Evaluation of fabrics' resistance for surgical aprons after washing-sterilization process

Avaliação da resistência de tecidos para aventais cirúrgicos após processo de lavagem e esterilização

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Talita Nicolau de Oliveira Vidal de Negreiros
Doutoranda em Engenharia Têxtil na Universidade do Minho
Instituição: Universidade do Minho
Endereço: Departamento de Engenharia Têxtil, Universidade do Minho, Campus de Azurém, 4800-058 Guimarães, Portugal
E-mail: tali_nicolau@hotmail.com

Katiany do Vale Abreu
Doutora em Biotecnologia pela Universidade Estadual do Ceará
Instituição: Universidade Estadual do Ceará
Endereço: Departamento de Química, Campus do Itapery, Av. Dr. Silas Munguba, 1700 - Itaperi, Fortaleza - CE, 60714-903, Brasil
E-mail: katianyabreu@yahoo.com.br

Núbio Vidal de Negreiros Gomes Filho
Doutorando em Gestão pela Universidade do Minho
Instituição: Universidade do Minho
Endereço: Escola de Economia e Gestão, Rua da Universidade, 4710-057 Braga, Portugal
E-mail: nubiovidal@hotmail.com

Carlucio Roberto Alves
Doutor em Química pelo Instituto de Química de São Carlos da Universidade de São Paulo
Instituição: Universidade Estadual do Ceará
Endereço: Departamento de Química, Campus do Itapery, Av. Dr. Silas Munguba, 1700 - Itaperi, Fortaleza - CE, 60714-903, Brasil
E-mail: alvescr@yahoo.com

Luciana França Jorge
Mestranda em Saúde Coletiva pela Universidade de Fortaleza
Instituição: Universidade de Fortaleza - UNIFOR
Endereço: Av. Washington Soares, 1321, Bairro Edson Queiroz, Fortaleza - CE, 60811-905
E-mail: lucianafjorge@gmail.com

Antonia Debora Sales
Doutora em Biotecnologia pela Universidade Estadual do Ceará
Instituição: Centro Universitário Estácio do Ceará
Endereço: Av. Duque de Caxias, 101 - Centro, Fortaleza - CE, 60035-111
E-mail: debissales@gmail.com
ABSTRACT
A surgical center is a unique place for biochemical risks, specially concerning surgical aprons, as they tend to be soaked with fluids from both patients and its users during procedures. This study evaluates how the common fabrics used as raw material in reusable surgical aprons behave after washing-sterilization process. In order to perform such analysis, this study uses three types of fabrics: 100% cotton, mixed (67% cotton and 33% polyester), and 100% polyester. The variables “grammage,” “pore area,” and “bacterial growth” were evaluated in three different moments. The variables “grammage” and “pore area”, presented less wear out in cotton fabric. However, it is noticed that textiles with natural fibers (e.g., cotton or mixed) had a higher incidence of bacterial growth. Even though cotton fabric presented one of the highest incidences of bacterial infestation, it was chosen as the best raw material for surgical aprons. To solve this problem mentioned above, we suggested using antibacterial finishes, which are common while manufacturing reusable surgical aprons.

Keywords: Surgical apron, Textile fiber, Antimicrobial barrier.

RESUMO
O centro cirúrgico é um ambiente único para riscos bioquímicos, especialmente considerando aventais cirúrgicos, os quais tendem a ficar molhados de fluidos tanto dos pacientes quanto dos seus usuários durante os procedimentos. Este estudo avalia como as principais composições têxteis, utilizadas como matéria-prima dos aventais cirúrgicos reutilizáveis se comportam após o processo de lavagem-esterilização. A fim de fazer tal avaliação, este estudo utilizou três tecidos: 100% algodão, misto (67% algodão e 33% poliéster) e 100% poliéster. As variáveis “gramatura”, “área do poro” e “crescimento bacteriano” foram avaliadas em diferentes momentos. As variáveis “gramatura” e “área do poro” apresentaram menor desgaste para tecidos 100% algodão. Contudo, tecidos com fibras naturais (misto e 100% algodão) apresentam resultados piores neste quesito. Apesar dos tecidos de algodão apresentarem maiores incidências bacterianas, este tecido representa uma escolha mais acertada como matéria-prima dos aventais cirúrgicos reutilizáveis. A fim de resolver o problema da infestação de microrganismos, é possível o uso de acabamentos germicidas, os quais são comumente utilizados em aventais cirúrgicos reutilizáveis.

