JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Concomitant viral and bacterial pneumonia among patients in ICU with mechanical respiratory support

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Abstract

Introduction: The use of mechanical ventilators in the intensive care unit (ICU) is often associated with higher risk of respiratory tract infections, including ventilator-associated pneumonia (VAP). Concomitant bacterial-viral infection was reported to worsen patient's clinical condition. This study evaluated the rate of concomitant bacterial-viral infections in patients with VAP and analyzed their clinical outcomes.

Methodology: In this retrospective observational study 107 patients diagnosed with VAP and admitted in ICU with mechanical ventilator support between April 2018 and May 2019 in the Department of Respiratory Medicine, Dachang Hospital, Shanghai, China were included. 27 most commonly involved lower respiratory tract infection (LRTI) pathogens (bacteria and virus) and seven genetic markers of antibiotic resistance were detected and analyzed using Biofire® FilmArray® Pneumonia Panel plus (bioMérieux SA, Paris, France).

Results: Of the 107 patients, 45 (42.1%) patients had bacterial infection alone (bacterial group), 26 (24.3%) had virus infection alone (viral group) and 24 (22.4%) patients had concomitant bacterial-viral infection (mixed group). Sixty-nine (64.5%) and 50 (46.7%) patient samples were positive for bacterial (bacterial and mixed groups) and viral (viral and mixed groups) detection, respectively. *Streptococcus pneumonia* (11.2%) and Influenza A (17, 15.9%), were the predominantly identified bacterial and viral species. The blaCTX-M (21.5%) was the predominant resistance gene detected. Twenty-four (22.4%) patients were positive for concomitant bacterial-viral infection; *Staphylococcus aureus* and Influenza A were the most common bacterial-viral combination identified.

Conclusions: Concomitant bacterial-viral infection was higher compared to previously published studies and the increased duration of mechanical ventilation was associated with increased disease severity.

Key words: Concomitant infection; Biofire® FilmArray® Pneumonia Panel; VAP.

J Infect Dev Ctries 2022; 16(9):1482-1489. doi:10.3855/jidc.12999

(Received 09 May 2020 – Accepted 13 September 2020)

Introduction

A mechanical ventilator is essential for the treatment of patients admitted to intensive care units (ICUs) [1]. Patients admitted in ICUs are at increased risk of mortality, not only due to critical illness but also due to secondary nosocomial infections [2]. Pneumonia is one of the most common nosocomial infections among critically ill patients and 86% of nosocomial pneumonia is associated with the use of mechanical ventilators [2,3]. The use of mechanical ventilators in ICUs is often associated with a higher risk of respiratory tract infections, including ventilator-associated pneumonia (VAP) and ventilator-associated respiratory infections [1]. VAP is most common in patients under mechanical ventilators and the second most common nosocomial infection in ICUs [4,5]. About 50% of the cases of hospital-acquired pneumonia were due to VAP and were estimated to occur in 9-27% of the patients who were under mechanical ventilators [6,7]. In the United States, the incidence rate of VAP ranged from 5-10 cases per 1000 hospital admissions, and about 250,000-300,000 cases per year [2,3]. Another study reported that the incidence rates (5-67%) would vary depending on the case-mix and the diagnostic criteria; with a higher rate in surgical, immunocompromised, and elderly patients [8,9]. VAP poses serious concerns in endotracheally intubated adult patients who were admitted in ICUs due to increased risk of adverse events, length of ICU stay (LOS) and cost of treatment [10]. Higher mortality rates have been reported in late-onset VAP than early-onset VAP [9].

In general, the etiology of VAP was assumed to be bacterial; however, the evolving diagnostic techniques shifted the interest towards the epidemiology, pathogenesis, presentation and prognosis of viral pneumonia. Ventilator-associated bacterial pneumonia is well documented [4,7,10]. The most common bacterial species associated with ventilator-associated

bacterial pneumonia include *Streptococcus pneumonia*, *Hemophilus influenza*, and *Staphylococcus aureus* [4,10]. Herpes simplex virus, cytomegalovirus, influenza virus and enterovirus were the reported viral pathogens among ventilator-associated viral pneumonia patients [11,12]. Viral and bacterial co-infection in pneumonia is gaining importance, particularly after the H1N1 pandemic occurred in 2009. This was best described among patients with influenza. The most commonly isolated bacterial species among influenza patients include *S. pneumoniae*, *S. aureus*, *S. pyogenes* and *H. influenza* [13]. Viruses associated with bacterial co-infection include parainfluenzavirus (PIV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), rhinovirus, and adenovirus [13]. Bacterial-viral co-infection was reported to worsen the patient's clinical condition, including severity and mortality [14]. Few studies have reported on the bacterial-viral co-infection among patients with pneumonia; however, all these studies were on community-acquired pneumonia (CAP), and there were no studies on VAP [13,15-17].

