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MICROBIOLOGICAL EVALUATION OF THE CLEANING CONDITIONS OF THE MECHANICAL VENTILATION CIRCUITS USED IN UNIVERSITY HOSPITAL

AVALIAÇÃO MICROBIOLÓGICA DAS CONDIÇÕES DE LIMPEZA DOS CIRCUITOS DE VENTILAÇÃO MECÂNICA UTILIZADOS EM UM HOSPITAL UNIVERSITÁRIO

EVALUACIÓN MICROBIOLÓGICA DE LAS CONDICIONES DE LIMPIEZA DE LOS CIRCUITOS DE VENTILACIÓN MECÁNICA UTILIZADOS EN UN HOSPITAL UNIVERSITARIO

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ABSTRACT

Objective: To verify the incidence of potentially pathogenic microorganisms isolated before and after mechanical ventilator respiratory circuit processing methods and to analyze the blood cultures results of tracheostomized patients during the study period. **Methodology:** Cross-sectional, descriptive study, with qualitative-quantitative approach, carried out in a public teaching hospital, in Southeastern Brazil. Microbiological analyzes of the reprocessed respiratory circuits of the mechanical ventilation apparatus were carried out. The data of the medical records were analyzed for the following aspects: patient gender, tracheostomy, isolated microorganisms and the bacterial resistance profile to the antimicrobial tested. **Results:** *Staphylococcus aureus, Staphylococcus* sp., Was detected in the different stages of the study. coagulase negative, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Enterococcus.* **Conclusion:** it was possible to evidence the presence of bacterial contamination in the different stages of the study and also the presence of these agents in blood culture tests.

Descriptors: Respiration; Artificial; Equipment Contamination; Cross Infection.

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RESUMO
Objetivo: verificar a incidência de micro-organismos potencialmente patogênicos isolados antes e após os métodos de processamento dos circuitos respiratórios dos ventiladores mecânicos e analisar os resultados das hemoculturas dos pacientes traqueostomizados durante o período do estudo. **Metodologia**: estudo transversal, descritivo, com abordagem quali-quantitativa, realizado em um hospital público de ensino, do Sudeste do Brasil. Realizaram-se análises microbiológicas dos circuitos respiratórios reprocessados dos aparelhos de ventilação mecânica. Foram analisados os dados dos prontuários quanto aos seguintes aspectos: sexo do paciente, realização de traqueostomia, micro-organismos isolados e o perfil de resistência bacteriana ao antimicrobiano testado. **Resultados:** foi detectada a presença de bactérias nas diferentes etapas do estudo, sendo caracterizados como *Staphylococcus aureus, Staphylococcus* sp. coagulase negativa, *Pseudomonas aeruginosa, Enterobacter cloacae,* e *Enterococcus.* **Conclusão:** foi possível evidenciar a presença de contaminação bacteriana nas diferentes etapas do estudo e também a presença desses agentes nos exames de hemocultura.
Descritores: Respiração artificial; Contaminação de Equipamentos; Infecção Hospital.

RESUMEN
Objetivo: verificar la incidencia de microorganismos potencialmente patógenos aislados antes y después de los métodos de procesamiento de los circuitos respiratorios de los ventiladores mecánicos y analizar los resultados de los hemocultivos de los pacientes traqueostomizados durante el período del estudio. **Metodología:** Estudio transversal, descriptivo, abordaje cualitativo cuantitativo, realizado en un hospital público, de Sudeste de Brasil. Se realizaron análisis microbiológicos de los circuitos respiratorios reprocesados de los aparatos de ventilación mecánica. Se analizaron los datos de los prontuarios en cuanto a los siguientes aspectos: sexo del paciente, realización de traqueostomía, microorganismos aislados y el perfil de resistencia bacteriana al antimicrobiano probado. **Resultados:** De las muestras analizadas, se detectó la presencia de bacterias en las diferentes etapas del estudio, siendo caracterizados como *Staphylococcus aureus, Staphylococcus* sp. coagulasa negativa, *Pseudomonas aeruginosa, Enterobacter cloacae*, y *Enterococcus*. **Conclusión:** fue posible evidenciar la presencia de contaminación bacteriana, en las diferentes etapas del estudio y también la presencia de esos agentes en los exámenes de hemocultura.
Descriptores: Respiración Artificial; Contaminación de Equipos; Infección Hospitalaria.

