostas em tubos de ensaio, contendo 1mL de solução salina, que foram agitadas por cinco minutos e submetidos a técnica de alça calibrada.
Resultados: Houve aumento da carga microbiana na ordem de uma grandeza na escala logarítmica a cada 12 horas ($p<0,05$), nas condições de limpeza e armazenamento proporcionados pela instituição, nos grupos experimental e controle positivo, e não houve diferença quando comparados o meio e extremidades dos tubos de silicone ($p>0,05$) nos períodos zero, 12 e 24 horas.
Conclusão: A depender da carga microbiana inicial, o aumento da ordem uma grandeza pode resultar no insucesso da esterilização, achados que ratificam a não permanência de PPS na área limpa aguardando o processamento.

Resumen
Objetivo: Determinar la carga microbiana de tubos de silicona inmediatamente después de su limpieza e en diferentes intervalos de almacenamiento.
Métodos: Estudio experimental en el que se analizaron tubos de silicona propios de la asistencia al paciente quirúrgico, en el período de septiembre a noviembre de 2015. Los tubos provenían del Centro de Material y Esterilización (CME) de un hospital general de gran tamaño de la región Centro-Oeste de Brasil. Los tubos fueron segmentados así: extremo 01, 02 y medio y nuevamente segmentados según intervalos de tiempo preestablecidos en cero, 12 y 24 horas. Los fragmentos se llenaron con agua estéril, fueron sellados y sometidos a cinco minutos de sonicación. El agua fue filtrada en Millipore 0,45 µm y las membranas incubadas a 35ºC por 24 horas en agar nutriente. Las membranas fueron removidas y dispuestas en tubos de ensayo que contenían 1mL de solución salina, fueron agitados durante cinco minutos y sometidos a técnica de alza calibrada
Resultados: Se observó un aumento de la carga microbiana en el orden de una magnitud en la escala logarítmica cada 12 horas ($p <0,05$), en las condiciones de limpieza y almacenamiento proporcionadas por la institución, en los grupos experimental y de control positivo. No hubo diferencia cuando se compararon el medio y los extremos de los tubos de silicona ($p> 0,05$) en los períodos cero, 12 y 24 horas.
Conclusión: Dependiendo de la carga microbiana inicial, el aumento del orden de una magnitud puede resultar en el fracaso de la esterilización. Estos hallazgos ratifican la no permanencia de PPS en el área limpia mientras se aguarda el procesamiento.

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Introduction

The cleaning of healthcare products (HP) is defined as a fundamental step in the sterilization process, and is proposed to remove waste from the surface of HP.[1] The presence of organic and inorganic waste compromises the effectiveness of material processing in a number of ways; in particular, it acts as a physical barrier and prevents the action of the disinfecting and sterilizing agent.[2,3] Efficient cleaning removes organic matter from the product, and consequently reduces the initial microbial load.

Several factors may compromise the cleaning step, including PPS characteristics such as tubular material including silicone tubing, which are processable and not specified as single use HP, as established by national legislation.[4] They consist of elastic, flexible, and resistant materials, are durable, even when successively submitted to temperatures ranging between -20ºC and 200ºC.[5]

The extension of these tubes, which can be up to two meters in length, depending on their purpose, is a factor that hinders direct friction. Nevertheless, they are widely used both in complex procedures such as cardiac catheterization, aspiration of organs and body cavities, blood transfusions and drainage, which makes them critical HPs.[6] In addition, they can be used in oxygen therapy and routinely are used as intermediaries between equipment, conducting and transporting gases, and under these conditions are considered semi-critical products.[6] The silicone tubes are sterilized by saturated steam under pressure, regardless of their use.

