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Original Article

16S rRNA gene sequencing is a non-culture method of defining the specific bacterial etiology of ventilator-associated pneumonia

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Abstract: Ventilator-associated pneumonia (VAP) is an acquired respiratory tract infection following tracheal intubation. The most common hospital-acquired infection among patients with acute respiratory failure, VAP is associated with a mortality rate of 20-30%. The standard bacterial culture method for identifying the etiology of VAP is not specific, timely, or accurate in identifying the bacterial pathogens. This study used 16S rRNA gene metagenomic sequencing to identify and quantify the pathogenic bacteria in lower respiratory tract and oropharyngeal samples of 55 VAP patients. Sequencing of the 16S rRNA gene has served as a valuable tool in bacterial identification, particularly when other biochemical, molecular, or phenotypic identification techniques fail. In this study, 16S rRNA gene sequencing was performed in parallel with the standard bacterial culture method to identify and quantify bacteria present in the collected patient samples. Sequence analysis showed the colonization of multidrug-resistant strains in VAP secretions. Further, this method identified Prevotella, Proteus, Aquabacter, and Sphingomonas bacterial genera that were not detected by the standard bacterial culture method. Seven categories of bacteria, Streptococcus, Neisseria, Corynebacterium, Acinetobacter, Staphylococcus, Pseudomonas and Klebsiella, were detectable by both 16S rRNA gene sequencing and standard bacterial culture methods. Further, 16S rRNA gene sequencing had a significantly higher sensitivity in detecting Streptococcus and Pseudomonas when compared to standard bacterial culture. Together, these data present 16S rRNA gene sequencing as a novel VAP diagnosis tool that will further enable pathogen-specific treatment of VAP.

Keywords: Ventilator-associated pneumonia (VAP), lower respiratory tract, oropharynx, 16S rRNA gene sequencing, 16S rDNA

Introduction

Ventilator-associated pneumonia (VAP), a pulmonary infection occurring after tracheal intubation, is associated with a mortality rate of 20-30% [1]. VAP prognosis is largely dependent on the accurate identification of the pathogenic bacteria and the early treatment of antibacterial drugs [2]. The standard method to identify VAP-causing bacterial pathogens is via bacterial culture of lower respiratory tract secretions. However, the standard culture method is imprecise due to prolonged culture incubation periods, the use of empirical antibacterial therapy, and the potential presence of a fungal infection or a complex infection involving bacteria, fungi, and viruses. Often, the standard bacterial culture method provides false negative results and fails to identify the disease-causing bacterial pathogens [3].

Recently, broad-range 16S rRNA gene polymerase chain reaction (PCR) coupled with metagenomic sequencing has been used to detect bacterial pathogens [4]. The gene encoding ribosome 16S rRNA is present among all prokaryotic organisms and is relatively short at 1.5 kb, which allows for rapid sequencing results [5]. The 16S rRNA gene consists of highly conserved regions interspaced with hypervariable regions (V1-V9), making it a strong candidate target for bacterial species identification

[6]. Therefore, PCR primers that bind the known conserved regions enable PCR amplification and metagenomic sequencing of species-specific hypervariable regions [6]. This study defined the novel application of 16S rRNA gene sequencing to identify and measure the pathogenic bacterial infections causing VAP. 16S rRNA gene sequencing detected the bacterial populations colonized in lower respiratory tract and oropharyngeal samples collected from 55 VAP patients. Standard bacterial cultures were performed in parallel to 16S rRNA gene sequencing for data comparison. Further clinical application of 16S rRNA gene sequencing could be used to accurately define the bacterial etiology driving VAP, which would enable species-specific treatment therapy and improved VAP outcomes.

Materials and methods

Patient population

Samples were collected from 55 VAP patients admitted into the comprehensive intensive care unit (ICU) of Yancheng First Peoples' Hospital. The patients had been on mechanical ventilation for more than 48 hours before sample collection. Patients that had preexisting lower respiratory infections before mechanical ventilation were excluded. In total, 29 men and 26 women, ages 34-86 years (mean age, 61±14 years), were enrolled in this study.

