British Microbiology Research Journal
4(6): 598-606, 2014

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Bacterial Colonization of Mechanical Ventilation Circuits in a Pediatric Intensive Care Unit

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Authors' contributions

This work was carried out in equal collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 27th July 2013
Accepted 25th January 2014
Published 18th February 2014

ABSTRACT

To identify the onset time of bacterial proliferation in mechanical ventilator circuits in a pediatric intensive care unit (PICU) and the colonizing agents involved, as well to verify any possible pre-use contamination, we conducted a prospective microbiological analysis of the circuits of 30 patients, with a total of 342 cultures. Bacterial colonization of the mechanical ventilator circuits in the studied PICU was confirmed. Microorganisms were present in 39% of expiratory branch cultures: *K. pneumoniae* (77%), *A. baumannii* (65%), and *S. aureus* (42%). The inspiratory branch registered a smaller number of positive culture: *A. baumannii* (51%), *S. aureus*, coagulase-negative (38%) and *K. pneumoniae* (32%). As to the colonization onset time, 23% of the cultures collected on the 1st day from the inspiratory branch were positive, versus 30% of those collected from the expiratory branch, which lead us to conclude that bacteria can be present within the first hours of use. The sequential analysis of bacterial colonization over the course of circuit usage and the observation that the number of positive cultures becomes progressively higher on the 5th, 6th and 7th days in the expiratory branch both suggest that the systems should be replaced more often.

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Keywords: Bacterial infections; mechanical ventilation; pediatric intensive care.

1. INTRODUCTION

Mechanical ventilation stands out amongst the technologies that directly assist life support of seriously ill patients. However, it is an invasive procedure, sometimes used for prolonged periods of time, which exposes patients in need of intensive care to diverse risks, especially that of infection [1,2].

In the physiopathology of intensive care unit infections originated from endogenous or exogenous reservoirs, the preponderant initial event is the microbial colonization of the organic epithelia in devices or dispositives used for the monitoring and clinical handling of patients. Such event will determine the infection type and its prognosis [2-4].

Ventilator-Associated Pneumonia (VAP) represents a 20% incidence in the population undergoing pediatric intensive care. In seriously ill children, the most frequent infection site is considered to be the blood stream, followed by the pulmonary system and the urinary tract [4,5].

Bacterial colonization refers to the presence of bacteria at a given site in the host's organism without eliciting its active response to their presence. Pulmonary colonization in children undergoing ICU treatment may occur due to invading microorganisms originating from sites such as the oropharynx, sinuses, dental plaque, gastrointestinal tract, mechanical ventilator's circuits, catheters and other patients [5].

The frequency of hospital-acquired pneumonia caused by gram-negative bacilli has increased in recent years. Several pneumonia epidemics caused by this class of microorganisms associated to the use of mechanical ventilators have been reported [6]. Inhalation of aerosols containing bacteria is one of the main routes of entry for microorganisms into the lower respiratory tract. The mechanical ventilation circuits can act as a reservoir for those microorganisms [7-9].

Such factors expose the ICU patient to the risk of prolonged hospitalization, sepsis, death and, consequently, they cause a raise in the costs for the hospital institution, lower the number of ICU beds available, and lead to queries as to the reliability and quality of the services provided. Bacterial colonization of respiratory circuits could be part of the pathophysiology of respiratory infections in mechanically ventilated patients [7-10].

Respiratory circuits are products or equipment that come in contact with intact colonized mucosae, being regarded as semicritical devices and thus requiring a high level disinfection. According to CDC guidelines, it is recommended that such equipment be withdrawn and sent for reprocessing only in the event of visible soiling or if there is known contamination of the circuit [10].

The objectives of the current study were to assess bacterial colonization in mechanical ventilator circuits in a Pediatric ICU and the presently involved agents thereof to investigate the occurrence of pre-use contamination and to determine the colonization onset time. In accordance with the results obtained, one additional objective was to present a proposal for a routine replacement of the mechanical ventilation circuits.

