Can Humidifier Reservoir Bacteria Colonize the Circuit During Mechanical Ventilation?

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Research Article

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Abstract

Background:

Although the circuit condensate, an ideal bacterial reservoir, may flow into the humidifier reservoir (HR), no study has investigated if HR-colonized bacteria colonize other circuit locations with airflow. Therefore, the objective of this study was to explore if bacterial growth in the HR leads to bacterial colonization in the ventilator circuit.

Methods:

A randomized controlled experiment was performed in a public tertiary hospital in Guangdong Province, China. In vitro mechanical ventilation models \((n = 60)\), divided into sterile water samples \((n = 30)\) and broth samples \((n = 30)\), were established. Sterile water was used for humidification in the ventilation models. The sterile water group contained either *Acinetobacter baumannii* \((n = 15)\) or *Pseudomonas aeruginosa* \((n = 15)\) in humidifier water. The broth group was similar to the sterile water group, but brain heart infusion broth was added to the HR. After 24, 72, and 168 h of continuous ventilation, bacteria in the humidifier water and at different circuit locations were sampled and cultured, and the results were analyzed by the Chi-square test. The difference in bacterial concentration at the HR outlet was analyzed by the F test, and \(P < 0.05\) was considered statistically significant.

Results:

Bacterial culture results of the sterile water samples were negative. Bacteria in the humidifier water continued to proliferate in the broth group, and the bacterial concentration at different times was not significantly different \((P > 0.05)\). With prolonged ventilation, the bacterial concentration at the HR outlet increased \((P < 0.05)\). During continuous ventilation, no bacterial growth occurred at 10 cm from the HR outlet and the Y-piece of the ventilator circuit. The bacterial concentration at the HR outlet was higher in the *P. aeruginosa* group than in the *A. baumannii* group \((P < 0.05)\).

Conclusions:

Sterile water in the HR was not conducive to bacterial growth. Although bacteria grew in the HR and could reach the HR outlet, colonization of other circuit locations was unlikely.

Background

Invasive mechanical ventilation therapy is based on the artificial airway, but the warming and humidifying effects provided by the upper respiratory tract on inhaled air are lost. In 2012, the American Association for Respiratory Care recommended that patients undergoing invasive mechanical ventilation therapy be provided with airway humidification \([1]\). In the clinical setting, a humidifier is commonly used, but the air heated and humidified by the humidifier is easily cooled in the circuit and forms a condensate.
Studies have found that the condensate is an ideal bacterial reservoir [2, 3]. In the past, the condensate collected in a water trap located in the middle of the circuit. However, this process damaged the closed-circuit system and easily caused condensate splash. At present, a disposable ventilator circuit with heating guidewire and without a water trap is commonly used in the clinical setting. Although this circuit can reduce condensate production, a small amount of condensate is still produced. Directly disconnecting the circuit to discard the condensate will not only interrupt respiratory support in patients but also increase the risk of exposure to bioaerosols in medical staff [4].

Can the condensate be poured into the humidifier reservoir (HR)? According to the clinical practice guidelines for the prevention of ventilator-associated pneumonia (VAP) by the Infectious Diseases Society of America, the condensate is an infectious waste and cannot be poured into the HR [5]. However, the HR is located at a position lower than the circuit, and the condensate with bacteria can easily flow back into the HR.

If bacteria grow in the HR, will they colonize other circuit locations with airflow? In 2005, Wenzel et al. added isotopes to the HR and controlled the airflow at different flow rates (2–46 L/min) [6]. They used a filter at the HR outlet to collect the isotopes. No isotopes collected in the filter; thus, they inferred that bacteria could only exist in the HR [6]. However, as isotopes are different from bacteria, this inference is not reliable. Furthermore, no research has investigated if HR-colonized bacteria colonize other circuit locations with airflow. In this study, we investigated if bacterial growth in the HR leads to bacterial colonization in the ventilator circuit.

Methods

Study design and setting

We performed a randomized controlled experiment in a public tertiary hospital in Guangdong Province, China, using in vitro mechanical ventilation models (n = 60) divided into sterile water samples (n = 30) and broth samples (n = 30).