In order to facilitate the processing of these HP, it is indicated that the cleaning is initiated close to the location of their use, to prevent adherence of organic matter.[7] Initially, a manual cleaning is performed, using brushes appropriate for the length and diameter of the lumen, followed by automated cleaning using ultrasonic equipment, which aims to reduce the initial microbial load, or bioburden.[3,8]

Bioburden is defined as the amount of microorganisms present in the HP to be sterilized, which must not exceed the maximum acceptable microbial load; in laboratory conditions this is defined by 106 colony-forming units (CFU), similar to that present in the biological indicators used for monitoring of saturated steam under pressure autoclaves.[1,3,7]

The sterilization process of a HP must be executed after the cleaning step, and stored in a appropriate place that is free from external contamination, due the risk of increasing the initial microbial load,[9] and restricting the sterilization process. In the case of tube-shaped HP, the difficulty in the cleaning process and the waiting time for packaging in the clean area, a frequent practice in health services, led to the proposition of this study. Thus, the objective of this research was to determine the microbial load of silicone tubes, immediately after cleaning, and at different storage intervals.

Methods

This was an experimental study that used randomly selected silicone tubes as samples, obtained from surgical patient care during the intraoperative period, which were collected from the Central Processing Department (CPD) of a large general hospital in the West Central region of Brazil, from September to November of 2015. The silicone tubes were designed according to a diameter (0.6 cm) and length (150 cm) pattern, and were used for surgical procedures as well as for aspiration of organs and body cavities, as well as for oxygen therapy procedures. During the period of data collection, the hospital did not adopt a method of identifying these tubes according to the purpose of use.

Method Validation

Previous studies that performed a microbiological analysis on the whole extension of silicone tubes were not found, which justified the previous validation of the method. Sterilized water flushes were used, as described by other studies, to extract microbial load in long, tube-shaped tubing.[10-13]

For validation of the methodology of microbial load quantification, new silicone tubes, sterilized in a steam saturated, under pressure autoclave at 134º C, were used. The tubes had the same characteristics as those used in the experimental group, and

were carried to the Laboratory of Microbiological Analysis in Health of the Institute of Pathology and Public Health of the Federal University of Goiás (LAMSA/IPTSP /UFG) for artificial contamination. All procedures were performed by the researcher, properly dressed (mask, gloves, and lab coat) using a class II biological safety cabin.

The broth preparation containing 106 bacterial spores of Geobacillus stearothermophilus (ATCC 7953®) was obtained from a biological indicator unit containing the microorganism that was isolated and inoculated into Brain Heart Infusion (BHI) broth, sterilized and incubated in an oven at 35ºC for 24 hours. Positive broth controls were performed by visual confirmation of turbidity after 24 hours of incubation.

The bacterial solution was injected into the silicone tubes for induced microbiological contamination, using the calculation of the volume of geometric solids of cylindrical conformation. [14] Thus, the volume of solution to be injected was estimated considering the area of the tube, so that the liquid completely filled the internal area of the tubes.

Thus, a total of 42 mL of microbiological solution was used. The two ends of the tubes were sealed with plastic seal, the tubes were conditioned at room temperature for 80 minutes, to contaminate the inner walls of the lumen.[15] The seals were removed and the microbiological solution was autoclaved and discarded.

Then, a flush of distilled water was injected into the contaminated tubes, which were sealed again and subjected to five minutes of sonication,[16] in a 40 kHz frequency ultrasonic washer. The water injected into the lumen was collected and subjected to 0.45µm Millipore membrane filtration, using a syringe holder device. The flushes performed on the lumen of the tubes, and the recovery of the solutions, was performed using 60 ml syringes. The membrane was then removed from the device and placed on the surface of the nutrient agar plate, and maintained in an oven at 35 ° C for 24 hours.

After incubation, a growth of colony forming units (CFU) on the membrane surface was verified; however, due to the large amount of CFU on the membrane surface, counting was not possible. Thus, they were removed and placed in test tubes containing 1 mL of sterile 0.9% saline solution. The membranes were mixed by vortexing for five minutes, and then the calibrated-loop method (0.01 mL) was used for inoculation of the solution on the surface of the nutrient agar, followed by incubation for 24 hours at 35°C.[17,18] The procedure was able to recover the amount of microorganisms injected during the artificial contamination, demonstrating the effectiveness of the method used.

Experimental Group

Ten silicone tubes used in the care of patients in the transoperative period, from September to December of 2015, were included. These were gathered immediately after cleaning, and excluded those with visible grooves and signs of deterioration. The tubes were transported to the preparation area in sterile bags.