2. METHODS

The prospective study was conducted in the Pediatric Intensive Care Unit at Santa Marcelina Hospital – Itaquera, Brazil, from September 2011 through September 2012, in a single ward with eight beds – and another two isolation beds, where care and support are provided for patients in various specialties including Neurosurgery, Cardiac Surgery, Orthopedics, Pediatric Surgery, Pediatric Oncology and general clinical critical care cases such as respiratory failure and sepsis.

After the approval from the hospital Ethics Committee, the research was conducted using as exclusion criteria patients with chronic respiratory diseases, immunodeficiencies (HIV) and children in a pathological or a chemotherapy-induced immunodepression state. Those responsible for the patients who were research subjects filled out the Voluntary and Informed Consent form.

Thirty patients undergoing supportive mechanical ventilation were prospectively studied. The respiratory systems (inspiratory and expiratory branches) were investigated at the following times: zero hour or day one (first 24 hours post-intubation) and daily thereafter for six days, totaling seven samples, ending on the seventh day. Types of tubes, ventilators, suctioning systems and nebulizers varied among included patients.

Samples were collected from the inspiratory and respiratory branches' tracheas – the four system extensions were used for sample collection on one of the collection days, the proximal branches were used on the next day, the distal ones were used. This procedure, developed by our group was adopted in order not to completely eliminate the bacteria present therein and to allow a period of at least 24 hours for their proliferation or for causing no significant changes in the bacterial biofilm.

The investigators adopted sterile techniques while collecting the samples. Each day involved the collection of one swab followed by one wash from one trachea in each branch (inspiratory and expiratory), totaling four daily samples.

The trachea's disconnection site was rubbed with gauze previously soaked in an antiseptic aqueous or alcoholic chlorhexidine solution, after which it was removed and the procedure was then initiated by rubbing a long stem with a cotton tip (culturette) over the wall and villosity along the extension of the circuit. Regular insertion and withdrawal movements were used, with the stem being inserted up to its support handle, for approximately five to seven seconds. Storage was done in sterile acrylic test tubes.

Subsequently, the same extension was washed by instilling 5 ml of sterile 0, 9% saline into the internal part (lumen) of the circuit with a syringe. "Back and forth" movements kept for the same period of time (five to seven seconds) ensured that the liquid would wash the trachea along all of its extension. The collected wash was then stored in the same test tube in which the cotton swab had been kept. Finally, the lid was closed and the trachea reconnected back for use. The same procedure was repeated in the opposite branch.

The collected samples, kept in plastic containers at room temperature, were shortly sent thereafter for proper storage at the ideal temperature in the institution's laboratory.

After collection, the samples were seeded over Azide Blood Agar base in order to search for gram-positive microorganisms; Mac Conkey Agar for gram-negative microorganisms and

Chococlate Agar for *Streptococcus, Neisseria* and Haemophilus. The enriched BHI (Brain Heart Infusion) broth was used with the purpose of improving analysis and reading when previous analyses had revealed low bacteria count.

Plates were incubated at 35ºC. The minimum incubation period for considering them negative was 48h.

In order to identify previous contamination, cultures from 24 sterile systems prior to their use were obtained.

Bacterial identification was performed according to recommended criteria [11].

Results were described as frequencies and percentages versus collection time. The convenience sample size was defined as 30 patients.

3. RESULTS

Seventeen patients completed the whole seven days of study. The cultures from the mechanical ventilation circuit's tracheas were grouped according to the branch (inspiratory and expiratory) and collection time (from the 1st through the 7th days) Fig. 1. Altogether, 342 cultures were obtained, 171 from each branch.