Experimental equipment and consumables

Equipment and consumables used in the experiment were as follows: two ventilators (Savina and Evita XL; Dräger Co., Ltd.), humidifier (MR850; Fisher & Paykel), disposable double heating guidewire circuit (RT308; Fisher & Paykel), lung simulator (MP02400; Dräger), temperature-controlled box (WTFK-3701; Tianchang Wantian Fukang Electronics Factory), sterile water (500 ml; Guangdong Aixide Pharmaceutical Co., Ltd.), vortex mixer (MX-S; Lianyungang Qingfeng Technology Instrument Company), automated microbial identification system (VITEK-2; BioMerieux), and autoclaved brain heart infusion broth prepared by the hospital microbiology department.

In vitro mechanical ventilation model
After hand hygiene, the operator connected the ventilator circuit with the lung simulator. The lung simulator was placed in a temperature-controlled box set at 38°C. The operator connected the sterile water bottle with the HR, turned on the ventilator and humidifier, set the ventilator mode to synchronized intermittent mandatory ventilation (parameters: tidal volume 500 ml; frequency 14 bpm; fraction of inspired oxygen 45%; pressure support ventilation 12 mbar), and connected a filter to the front end of the exhalation valve of the ventilator. When the humidifier reached the normal temperature range and stopped rising, the operator injected 100 ml broth into the HR. By following this procedure, 60 in vitro mechanical ventilation models were established.

**Bacteria selection and cultivation**

The main pathogens in VAP are Gram-negative bacteria, among which *Pseudomonas* and *Acinetobacter* species are very common [7, 8]. *P. aeruginosa* and *A. baumannii*, which easily survive in the environment, were selected as experimental bacteria. Each of the 15 samples in the sterile water group and the broth group contained the same bacterial species. The ventilator was temporarily kept on standby mode, and 1500 cfu/ml of *P. aeruginosa* or *A. baumannii* was injected into the HR using a 5-ml syringe. Ventilation was then resumed.

**Sampling**

After 24, 72, and 168 h of continuous ventilation, samples collected at the HR outlet (where the humidifier connects with the circuit) and the Y-piece of the ventilator circuit as well as the humidifier water (water in the HR) were sent for bacterial culture. After 168 h of continuous ventilation, the operator cut off the ventilator circuit with a pair of sterile scissors and collected samples at a distance of 10 cm from the HR outlet (Fig. 1). These samples were also sent for culture.

**Statistical methods**

All data were imported into Microsoft Excel, and SPSS 25.0 software was used for statistical analysis. Measurement data were described as mean ± standard deviation (\( \bar{x} \pm S \)), and count data were presented as percentage (%). The results of bacterial culture at different times and sampling locations were analyzed by the Chi-square test. The difference in the concentration of the two bacterial species at the HR outlet was analyzed by the F test. A value of \( P < 0.05 \) was considered statistically significant.

**Results**

In this study, a culture result of 0–5 cfu/ml was considered negative and that greater than 5 cfu/ml was considered positive.

**Bacterial culture results of the sterile water group**

After 24, 72, and 168 h of continuous ventilation, the sampling results of the sterile water samples (\( n = 30 \)) at all locations were negative (Table 1).
Table 1. Bacterial culture at different sampling locations in the sterile water group.

<table>
<thead>
<tr>
<th>location</th>
<th>Humidifier water n(%)</th>
<th>HR outlet n(%)</th>
<th>Y-piece n(%)</th>
<th>HR outlet 10 cm n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>negative</td>
<td>90(100)</td>
<td>90(100)</td>
<td>90(100)</td>
<td>30(100)</td>
</tr>
</tbody>
</table>

Note: “n” indicates the number of cases.

**Bacterial culture results of the broth group**

The bacterial concentration in the humidifier water and at the HR outlet in the broth group were 90% and 36.7% after 24 h of continuous ventilation, 93.3% and 63.3% after 72 h of continuous ventilation, and 93.3% and 76.7% after 168 h of continuous ventilation (Figs. 2 and 3). The results of the bacterial culture at the Y-piece of the ventilator circuit and 10 cm from the HR outlet were negative (Table 2).

Table 2. Distribution of bacterial concentration at different times and sampling locations in the broth group.

<table>
<thead>
<tr>
<th>time</th>
<th>0-5 cfu/ml</th>
<th>5-1000 cfu/ml</th>
<th>&gt;1000 cfu/ml</th>
<th>0-5 cfu/ml</th>
<th>5-1000 cfu/ml</th>
<th>&gt;1000 cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>72 h</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>168 h</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: 5–1000 cfu/ml is light contamination, and >1000 cfu/ml is heavy contamination.