This study was approved by the ethical committee of Yancheng First Peoples' Hospital. Informed consent was obtained from all patients enrolled in this study.

Sample collection

Pulmonary computed tomography (CT) scans were performed to identify the pulmonary segments with severe inflammation. Pulmonary segments with the most inflammation were selected for bronchovesicular wash. Secretions from the oropharynx and aspirates from the lower respiratory tract, including the trachea, bronchi, bronchioles, and alveoli, were collected by bronchovesicular wash. Two samples were collected from each patient to perform the standard bacterial culture method and 16S rRNA gene sequencing analysis in parallel.

DNA extraction

DNA was extracted from patient samples using TRIzol (Life Technologies) and a DNA extraction reagent kit (Quiagen), both according to manufacturers' instructions. Extracted DNA was stored at -80°C.

Primer design and PCR amplification

Primers were designed from the Genbank bacterial genome database to hybridize to the conserved sequences of the 16S rRNA gene. The expected amplicon size was 637bp and included hypervariable regions V3-V5. Primer synthesis was performed by Biological Engineering Technology Service (Shanghai, China). The DNA extracted from patient samples were used as the PCR templates. The PCR amplification kit (Fermentas) included 12.5 μL of a premix solution, 1 μL of forward primer, 1 μL of reverse primer, 1 μL of template DNA, and 9.5 μL of deionized water. The PCR amplification was performed at the following conditions: 95°C for 3 min, 95°C for 30 s, 56°C for 30 s, 72°C for 30 s, and repeated for a total of 35 cycles. PCR products were separated on a 2% agarose electrophoresis gel. Following PCR amplification, 49 out of the original 55 patient samples had sufficient product yield to undergo 16S rRNA gene sequencing.

16S rRNA gene metagenomic sequencing

Rapid library adapters were ligated to PCR products to aid in the purification, quantification, amplification, and sequencing steps of the high throughput pyrophosphate sequencing protocol. High-throughput pyrophosphate sequencing was performed on tagged PCR products in detail the high throughout pyrophosphate sequencing protocol (454 Sequenator, Roche). The 16S rRNA gene metagenomic sequencing was performed by the BGI Research Institute (Shenzen, China).

16S rRNA gene sequencing analysis, tag species annotation, and OTU analysis

The sequencing data from the 49 patient samples were aligned to the Ribosomal Database Project (RDP) using Mothur (v1.49) software, and bacterial species were identified using operational taxonomic units (OTU) and the

Table 1. The distribution and abundance of bacterial genus in 49 lower respiratory tract secretions samples from 55 VAP patients

