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Monitoring the effect of microbial culture on cleaning and sanitizing of the external ventilator circuit

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ABSTRACT

This research aimed to investigate the microbial spectrum of microorganisms that cause ventilator-associated pneumonia (VAP) among ICU patients in the selected hospital, antimicrobial susceptibility, genetic diversity of common isolates, and the monitoring effect of microbial culture on cleaning and sanitizing of external ventilator circuits in order to reduce the occurrence of hospital infections. For this purpose, endotracheal aspirate (ETA) specimens were sampled from ICU patients with clinically suspected VAP in the hospital between August 2020 and August 2021 and then investigated for microbiological content. This was followed by Kirby-Bauer testing for determining drug sensitivity and ERIC-PCR for genotyping. Afterward, microbial culture was performed on cleaned, sanitized and dried ventilator external ventilator pipelines and those stored aseptically for 4 weeks to evaluate the cleaning and disinfection effect and measure the bacterial content. Results showed that in the 64 confirmed VAP cases, Klebsiella was the most frequently isolated organism, followed by P. aeruginosa and Acinetobacter baumannii, while Candida is the most widely isolated fungus. The antimicrobial susceptibility spectrum revealed that 40% of the isolates were multidrug-resistant (MDR). ERIC-PCR showed no genetic relationship between pneumococcal isolates. Through microbial culture, no pathogenic bacteria were detected among cleaned and sanitized ventilator external ventilator pipelines and those stored aseptically for 4 weeks, indicating a 100% pass rate. It was concluded that ventilators in intensive care units (ICU) are susceptible to contamination, exposing patients to bacterial contamination and other comorbidities. Gram-negative bacteria are the main pathogens of VAP, which are mostly multidrug-resistant. Clinical care measures for ventilators should be strengthened to reduce the incidence of ventilator microbial contamination and to improve accurate clinical diagnosis and correct antimicrobial therapy.

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Introduction

Ventilators are common first-aid and life-support equipment in modern clinics to maintain a normal respiratory process, reduce symptoms and prolong survival through assisted mechanical ventilation. However, ventilator-associated pneumonia (VAP) has seen an increasing incidence year by year due to its wider clinical application (1). Mechanical ventilation creates a closed loop between the ventilator circuit and the respiratory tract, and bacteria that colonize the lungs contaminate the ventilator circuit as the patient breathes and coughs. Such contamination is the main factor underlying VAP, so conducting strengthened management of ventilator circuit cleaning & disinfection and doing regular testing is of great significance to control VAP (1,2).

VAP denotes inflammation of lung parenchyma caused by a microbial pathogen(2) and represents a significant subset of hospital-acquired pneumonia (HAP). It is defined as pneumonia that occurs 48 hours or more after mechanical ventilation, causing a healthcare cost burden and severely affecting prognosis (3). VAP is mainly characterized by new pulmonary infiltrates, signs of systemic infection, altered sputum appearance, leukocytosis and decreased oxygenation (4,5).

Aetiology detection is crucial in the diagnosis of VAP. Specifically, lower respiratory tract samples were pooled by either invasive (protected specimen brush [PSB] or bronchoalveolar lavage [BAL]) or non-invasive (endotracheal aspiration [ETA]) techniques and cultured quantitatively or semi-quantitatively. As recommended in the American Thoracic Society (ATS) guidelines, quantitative

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culture may be performed on ETA or transbronchoscopically or non-bronchoscopically collected (6) specimens. Thus, microbial differentiation between VAPs is relevant to broad-spectrum antimicrobial coverage of MDR pathogens. As an appropriate antimicrobial therapy significantly improves the prognosis of VAP patients, more rapid identification of infected patients and accurate antimicrobial drug selection are important clinical goals (7).

In this study, the cleaning and disinfection effect of external ventilator circuits was quantitatively evaluated by measuring bacterial residues in the circuits and analyzing endotracheal aspirates of patients with clinically suspected VAP, providing a scientific basis for rapid clinical diagnosis and antimicrobial treatment of VAP.