The ultrasonic tub was not used for cleaning silicone tubes, although it existed in the institution. The routine established for these tubes followed the flowchart: pre-rinse in running water, followed by immersion in enzymatic detergent (5 minutes, according to the manufacturer) and aspiration of the product until the lumen was filled, using a 20 mL syringe. Subsequently, the tubes were attached to a faucet adapter, rinsed and dried by compressed air, or arranged in a drain holder.

The silicon tubes of the experimental group were transported to the LAMSA/IPTSP/UFG, where analyses were conducted by a researcher, properly trained as described in the validation method. For the processing of the samples, the tube was initially fragmented using a sterile scalpel blade, and identified in three equal parts of 50 cm in length, noted as ends (E1 and E2) and middle (M).

Each of the three fragments of 50 cm was again fractionated into three other equal portions, constituting nine portions of 16.7 cm, in order to verify, more precisely, the distribution of the microbial contamination in the lumen of the tube, therefore, the sample consisted of 90 fragments (10 tubes x 9 parts). The fragments of the tubes were submitted to analysis, according to pre-established time intervals: zero, 12 and 24 hours.

The zero time portions were immediately analyzed, and the 12 and 24 hour time portions were stored under the same conditions as in the unit: in a 200 liter plastic basket with lid, in low light and non-refrigerated environment, simulating the conditions under which these tubes regularly remain in the CPD, awaiting the packaging step.

Negative control group

The negative control group was composed of new tubes not submitted to the cleaning step (n = 3), to verify contamination prior to use.

Comparison group

For establishment of acceptable contamination, tubes used in hospital care were used, randomly collected, in triplicate (n=3), under the same described conditions, but with the cleaning step done by the researcher.

For cleaning process, the following steps were performed: pre-rinsing with pressure filtered water with the aid of a water pistol system followed by immersion in enzymatic detergent for five minutes, with negative pressure in the lumen with a 20 mL syringe, as indicated by the manufacturer. The tubes were scrubbed with a brush compatible with the diameter of the lumen, and were subsequently removed from the solution and attached to the adapters in the ultrasonic washer for cleaning. After the cycle was completed, they were removed and externally dried, using gauze and compressed air inside the lumen. The values found were used to estimate, comparatively, the contamination of the tubes of the experimental group.

Positive control group

In order to confirm previous contamination prior to use, another group was created with three silicone tubes used in transoperative care, containing visible organic matter, immediately after arrival at the CPD, and preceding any cleaning steps.

Determination of the microbial load

For the analysis of the results and quantification of the microbial load in the tubes of the experimental and control groups, it was necessary to consider the microbial load present in the total length of the tube (150 cm). For this calculation, the mean of the microbial load between the three portions (E1, M, and E2) of 16.7 cm of each tube was used, for each of the time intervals established (zero, 12 and 24 hours).

The calculation of the mean of E1, M, and E2 enabled the identification of a representative value of CFU in the distribution of the fragment (16.7 cm). To estimate the total CFU value of the tube, the value found multiplied by the total tube length (150 cm) was used.

Data were processed using the IBM® SPSS® software - version 21.0, using descriptive and inferential statistics (Student's t-test), adopting $p<0.05$. The study was approved by Ethics Committee (protocol no. 1,277,077) and for its development, all the recommendations established at the Resolution 466/12 of the National Health Council (BRASIL, 2012) were met.

Results

In the negative control composed of new tubes, not undergoing the cleaning step, no microbial growth was identified. Table 1 presents the results of the comparison groups and positive control groups.

Table 1. Microbial load of silicone tubes of the comparison group and positive control group, according to the different time intervals

n Tube	CFU (T0)*	CFU (T12)**	CFU (T24)***
Comparison group			
1	$3.9.10^2$	$3.1.10^2$	$3.3.10^2$
2	$5.9.10^2$	$4.0.10^2$	$4.6.10^2$
3	$5.8.10^3$	$4.9.10^3$	$4.5.10^3$
Positive control group			
1	$4.8.10^6$	$4.0.10^7$	$3.7.10^8$
2	$5.3.10^5$	$4.7.10^6$	$4.6.10^7$
3	$5.8.10^5$	$5.4.10^6$	$5.0.10^7$

*T0 - Zero time **T12 –After 12 hours ***T12 - After 24 hours

In the experimental group, the increase of the microbial load found in the lumen of the silicone tubes (n = 10) of the experimental group, at the dif-

ferent time intervals, was of the order of one magnitude in a logarithmic scale every 12 hours.