Palavras-chave: Aventais cirúrgicos, Fibra têxtil, Barreira Antimicrobiana.

1 INTRODUCTION
A surgical center is a unique place where biochemical risks may happen. Thus specific textiles adequate for such particularities and needs are necessary, since the use of inappropriate material contributes to eventual contamination during surgical procedures (Kieser et al., 2018; Kishwar & Ali, 2017; Moradi et al., 2019), especially in clothing (Maqsood et al., 2016; Rogina-Car, 2018).

Surgical vestment comprehends a set of physical barriers that prevent microorganisms from spreading in the operative wound (Kieser et al., 2018; Maqsood et al., 2016) and avoid professionals’ exposure (Khomarloo et al., 2019; Maqsood et al., 2016; Moradi et al., 2019). Several pieces and different materials compose the vestment, each with its peculiarities (Cataneo et al., 2004; Gutarowska & Michalski, 2012; Teixeira et al., 2014), then it is necessary to study each equipment separately; therefore, this study focus on the surgical apron, precisely the reusable types.
Surgical aprons need to use wear-resistant materials (Rogina-Car, 2018) that eliminate as little residue as possible to minimize the release of particles in the wound and the operating room. On the other hand, they should also allow steam passes through them during sterilization processes and resist various reprocessing rounds, meaning the washing and sterilization processes.

Some studies explain that a wet apron completely loses its barrier against bacteria, as they benefit from a liquid environment to move. These studies also claim the importance of fabric inspection after each sterilization cycle to ensure its effectiveness as a physical barrier (Boryo, 2013; Leonas, 1998; Stanewick & Kogut, 1997; Teixeira et al., 2014).

Among the used fibers, cotton is the most susceptible to attack by fungi and bacteria, because its structure retains water, oxygen, and nutrients, favoring the growth of microorganisms (Magalhães, 2015).

In Brazil, although there are norms determining testing parameters to be followed during the manufacture of surgical aprons (ABNT NBR, 2020), there are still many caveats in these standards, as they lack suitable textiles and fibers; reprocessing, washing-sterilization, cycles (parameters and maximum amount); a range for pore size (area or diameter) for the textile be useful a physical barrier. Additionally, the apron becomes more soaked as more invasive the procedure is, leaving its user exposed and uncomfortable. Therefore, this study evaluates how the common fabrics used as raw material in reusable surgical aprons behave after washing-sterilization process.

2 METHODS

As this study aims to evaluate how the common fabrics used as raw material in reusable surgical aprons behave after washing-sterilization process, this section describes the methods used to reach our results. First, in section 2.1, there is a description regarding sample, what the rounds crucial to the analysis are, and the variables used to evaluate the raw materials behavior after washing-sterilization process. Section 2.2 describes which laboratory methods were used, while section 2.3 defines the statistical methods used in this raw materials' behavior analysis after washing-sterilizing process.

2.1 SAMPLES, ROUNDS, AND VARIABLES

This study uses three different types of fabrics: 100% cotton, mixed (67% cotton and 33% polyester) and 100% polyester. From each one, there were four samples, coded with an alphanumerical code composed by the fabrics initial (C, M, and P) and a value of 1 to 4 in order to identify them during rounds better.
Each round was numbered from 1 to 3, being: 1) the moment before any procedure; 2) after five complete washing-sterilization processes; and, 3) after completing the 10th cycle washing-sterilization processes.