INTRODUCTION

The influence of technological innovation in health care has become increasingly more evident today, both in terms of availability of equipment or new care techniques, reflecting improvements in health care for population.[1]

Nevertheless, even in the face of all progress, some old problems such as infections Related to Health Care (IRAS) still show prevalent, promoting a major impact across the hospital mortality, influencing hospital stay and, hence, the cost for the treatment.[2]

The mechanical ventilation (MV) or also known as ventilatory support consists of a support method for the treatment of patients with acute or chronic respiratory failure, acute exacerbation, for replacing wholly or partially spontaneous ventilation. Its goal is to provide adequate gas exchange, reduce the work of the respiratory muscles and alleviate the metabolic demands of the patients using it.[3]

To perform this method, it is necessary the use of mechanical ventilators, which are composed of a set of accessories such as respiratory circuits. These consist of a set of reusable trachea leaving the MV set and take oxygen gas and compressed air into the patient in need of support. The process of handling these devices covers steps from its use until its return in ideal conditions of reuse.[3]

The Brazilian Society of Thoracic Association and the Brazilian Critical Care Association rate the circuits used in the MV as semi-critical due to the potential risk of transmitting infections. In this sense, the prevention and control of transmission of microorganisms present themselves essential to the health and safety of patients who need to use these equipments.[4]

Among the main preventive measures, there is the reprocessing of devices used in hospital care. This equipment must be in safe condition and free of viable micro-organisms before a next use, thus minimizing the risk of contamination.[5]

The MV patients belong to an increased risk for acquiring IRAS, at which the main predisposing factors are basically advanced age, low level of consciousness, intubation and reintubation, lagged immunity conditions, use of immunosuppressive drugs, MV time greater than seven days, impaired nutritional status, exogenous contamination, antibiotics, microbial colonization, prolonged surgery, suction of the contaminated condensate of the fan circuits used, aspirations of contaminated secretions, gastric colonization and its aspiration.[6]

Ventilator-Associated Pneumonia (VAP) it is the most prevalent infectious complication in patients requiring ventilatory support. The main components for the diagnosis of VAP at the bedside take into account a combination of findings such as chest X-ray, clinical signs and symptoms, and laboratory tests. A recent study shows that in addition to the

apparent symptoms, patients with suspected infection should be immediately subjected to microbiological studies.[7]

Thus, early identification of the pathogen by microbiological analysis in blood and other bodily fluids is predictive value when diagnosis, monitoring and screening of pathogens, since the development of resistance of microorganisms to antimicrobial agents proves to be a constant problem. Among the laboratory criteria, microbiological confirmation is critical to achieving the proper treatment being performed from the result of a positive blood culture without further focus of apparent infection, culture positive pleural fluid, culture alveolar lavage or tracheal bronchoconstriction, histopathological examination with evidence of pulmonary infection, urinary antigen and other positive laboratory tests for respiratory pathogens.[8]

Considering the magnitude of IRAS, knowing the microbiota and the main sources of infection is necessary for the prevention and control of infections related to health care. Furthermore, the investigation of the processing ventilation therapy devices, and other medical equipment, is of extreme importance, since the MV-associated infections have achieved significant numbers in hospital institutions.[9]

The objective of this study is to assess the incidence of potentially pathogenic microorganisms isolated before and after processing methods of respiratory circuits of mechanical ventilators, and analyzing the results of blood cultures from patients using mechanical ventilation during the study period.

METHOD

This is a descriptive cross-sectional study, with non-probabilistic sample, with qualitative and quantitative approach, in which the occurrence of the outcome was observed in a single period of time, in the period from September to November 2017, in a public teaching hospital Southeastern Brazil.