The application of the t-test to verify the correlation between the increase of the microbial load of the silicone tube at the different time intervals (n=10) presented a statistical difference (p<0.05) between the increase of the microbial load in the silicone tubes, in relation to all the time intervals tested, as shown in table 2.

Table 2. Mean (M), standard deviation (SD) and t-test for paired samples between total microbial loads of silicone tubes (n=10) used in hospital care, according to time intervals, zero, 12 and 24 hours

	T0*	T12**	T0	T24***	T12	T24
M	1291.20	1546	1291.20	94550,70	1546	94550,70
SD	(772.69)	(822.96)	(772.69)	(45050,13)	(822,96)	(45050,13)
	p = 0.03		p< 0.01		p< 0.01	

*T0 - Zero time **T12 –After 12 hours ***T12 - After 24 hours

Table 3 shows the p-value between E1 and M, M and E2, and E1 and E2, in the tubes analyzed for the comparison of CFUs between the different portions of the tube, and the time intervals. There was no correlation between the microbial loads of the different portions of the tube for the same time interval (p <0.05).

The results show a statistically significant difference between the means of the E1, M, and E2 portions of the analyzed tubes, when submitted to different time intervals (zero, 12 and 24 hours), evidencing an increase in the microbial load between the portions stored at different times. There was no correlation between the microbial loads of E1, M, and E2 when analyzed in the same time interval, which shows a uniform contamination inside the tube.

Discussion

For the establishment of acceptable contamination of silicone tubes for this study, a comparison group was proposed using previously established gold standard cleaning.[3] In this group, microbial load up to 103 was found, which was considered standard for the comparison, as the level of contamination of a HP depends on its use.[3] In the experimental group, at time zero, acceptable microbial loads were found, ranging from 102 to 103.

There were no statistically significant differences when comparing the middle and the ends of the tube (Table 3), findings that are in agreement with a study that, when evaluating the sterility of ready-to-use silicone tubes, also did not find any difference.[19] This contests the initial hypothesis that the middle part could be more contaminated by presenting greater difficulty for direct mechanical friction with the use of brushes during cleaning.

An increase of the microbial load was verified, in the order of magnitude in the logarithmic scale every 12 hours, and statistical significance (p<0.05) was found between the initial microbial load and those found 12 and 24 hours later. No studies were found to evaluate the relationship between increased microbial load and post-cleaning storage time in HP, making it difficult to compare these results. An experimental study aimed to determine the relationship between elapsed time and increased microbial load on surgical instruments prior to the cleaning step. The surgical instruments were artificially contaminated, incubated for two, four, six, eight, 12, 24, 36 and 48 hours, and underwent microbial load analysis. The results showed a significant increase of the microbial load,

Table 3. Mean (M), standard deviation (SD) and t-test for paired samples for comparison of microbial load in the different portions of the silicone tube (n = 10)

Time		Total	E1* (CFU)	M***(CFU)	E2** (CFU)	$\rho_{(E1,M)}$	$\rho_{(M,E2)}$	$\rho_{(E1,E2)}$
0	M	1291.20	144.10	145.10	142.10	0.876	0.605	0.693
	SD	(772.69)	(85.59)	(88.28)	(86.06)			
12	M	13595.60	1481.00	1514.00	1546.00	0.632	0.580	0.273
	SD	(7370.40)	(815.52)	(844.43)	(822.96)			
24	M	94550.70	10320.00	10900.00	10360	0.370	0.235	0.951
	SD	(45050.13)	(5103.33)	(5275.52)	(4978.88)			

*E1 – End 1 **E2 – End 2 *** M - Middle of the tube

with a growth peak and consequent increase after the first six hours of use. [20] This is explained by the exponential increase of the population as the cells are divided.