Materials and methods

Microbiological monitoring in external ventilator circuits after cleaning and storage for 4 weeks

The external circuit of the ventilator was disassembled to the smallest unit for cleaning and disinfection using a double door washer-disinfector. After the circuit was dried, a sterile cotton swab soaked with stroke-physiological saline solution was applied in a circular pattern at the open end of the threaded pipe towards the distal end, and then repeatedly smeared on the inner and outer surfaces of the threaded pipe, the tee pipe, the connecting pipe, the humidification tank and the inner surface of the water collector to collect samples for a total bacterial count test. As per the updated hygiene industry standards, the total bacterial count was <20 cfu/cm^2 after disinfection, and no pathogenic bacteria could be detected (8).

Microbiological & drug sensitivity testing of ETA specimens and genotyping of common isolates

Study population and inclusion & exclusion criteria

The study population consisted of patients with clinically suspected VAP admitted to the ICU from August 2020 to August 2021. Inclusion criteria: (i) the patients developed symptoms after more than 48 hours of mechanical ventilation; (ii) the chest X-ray showed infiltrative shadows or new inflammatory lesions in the lungs after mechanical ventilation; (iii) signs of pulmonary consolidation and/or breath sounds and moist rale heard during auscultation of the lungs. Additionally, one of the following three conditions were satisfied: white blood cells (WBC) >10.0 x 109/L or <4 x 109/L with or without nuclear metastases; fever with temperature >37.5°C and copious purulent secretions from the respiratory tract; new pathogenic organisms were cultured on post-onset sputum specimens. Exclusion criteria: those with clinical and radiological signs suggestive of pneumonia upon admission; those with pulmonary conditions such as tuberculosis, pulmonary tumors and pulmonary atelectasis.

Collection and processing of ETA specimens

A total of 64 endotracheal aspirate (ETA) specimens were collected from the subjects using bronchoalveolar lavage in the following manner:(9) ETAs were pooled from each patient with a 22-inch suction catheter that was gently introduced through the endotracheal airway at a distance of approximately 25 - 26 cm. Gentle suctioning was then performed without injecting saline, and the catheter was removed from the endotracheal tube. Afterward, the portion of the catheter containing the aspirate was cut open, placed in a sterile container and immediately transported to the microbiology laboratory.

Determination of antimicrobial susceptibility

The susceptibility of microbial isolates to different antimicrobial agents was determined by the Kirby-Bauer disc diffusion method (10) on Mueller-Hinton (MH) agar. Antimicrobial discs were stored at 4°C and used after reaching room temperature. The antimicrobial agents used in this study, targeting different bacterial and fungal species, represented different classes of antibacterial and antifungal drugs. The antibacterial tablets included amikacin (30 μg), amoxicillin (30 μg), amoxicillin/clavulanic acid (20/10 μg), azithromycin (15 μg), aztreonam (30 μg), cefazolin (30 μg), cefepime (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), clarithromycin (15 μg), clindamycin (2 μg), colistin (10 μg), doxycycline (30 μg), gentamicin (10 μg), levofloxacin (5 μg), novobiocin (30 μg), oxacillin (1 μg), piperacillin (100 μg), tobramycin (10 μg), trimethoprim (25 μg) and vancomycin (30 μg). The

antifungal tablets used in this study included amphotericin B (100 µg), nystatin (100 µg), clotrimazole (50 µg), ketoconazole (50 µg), fluconazole (25 µg), griseofulvin (10 µg), itraconazole (50 µg) and terbinafine (30 µg). Isolates showing resistance to at least three different antimicrobial drugs were considered multidrug-resistant.

Identification of pneumococcal isolates by ERIC-PCR to determine genetic diversity

To perform ERIC-PCR amplification, genomic DNA was extracted from the examined pneumococcal isolates using the GeneJet Genomic DNA Purification Kit as indicated by the manufacturer (Thermo Scientific, Waltham, Massachusetts, USA-K0721). The total volume of the PCR reaction was 25 µL, containing approximately 10 ng of template DNA, 10 pmol of ERIC-1 primer (5´-ATGTAAGCTCCTGGGGATTCAC-3´), 12.5 µL of PCR masterbatch (2X) (Promega, Madison, Wisconsin, USA) that was added with nuclease-free water to a volume of 25 µL. PCR amplification was performed on a 96-well thermal cycler (Applied Biosystems, Foster City, California, USA), programmed for initial denaturation at 95°C for 5 minutes. This was followed by 40 cycles of 1-minute denaturation at 95 ºC, primer annealing at 45°C for 1 minute, extension at 72 °C for 8 minutes, and a final extension at 72°C for 10 minutes (11). PCR products were examined by Tris, acetate and EDTA (TAE) agarose gel electrophoresis.