**Bacterial culture at different times and sampling locations**

After 24, 72, and 168 h of continuous ventilation, the bacterial concentration in the HR was high, but no significant difference was observed between the three ventilation times ($P > 0.05$). The bacterial concentration at the HR outlet increased with ventilation time, and the difference between the three times was statistically significant ($P < 0.05$). It was significantly lower at the HR outlet than in the humidifier
water after 24 and 72 h of ventilation \((P < 0.05)\). However, after 168 h of continuous ventilation, the bacterial concentration at the HR outlet increased but was not significantly different from that of the humidifier water \((P > 0.05; \text{Table 3})\).

**Table 3.** Bacterial culture at different times and sampling locations in the broth group.

<table>
<thead>
<tr>
<th>location</th>
<th>time</th>
<th>Humidifier water n(%)</th>
<th>HR outlet n(%)</th>
<th>Y-piece n(%)</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>3</td>
<td>27(90.0)</td>
<td>-</td>
<td>19</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>2</td>
<td>28(93.3)</td>
<td>-</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>168 h</td>
<td>2</td>
<td>28(93.3)</td>
<td>-</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>(\chi^2)</td>
<td>0.27</td>
<td></td>
<td>10.28</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P)</td>
<td>0.86</td>
<td></td>
<td>0.01</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: "−" represents negative results, and "+" represents positive results.

**Comparison of the bacterial concentration at the HR outlet**

After 24, 72, and 168 h of continuous ventilation, the bacterial concentration at the HR outlet was consistently higher in the *P. aeruginosa* group than in the *A. baumannii* group, and the difference was statistically significant \((P < 0.05; \text{Table 4})\).

**Table 4.** Comparison of the concentration of the two bacterial species at the HR outlet.

<table>
<thead>
<tr>
<th>bacteria</th>
<th>(\bar{x} \pm s), (cfu/ml)</th>
<th>(\bar{x} \pm s), (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(P. aeruginosa)</td>
<td>(A. baumannii)</td>
</tr>
<tr>
<td>24 h</td>
<td>13.1±31.1</td>
<td>34.2±56.7</td>
</tr>
<tr>
<td>72 h</td>
<td>20.8±28.6</td>
<td>91.83±82.6</td>
</tr>
<tr>
<td>168 h</td>
<td>46.2±62.1</td>
<td>207.8±173.4</td>
</tr>
<tr>
<td>(F)</td>
<td>12.890</td>
<td></td>
</tr>
<tr>
<td>(P)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Note: "\(\bar{x} \pm s\)" represents mean ± SD.

**Discussion**
Sterile water in the HR was not conducive to bacterial growth

Sterile water does not contain nitrogen, carbon, inorganic elements, and a suitable pH value. Therefore, sterile water is not ideal for bacterial growth. The diameter of water vapor molecules is about 0.0001 μm, whereas the diameter of bacteria is 0.2–10 μm and that of viruses is 0.017–0.3 μm. Thus, theoretically, water vapor molecules cannot carry bacteria or viruses [9]. The sampling results for the humidifier water and different locations of the ventilator circuit were negative in the sterile water group. This confirmed that sterile water in the HR was not conducive to bacterial growth and that bacteria could not colonize other circuit locations during ventilation.

Will bacteria in the HR colonize other circuit locations?

Although sterile water was not conducive to bacterial growth, the reflux condensate may contain respiratory secretions and nutrients conducive to bacterial growth. Studies have indicated the presence of bacteria in the humidifier water during ventilation [10, 11]. Therefore, to confirm if bacteria colonize other circuit locations with aspiration after they reproduce in the humidifier water, brain heart infusion broth, which can promote bacterial proliferation, was added to the humidifier water in the broth group.

Under the influence of the broth, bacteria could reproduce in the HR as expected (Figs. 2 and 3). After proliferation in the humidifier water, bacteria spread in the ventilator circuit in the direction of airflow and reached the HR outlet, but they did not reach a distance of 10 cm from the HR outlet or the Y-piece of the circuit (Table 3).

Bacteria can reach the HR outlet

Bacterial movement

*P. aeruginosa* has a flagellum at one end. Flagella, the locomotory organs, help bacteria to swim quickly in water or slide on the surface of objects [12].