In each round, the variables “grammage” and “pore area” help to observe the fabric’s structural damage during washing-sterilization process. As the fabrics wear out, there is a reduction in their physical antimicrobial barriers (Khomarloo et al., 2019). The variable “grammage”, followed the formula presented at NBR 10591/08 with a transformation, once our samples had areas four times bigger than the described in it. The third variable “bacterial growth” was evaluated at rounds 2 and 3, by contaminating samples 3 and 4 of each type with bacteriological broth composed of two pathogenic bacteria: *Staphylococcus aureus* ATCC27664 and *Bacillus sp.* In contrast, samples 1 and 2 were submitted to a bacteria-free broth (white). This process was repeated before rounds 2 and 3. Both bacteria are common in human skin microbiota, being the first (*Staphylococcus aureus*) generally used in this kind of research, whereas *Bacillus sp.* are allegedly more resistant to this process for producing spores.

### 2.2 LABORATORY METHODS

For activating the strain, the bacterium *Staphylococcus aureus* ATCC27664 was obtained from EMBRAPA Tropical Agroindustry, while *Bacillus sp.* was available in the laboratory. The bacterial strains were activated in the TSA culture environment (Tryptic Soy Agar) sterilized in an autoclave at 121°C for 15 minutes. Maintenance and peal of isolated cultures were done in Petri dishes containing TSA environment, autoclaved at 121°C for 15 minutes. The TSA spiked plates were incubated in a bacteriological oven at 30°C for 24 hours to obtain the culture for fermentative assays with the purpose of cell growth.

The inoculum’s culture carbon source was 10.0 g/L of glucose in mineral environment with reactive analytic pattern, 1.0 g/L of (NH₄)₂SO₄ as source of nitrogen, 5.0 g/L of yeast extract, 6.0g/L of Na₂HPO₄, 3.0g/L of KH₂PO₄, 2.7g/L de NaCl e 0.6g/L of MgSO₄. 7H₂O with 0.1% micronutrients solution: ZnSO₄ 10.95g/L, FeSO₄ 5.0g/L, MnSO₄g/L, CuSO₄ 0.39g/L, Co(NO₃)₂ 0.25g/L, Na₂B₄O₇ 0.17g/L, EDTA 2, 5.0g/L sterilized at 110°C for 10 minutes. Three growth loops in the exponential growth phase were transferred to 250 ml Erlenmeyer flasks containing 50 ml inoculum culture environment, incubated at 150 rpm, 30°C for 24 hours on a rotary shaker. The inoculum was adjusted to a range of 0.1 to 0.2 in a spectrophotometer (at 600 nm). The fabrics were contaminated with a bacterial solution, as mentioned before, using a spray. After the procedure, they were dried in an air circulating heating chamber at 30°C until completely dried.
The washing process was performed through a Brazilian public hospital, which outsources it. The samples were submitted to this process along with surgical aprons and other parts used in their surgical center to simulate a standard procedure that a surgical apron passes. The sterilization, on the other hand was carried out after each washing process, using the following parameters: 121ºC for 15 minutes as in (Khomarloo et al., 2019). A total of 10 reprocessing cycles (washing and sterilization) were carried out at each until contamination appeared.

After every five washing cycles, the samples were smeared with a sterile swab in sterilized Petri dishes with APGE culture environment (Agar, Peptide, Glucose, and Yeast Extract) in a laminar flow cabinet and then incubated in a bacteriological oven at 30ºC for 48 hours to check bacterial growth.

2.3 STATISTICAL METHODS

For better determining inferential techniques, a Shapiro-Wilk's normality test were used in "pore area", and "grammage", since it has a good performance with small samples (Razali & Wah, 2011).

The "pore area" variable was analyzed by One-way ANOVA for repeated measurements, because it allows the analysis of multiple individuals in contrast with them in other moments, making it possible to measure the effect of the rounds on the pore size in each of the fabric (A. J. Rodrigues, 2017; Viali, 2007). To perform this test, homoscedasticity should be also evaluated, then we used Mauchly's sphericity test (Diazaraque, 2015; Malheiros, 2012). When the ANOVA's null hypothesis was rejected, a post-hoc test was used to define the estimated mean difference between the results of each of the periods, and the confidence intervals corrected by the Bonferroni's method, which is more conservative and controls the type-I error (Diazaraque, 2015).

Regarding the wear's hypothesis and, consequently, the relationship between "grammage" and "mean area of the pore of each fabric" a Pearson's correlation was performed to verify if such a relationship exists and its strength.