Results and discussion

Pass rate after cleaning, disinfection, drying and aseptic storage for 4 weeks

The cleaned and dried ventilator circuits and those stored aseptically for 4 weeks were microbially cultured. It was found that the total bacterial count <20 cfu/cm^2, and no pathogenic bacteria were detected, with a pass rate of 100%. Sampling values showed high-efficiency disinfection levels, which conformed to the Ministry of Health's Regulation of Disinfection Technique in Healthcare Settings for external ventilator circuits (Table 1).

Microbiological test results of ETA specimens

Most VAP patients tested positive for a variety of aerobic bacteria (Gram-positive and Gram-negative) and predominantly Gram-negative fungi. K. pneumoniae was the predominant pathogenic species among all isolates (25%) (Table 2).

Table 1. Pass rate (%) after cleaning, disinfection and drying

Items	Testing time	CFU (cfu/cm^2)	Pass rate
Water collection cups	After cleaning & disinfection;	0	100%
	storage for 4 weeks	1	100%
Y-shaped pipe	After cleaning & disinfection;	0	100%
	storage for 4 weeks	0	100%
U-shaped pipe	After cleaning & disinfection;	0	100%
	storage for 4 weeks	0	100%
Circuit	After cleaning & disinfection;	0	100%
	storage for 4 weeks	0	100%
Humidification tank	After cleaning & disinfection;	0	100%
	storage for 4 weeks	0	100%
Filtration paper	After cleaning & disinfection;	0	100%
	storage for 4 weeks	0	100%
Threaded pipe	After cleaning & disinfection;	1	100%
	storage for 4 weeks	1	100%

Table 2. Bacteria and fungi detected in ETA specimens

Microbial species	Early-onset VAP	Late-onset VAP	Isolate count
Gram-negative bacteria			
K. pneumoniae	10	6	16 (25)
P. aeruginosa	5	7	12 (18.75)
Acinetobacter baumannii	4	3	7 (10.93)
E. coli	1	2	3 (4.68)
Burkholderia cepacia	0	3	3 (4.68)
Stenotrophomonas maltophilia	3	1	4 (6.25)
Salmonella pneumoniae	1	1	2 (3.12)
Bacillus thuringiensis	0	3	3 (4.68)
Gram-positive bacteria			
Staphylococcus aureus	2	3	5 (7.81)
Enterococcus faecalis	2	2	4 (6.25)
Fungi			
Candida tropicalis	1	2	3 (4.68)
Candida albicans	1	1	2 (3.12)
Total	30 (46.88)	34 (53.12)	64 (100)

Drug resistance of microbial species in VAP patients

The isolated bacterial and fungal species showed high resistance to the examined antimicrobial classes. Gram-negative bacteria, with the exception of Salmonella and Borrelia, had all isolates sensitive to clonidine. Klebsiella spp. isolates were sensitive to amikacin, meropenem and azithromycin. P. aeruginosa isolates were sensitive to azithromycin and clarithromycin. Gram-positive isolates were sensitive to teicoplanin, doxycycline and sulfamethoxazole. Antifungal sensitivities suggested that all isolates were resistant to the tested antifungal drugs, while Candida spp. were sensitive to itraconazole (Table 3).