Water droplets were found on the inner wall of the HR throughout the 7-day ventilation period. The liquid level of the humidifier water was about 5 cm from the HR outlet. Therefore, within a certain period, *P. aeruginosa* in the humidifier water can swim to the HR outlet through the water on the inner wall of the HR. Thus, as the ventilation time was extended, the bacterial concentration at the HR outlet in the broth group continued to increase.

In response to internal and external stimuli, bacterial cells produce fimbriae by activating the BFMRS system to promote the expression of the CSUA/B/D/E gene cluster [13].
Although *A. baumannii* lacks flagella, it can use fimbriae to move on wet surfaces by the depolymerization of type 4 fimbriae [14]. Therefore, after 168 h of continuous ventilation, the bacterial concentration at the HR outlet in the *A. baumannii* group also increased. However, because motility by fimbriae is far lower than that by flagella, the concentration of *A. baumannii* was always significantly lower than that of *P. aeruginosa* at the HR outlet (Table 4).

**Bacterial aerosol production**

In addition to movement, is there another way for bacteria to colonize the HR outlet? Studies have shown that after the humidifier water is contaminated by bacteria, a bacterial aerosol may be formed, but the probability of producing high levels of bacterial aerosol is low [15]. If this hypothesis is true, bacteria in the HR produce a bacterial aerosol during the aeration process, and the aerosol may carry bacteria from the humidifier water to the HR outlet.

Although *A. baumannii* has a relatively weak motive force, its concentration at the HR outlet was low (Table 4). It is not certain whether this concentration was achieved by the motive force of the bacterial species alone. If the bacterial aerosol hypothesis is established, in addition to bacterial movement, the presence of aerosol will further promote the bacteria in the HR to reach the HR outlet, which better explains the results in Table 4.

**Bacteria cannot colonize further**

In the broth group, the bacterial concentration was negative at a distance of 10 cm from the HR outlet and at the Y-piece. First, if aerosols were produced in the HR during ventilation, high levels of bacterial aerosol were not produced. Thus, the spread of the aerosol was limited, and bacteria could not colonize further locations. Second, ion hydration is a chemical reaction between substances and water. According to molecular dynamics theory, the higher the temperature and concentration, the more active the ion hydration of the solution. The temperature, humidity, and bacterial concentration 10 cm below the HR outlet were higher than the position above the HR outlet. Therefore, the water molecules and ions carried by the aerosol are more likely to undergo ion hydration at the position below the outlet.

Ion hydration will cause aerosol particles to be surrounded by water and form a solvation film [16]. This not only reduces the surface energy of colloidal particles and prevents them from colliding but also enhances colloid stability. It improves the deposition rate and reduces the transport rate of aerosols. Hence, the aerosol bacteria colonized 10 cm below the HR outlet but could not colonize the ventilator circuit 10 cm above the outlet.

**Limitations**

In this study, the number of samples was limited because of the limitation of experimental conditions, and research was not performed on *A. baumannii* movement. Moreover, whether *A. baumannii* can reach the HR outlet only by fimbriae could not be verified. Thus, the presence of a bacterial aerosol could not be confirmed. Finally, based on the results, pouring the condensate from the circuit into the HR may be feasible for condensate treatment.
Conclusions

In mechanical ventilation, sterile water in the HR was not conducive to bacterial growth and reproduction, but other factors in the clinical setting, such as condensate reflux, lead to the bacterial concentration in the humidifier water. However, even if bacteria reproduce in the HR in the clinical setting, they can only colonize the HR outlet during ventilation, and colonizing locations more than 10 cm from the HR outlet was difficult.

Abbreviations

| HR | Humidifier reservoir |
| VAP | Ventilator-associated pneumonia |

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the University of Hong Kong-Shenzhen Hospital, and the review approval number is Lun [2018]06.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors report no conflicts of interest relevant to this article.

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Author’s contributions

PXZ: Conceptualization, Methodology, Writing - review and editing, Formal analysis, Resources, Supervision. LTR: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. SLP: Validation, Writing - review and editing. JYG: Investigation, Resources. RWTL: Validation, Resources. All authors read and approved the final manuscript.

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References


**Figures**

**Figure 1**

*In vitro* mechanical ventilation model.

**Figure 2**

Bacterial concentration in humidifier water.

**Figure 3**

Bacterial concentration at the HR outlet.