Samples	Abundance value								
	Corynebacterium	Prevotella	Streptococcus	Sphingomonas	Aquabacterium	Limnohabitans	Klebsiella	Acinetobacter	Pseudomonas
T1	0	1.20	38.04	2.00	0	0	0.24	4.42	0
T2	13.98	-	0	0	0	0	0	0.99	0
T3	0	0	3.26	0	0	0	0	0	0
T4	0	3.53	43.21	0.39	0	35.59	0	0	0
T5	0.24	2.11	21.08	0	44.10	25.09	0	4.28	0
T6	1.12	0.58	0.34	0	34.60	2.06	22.03	0.43	0
T7	0.99	0	6.63	0	0	0.53	32.53	0	1.04
T9	0	0.55	0.22	12.96	0	0	0	0	2.04
T10	0	0.08	1.38	0	0	0	0	0	0
T11	1.24	0	2.03	0	43.98	43.98	-	0	0
T12	0.44	0	6.33	0.23	0	0	0	3.26	0
T13	0	0	0.24	1.12	0	0	3.58	0	0.39
T14	0	0	0	0.99	0	0.24	2.41	0	0
T15	0	0	1.04	0	2.10	4.42	0.58	0.34	0
T16	0	35.59	0.04	0	0.27	0.99	0	6.63	0
T17	44.099	25.09	0.03	1.23	0.05	0	0.57	0.22	42.96
T18	34.60	2.059	22.03	0.43	0	0	0.08	0	0
T19	0	0.53	32.53	0	0	4.24	0	0	0
T20	0	0	56.09	0	0.09	0.44	0	6.33	0.23
T21	0	0	0.93	0	56.90	0	0	0.24	4.42
T22	0	0	0.46	0	0	0	0.00	23.05	0
T25	0.84	5.05	0	44.10	0	0	0	17.31	0
T26	0	1.43	0.63	24.60	0	0	0	11.05	0
T27	0.67	-	0.09	0	0	0	4.29	0	0
T28	43.39	-	0.91	0	0	2.11	4.42	0.57	0.24
T29	11.37	0	6.58	0	0	0.26	0.99	0	2.27
T30	0	44.10	25.08	0	1.23	0.05	0	0.56	0.22
T31	0	84.60	2.058	22.02	0.43	0	0	0.078	0
T32	0	0	0.53	82.53	0	0	4.29	0	0
T33	3.43	0	0.24	0	0	0.099	0.49	0	2.33
T35	2.11	0	30.31	0	0	52.90	0	0	0.25
T36	0.98	0	0.00	0	0	0	0	0.00	0

T37	0	0.79	5.05	0	44.09	0	0	0	16.81
T38	0.34	0	1.48	0.28	24.60	0	0	0	11.05
T39	0.09	0	0	1.21	0	2.01	0	0	0.28
T40	0	0	18.98		0	0	0	0	0
T41	0	32.92	0	0	8.23	0	0	0	0
T42	0	0	0.03	8.53	0	0.89	0	85.58	0
T43	0	0	0.29	2.11	0	0	44.10	25.08	0
T45	0	0.23	1.12	0.57	0.89	0	84.60	2.06	22.02
T46	34.99	4.42	0.99	0	2.28	0	0	0.53	82.53
T47	26.08	0	0.00	0.55	0.22	12.92	0	0	0
T48	2.09	0	0	0.08	0	0	0	0	0
T49	0.43	0	1.29	0	0	0	48.98	48.98	
T50	0		0.49	0	2.88	0.23	0	0	0
T52	0	4.42	2.32	0	0.28	1.12	0	0	8.57
T53	0	0.99	33.08	0	0.99	0	0.29	2.41	0
T54	4.04	0	45.30	0	0	2.11	4.42	0.57	0
T55	1.36	0	12.41	0	0	0.26	0.99	0	1.04

Table 2. The distribution and abundance of bacterial genus in oropharyngeal samples of 55 VAP patients

Samples	Abundance value								
	Corynebacterium	Prevotella	Streptococcus	Sphingomonas	Aquabacterium	Limnohabitans	Klebsiella	Acinetobacter	Pseudomonas
T1	9.82	0	0	0	0	0.05	0.89	0	2.88
T2	0	3.90	6.25	0	0	7.05	3.10	0	0
T3	2.11	0	0	0	0	52.40	0	0	0.28
T4	0.48	0	57.09	0	33.96	0	0	0.00	0
T5	0	0.78	5.05	0	88.04	0	0	0	13.81
T6	0.98	0	1.88	0.28	25.54	0	0	0	11.09
T7	0.09	0	5.08	1.21	0	2.00	0	0	0.28
T9	0	92.42	0.01	0	7.23	0	0	0	0
T10	0	0	0.40	8.53	0	0.84	0	5.58	0
T11	0	0	0.28	2.11	0	0	88.05	25.07	0
T12	34.09	56.04	0	3.05	4.06	7.06	3.05	0	2.05
T13	0	0.23	1.13	0.57	0.09	0	88.55	2.06	22.02
T14	12.48	8.83	0.44	0	2.28	0	0	0.53	82.53
T15	23.09	0	0.46	0.55	0.22	12.42	0	0	0

