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BACTERIAL COLONIZATION ON VENTILATOR SURFACES IN THE ICU OF GOVERNMENT HOSPITAL IN PONTIANAK

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Abstract

Introduction: Colonization on ventilator surface is causing a risk of pathogenic bacteria transmission, leading to Healthcare-Associated Infections (HAIs). Therefore, this study aimed to determine bacterial colonization on ventilator surface in the Intensive Care Unit (ICU) of Government Hospital in Pontianak. Methods: Two ventilators, designated A and B, were sampled by sterile cotton swabs moistened with NaCl at 7 sampling points, namely power, interface, and control button, as well as screen, handrail, inspiratory port, and expiratory port. Samples were plated in triplication using the spread plate method on tryptone soya agar (TSA) medium and then incubated for 24 hours. The growth colonies were counted, and the morphology was observed macroscopically and microscopically. Results and Discussion: The results showed colonization at all sampling points on both ventilator surfaces. Ventilator A had total average number of colonies of 97, which was significantly higher compared to B with a total average of 7. Gram-negative bacteria (GNB) were observed more than Gram-positive bacteria (GPB) in both ventilators, accounting for 58.75% and 41.25%, respectively. The handrail part showed the highest number of colonies, accounting for 546 and 35 in ventilator A and B, respectively, represent both GNB and GPB but dominated by Gram-Positive coccus. The morphological forms of bacterial cells found were Gram-negative bacillus (GNB), Gram-positive coccus (GPC), Gram-negative coccus (GNC), and Gram-positive bacillus (GPB), with percentages of 37.50%, 27.50%, 21.25%, and 13.75%, respectively. Conclusion: This study showed colonization on the surfaces of two ventilators used in the ICU.

INTRODUCTION

Infectious diseases are the leading cause of high morbidity and mortality rates in hospitals in developing countries, such as Indonesia. These diseases are better known as nosocomial infections, also called Healthcare-Associated Infections (HAIs) (1–4). The annual direct costs of HAIs in developed countries, such as the UK is at £503 million and cause 58,010 days of extended hospitalization (5). In developing countries, such as India, there were 515 patients suffering from HAIs with a mortality rate of 8.75%. The increased cost was reported to be 4611 USD, leading to 23 days of extended hospitalization (6).

HAIs are infections that patients acquire

from a hospital, regardless of initial health status or existing infection (7–9). HAIs can be caused by bacterial colonization from the hospital environment, which contaminates medical equipment and facilities or equipment in the hospital (10,11). Several studies reported that the prevalence of healthcare-associated infections (HAIs) in the Intensive Care Unit (ICU) is higher compared to other wards (4,12–14). The infections acquired from the ICU in developing countries, such as Indonesia are higher than in high-income countries (15– 17).

Patients in the ICU have critical conditions, thereby increasing the risk of experiencing infection. Colonization of bacteria on the surface of medical equipment, such as

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ventilators can cause cross-transmission of pathogenic bacteria to patients. Ventilator surface is often touched by health workers who then come into direct contact with patients. This situation increases the risk of transmitting potentially pathogenic bacteria and causes infection from one patient to another (18). An example of health-related infections associated with ventilator use is Ventilator Associated Pneumonia (VAP). According to a previous study, the reported incidence of VAP ranges from 5% to 40% (19). This infection is associated with high rates of mortality and morbidity, as well as increases the cost of care and stay in the ICU (20–22).

A study in the Veterans Hospital Baltimore showed that there was bacterial colonization on the surfaces of 20 ventilators in the ICU (23). The proportion of Gram-positive bacteria was more significant (89.3%) than Gram-negative (10.7%). Another study conducted in Hospital Taipei found bacterial colonization on the surface of 17 ventilators, with an average number of 122 CFU, 8 CFU, 8 CFU, 32 CFU, and 22 CFU in faceplates, silence buttons, shuttle buttons, ventilator plates, and handrails, respectively (24). There has been no study regarding bacterial colonization on ventilator surface in the ICU of Government Hospital in Pontianak. Therefore, this study aimed to determine bacterial colonization on ventilator surface in the ICU of the Government Hospital in Pontianak.

METHODS

The protocol was peer-reviewed by the Ethics Committee of the Faculty of Medicine of Universitas Tanjungpura with an ethical clearance number of 1937/ UN22.9/PG/2023.