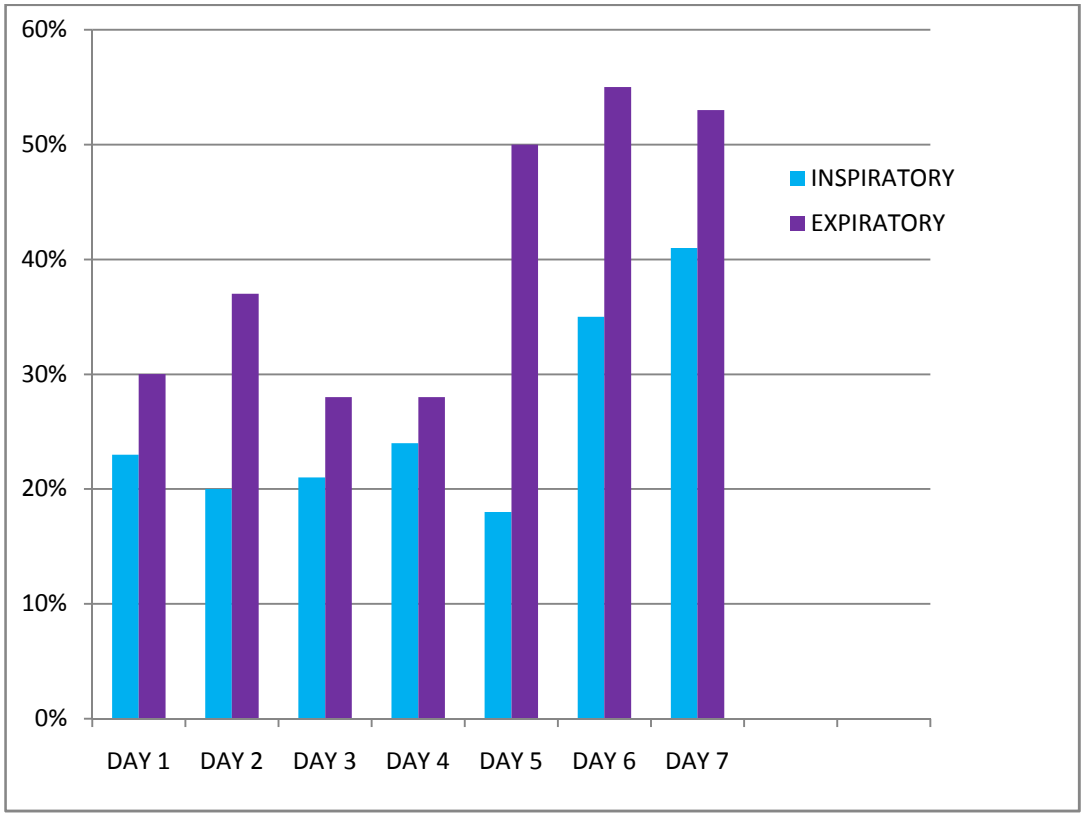

Fig. 1. Circuit colonization/collection day ratio: inspiratory and expiratory branches

Considering the 1st collection day, bacterial growth was observed in the inspiratory branch in the circuits from seven patients (23%), with the predominance of coagulase-negative *Staphylococci* (10%) and Enterococcus (7%); whereas in the expiratory branch, positive cultures were obtained from 9 patients (30%), with the prevalence of *S. aureus*, coagulase-negative (10%) and *P. mirabilis* (7%).

In the inspiratory branch, the highest bacteria counts were registered for the 6th and 7th days: seven patients with positive cultures (23%) for both days. The most prevalent bacteria on the 6th day were *S. aureus*(10%); coagulase-negative *Staphylococci* (10%) and *A. baumannii* (10%); whereas on the 7th day, *A. baumannii* (23%) and *S. maltophilia* (12%) were the most prevalent ones Table 1.

Table 1. Quantitation of identified bacteriaover their time of appearance: inspiratory branch

	Day 1 (n = 30)	Day 2 (n = 30)	Day 3 (n = 28)	Day 4 (n = 25)	Day 5 (n = 22)	Day 6 (n = 20)	Day 7 (n = 17)
S. aureus	0	0	0	1 (4%)	1 (4%)	2 (10%)	1 (6%)
coagulase-negative *Staphylococci*	3 (10%)	2 (7%)	1 (3%)	2 (8%)	0	2 (10%)	0
A. baumannii	0	2 (7%)	2 (7%)	0	1 (4%)	2 (10%)	4 (23%)
Enterococcus	2 (7%)	0	0	0	0	1 (5%)	0
Pseudomonas	0	0	0	2 (8%)	0	0	1 (6%)
C. parapsilosis	0	0	0	0	0	1 (5%)	0
E. cloacae spp	0	0	0	0	0	1 (5%)	1 (6%)
K. pneumoniae	1 (3%)	1 (3%)	2 (7%)	1 (4%)	1 (4%)	1 (5%)	1 (6%)
P. mirabilis	0	0	0	0	0	0	0
S. marcescens	0	0	0	0	0	0	0
S. maltophilia	0	0	0	0	0	1 (5%)	2 (12%)
Other**	1 (3%)	1 (3%)	1 (3%)	0	1 (4%)	0	0