Processing circuits and sample collection

The process of breathing circuits was performed in a centralized manner in the sector known as Equipment Center, in the following sequence: immersion in enzyme solution (according to the manufacturer's instructions), manual cleaning and rinsing. Once clean, the tracheas were placed to drain and dry in plastic boxes, subjected to

compressed air and, subsequently, forwarded to a third party to be sterilized with ethylene oxide. During the operational steps of the processing methods, there was no interference of the researchers.

The sample collection, considering the circuits ready for use, was carried out within the restricted area of the equipment and devices sterilized by aseptic technique with prior preparation of the surfaces, which rules out the possibility of contamination at the time of collection.

For quantitative and qualitative analyzes, samples collected were divided into three stages, according to the processing methods used by the research institution. The subjects of the sample by convenience had a total of 276 samples (30 samples from the quantitative analysis and 246 from the qualitative analysis), according to demand reuse of such equipment and devices.

Step 1 represents the group of samples obtained from mechanical ventilators circuits of care units, which were used in extubated patients due to death or weaning procedure. The circuits collections were performed in the expiratory branch and the analysis is conducted aseptically, ensuring the sterility of the sample. After the circuits are submerged in enzyme solution, and dried with compressed air, the second step started, when 82 samples were collected.

At these stages, a total of 82 devices were taken at the site known as purging or dirty area, and control of mechanical ventilators included in the survey was based on the number of assets assigned by the institution, in order to avoid possible exchanges, since the same equipment can enter the reuse cycle several times.

For this procedure, 50 ml of phosphate buffered saline sterile 0.1% were added in the branch circuit. Homogenization was performed three times in the pipe and then was collected into a sterile Falcon tube and transported to the laboratory.

Subsequently, the circuits were sent to a third party responsible for sterilization with ethylene oxide. After the return of the equipment and devices, step 3 started. In this step, collection was performed at two different times: (1) after opening the sterile package (41 collections); (2) after assembly of the circuit in mechanical ventilators (41 trials) for later release to assistance units, totaling 82 samples. During this step, the circuits used in the

study were directed to a new processing cycle.

Qualitative Microbiological analysis

In the laboratory, samples were plated in saline, using the qualitative seeding technique in culture medium Mueller-Hinton agar and incubated at 37°C for 48 hours. Then, five different colonies were isolated from each plate and inoculated in Eppendorf tubes containing 1 ml of Brain Heart Infusion agar (BHI), which was incubated at 37°C for 24 hours.

Samples with detection of microbial growth on blood agar were isolated and those that grew were characterized macroscopically and microscopically (Gram stain). For the identification of Gram positive bacteria, the catalase and coagulase tests were performed. In the case of Gram-negative, direct biochemical procedures were employed as motility, Indole and sulfate, Agar Triple Sugar Iron (TSI), Citrate and Urea.

Data analysis

To analyze the efficiency of the processing methods of the mechanical ventilation circuits, a quantitative study was carried out, based on pour-plate counting, 10 samples at each stage, totaling 30 samples. To calculate the efficiency, it was used the following equation (Equation 1)[10] wherein initial X is the sum of the colony forming units count (CFU) present in circuits before the application of enzymatic detergent and $_{final}$ X is the sum of the CFU count still present in the circuit after application of the detergent enzyme. The detergent used in the research institution (Indazyme plus 6 - Indalabor®) consists of lipolytic enzyme (lipase), proteolytic enzymes (protease, peptidase) and amylolytic enzymes (carbohydrase, cellulase and alpha-amylase).

$$Eficiência\,(\%) = \frac{100 \times (X\ inicial - X\ final)}{X\ inicial} \quad (1)$$

Analysis of records

The ventilatory assistance method performed in different hospital treatment units provided important information on the epidemiological profile of patients during the treatment period. For this, one used data in the medical records of patients, extracted from the medical records department and Statistical Laboratory and Database Analysis and Clinical Anatomy, without direct involvement of the patient. For the composition of the samples of this study, subjects were selected, who underwent blood culture in the period studied and carried out retrospective analysis of medical records, the sample number being determined by non-probabilistic sampling (convenience). The patients were not monitored for the presence of VAP, and the result of blood culture in this study does not reflect on their possible diagnosis.

