Avaliação microbiológica realizada em equipamentos de aerosolterapia hospitalar
Microbiological evaluation performed in hospital aerosoltheraphy equipment
Evaluación microbiológica realizada en equipos hospitalarios de terapia de aerosol

Resumo
A investigação da contaminação microbiológica em equipamentos de uso hospitalar permite avaliar se os processos de descontaminação adotados pelo serviço de saúde são adequados e contribui para minimizar os riscos de infecções relacionadas à assistência à saúde. Com base nisso, este estudo teve como objetivo avaliar a contaminação microbiológica de nebulizadores em três serviços de saúde e fazer uma breve associação entre métodos de contaminação e reprocessamento. Dez amostras de nebulizadores (máscara e copo reservatório) foram coletadas, com swabs umedecidos em solução salina e semeado em meio de cultura seletivo.
para bactérias e fungos. Após 24-48 horas de incubação a 37 °C, foi realizada a identificação por testes bioquímicos de culturas positivas. Também foi realizada uma análise dos protocolos de reprocessamento das unidades de saúde. 40% das amostras analisadas apresentaram contaminação por algum tipo de microrganismo. *Enterobacter* sp., bacilos Gram-positivos e *Staphylococcus* coagulase-negativos foram as amostras encontradas neste estudo. Apenas um hospital, que seguiu o recomendado para o produto de pré-lavagem e desinfecção, apresentou todas as suas amostras sem contaminação microbiológica. Assim, pode-se concluir que há fragilidade no reprocessamento desses equipamentos contaminados, cujas falhas na desinfecção impõem riscos à saúde do usuário.

**Palavras-Chave:** Infecção hospitalar; Doenças respiratórias; Contaminação microbiológica.

**Resumen**

La investigación de la contaminación microbiológica en los equipos para uso hospitalario nos permite evaluar si los procesos de descontaminación adoptados por el servicio de salud son adecuados y contribuye a minimizar los riesgos de infecciones relacionadas con la atención médica. En base a esto, este estudio tuvo como objetivo evaluar la contaminación para bactérias e fungos. Após 24-48 horas de incubação a 37 °C, foi realizada a identificação por testes bioquímicos de culturas positivas. Também foi realizada uma análise dos protocolos de reprocessamento das unidades de saúde. 40% das amostras analisadas apresentaram contaminação por algum tipo de microrganismo. *Enterobacter* sp., bacilos Gram-positivos e *Staphylococcus* coagulase-negativos foram as amostras encontradas neste estudo. Apenas um hospital, que seguiu o recomendado para o produto de pré-lavagem e desinfecção, apresentou todas as suas amostras sem contaminação microbiológica. Assim, pode-se concluir que há fragilidade no reprocessamento desses equipamentos contaminados, cujas falhas na desinfecção impõem riscos à saúde do usuário.

**Palavras-Chave:** Infecção hospitalar; Doenças respiratórias; Contaminação microbiológica.

**Abstract**

The investigation of microbiological contamination in hospital use equipment makes it possible to assess whether the decontamination processes adopted by the health service are adequate and contribute to minimize the risks of Health Care-Related Infections. Based on this, this study aimed to evaluate the microbiological contamination of nebulizers in three health services and also to make a brief association between contamination and reprocessing methods. Ten samples of nebulizers (mask and reservoir cup) were collected, with swabs moistened with saline solution and sown in culture media selective for bacteria and fungi. After 24-48 hours of incubation at 37 °C, identification by biochemical tests of positive cultures was performed. An analysis of the health unit reprocessing protocols was also carried out. 40% of the analyzed samples presented contamination by some type of microorganism. *Enterobacter* sp., Gram positive bacilli and coagulase-negative *Staphylococcus* were the specimens found in this study. Only one Hospital, which followed the recommended for pre-washing and disinfection product, presented all its samples without microbiological contamination. Thus, it can be concluded that there is weakness in the reprocessing of these contaminated equipment, whose failures in disinfection impose risk to the user's health.