Culture Medium Preparation

A total of 40 grams of TSA powder was weighed using an analytical scale. This powder was then dissolved in 1,000 mL of distilled water in a Duran bottle and stirred on a hotplate stirrer at 60°C until boiling. Tryptic Soy Agar is a nutrient-rich medium commonly used for cultivating a wide range of microorganisms (25).

Tools and Materials Sterilization

Tools used in this study include Petri dishes, test tubes containing 0.9% NaCl, and Duran bottles with TSA medium closed tightly with paper. One ml microcentrifuge tubes were placed in a beaker and covered with aluminum foil. The tools and materials were sterilized in an autoclave at 121°C for 20 minutes.

Sampling

Two ventilators of the Intensive Care Unit (ICU)

in one of the Government Hospital in Pontianak were sampled at 7 points, with a total of 14 samples. The points sampled were the power, interface, and control buttons, as well as the screen, handrail, inspiratory, and expiratory port, as shown in Figure 1. Samples were taken when ventilator was not attached to the patient and had been cleaned. Before cleaning, ventilator A was in use, while B was not in use by any patient. At the time of sampling, ventilator A was near the bed occupied by the patient, while ventilator B was not. A sterile cotton swab that has been moistened with sterile 0.9% NaCl was applied to each of the 7 predetermined points by rotating the sterile cotton swab to make contact with the sample surface. The sterile cotton swab was then broken into a microcentrifuge tube containing 1 ml of sterile 0.9% NaCl. The samples were coded, placed in a box in an upright position, and taken to the Microscopic Laboratory, Faculty of Medicine, Tanjungpura University, Pontianak. All ventilator surface swab samples were collected on the same day.



Description: 1. Power button, 2. Interface button, 3. Control button, 4. Screen, 5. Handrail, 6. Inspiratory port, 7. Expiratory port.

Figure 1. Hamilton-C1 Ventilator Surface Sampling Points

Bacteria Culture

Each sample was centrifuged for 1 minute, pipetted at 250 μ L (triplicate), and spread with an L rod until evenly distributed (spread plate) on agar medium. Each petri dish was incubated at 37°C for 24 hours. This study used a sterile TSA medium as a control to ensure the accuracy and reliability of the result.

Calculation of Number of Colonies

The colonies were counted using a manual colony counter (Interscience Scan 100[®]) and the results were added and averaged. The calculation results are expressed in colonies.

Macroscopic and Microscopic Observation

Macroscopic observations were carried out to observe the shape, edges, elevation, and color of the growing colonies. Subsequently, colonies of different types were taken and Gram-stained. Microscopic observations were carried out using Gram staining with objective lense 100x and immertion oil.

Data Analysis

Data were analyzed descriptively by describing the number of colonies, macroscopic and microscopic observations, and presented in figures and tables. Macroscopic observations include the shape, edges, elevation, and color of growing bacterial colonies. Microscopic observations include cell shape and color of bacterial cell walls (gram type).

RESULTS

Number of Colonies

The results of this study showed that bacterial colonies grew at all sampling points. The number of bacterial colonies at each sampling point was different, as shown in Table 1. However, samples obtained from ventilator A showed more colonies than B at all sampling points. On ventilator A, the highest number of colonies were found at handrail (VA.5) (546 colonies), control button (VA.3) (48 colonies), and expiratory port (VA.7) (39 colonies). The highest number of colonies on ventilator B was found on handrail (VB.5) (35 colonies), inspiratory port (VB.6) (7 colonies), and expiratory port (VB.7) (4 colonies). Based on the result, this study showed that handrail was the most considerable colony growth in both ventilator units, as shown in Figures 2A and 2B.