The analysis involved the following information: patient's gender, tracheostomy (TQT), microorganisms isolated and the profile of bacterial resistance to the antimicrobial tested.

Statistical analysis

Statistical analysis was performed by distribution of absolute frequencies and percentages for nominal variables and discrete numerical variables. Descriptive analysis was performed using the GraphPad Prism software program. The Kolmogorov-Sminorv with Dallal-Wikinson-Liliefor was applied to verify if the data followed normal or non-normal distribution. For non-normal data, we applied the nonparametric Friedman test-paired. The significance level was 5%, ie, $p < 0.05$.

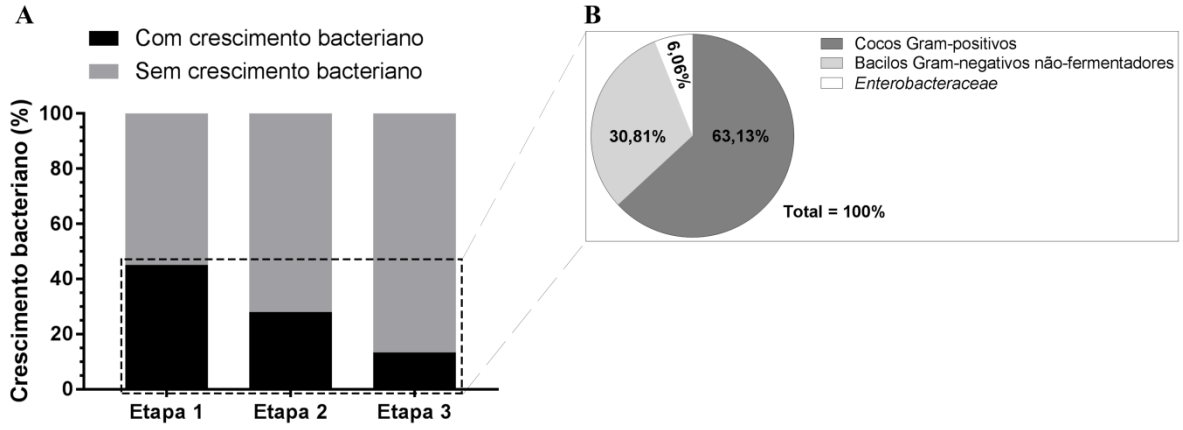
The project was approved by the Ethics in Research of the Federal University of Triângulo Mineiro Committee under number 2655145/2018 and authorized by the Management of Education and Research.

RESULTS

Of the samples relating to the qualitative approach, 45.12% (37/82) showed positive growth before starting the processing method (step 1). After processing enzyme solution (step 2), 28.04% showed bacterial growth (23/82). And after being sterilized by ethylene

oxide (step 3), 13.41% showed positive growth (11/82) (Figure 1A). In total, 198 microorganisms were isolated, of which 125 samples (63.13%) were identified as Gram-positive cocci, 61 specimens (30.81%) and Gram-negative bacilli and 12 non-fermenting samples (6.06 %) as belonging to the family Enterobacteraceae (Figure 1B).

Figure 1 - Frequency distribution of positive and negative samples (A), and percentage of microorganisms isolated from the cultures of positive expiratory limb of the circuit (B) found in the different stages analyzed.