**Keywords:** Hospital infection; Respiratory diseases; Microbiological contamination.
microbiológica de los nebulizadores en tres servicios de salud y hacer una breve asociación entre la contaminación y los métodos de reprocesamiento. Se recogieron diez muestras de nebulizadores (mascarilla y copa de depósito), con hisopos humedecidos en solución salina y sembrados en un medio de cultivo selectivo para bacterias y hongos. Después de 24-48 horas de incubación a 37 °C, se realizó la identificación mediante pruebas bioquímicas de cultivos positivos. También se realizó un análisis de los protocolos de reprocesamiento de la unidad de salud. El 40% de las muestras analizadas presentaron contaminación por algún tipo de microorganismo. Enterobacter sp., Bacilos grampositivos y Staphylococcus coagulasa negativo fueron las muestras encontradas en este estudio. Solo un hospital, que siguió lo recomendado para el producto de prelavado y desinfección, presentó todas sus muestras sin contaminación microbiológica. Por lo tanto, se puede concluir que existe una debilidad en el reprocesamiento de este equipo contaminado, cuyas fallas en la desinfección imponen riesgos para la salud del usuario.

**Palabras clave:** Infección hospitalaria; Enfermedades respiratorias; Contaminación microbiológica.

1. **Introduction**

During the provision of hospital services, failures can occur, which lead to a direct worsening of the patient's health and their perception of hospital care. As a result, an acquired condition may arise, which was not determined in the patient's basic clinical condition, and may progress to death, definitive and transient sequelae, as well as psychological distress, in addition to raising the cost of assistance services. In a characterization of the most frequent acquired conditions, it was observed that 40.7% of the occurrences were caused by general devices for hospital use such as nebulizers (Couto, 2018).

People with respiratory illnesses like asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis usually need aerosol therapy equipment. However, as these devices come into contact with respiratory system secretions, in addition to microbial contamination of the skin and mucous membranes, they can be characterized as vehicles of cross-infection (Gaetti, et al., 2010). It is known that any pathogens in the respiratory tract of users of these devices they can survive on inanimate surfaces such as masks and nebulizer nozzles and contaminate the hospital environment (Zuckerman, et al., 2009).

About 235 million people are affected by asthma in the world, constituting the most common chronic disease of childhood, although it affects all age groups, with high
prevalence, morbidity and mortality worldwide. Brazil follows this worldwide trend with a high prevalence of asthma in children, with high rates of severe asthma, causing a representative number of hospitalizations (Cardoso, et al., 2017; Solé, Aranda & Wandalsen 2017).

According to the World Health Organization (WHO), there are about 65 million people with moderate to severe chronic obstructive pulmonary disease worldwide. The Organization estimates that this disease will be responsible for the third leading cause of death worldwide by 2030. Most of the information on COPD-related prevalence, morbidity and mortality comes from developed countries, although about 90% of deaths occur in poor countries or emerging (Mathers, 2011).

Between 2009 and 2016, in Brazil, 4,654 cases of cystic fibrosis were registered. The number of records and follow-up grows. Despite this increase, more than 60% of patients have at least three years of follow-up and 76.5% have at least 2 years of follow-up in this data survey. During this period, 249 deaths were observed (GBEFC, 2016).

The use of aerosol therapy is often done by patients with chronic respiratory diseases, and lung disorders associated with tobacco. The therapeutic option by inhalation is preferred for the administration of bronchodilators, which form the basis for the symptomatic treatment of obstructive pulmonary diseases (Gaetti, et al., 2010; Coelho, et al., 2011; Gold, 2019).

The investigation of possible microbiological contamination in equipment for hospital use is necessary, since it makes it possible to evaluate whether the decontamination processes adopted by the health service are adequate, contributing to minimize the risks of Infections Related to Health Care. In this context, the relevance of this research in relation to public health is highlighted, as it evaluates the microbiological quality of health care equipment for aerosol therapy from three hospitals, as well as whether reprocessing methods are effective for safe reuse.

2. Methods

In this experimental research, a total of ten nebulizer samples were collected from three Brazilian hospital units: two in the state of Paraíba that will be called “A” and “B”, as well as one in Rio Grande do Norte (C). These aerosol therapy equipment disinfected (reprocessed) by the hospital units, were available for immediate use by patients. At the time of collection, sterile swabs were used, moistened in 0.9% sodium chloride saline solution, which were rubbed on the inner and outer surfaces of the equipment's masks and reservoirs.
(cups). After that, these swabs were packed in a tube with 0.9% saline solution and transported in a thermal container with ice (Blau, et al., 2007).