The FilmArray® Pneumonia Panel plus (bioMérieux SA, Paris, France), a recently Food and Drug Administration (FDA) approved system, was used to detect bacteria and viruses in our study. The FilmArray® Pneumonia Panel plus is a combination of real-time Polymerase Chain Reaction (PCR) and nested multiplex PCR. This is an entirely automated system wherein nucleic acid extraction, amplification, detection, and data analysis are performed in a single disposable pouch system to provide a semi-quantitative report. This system can aid in the diagnosis of lower respiratory tract infections (LRTI) by identifying 33 respiratory targets within 1 hour. To the best of our knowledge, this is the first study on bacterial-viral co-infection in patients with VAP. This study evaluated the rate of co-occurrence of bacterial and viral infections in patients with VAP and analyzed their clinical outcomes.

Methodology

Patients and Data Collection

In this retrospective observational study, 107 patients admitted in ICU, who were under mechanical ventilator support and diagnosed with VAP between April 2018 and May 2019 in the Department of Respiratory Medicine, Dachang Hospital, Shanghai, China were included. VAP was defined as the occurrence of pneumonia after intubation and determined not to have occurred before an artificial airway was put into place [18]. As all the patients were intubated, endotracheal aspirates were collected from them and transported to the laboratory for microbiological analysis. Informed consent was obtained from all legal heirs of the patients after explaining the nature of the study. The institutional review board approved the study (IRB number: M-106-013). Data including demographics, comorbidity and medication were collected during the ICU stay. The 30-day mortality, defined as the death due to any cause

Table 1. Pathogens and genetic markers of antibiotic resistance detected through BIOFIRE® FILMARRAY® Pneumonia Panel Plus.

Bacteria (semi-quantitative)	**Antibiotic Resistance Genes**
Acinetobacter calcoaceticus-baumannii complex	**ESBL**
Enterobacter cloacae	CTX-M
Escherichia coli	
Hemophilus influenzae	**Carbapenemases**
Klebsiella aerogenes	KPC
Klebsiella oxytoca	NDM
Klebsiella pneumoniae group	Oxa48-like
Moraxella catarrhalis	VIM
Proteus spp.	IMP
Pseudomonas aeruginosa	
Serratia marcescens	**Methicillin Resistance**
Staphylococcus aureus	mecA/mecC and MREJ
Streptococcus agalactiae	
Streptococcus pneumoniae	
Streptococcus pyogenes	
Atypical Bacteria (Qualitative)	**Viruses**
Legionella pneumophila	Influenza A
Mycoplasma pneumoniae	Influenza B
Chlamydia pneumoniae	Adenovirus
	Coronavirus
	Parainfluenza virus
	Respiratory Syncytial virus
	Human Rhinovirus/Enterovirus
	Human Metapneumovirus
	Middle East Respiratory Syndrome Coronavirus (MERS-CoV)*
	* MERS-CoV will only be available on the Pneumonia Panel *plus*

within 30 days of hospital admission, was also analyzed. Pneumonia Severity Index (PSI) and the Simplified Acute Physiologic Score (SAPS) II were used to assess the severity of pneumonia [19,20].

FilmArray® Pneumonia Panel Assay

The Biofire® FilmArray® Pneumonia Panel plus (bioMérieux SA, Paris, France) is a cartridge-based completely automated multiplex PCR which enables simultaneous detection for 27 of the most common pathogens involved in lower respiratory tract infection (LRTI) and seven genetic markers of antibiotic resistance (Table 1). The integrated sample preparation makes the process easier. The samples were loaded into the Pneumonia Panel plus (bioMérieux SA, France) as per the manufacturer's instructions and the multiplex PCR was performed using the Biofire® FilmArray® (bioMérieux SA, Paris, France) machine. The machine has an inbuilt software which can automatically analyze the results and provide the output as one single easy to read report. The results were obtained in approximately one hour from the time of sample loading. Although some of the bacteria and the resistance genes were detected semi-quantitatively, we have considered them as qualitative results for our analyses.