Table 3. Drug resistance of VAP microorganisms

Microbial species	Antimicrobial resistance
Gram-negative bacteria	
Klebsiella	AK (25)[1], AM (100), AMC (68), AZM (22), ATM (62), CZ (100), FEP (68), CTX (70), CAZ (70), CXM (78), FOX (86), CRO (87), CIP (67), CLR (80), DA (92), CT (0), DO (65), CN (60), LEV (65), MEM (25), PRL (100), TPZ (65), TOB (62), SXT (75)
P. aeruginosa	AK (45), AM (100), AMC (100), AZM (0), ATM (85), CZ (100), FEP(100), CTX(100), CAZ (100), CXM (100), FOX (100), CRO (100), CIP (56), CLR (74), DA (85), CT (0), DO (16), CN (72), LEV (65), MEM (56), PRL (100), TPZ (75), TOB (75), SXT (72)
Acinetobacter baumannii	AK (35), AM (100), AMC (100), AZM (19), ATM (100), CZ (100), FEP(100), CTX(100), CAZ (100), CXM (100), FOX (100), CRO (100), CIP (68), CLR (87), DA (100), CT (0), DO (43), LEV (73), MEM (93), PRL (100), TPZ (93), TOB (87), SXT (63)
E. coli	AK (53), AM (100), AMC (100), AZM (63), ATM (100), CZ (100), FEP(100), CTX (100), CAZ (100), CXM (100), FOX (100), CRO (100), CIP (87), CLR (57), DA (100), CT (0), DO (43), LEV (57), MEM (56), PRL (100), TPZ (58), TOB (62), SXT (100)
Maltophilia	AK (0), AM (100), AMC (60), ATM (100), CZ (100), FEP (100), CAZ (100), FOX (100), CRO (100), CIP (60), CT (0), DO (0), LEV (0), MEM (0), PRL (100), TOB (0), SXT (0)
Salmonella	AK (0), AM (100), AMC (100), AZM (100), ATM (0), CZ (100), CXM (100), FOX (100), CRO (72), CIP (100), CLR (100), DA (83), CT (100), DO (0), CN (100), LEV (100), MEM (100), PRL (100), TOB (100), SXT (100)
Gram-positive bacteria	
Staphylococcus aureus	AM (100), AMC (57), AK (46), AZM (68), CRO (100), CRT(82), FOX (65), FEP (77), CIP (68), CLR (67), DA (59), DO (0), LEV (64), LNZ (38), NV (0), OX (67), PRL (100), TPZ (67), TEC (0), SXT (32), VA (46)
Enterococcus faecalis	AM (0), AMC (0), AK (100), AZM (100), CRO (0), CIP (100), DO (0), LEV (100), LNZ (100), TPZ (0), TEC (0), TOB (100), SXT (0), VA (0)
Fungi	
Candida	AB (100), NY (100), CTR (100), KET (50), FCA (100), GRS (100), ITR (0), TER (100)

Note: 1. The percentage of resistant isolates correlates with the number of isolates within each species. AK, amikacin; AM, amoxicillin; AMC, amoxicillin-clavulanate; AZM, azithromycin; ATM, aztreonam; CZ, cefazolin; FEB, cefepime; CAZ, ceftazidime; CTX, cefotaxime; CXM, cefuroxime; FOX, cefoxitin; CRO, ceftriaxone, IP, ciprofloxacin; C, chloramphenicol; CLR, clarithromycin; DA, clindamycin; CT, colistin; DO, doxycycline; CN, gentamicin; LNZ, linezolid; LEV, levofloxacin; MEM, meropenem; NV, novobiocin; OX, oxacillin; PRL, piperacillin; TPZ, piperacillin/tazobactam; TEC, teicoplanin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; VA, vancomycin; AB, amphotericin B; NY, dicentrine; CTR, clotrimazole; KET, ketoconazole; FCA, fluconazole; GRS, griseofulvin; ITR, itraconazole; TER, terbinafine.

ERIC-PCR results of K. pneumoniae

Genotyping of 16 pneumoniae isolates detected by ERIC-PCR revealed significant molecular heterogeneity in diplococcus pneumoniae isolates, exhibiting 16 different base ERICs (Figure 1).