The samples were sown within 3 hours after the material was collected, at the “Laboratório de Microbiologia” of the “Centro de Educação e Saúde – CES”, in “Universidade Federal de Campina Grande (UFCG)”, Cuité/PB campus. For this purpose, four culture media were used: MacConkey Agar (MCA) selective for Gram negatives, Mueller Hinton Agar (MHA) as a non-selective medium, Salty Mannitol Agar (SMA) recommended for isolation of the genus *Staphylococcus* and Sabouraud Dextrose Agar (SDA) to check for possible contamination by fungi. All media were prepared according to the manufacturer's recommendations. The collected material was inoculated into the media through the swab itself. Subsequently, the cultures were incubated in a microbiological oven at 37 °C for a period of 24-48 hours.

After this period, positive cultures were evaluated to identify the colonies, using Gram stain, biochemical tests of identification (urea, citrate, lysine decarboxylase, TSI (Triplice Sugar Iron) and MIO medium (Motility, Indole, Ornithine), in addition to observation macro and micromorphological characteristics. The methodological details can be seen in the flowchart of Figure 1.

Since this research involved microorganisms, its access activity was registered in the “Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado” (SisGen) in compliance with Law No. 13,123/2015, under A581B57 registration number.
Figure 1. Flowchart of processing samples collected from aerosol therapy equipment.

Source: Authors.

3. Results

Ten samples of nebulizers (reservoir cup and mask) were evaluated, four from Hospital “A”, two from Hospital “B” and four from Hospital “C”. All samples had been reprocessed and were available for immediate use by patients.

After the incubation period, contamination by bacteria was observed in 4 samples (40%), as shown in Table 1. There was no fungal growth in the culture media used.
Table 1. Culture results of samples collected in nebulizers for hospital use.

| HOSPITAL | SAMPLE | MHA  | SMA  | MCA | SDA  | RESULT                      |
|----------|--------|------|------|-----|------|-----------------------------|
| A        | 1      | -    | -    | -   | -    | -                           |
| A        | 2      | +    | -    | +   | -    | *Enterobacter* sp.          |
| A        | 3      | -    | -    | -   | -    | -                           |
| A        | 4      | -    | -    | -   | -    | -                           |
| B        | 5      | -    | -    | -   | -    | -                           |
| B        | 6      | -    | -    | -   | -    | -                           |
| C        | 7      | -    | -    | -   | -    | -                           |
| C        | 8      | +    | +    | -   | -    | Coagulase (-); *Staphylococcus* |
| C        | 9      | +    | +    | -   | -    | Gram positive bacillus      |
| C        | 10     | +    | +    | -   | -    | Coagulase (-); *Staphylococcus*; Gram positive bacillus |

Caption: + (presence of microorganism); - (absence of microorganism). Culture media: MHA - Muller Hinton Agar; SMA - Salted Mannitol Agar; MCA - MacConkey Agar; SDA - Sabouraud Dextrose Agar.
Source: Authors.

In view of a comparative analysis between the results obtained and the disinfection protocols that instructed about the method of reprocessing the nebulizers, available for consultation at the locations of the collected samples, it can be inferred that there are weaknesses in these methods. Table 2 shows this comparison.

Table 2. Comparison between previous cleaning, disinfectant and bacterial development.

| Health Unit | Previous cleaning | Disinfectant          | Bacterial development |
|-------------|-------------------|-----------------------|-----------------------|
| A           | No                | Trisodium orthophosphate | Present              |
| B           | Yes               | Sodium hypochlorite   | Absent                |
| C           | No                | Sodium hypochlorite   | Present               |

Source: Authors.
Hospital "A" did not use a disinfectant solution and did not perform previous cleaning. The disinfection was performed with a substance classified as detergent, a fact that may justify bacterial development in one of the samples in this unit.