T16	2.05	0	0.00	0.078	0	0	0	0	0
T17	0	0	0.29	1.13	0	0	9.58	0.01	0.94
T18	0	0	0.00	0.44	0	0.30	2.81	6.01	0
T19	0	0	0.91	0	2.11	8.83	0.58	1.00	0
T20	0	45.59	3.59	0	0.28	0.44	0	3.40	0
T21	18.05	25.09	55.03	1.22	0.09	0	0.57	0.22	82.43
T22	32.55	2.06	22.02	0.82	0	0	0.09	0	0
T25	19.49		0	0	0	0	0	0.44	0
T26	0	0	9.24	0	0	0	0	0	0
T27	0	9.53	0	0.94	0	0. 35	0	0	0
T28	0.30	2.11	0	0	88.05	25.09	0	8.27	0
T29	1.13	0.58	0.97	0	98.55	2.06	22.02	0.82	0
T30	0	0.23	1.13	0.57	0.09	0	32.55	2.06	6.09
T31	0.44	0	3.39	0	0	0.53	3.53	0	1.10
T32	0	0.55	0.22	12.43	0	0	0	0	2.09
T33	0	0.09	0	0	0	0	0	0	0
T35	0.90	0	3.99	0.23	0	0	0	3.24	0
T36	0	0	0.29	1.13	0	0	9.58	0	0.94
T37	0	0	0	0.44	0	0.30	2.81	0	0
T38	0	0	0.43	0	2.11	6.43	0.58	0.40	0
T39	0.38	3.10	0.06	0	0	0	8.28	0	0
T40	0	0	0	0	0	2.11	8.83	0.57	0.89
T41	11.98	0	3.58	0	0	0.24	0.44	0	2.28
T42	0	8.05	25.08	0	1.22	0.09	0	0.53	0.22
T43	0	21.54	2.06	22.02	0.82	0	0	0.08	0
T45	9.82	0	0.03	0	0	0.05	0.89	0	2.88
T46			2.04						
T47	2.11	0	5.03	0	0	52.40	0	0	0.28
T48	0.48	0	7.05	33.04	0	0	0	0.00	0
T49	0	0.78	5.05	0	88.04	0	0	0	13.81
T50	0	3.49	0.49	0	2.88	0.23	0	0	0
T52	0	4.42	5.20	0	0.28	1.12	0	0	8.57
T53	0	0.99	6.31	0	0.99	0	0.29	2.41	0
T54	4.04	0	0.04	0	0	2.11	4.42	0.57	0
T55	1.36	0	0.43	0	0	0.26	0.99	0	1.04