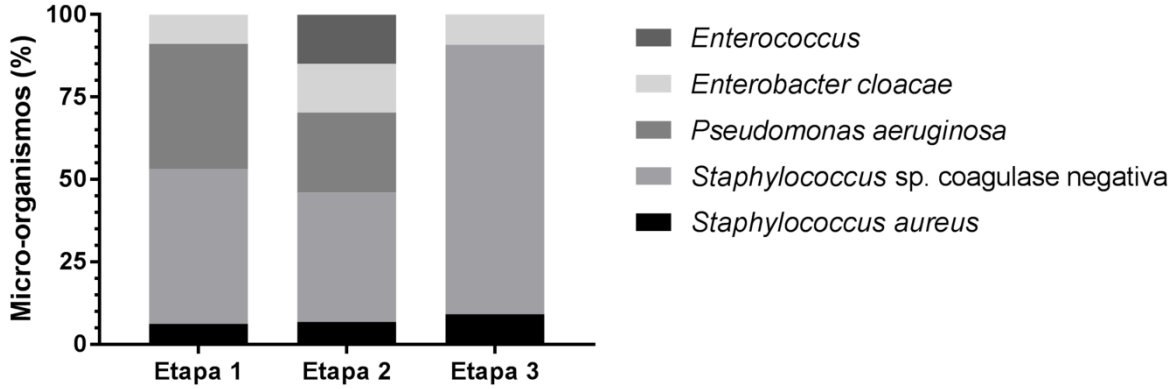


In step 1, the positive collected 113 samples were positive for 6.19%

Staphylococcus aureus, 46.9% for Staphylococcus sp. coagulase negative 38.05% for Pseudomonas aeruginosa and Enterobacter cloacae to 8.85%. Step 2 of the 74 positive samples, we can see a greater variation of bacterin population compared to other stages, with 6.76% positive for Staphylococcus aureus, 39.19% for Staphylococcus sp. coagulase negative, 24.32% for Pseudomonas aeruginosa, Enterobacter cloacae, 14.86% and 14.86% for Enterococcus. Finally, in step 3, the 11 samples found positive, it is possible to observe positivity 9.09% Staphylococcus aureus and Enterobacter cloacae and 81.82% for Staphylococcus sp. coagulase negative. It is notorious the high prevalence of Staphylococcus sp.

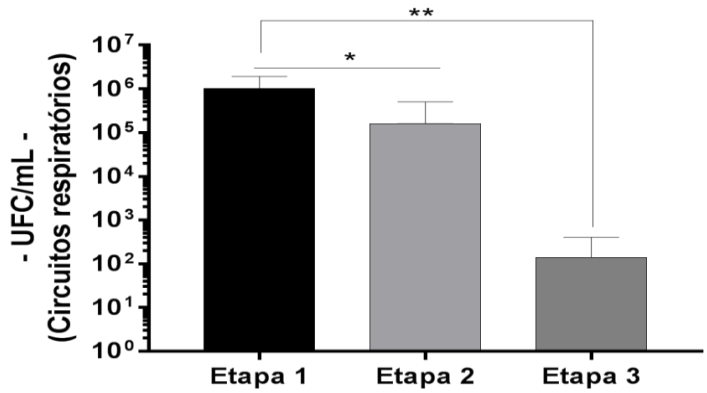
coagulase-negative (45.96%) and the standard positive for Staphylococcus aureus analyzed in three steps (Figure 2).

Figure 2 - Frequency (%) of positive expiratory branch circuits of samples for each isolated etiologic agent during step 1, 2 and 3.