3 RESULTS

This section presents the main results found in this evaluation on how the common fabrics used as raw material in reusable surgical aprons behave after washing-sterilization process. The first results presented are descriptive (Table 1), mean (M), and standard deviation (SD). Regarding the variable “pore area” it is possible to notice that, before any rounds, the polyester's samples had larger pore average area and lesser grammage than the others. As the rounds follow, this trend maintains. The results are already worrying, since a bacterium measures between 0.5 to 10mm² (Stanewick & Kogut, 1997; Teixeira et al., 2014). On the other hand, for the variable “grammage”, it is possible to notice
that all the fabrics presented reduction as the rounds continue. However, after the complete cycles and even dry appearance, they first had presented larger grammages, this happens because fibers absorb water vapor, increasing their mass and re-adjusting filaments, generating values up to 25% larger (E. Rodrigues et al., 2006). From this result, these results were computed only after processing in an air circulating greenhouse and using the silica drying method.

| Cycles | Cotton | Mixed | Polyester |
|--------|--------|-------|-----------|
|        | Area M (DP) | Gram. M (DP) | Area M (DP) | Gram. M (DP) | Area M (DP) | Gram. M (DP) |
| 0      | 1.69 (0.60) | 726.13 (10.47) | 1.05 (0.38) | 516.31 (9.22) | 3.92 (1.74) | 440.25 (11.47) |
| 5      | 1.77 (0.50) | 720.88 (10.15) | 1.54 (0.37) | 514.31 (9.28) | 4.34 (1.25) | 439.44 (11.12) |
| 10     | 2.05 (0.28) | 703.69 (9.91) | 2.81 (0.51) | 513.63 (9.08) | 6.72 (1.09) | 439.25 (10.88) |

Source: IBM® SPSS v. 23 (2020). Observation: The “Area” was measured in µm², while “Gram.”, representing Grammage is given in g/m².

Concerning the normality test, fabrics variables (area and grammage) every round did not reject normality, being the lowest p-value 0.275, and the highest, 0.883. From Mauchly’s sphericity test (Table 2), the homoscedasticity hypothesis was not rejected in any fabric. Cotton’s pore area (Table 2) are not statistically different (F(2,10) = 2.302, p: 0.124) as rounds pass, meaning that, even though there has been a descriptive change in its values, it is not possible to guarantee that the results are different for population, a good aspect for such fiber. Differently the changes in both mixed and polyester fabrics are extremely significant (F(2,10) = 57.861, p: 0.000 and F(2,10) = 17.734, p: 0.001, respectively).

| Textile | Mauchly’s test | Multivariable tests |
|---------|----------------|---------------------|
|         | p-value | df | F | df (Rounds) | df (Error) | p-value |
| Cotton  | 0.164    | 2  | 2.302 | 2  | 10  | 0.124 |
| Mixed   | 0.340    | 2  | 57.861 | 2  | 10  | 0.000 |
| Polyester | 0.523 | 2  | 17.734 | 2  | 10  | 0.001 |

Source: IBM® SPSS v. 23 (2020). Observations: “Mauchly’s test” represents the Mauchly’s sphericity test.
Therefore, we performed a posthoc test to evaluate such differences within rounds. For mixed fabrics (Table 03), every round was statistically different from the others, but especially from rounds 2 to 3, it is expected, with 95% confidence and Bonferroni’s adjustment, that a random pore’s area augments from 0.792 mm² until 1.748 mm². This result is noteworthy because, despite pore’s area after the 3rd round, the smallest microorganisms will be able to pass these fabric’s physical barriers.

| Round (I) | Round (J) | Mean difference (I-J) | p-value | Confidence Interval* |
|-----------|-----------|-----------------------|---------|----------------------|
| 0         | 5         | -0.485                | 0.004   | -0.802               |
|           | 10        | -1.755                | 0.000   | -2.198               |
| 10        | 5         | 1.270                 | 0.000   | 0.792                |

Source: IBM® SPSS v. 23 (2020). Observations: The results for the three first columns are in μm². "*" means Bonferroni’s adjusted.