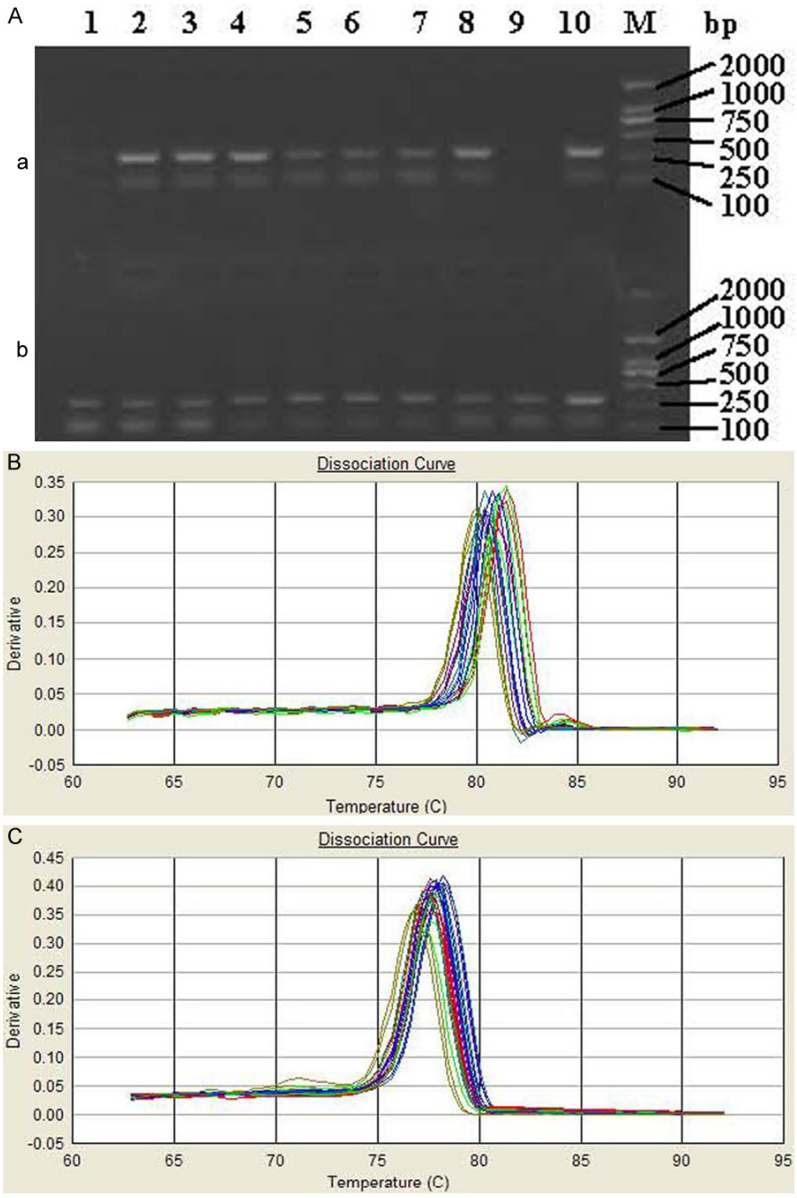

Figure 1. The Alpha diversity distribution of secretion samples isolated from lower respiratory tract of 49 VAP patients had a Shannon index of 1.1 and a Simpson index of 0.44. This showed that the bacterial species isolated from the lower respiratory tract were abundant and relatively complex.

diversity of bacterial species was classified according to Tags of gene expression. Sequences that shared 97% or greater similarity were considered as originating from the same bacterial species [7]. Sequences that shared 95% or greater similarity were grouped into the same bacterial genus [7]. Bacterial genera or species were considered to be abundant when that particular bacterium was identified in more than 14 separate patient samples.

Shannon and Simpson indexes were used to analyze the Alpha diversity of species within a single sample. The quantity of species-specific tags from each sample was summarized in a species overview table. Thus, significant differences were identified in bacterial species between the culture method and sequencing method patient samples.

Bacterial culture

Patient samples were inoculated onto blood agar plates and aerobic incubation was carried out at 37°C for 3 to 5 days. Bacterial colonies were categorized using Gram staining and VITEK2 bacterial analysis(BiomerieuxDiagnostics) according to a published protocol [8].

Statistical analysis

All results were analyzed with SPSS 17.0 (SPSS 17.0 Software, USA). The paired chi-square tests were used to analyze the results between bacterial culture and 16S rRNA gene sequencing. Sequences were clustered and assigned to operational taxonomic units (OTUs) using the Quantitative Insights into Microbial Ecology (QIIME) implementation of cd-hit according to a threshold of 97% pairwise identity. The OTU of every sample and the number of sequences of every OTU were counted after the OTU output. The taxonomy information of every OTU was obtained by searching the most similar species. The Chao1 and ACE abundance indexes, and the Simpson and Shannon diversity indexes were calculated using Mothur software (http://www.mothur.org/) to analyze Alpha diversity.