Although there was progressive increase of the microbial load in the tubes, the evaluation after 24 hours showed that the levels of contamination varied between 10^4 and 10^5 CFU, remaining below 10^6 in the established time intervals, presenting maximum allowed microbial load for a PPS that will undergo sterilization. This study did not evaluate the behavior of the microorganisms present in the lumen after 24 hours of storage, and it was not possible to confirm if the storage after this period could cause subsequent increases, overlapping the limit of 10^6. However, it is possible to state that, depending on the microbial load achieved through cleaning, microbial growth after 24 hours in the tube, in the storage place, can lead to sterilization failure. The findings of this research reinforce the recommendation of not maintaining HP in the clean area, awaiting processing.[3]

In the negative control group, different behavior was detected when compared to the experimental group and the positive control, as the microbial load did not increase. It is known that the main purpose of cleaning in HP is to remove organic matter, allowing the penetration of the vapor and, consequently, the effectiveness of the sterilization process.[3] The increase of CFUs in the lumen of the tubes exclusively occurred in the experimental and positive control groups, indicating the probable presence of organic matter and flaws in the cleaning process of these silicone tubes; different behavior was found in the comparison group. It is worth mentioning that in this group, cleaning followed the gold standard recommendation for tubular HP. [1,3]

During the study, the routine established at the institution did not use cleaning by automated methods, even though the hospital had an ultrasonic washer, which may have been determinant for the failure in the cleaning process, especially by the presence of lumen. Studies have shown that the removal of organic waste and the reduction of bioburden were more effective when automated cleaning was used, especially in tube-shaped HPs.[21,22]

Healthcare services must provide a mechanism for safe reprocessing of HPs in use; and in case of silicone tubes, their reuse should be dependent on the functioning of the ultrasonic washer. In addition, it is necessary that the health services have the means to maintain control of the reuse of these HPs, validating the frequency of changing the tubes, according to each use. It is important to note that damage inside the lumen is not possible to be verified by visual inspection during the work routine. Hence, the damage caused by multiple reprocessing and inadequate cleaning are both factors that promote the formation of a biofilm, and compromise the sterilization process.[1,3,21,22]

The storage conditions may also be directly related to the progression of the load, as most microorganisms grow at temperatures ranging from 25°C to 40°C, i.e., at room temperature. [18]

In this sense, although it seems to be outside the scope of this study, but considering the possible relationship with the life of the tube, it is necessary to contest the practice of using the silicone tubes for different purposes. In the market silicone tubes are already available with colored longitudinal lines that facilitate the monitoring process within the sectors of the hospital institution, avoiding the use of tubes for suctioning in surgical procedures that are destined to other sectors. These tubes must be separated according to their purpose, in order not to mix different microbial loads during reprocessing. Stages of processing, such as immersion in the same enzymatic solution, and using the same brushes for mechanical friction process may, unnecessarily, expose a tube used in ventilatory care with blood in a tube used for suctioning the abdominal cavity.

Conclusion

An increase in the microbial load was identified, in the order of a logarithmic magnitude, every 12 hours ($p<0.05$), in the cleaning and storage conditions provided by the institution, both in the experimental and positive control groups. No statistically significant difference was identified when comparing the middle and the end parts of the material at periods zero, 12 and 24 hours, demonstrating that

the microbial load is uniform through the lumen extension. Even with the consecutive increase, the microbial load remained below the limit of 10^6 CFU for up to 24 hours. However, storage for longer times does not mean a higher level of contamination, requiring further studies that aim to verify microbial behavior at longer intervals. Depending on the initial microbial load, the increase identified in this study can result in unsuccessful sterilization, and therefore, CPD technicians are recommended to plan and supervise, to perform the steps subsequent to cleaning with agility, as a quality control measure for the sterilization processing of silicone tubes, and probably all tubular HPs.

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Collaborations

Trindade JPA, Vasconcelos LSNOL, Ribeiro EL, Watanabe E and Tipple AFV contributed to the study design, analysis, data interpretation, article writing, and final approval of the version to be published.

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