*Other**: Corynebacterium spp; gamma-hemolytic Streptococci; Micrococcus; Providencia stuartii; Chryscobacterium indologenes*

In the expiratory branch, the highest bacterial counts were registered on 5th, 6th, and 7th days, considering the number of patients still undergoing mechanical ventilation at these collection times. The most commonly found bacteria on the 5th day were *K. pneumoniae* (14%), and *S. aureus* (9%); on the 6th day, *S. aureus*, *A. baumannii, E. cloacae*, and *K. Pneumoniae* (all these microorganisms found in 10% of the cultures) and on the 7th day, *A. baumannii* (23%); *K. pneumoniae* (18%) and Enterococcus (12%) Table 2.

In all cultures collected, the inspiratory tracheas accounted for 43 positive cultures, whereas the expiratory tracheas totaled 66 positive cultures, which is equivalent to a 22% difference.

Six microbiological findings (20%) could be related, in blood cultures or catheter tip cultures, with concomitant circuit colonization in one or both of the mechanical ventilation system's ducts. In 33% of these samples, the same bacterial species (*A. baumannii* and *S. aureus*) were observed in all ducts analyzed, including the inspiratory and expiratory branches.

Colonization in the expiratory branch was observed in 100% of the concomitant findings, 50% of which presented identical culture results on 3 of the days analyzed.

Table 2. Quantitation of identified bacteria over their time of appearance: expiratory branch

	Day 1 (n = 30)	Day 2 (n =30)	Day 3 (n = 28)	Day 4 (n = 25)	Day 5 (n = 22)	Day 6 (n = 20)	Day 7 (n = 17)
S. aureus	1 (3%)	1 (3%)	1 (3%)	2 (8%)	2 (9%)	2 (10%)	1 (6%)
coagulase-negative *Staphylococci*	3 (10%)	2 (7%)	0	0	1 (4%)	1 (5%)	1 (6%)
A. baumannii	1 (3%)	2 (7%)	4 (14%)	1 (4%)	1 (4%)	2 (10%)	4 (23%)
Enterococcus	0	1 (3%)	0	0	1 (4%)	1 (5%)	2 (12%)
Pseudomonas	0	0	0	1 (4%)	0	1 (5%)	1 (6%)
C. parapsilosis	0	0	0	0	0	1 (5%)	0
E. cloacae spp	0	0	0	0	1 (4%)	2 (10%)	1 (6%)
K. pneumoniae	1 (3%)	4 (13%)	2 (7%)	3 (12%)	3 (14%)	2 (10%)	3 (18%)
P. mirabilis	2 (7%)	1 (3%)	0	0	0	0	0
S. marcescens	1 (3%)	1 (3%)	1 (3%)	0	1 (4%)	1 (5%)	1 (6%)
S. maltophilia	0	0	0	0	0	1 (5%)	1 (6%)
Other**	0	1 (3%)	0	0	2 (9%)	1 (5%)	0

*Other**: Corynebacterium spp; gamma-hemolytic Streptococci; Micrococcus; Providencia stuartii; Chryscobacterium indologenes.*

The most prevalent gram-negative bacteria found throughout the study were *K. pneumonia* and *A. baumannii*. However, the presence of coagulase-negative *Staphylococci* was frequent in the inspiratory branch, as well as that of *S. aureus* in the expiratory branch, which was the 3rd most commonly found bacteria therein Table 2.

In 20% of all patients analyzed, no bacterial growth was observed on any collection day. Their average intubation time was four days. In half of the patients in this group, collection had to be interrupted due to extubation.