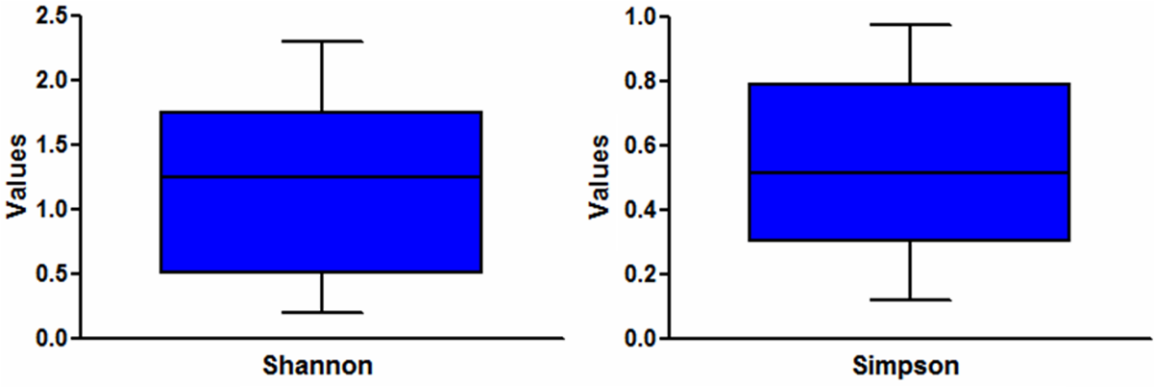

Figure 2. The Alpha diversity distribution of secretion samples isolated from the oropharynx of 49 VAP patients showed a Shannon index of 1.2 and a Simpson index of 0.52. This indicated that that the bacterial species isolated from the oropharynx were abundant and relatively complex.

Results

DNA extraction and 16S rRNA gene PCR amplification from VAP lower respiratory tract and oropharynx samples

Oropharyngeal and lower respiratory tract samples were collected by bronchovesicular wash of 55 VAP patients. DNA was extracted from patient samples and the absorbance value (A260/A280) ranged from 1.8 to 2.0, indicating that the purity of the extracted DNA was high. The mean concentration of DNA was 35.56 μg/μL, indicating that the DNA quality in the samples was not degraded. Therefore, the methods of sample collection and DNA purification performed in this study maintained DNA purity and integrity, enabling further testing. After 16S rRNA gene PCR amplification of the 55 patient samples collected, 49 lower respiratory tract samples generated robust PCR products at the expected size.

16S rRNA gene metagenomic sequencing data suggested diverse and abundant bacterial species in VAP samples

High-throughput pyrophosphate sequencing was performed on the PCR products using the Roche 454 platform. The 16S rRNA gene sequencing data showed a total of 99,439 sequences. The sequences were aligned to the RDP to identify the bacterial genera detected in the 49 patient samples. The specific bacterial genera identified in the VAP lower respiratory tract and oropharyngeal samples were diverse and abundant (**Tables 1**, **2**). 638 OTUs were classified after sequencing analysis. Following OTU classification, 638 OTUs are placed into Genera. Finally, 55.5% of OTUs were identified as specific bacterial species.

The Alpha diversity of the 49 lower respiratory tract samples displayed a Shannon index of 1.1 and a Simpson index of 0.44. This indicated that the bacterial species colonized in the lower respiratory tract of VAP patients were abundant and complex (**Figure 1**). Similarly, 49 oropharynx samples showed a Shannon index of 1.2 and a Simpson index of 0.52, indicating that the bacterial species isolated from the oropharynx of VAP patients were abundant and relatively complex (**Figure 2**).