Table 1. Number of Bacterial Colonies on VentilatorSurfaces in the ICU of Government Hospital in Pontianak

Ventilator	Sampling Point	Average Number of Colonies	Total Average Number of Colonies
A	Power button (VA.1)	9 colonies	
	Interface buttons (VA.2)	3 colonies	
	Control button (VA.3)	48 colonies	
	Screen (VA.4)	23 colonies	97 colonies
	Handrail (VA.5)	546 colonies	
	Inspiratory port (VA.6)	11 colonies	
	Expiratory port (VA.7)	39 colonies	
В	Power button (VB.1)	1 colony	
	Interface buttons (VB.2)	1 colony	
	Control button (VB.3)	1 colony	7 colonies
	Screen (VB.4)	1 colony	
	Handrail (VB.5)	35 colonies	
	Inspiratory port (VB.6)	7 colonies	
	Expiratory port (VB.7)	4 colonies	



Figure 2. Bacterial Colonies on Handrail of Ventilator A (2A) and Ventilator B (2B)

Macroscopic and Microscopic Observations

The shape of bacterial morphology was punctiform, circular, and irregular. Colony elevations were flat, raised, convex, pulvinate, umbonate, and crateriform. The edges of the colony were entire, undulate, lobate, and erose, with colors of white, yellow, milky white, gray-white, and semi-trans. Furthermore, the distribution of Gram-negative bacteria at 7 sampling points from both ventilators was 58.75%, relatively higher than the Gram-positive of 41.25%. The predominant bacterial morphology observed was Gram-negative bacillus (GNB), accounting for 37.50%, followed by Gram-positive coccus (GPC) (27.50%), Gram-negative coccus (GNC) (21.25%), and Gram-positive bacillus (GPB) (13.75%), as shown in Table 2.

 Table 2. Distribution of Bacterial Patterns on Ventilator

 Surface in the ICU of Government Hospital in Pontianak

Gram Type	Coccus	Bacillus	Ν
Positive	22 (27.50%)	11 (13.75%)	33 (41.25%)
Negative	17 (21.25%)	30 (37.50%)	47 (58.75%)
Ν	39 (48.75%)	41 (50%)	80

DISCUSSION

Bacterial colonization was significantly higher on ventilator A compared to ventilator B. Particularly, handrail on both ventilators exhibited the highest bacterial colony counts.

Two ventilators designated A and B were used in the ICU setting. Ventilators are situated in the patient zone, typically close to the patient during treatment (26). The treatment process, combined with frequent hand contact by healthcare personnel on frequently touched ventilator surfaces, can facilitate rapid microbial contamination (27). Consistent with the report of the previous investigation, this study identified handrail on both ventilators as the sampling point harboring the highest bacterial colony counts, as shown in Table 1. These results were different from the report of previous studies conducted in Taipei hospitals that the highest average bacterial colony counts were found on ventilator screens (faceplates) (122 CFU) (24). The differences may be attributed to variations in cleaning protocols implemented at different healthcare facilities.

Handrail, a frequently touched surface on ventilator, exhibited the highest bacterial colony counts. This observation is consistent with the statement that frequent hand contact by healthcare personnel can increase bacterial burden on the surfaces (28-29). Significantly higher bacterial colony counts were recovered from all sampling points on ventilator A compared to B, reflecting the positioning of ventilator during the period. Ventilator A was situated adjacent to a bed occupied by a patient, while B was positioned next to an unoccupied bed. This spatial arrangement would presumably lead to more frequent contact with ventilator A by healthcare personnel during patient care activities or by direct skin shedding of the patient (30). In addition to patient occupancy, the history of ventilator use and its associated cleaning protocol may also impact bacterial colony counts at various sampling points.

Referring to the WHO Care, Cleaning, Disinfection and Sterilization of Respiratory Devices document, ventilators should be cleaned and sterilized after used and stored in a clean environment before being used by the next patient. Ventilator cleaning is carried out in a well-ventilated area, away from the patient area and other equipment. Staff who are tasked with cleaning the ventilator surface must perform hand hygiene and wear Personal Protective Equipment (PPE), including surgical masks/respirators, goggles or face shields, medical gowns, rubber gloves, and boots or closed work shoes. The ventilator surface from top to bottom (including controls, body, trolley and handrails, screen, power cord, and gas supply hose) is cleaned with a disposable cloth moistened with detergent and clean water, then any remaining detergent residue is wiped with a dry lint-free cloth. After the cleaning process with detergent, the ventilator surface from top to bottom is wiped with a new cloth or disposable cloth moistened with a suitable disinfectant, according to the instructions for use on each ventilator. Disinfectants that can be used are ethanol, isopropyl alcohol, mikrobac forte 0.25%-4%, perform 3% and terralin protect 2% (31). However, due to the limitations of the current study design, the specific impact of these factors could not be definitively assessed.