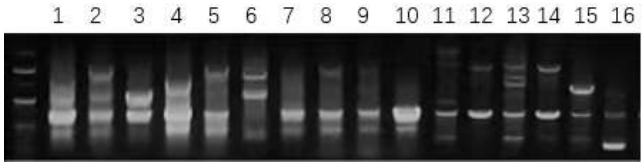

Figure 1. ERIC-PCR spectrum of K. pneumoniae

The application of mechanical ventilation has modernized the management of critically ill patients with respiratory failure. The use of ventilators has increased several times since they were first described in the 1950s(12). It has become an essential feature of modern critical patient care but is accompanied by complications across airway injury, ventilator-induced lung injury, pulmonary atelectasis, and especially VAP(13). Tracheal intubation compromised the natural barrier between the oropharynx and the trachea, helping bacteria to enter the lungs through aspiration and exudation of contaminated secretions around the tracheal catheter cuff. A systematic review by Sanjeev Kharel et al (14) indicated that VAP rates in Southeast Asia ranged from 2.13 to 116 per 1,000, with variation between countries. Significant mortality rates ranging from 16.2% to 74.1% were observed in 13 studies. Therefore, there is an urgent need for cost-effective control and prevention measures such as intervention studies and staff training, hand hygiene, awareness of antibiotic resistance and improved management of ventilator cleaning and disinfection.

The most common pathogens reported for VAP were aerobic Gram-negative bacteria including P. aeruginosa, E. coli, K. pneumoniae and Acinetobacter, and Gram-positive bacteria such as S. aureus (15-17). In agreement with the above data, Gram-negative bacteria accounted for 78.12% (50/64) of the microorganisms isolated from ETA specimens in this study, followed by Gram-positive bacteria (14.06% (9/64)) and fungal isolates (7.81% (5/64)). The most common gram-negative bacterial species were Klebsiella (25%), P. aeruginosa (18.75%) and Acinetobacter baumannii (10.93%).

VAP triggered by MDR pathogens was significantly associated with high mortality (18-20). In the present study, the high resistance profile of Gram-negative strains to cefazolin, piperacillin, cefoxitin, ceftriaxone and clindamycin suggested that these antimicrobials were not suitable for early empirical treatment of VAP cases. In contrast, the

susceptibility of meropenem, amikacin and doxycycline make their possible alternatives. In addition, mucomycin and azithromycin among other drugs presenting better efficacy against Klebsiella, P. aeruginosa, and Pseudomonas baumannii may be given to patients with severe complications. Therefore, these drugs may be considered when determining empirical treatment. Teicoplanin, doxycycline and vancomycin may be used as possible options for the treatment of Gram-positive bacteria. Of the fungal isolates, itraconazole is the most effective drug.

As *K. pneumoniae* was the most common organism isolated from confirmed VAP cases, ERIC-PCR was performed on 16 K. pneumoniae strains to investigate their genetic correlation and further determine disease acquisition and transmission of VAP. ERIC data showed that 16 *K. pneumoniae* strains had 16 different banding patterns and these bacteria were not transmitted between ICU patients because there was no similarity in the bands of strains isolated from different patients. This suggested that VAP cases may be of endogenous origin. These findings were consistent with the study by Heo et al.(21) in which each patient in the ICU had a unique banding pattern.

As a commonly used device in clinical intensive care, the respiratory circuit is a multi-use apparatus, whose cleaning and disinfection effectiveness is directly associated with its sterilization quality and in turn, affects the anti-infection treatment. At present, monitoring the cleaning and disinfection effect is a key part of the medical device quality control process (22-25). Microbial culture is now a highly accurate, common and simple clinical method for evaluating the effectiveness of cleaning and disinfection. Gao et al. (26) found that microbial culture can be used to evaluate the cleaning and disinfection effect of external ventilator circuits, producing highly consistent results with the ATP method. Consistent with these research results, no pathogenic bacteria were detected in the external ventilator circuits that were disinfected or aseptically stored for 4 weeks in this study, with a 100.0% pass rate by microbial culture. It indicated that this microbial approach was effective in monitoring the cleaning and disinfection effect of the external ventilator circuits.

To put together, Gram-negative bacteria are the main pathogens of VAP, which are mostly multidrug-resistant. Clinical awareness of antibiotic resistance and management of ventilator cleaning and disinfection should be strengthened, regular monitoring of VAP pathogens and their drug sensitivity patterns should be performed, and appropriate antimicrobial therapy should be initiated rapidly.

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None.

Conflict interest

The authors declare no conflict of interest.

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