16S rRNA gene metagenomic sequencing showed higher sensitivity and quantification compared to a standard bacterial culture method

16S rRNA gene metagenomic sequencing detected Prevotella, Proteus, Aquabacter and Sphingomonas, which were not detected by standard bacterial culture. There were 7 bacterial genera that were detected by both 16S rRNA gene sequencing and standard bacterial culture methods, i.e., Streptococcus, Neisseria, Corynebacterium, Acinetobacter, Staphylococcus, Pseudomonas, and Klebsiella. However, the positive rates of 16S rRNA gene sequencing identifying Streptococcus, Klebsiella, Acinetobacter, Corynebacterium, and Pseudomonas were higher than that of the standard bacterial culture ($P<0.05$). Interestingly, there was no difference in the detection

Table 3. A comparison of bacterial detection rates between 16S rRNA gene metagenomic sequencing and standard bacterial culture methods of VAP lower respiratory tract secretions

Detection Methods	Number of samples	*Streptococcus*	*Neisseria*	*Corynebacterium*	*Acinetobacter*	*Staphylococcus*	*Pseudomonas*	*Klebsiella*
		% (portion)	% (portion)	% (portion)	% (portion)	% (portion)	% (portion)	% (portion)
Standard bacterial culture	49	18.3 (9)	6.1 (3)	12.2 (6)	32.6 (16)	8.2 (4)	16.3 (8)	8.2 (4)
Metagenomic sequencing	49	87.7 (43)	40.8 (20)	46.9 (23)	48.9 (24)	10.2 (5)	36.7 (18)	40.8 (20)
P value		0.000	0.050	0.050	0.004	1.000	0.019	0.000

Table 4. A comparison of bacterial detection rates between 16S rRNA gene metagenome sequencing and standard bacterial culture methods of VAP oropharynx secretions

Detection Method	Number of samples	Streptococcus	Neisseria	Corynebacterium	Acinetobacter	Staphylococcus	Pseudomonas	Klebsiella
		% (portion)	% (portion)	% (portion)	% (portion)	% (portion)	% (portion)	% (portion)
Standard bacterial culture	49	22.4 (11)	10.2 (5)	16.3 (8)	20.4.6 (10)	12.2 (6)	6.1 (3)	6.1 (3)
Metagenomic sequencing	49	81.6 (40)	51.0 (25)	44.9 (22)	53.0 (26)	16.3 (8)	40.8 (20)	48.9 (24)
P value		0.000	0.003	0.018	0.003	1.000	0.000	0.000

of Staphylococcus or Neisseria between the two methods (**Tables 3**, **4**).

Discussion

Approximately 90% of hospital-acquired pneumonia cases in ICUs require the use of mechanical ventilation [9]. The morbidity of VAP in developing countries is higher than in developed countries [10]. In the developing country of China, the VAP morbidity rate is as high as 40-55% [11]. In an attempt to improve the diagnosis of VAP, thereby enabling more suitable treatment, this study applied 16S rRNA gene metagenomic sequencing technology to identify the bacterial strains in lower respiratory tract and oropharynx secretions of VAP patients. The 16S rRNA gene sequencing was performed in parallel to the standard bacterial culture method. Lower respiratory tract and oropharynx samples were collected from 55 VAP patients by bronchovesicular wash. After PCR amplification of the V3-V5 hypervariable regions of the 16S rRNA gene, 49 sample outputs were robust and underwent high-throughput pyrophosphate sequencing. The metagenomic sequencing produced 99,439 sequences and 638 OTUs. Shannon and Simpson indexes were used to analyze the diversity and distribution of individual species within a sample population. In practice, a sample population that has both a large Shannon index value and a Simpson index that is close to 0 indicates species abundance in the sample population. The 16S rRNA gene sequencing data followed a trend consistent with species abundance within the samples. The lower respiratory tract samples had a Shannon index of 1.1 and a Simpson index of 0.44. Similarly, the oropharyngeal samples had a Shannon index of 1.2 and a Simpson index of 0.52. Together these data indicated that the bacterial species were abundantly represented in both VAP lower respiratory tract and oropharynx samples.