The same analysis may be performed to polyester fabric (Table 04) since there is also a difference between rounds 2 and 3, but in this fabric’s case, the pores are expected to enhance from 1.176 mm² until 3.582 mm², allowing even bigger microorganisms pass through it.

| Round (I) | Round (J) | Mean difference (I-J) | p-value | Confidence Interval* |
|-----------|-----------|-----------------------|---------|----------------------|
| 0         | 5         | -0.420                | 1.000   | -2.009               |
|           | 10        | -2.799                | 0.002   | -4.433               |
| 10        | 5         | 2.379                 | 0.000   | 1.165                |

Source: IBM® SPSS v. 23 (2020). Observations: The results for the three first columns are in μm². "*" means Bonferroni’s adjusted.
The wear hypothesis, a relationship between “grammage” and “pore area”, was analyzed by Pearson’s correlation, resulted in a moderate negative relation (-0.617), but this assumption can be held only with a 90% confidence level.

On bacterial growth, some impressive results have been found. The first and most important is that there was fabric contamination in the third round (after ten cycles). On this contamination, there was no pattern in its results, as expected. For this variable, however, tissues with natural fibers (cotton and mixed) were the ones with the highest bacterial growth, consistent with the literature because there are moisture, oxygen, and food for microorganisms to survive (Boryo, 2013; Gutarowska & Michalski, 2012; Khomarloo et al., 2019; Leonas, 1998; Magalhães, 2015; Oliveira et al., 2010).

It is also possible to notice that from the microscopic evaluation and Gram staining, they are gram-positive Bacillus, due to its rod format, spores, and final coloring (Image 1).

This finding is convergence with findings in other studies (Burgatti, J. C., Possari, J. F., Moderno, 2004; E. Rodrigues et al., 2006; Teixeira et al., 2014). It is worth noticing, though, that most studies use different sterilization methods according to internal norms of each institution (hospital laundry or industrial laundry services), lacking a standardization. For example, in hospital A, each sterilization cycle lasts for 17 minutes using temperatures ranging from 121°C to 131°C, hospital B uses temperatures of 121°C to 123°C for 30 minutes, in a third hospital C they used a sterilization process of 134°C for 45 minutes, while a fourth institution utilized 134°C for 12 minutes (Burgatti, J. C., Possari, J. F., Moderno, 2004; Neves et al., 2004; E. Rodrigues et al., 2006). These differences may influence on the quality of the sterilization processes.
4 CONCLUSION

Surgical infection control is directly related to the clothing and fields used during this procedure. These items are cardinal as antimicrobial barrier; therefore, the correct choice of suitable raw materials for their manufacture is the utmost.

This study evaluates how the common fabrics used as raw material in reusable surgical aprons behave after washing-sterilization process. To perform such behavior analysis, the three most common raw materials used in reusable surgical aprons were evaluated. From the results, the inadequacy of mixed and polyester fabrics is observed concerning the “area of the pore” and “grammage”, because as the washing-sterilization provoked physical changes, enabling the passage of microorganisms. After these results, the cotton denim fabric is the most appropriate, among the tested ones, as raw material for surgical aprons because it presents a larger grammage and a smaller pore size after ten cycles.

In the case of bacterial growth, all types presented growth after the tenth cycle. Compared to the previous variables, the results are reversed since polyester was adequate compared to the textiles having natural fibers (cotton and mixed). However, antimicrobial finishing is a possible solution for such a caveat, as the textiles after this treatment became a lesser attractive environment for bacterial growth, especially in natural fiber textiles.

Another crucial result is the inverse relationship between “grammage” and the average “area of the pore” since the fabrics due to the washing-sterilization processes go through physical processes that wear them out.

The third finding of this study is the lack of standardization in dry or wet antimicrobial barrier efficiency tests for hospitals or industrial laundries as the reuse happens. Most studies in the past only evaluated the permeability of the protection barrier while it was dry. Recently, the wet fabric’s barrier has been being considered, a common situation in the operating room.

For future studies, we suggest developing a surgical apron that meets the team’s real needs and the patient. Various auxiliary studies can be made from this, for example, fabric creation with novel fibers, non-toxic antimicrobial finishes that resist washing, diffusion of anti-flame treatment, and treatments that increase the hydrophobic capability of the apron and its comfort.

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