Colony Morphology

Bacterial colonies are visible aggregations of a single bacterial cell type on an agar plate. Colony morphology, including shape, elevation, edge, and color, aids in preliminary bacterial identification, such as colony picking for further identification (32-33). The limitation of this study is that specific strain types for colonies found were not identified.

Gram-negative was more determined than Grampositive bacteria with Gram-negative bacillus (GPC) most observed.

Analysis of bacterial isolates showed a predominance of GNC compared to GPC. In particular, the handrail sampling sites (VA.5 and VB.5) on both ventilators showed a dominance of GPC. At VA.5 and VB.5 (handrail sites with the highest colony counts), GPC was the most frequently isolated bacteria. This result suggested that handrail, frequently touched by healthcare personnel, could harbor a predominant commensal skin flora, primarily consisting of GPC (34). The results are consistent with the report of previous studies that Grampositive bacteria, particularly *Staphylococcus aureus*, were found on ventilator surfaces (18).

Moreover, our results demonstrated the growth colonies in both inspiratory and expiratory port of two ventilators that may contribute to HAIs. GNB represents a significant threat in HAIs, reported to be responsible for 2.3 times more cases compared to Gram-positive bacteria (35). This enhanced virulence is partly due to the presence of an outer membrane in GNB, which functions as a permeability barrier, hindering the penetration of antibiotics and disinfectants (36-38). GNB was the predominant bacterial isolate in the current study, constituting 37.50%. This isolate includes a diverse range of pathogens associated with ventilatorassociated pneumonia (VAP). The reported incidence of VAP varies from 5% to 40%. VAP is associated with high mortality and morbidity, increased costs of care and increased length of stay in the ICU (19-20). GNB associated with VAP was found on ventilator surfaces, including Pseudomonas aeruginosa and Acinetobacter baumannii (23-24,39-41). GPC represented the second-most prevalent bacterial group, accounting for 27.50%. The species of GPC associated with VAP, such as Staphylococcus aureus (18,23-24,42-44) were recovered from ventilator surfaces. The presence of GNC on ventilator surfaces has not been reported in previous studies and the association with VAP remains less explored in the current literature. Bacillus spp. were recovered from ventilators in previous investigations (23), but the association with VAP is unknown.

Due to limitations in the current study design, bacterial isolates were characterized solely based on Gram stain morphology, and definitive identification to the species level was not carried out. Further investigations using biochemical testing are needed to achieve a more precise taxonomic classification of the recovered isolates. This characterization is important as ventilator surfaces may harbor bacteria with the potential to cause HAIs, particularly VAP. Previous studies identified bacteria commonly found on ventilator surfaces as part of the human commensal flora. These results suggested that the potential sources of ventilator contamination included both patient shedding and direct contact with the hands of healthcare workers. Shedding refers to the expulsion of microorganisms from the human body through various mechanisms, including the release from skin and hair, as well as through respiratory secretions generated during breathing, speaking, and coughing (45). Meanwhile, the commensal flora represents the diverse microbial communities that reside on human skin and mucosal surfaces. Resident bacteria are typically non-pathogenic in healthy individuals but can act as opportunistic pathogens in immunocompromised patients, such as those admitted to the ICU (46).

AUTHORS' CONTRIBUTION

HI: Conceptualization, Methodology, Reviewing, and Editing. IMP: Methodology, Writing-Original draft preparation. DFL: Methodology, Reviewing, and Editing.

CONCLUSION

In conclusion, this study reported bacterial colonization at all sampling points in both ventilators with a distribution of 58.75% and 41.25% in Gram-negative and 41.25% of Gram-positive bacteria, respectively. The morphology of the colonies observed was punctiform, circular, irregular shapes, flat, raised, convex, pulvinate, umbonate, and crateriform elevations, entire, undulate, lobate, erose edges, white, yellow, milk white, gray-white, and semi-trans colors. Furthermore, the morphological forms of bacterial cells found were Gram-negative bacillus (GNB), Gram-positive coccus (GPC), Gramnegative coccus (GNC), and Gram-positive bacillus (GPB), with percentages of 37.50%, 27.50%, 21.25%, and 13.75%, respectively.

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