16S rRNA gene sequencing analysis was superior in its ability to identify biochemically inert, slow-growing bacteria, compared to the standard bacterial culture method. For example, Mycobacteria identification required comprehensive phenotypic identification after 6-8 weeks of growth in standard culture. However, the 16S rRNA gene sequencing method enabled the rapid identification of Mycobacteria within 1-2 days. The relatively fast bacterial identification time of 16S rRNA gene sequencing is revolutionary in enabling the timely administration of appropriate antibacterial therapies [12-20]. Further, we showed that 16S rRNA gene sequencing technology is a quantitative tool in calculating the percentages of bacterial species within a sample population. Inhibited bacterial growth due to antibacterial drug therapy is a common complication with the standard bacterial culture method that was circumvented with 16S rRNA gene sequencing. Diagnostic use of 16S rRNA gene sequencing removed the need for viable bacteria and long culture incubation times that the standard bacterial culture method requires.

The most abundant bacterial genera identified in the lower respiratory tract samples were Streptococcus, Corynebacterium, Acinetobacter, Proteus, Sphingomonas, Aquabacter, Pseudomonas, Klebsiella, and Prevotella. The rate of drug-resistant, non-fermentative bacterial infections in a hospital setting is gradually increasing, with Acinetobacter calcoaceticus infections being among the most common [21]. Of particular interest, this study detected additional known and unidentified pathogenic bacteria in the VAP lower respiratory tract samples. This indicated that the pathogenic bacteria colonizing the lower respiratory tract of VAP patients were diverse. There was an abundance of the multidrug-resistant strains Acinetobacter, Klebsiella, and Pseudomonas among the identified pathogenic bacteria. This suggested that the identified multidrug-resistant strains colonized the respiratory tracts of VAP patients before the onset of VAP. This could be due to therapeutic treatment with antibacterial drugs failing to remove the multidrug-resistant strains, which enabled the multidrug-resistant strains to become pathogenic [22]. This study showed that VAP is likely induced by a variety of bacterial species, including many multidrug-resistant strains.

Standard bacterial culture failed to identify Prevotella, Proteus, Aquabacter, and Sphingomonas, which could be detrimental to VAP treatment as Sphingomonas is pathogenic [23-26]. The standard bacterial culture method for bacterial species identification was dependent on bacterial biochemical characteristics and phenotypic traits and therefore required extended bacterial culture incubation periods before analysis could be initiated. 16S rRNA gene sequencing circumvented these limita-

tions by not requiring preliminary bacterial culture techniques. 16S rRNA gene sequencing detected 7 bacterial strains in common with the standard bacterial culture method. However, 16S rRNA gene sequencing was more sensitive to the quantities of bacteria identified and quantified these strains at higher levels.

The sequence of pathogenic bacteria colonization initiated in the oropharynx and then extended into the lower respiratory tract. This suggested that treating the pathogenic bacteria in the oropharynx could prevent colonization expansion into the lower respiratory tract, which could have a positive effect on VAP outcome.

A previous investigation revealed that 16S rDNA sequencing provided a cost- effective alternative in the identification of clinically relevant forms of probe- negative nontuberculous mycobacteria. Additionally, starting with a pure culture, the turnaround time to species identification is 1 to 2 days for 16S rDNA sequencing compared to 2 to 6 weeks for biochemical testing [27].

In summary, we described the novel application of 16S rRNA gene sequencing to identify the bacterial etiology of VAP. 16S rRNA gene sequencing of VAP lower respiratory tract and oropharynx secretions identified the colonization of diverse pathogenic bacterial species, many of which were multidrug-resistant. 16S rRNA gene sequencing showed superior sensitivity, enhanced bacterial specificity, and shorter procedural time compared to the standard bacterial culture method. Because the sequencing procedure can be performed rapidly and is cost effective, 16S rRNA gene sequencing can revolutionize personalized medicine and provide individualized VAP treatment. Further, it would be interesting to analyze the sequencing data from each patient sample as a proof-of-point; analyzing patient-specific data will show that empirical antibiotic therapy is not the best course of treatment for each patient.

Disclosure of conflict of interest

None.

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