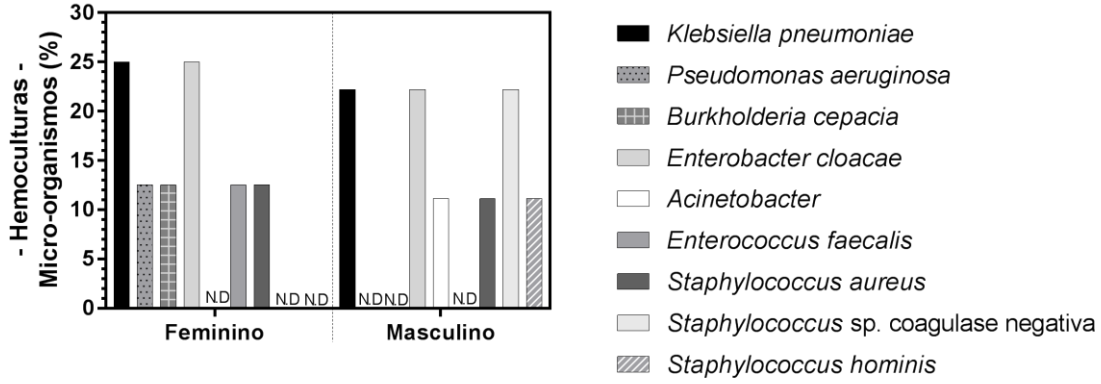
Evaluating quantitatively the microbial load in the three steps analyzed, it is possible to observe a significant reduction in the number of isolated colonies (CFU / mL) from step 1 and step 2 the isolated VM reducing circuits 70.9%. This reduction is more significant when held shows the comparison between step 1 and step 3, 99.9% by reducing the initial microbial load. Based on the results of the maximum values of micro-organisms found in each step, there is the influence of processing equipment across the microbial burden present in the circuits analyzed as follows: Step 1 3.3 × 106 CFU, step 2 9 6 × 105 CFU and during step 3 was 8.0 × 102 CFU.

Figure 3 -Quantitative analysis of the number of colony forming units per milliliter (CFU / mL) between steps before and after treatment of respiratory circuits enzyme solution. Statistical comparison test conducted by paired Fredman.



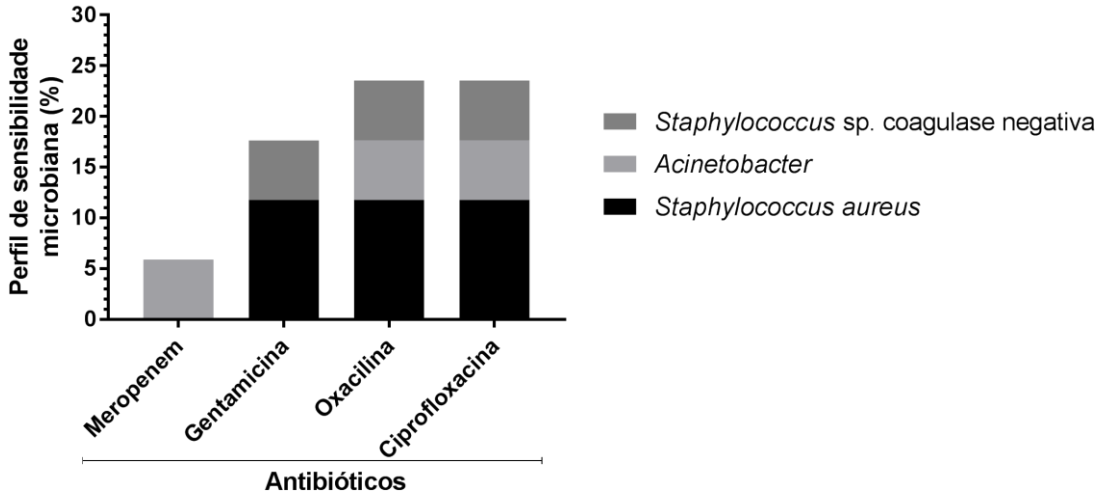
The corresponding analysis results of the step of screening blood cultures obtained from patients who underwent TQT procedure consisted of 17 patients, 9 (52.94%) and 8 females (47.06%) males. In Figure 4 are shown all microorganisms isolated and analyzed, at which time one observes a higher frequency of positive blood culture samples Klebsiella pneumoniae and Enterobacter cloacae, followed by Staphylococcus aureus and coagulase negative Staphylococcus.

Figure 4 -Distribution of microorganisms isolated from blood cultures of patients. ND: Not detected. Uberaba-MG, from September to November 2017.



Regarding microbial sensitivity, figure 5 shows the profile presented by micro-organisms Staphylococcus coagulase negative, Staphylococcus aureus, both the group of gram-positive and profile of Acinetobacter belonging to gram-negative bacteria group on the which oxacillin antimicrobial agents, meropenem and ciprofloxacin were able to act. You can see that the Acinetobacter spp. showed sensitivity to antibacterial agents from the group of beta-lactam antibiotics, synthetic quinolone and carbapenem meropenem, providing a high likelihood of responding to treatment due to the good pharmacokinetic profile / dynamic these classes.