Units “B” and “C” used sodium hypochlorite as a disinfectant, however unit “C” did not perform previous cleaning, and showed bacterial growth in most of its samples. Only unit “B” did not show bacterial development in its samples, as it was also the only one that performed the previous cleaning step, thus reaffirming the importance of this procedure before disinfection.

4. Discussion

The detection of bacteria in 40% of nebulizer samples was similar to the results found by Zuana, et al., (2014) in which 43.5% of the samples presented some bacterial contamination.

McLaurine, et al., (2019) report that contamination of this equipment is frequent after being repeatedly used and reprocessed, in view of the durability of these tools, they are not frequently replaced in the hospital environment.

However, the positivity shown in some samples by Coagulase-Negative Staphylococcus (CNS) ratifies a Brazilian study that found this, as the most frequent pathogen contaminating nebulizers (Cataneli & Cunha, 2014). Even though they are bacteria of the normal microbiota, considered non-pathogenic, their finding lights up a “signal alert”, on the need for improvements in the decontamination process for the reuse of this equipment.

Regarding the isolation of Enterobacter sp., It is noteworthy that this microorganism was found in a study carried out with nebulizers by Fernández-Huerta (2019). According to ANVISA, it is a microorganism of clinical importance, appearing as one of the main isolated enterobacteria and with high prevalence in Health Care Related Infections (Brasil, 2017). It appears that Enterobacter sp. it is not so uncommon in the microbiological findings of research carried out in hospitals, for example Barros, et al., (2015) found this species, among other microorganisms, in the water that supplied a public hospital in a city in Minas Gerais.

Anders, Tipple & Pimenta (2008) when performing microbiological analysis in an aerosol therapy kit reprocessed in a hospital in Goiânia-GO, also detected Gram positive bacilli. In our study, Gram positive bacilli were also isolated, although not identified, suggesting that there is some microorganism abundant in the human skin microbiota, such as Corynebacterium spp.
Equipment such as nebulizers are classified as semi-critical in accordance with RDC No. 15/2012/ANVISA (Brasil, 2012) which provides for good practice requirements for the processing of health products, and legislates that, “semi-critical health products used in care ventilation, anesthesia and inhalation therapy must be cleaned and, at least, disinfected at an intermediate level, with sanitizing products in compliance with sanitary regulations, or by a physical process of thermal disinfection, before use in another patient”.

In addition to the practices recommended by ANVISA, a study by Roseira, et al., (2016) highlighted the importance of prior cleaning of equipment before disinfection, this procedure involves simple washing, done with soap or detergent, using a non-abrasive sponge or soft bristle brush. This processing step is intended to reduce the microbial load, remove drug residues, physiological solutions, organic and inorganic materials, as well as some particulate material, leaving the equipment clean to the point of receiving it more efficiently the disinfectant substance.

Regarding the solution of choice for the nebulizer disinfection process, the most used was sodium hypochlorite, being used by two of the three health units analyzed. According to the Brazilian Society of Surgical Center Nurses (Sobec, 2013), a concentration of 10,000 ppm (1%) is required to disinfect semi-critical articles with sodium hypochlorite, for 30 minutes of exposure or 200 ppm (0.02%) for 60 minutes of exposure. Such facts justify the hypochlorite being widely used in the disinfection of semi-critical articles because it presents advantages such as lower cost, low toxicity, easy handling and wide antimicrobial action.

5. Final Considerations

Studies in this context are important, as it allows assessing which potential risks patients with chronic respiratory diseases such as asthma, cystic fibrosis and chronic obstructive pulmonary disease are being exposed. In addition, this analysis and guidance by health professionals on how to proceed with the best way to clean this equipment should be extended to home use.

Proper cleaning of nebulizers can have a clinical impact, because when done wrong, it can make the equipment a potential source of contamination, increasing the risks of Infections Related to Health Care. Given the facts presented, it is concluded that failures are occurring in the reprocessing of these aerosol therapy and/or contamination equipment after the process. It is necessary to track these failures, standardize norms and routines for better operationalization of the reprocessing of this equipment.
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Percentage of contribution of each author in the manuscript

Firmino Marcelino da Silva Neto – 25%
Ana Laura de Cabral Sobreira – 25%
Laísa Vilar Cordeiro – 25%
Egberto Santos Carmo – 25%