4. DISCUSSION

The number of patients included in the study was limited mainly due to the exclusion criteria adopted, and also due to the fact that the ICU needed to be isolated because of two chickenpox outbreaks during the study period, which comprised altogether approximately 42 days. Additionally, there was also a significant increase in the number of oncopediatric patients hospitalized.

The results demonstrated that the ventilation circuit's expiratory branch is the one that presented the highest bacterial colonization (39%). This suggests patients' participation in disseminating bacteria throughout the circuit as they expel secretions, in the form of droplets or aerosols, even if they are not infected.

The results also pointed out that circuits tend to remain colonized once colonization has begun, with bacteria then colonizing the opposite branch and thus favoring the growth of associated bacteria. This finding totaled 5% of the positive culture results and was observed from the 6th day onwards in both the inspiratory and expiratory branches, in which latter case it was more frequent. The most commonly found associated bacteria were *S. aureus* and *A. baumannii*.

Kusahara et al. [5], in a bacterial colonization and translocation study in children undergoing mechanical pulmonary ventilation demonstrated that, out of the 30 children analyzed, translocation was observed in 24 (80%). Assessing the need for replacing mechanical ventilation circuits is among the interventions to be implemented in order to minimize the risks posed by pathogenic and multiresistant bacteria.

In reviewing the literature, Calvo mentions avoiding routine replacement of the ventilator's circuits, nevertheless he recommends replacement if circuit damage or contamination is detected [12].

Taking into consideration the above-described risks associated to the respiratory care of seriously ill patients undergoing mechanical ventilation, one should add up the unresolved issues related to oral hygiene, respirator circuit replacement, cuff pressure, aspiration technique, to name a few, for which there are insufficient recommendations or consensus is still lacking.

The results obtained from the circuits' expiratory branch are indicative of colonization risk and possible infection or reinfection of a patient already receiving treatment. This is due to the predominance of gram-negative bacteria, which were also detected in the inspiratory branch on the 7^{th} day of use.

The method used in the study could not confirm infection arising from the use of colonized mechanical ventilation circuits in the involved patients. However, blood or catheter tip cultures were obtained from 6 patients who had been shown to be coincident with the growths observed on the circuits' tracheas.

The occurrence of VAP (Ventilator-Associated Pneumonia) varies amongst institutions primarily due to the diversity of the population assisted, but also as a result from the methods used for diagnosis and the laboratory capacity to process the tests conducted. It is important that each institution knows its own microbial profile, which includes microorganism type and antibiotic-resistance profile [2].

Prolonged mechanical ventilation time (periods greater than 7 days' time) and aspiration of contaminated condensate from the ventilator's circuits are among the risk factors for VAP [13]. A recent meta-analysis has shown that frequent circuit changes could be associated with a higher risk of VAP [14].

Institutional characteristics are relevant, especially with regard to the financial possibilities of each service. Adapting standards and regulations so as to comply with concepts defined by international organizations may create setbacks. Many Brazilian hospitals still do not have at their disposal equipment that is adequately set up for using circuits for prolonged periods of time in cases such as: tracheas less susceptible to the adherence of secretions; circuits with in-line heating, so as to minimize droplets; or even heaters capable of controlling "bubble" and aerosol elimination.

5. CONCLUSIONS

The present study confirmed the presence of bacterial colonization in the mechanical ventilation circuits in the Pediatric ICU investigated. The most prevalent agents were *A. baumannii, K. pneumoniae, S. aureus* and coagulase negative *Staphylococci*.

No bacterial colonization could be detected in any of the sterile circuits analyzed.

Some of the cultures collected as early as the 1st day from the inspiratory branch and the expiratory branch were positive, which leads us to conclude that bacteria can be present within the first hours of use.

The sequential analysis of bacterial colonization over the course of circuit usage and the observation that the number of positive cultures becomes progressively higher in the expiratory branch both suggest that the systems should be replaced weekly or before, in the presence of soiling or in the event of malfunctioning.

ACKNOWLEDGEMENTS

To Santa Casa de São Paulo School of Medicine, to Santa Marcelina Hospital – Itaquera, and to CAPES, for the scholarship granted.

COMPETING INTERESTS

None of the authors have any conflicts of interest with the content of this article.

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