Figure 5 - Percentage of sensitivity of the microorganisms to take the antibiogram tracheotomy patients. Uberaba-MG, from September to November 2017.



DISCUSSION

It established the fact that the surfaces of medical devices are a growing source of nosocomial infections in hospitals and clinics throughout the world.[5] The factors involved in this process relate to human error or mechanical that might occur during processing, implying possible compromise the sterility of devices providing thus an increased risk of HIV infections.

Works have reported that patients requiring MV for a long period of time are faced with a high probability of contracting PAV.11 The endotracheal tube used in the VM provides a favorable surface for bacteria adhere and form a biofilm, and permit formation of condensate within the respiratory circuit, which may undergo rapid colonization by pathogens and contribute to drug resistance antibacterianos.[11-12]

The study showed the presence of bacterial contamination in all stages of analysis of the breathing circuits, in addition to confirming the presence of these agents in blood culture tests performed in patients who underwent tracheostomy procedure. During the study, some tracheas showed the presence of organic matter (secretion and / or blood) after manual cleaning step (step 2). This corroborates the findings of the literature that highlight the importance of vigorously cleaning step for the disinfectant action is not prejudicada.[9,13]

For better efficiency in process quality after cleaning, detailed and exhaustive rinsing in running water must occur using taps with pressure nozzle for removing dirt and organics prior to occur drying of the material on the inner surface of the circuit. This action reduces the number of microbial contamination and, consequently, the formation of biofilme.[13]

Regarding the results obtained in step 3, quantitative method verified the presence of microbial load of up to 8.0 × 102 CFU. These results are similar to the study by Silva and Pinto[14] in that, after the process of cleaning and sterilization of equipment and devices, the authors noted the presence of microbial load of up to 103 CFU / ml. This same result was also confirmed macroscopic mode, the characteristic microbial growth in sterility testing. The authors concluded that although logarithmic reduction of microbial load the tubes showed no the absence of microbial and endotoxin load.

In relation to guidance and specific recommendations of the National Health Surveillance Agency (ANVISA) for the time that the circuit must be mounted on the equipment awaiting the admission of the patient are not yet well established fact that would enable better prevention and control of infections related However saúde.4 assistance to the quantitative analyzes of the samples from step 3 in this study refer to the number of days in which the sterile circuits were mounted on MV: 05 days, 10 days and 30 days values being

found concerning microbial load of 2.10 × 102 CFU / ml 3.80 × 102 CFU / ml and 8.00 × 102 CFU / ml, respectively. Assemblies of such circuits were performed with aseptic technique, as standards Equipment Center sector.

CONCLUSION

Evaluation of the effectiveness of the processing method showed a reduction in the total amount of organic matter present in the circuit, however, showed that the circuits of mechanical ventilators can harbor bacteria, leading eventually to cross-contamination. Were common microorganisms identified in blood cultures and the circuit samples, reinforcing the importance of acquiring an intra-hospital incisive policy in fighting infection, broadly, to all hospital service providers.

Preventive measures are essential, since the implementation of control strategies and standardization and conduct training to assist risk patients minimize transmission of microorganisms by the colonization of mitigation and its reservoirs.

The work in an Equipment Center has an important significance against the IH control, since the activities in this unit contribute to the reduction of adverse events. Thus, the main points raised in this study enabled the nurses of the unit were able to reflect on the problems related to the mechanical ventilation circuit processing method and, consequently, organize an action plan with objectives to improve and solve such problems .

It stands out that this study is not the limiting factor monitoring circuit using time according to the processing cycle. Still, it has shown that the quality control of the mechanical ventilation circuit processing is only obtained from the monitoring of steps involving cleaning, disinfection and sterilization. Thereby contributing to the improvement of indirect care in Central equipment and in other areas of assistance to the patient, attempting thus to its importance in favor of direct care.

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