Data presentation and Statistical Analysis

The patients were categorized, based on the presence of microorganisms, as bacterial group, viral group, mixed group, and no etiology group for data analysis. Data with categorical values were expressed as numbers and percentages; continuous values were expressed as medians and ranges. Mann Whitney test and Chi Square test were performed for non-parametric data. Univariate analysis was performed for the clinically relevant parameters to identify patients with concomitant bacterial-viral infections and deaths. The variables which were significant in the univariate analysis were used for multivariate analysis. A multivariate analysis was performed to identify the independent variable for bacterial-viral co-infections and deaths. All statistical tests were performed using SPSS software package (SPSS, version 13.5; SPSS Inc, Chicago, Illinois). A p value of < 0.05 was considered to be statistically significant.

Results

Out of the 107 patients, 59 (55.1%) were male and 48 (44.9%) were female; mean age was 55.2 ± 5.7 years. The majority of the patients had a fever (87, 81.3%), had the PSI Class III-V score (71, 66.4%), chronic obstructive pulmonary disease (COPD) (68, 63.6%) and SAPS II score (62, 57.9%). The mean ± SD hospital stay among the patients was 68.6 ± 31.7 days and the mean ± SD length of ventilation was 22.8 ± 14.3 days (Table 2).

Out of the 107 patients, 45 (42.1%) patients had bacterial infection alone (bacterial group), 26 (24.3%)

Table 2. Clinical characteristics and outcome of included patients.

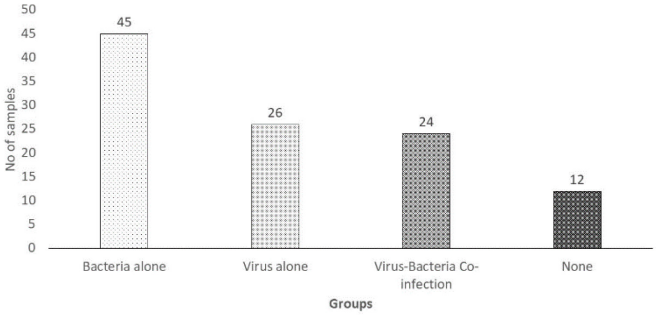
Characteristics	All Patients	Bacteria alone	Virus alone	Virus-Bacteria	None	*p* value
Male	59 (55.1%)	26 (24.3%)	15 (14.0%)	13 (12.1%)	5 (4.7%)	0.96
Female	48 (44.9%)	19 (17.8%)	11 (10.3%)	11 (10.3%)	7 (6.5%)	0.86
Age (mean ± SD years)	55.2 ± 5.7	59.7 ± 4.8	52.5 ± 2.5	49.2 ± 3.7	37.3 ± 4.1	NA
Smoking	38 (35.5%)	19 (17.8%)	9 (8.4%)	5 (4.7%)	5 (4.7%)	0.04
Fever (> 38°C)	87 (81.3%)	48 (44.9%)	24 (22.4%)	12 (11.2%)	3 (2.8%)	0.82
COPD	68 (63.6%)	23 (21.5%)	25 (23.4%)	14 (13.1%)	6 (5.6%)	0.32
PSI Class III-V	71 (66.4%)	22 (20.6%)	27 (25.2)	12 (11.2%)	10 (9.3%)	0.41
SAPS II score	62 (57.9%)	27 (25.2%)	18 (16.8%)	8 (7.5%)	9 (8.4%)	0.04
Length of hospital stay (mean ± SD days)	68.6 ± 31.7	63.5 ± 36.3	85.7 ± 27.6	70.8 ± 42.7	23.1 ± 6.5	0.12
Mean length of ventilation (mean ± SD days)	22.8 ± 14.3	23.7 ± 13.4	24.1 ± 8.9	27 ± 14.3	14.3 ± 9.1	0.08
Antibiotics before ICU admission	56 (52.3%)	8 (7.5%)	19 (17.8%)	6 (5.6%)	23 (21.5%)	0.01
Immunocompromised	31 (29.0%)	6 (5.6%)	15 (14.0%)	6 (5.6%)	4 (3.7%)	0.08
Coronary diseases	21 (19.6%)	12 (11.2%)	3 (2.8%)	0 (0%)	6 (5.6%)	0.03
Cancer	16 (15.0%)	5 (4.7%)	7 (6.5%)	1 (0.9%)	3 (2.8%)	0.24
Organ transplantation	5 (4.7%)	1 (0.9%)	2 (1.9%)	2 (1.9%)	0 (0%)	0.75
Renal disease	24 (22.4%)	13 (12.1%)	8 (7.5%)	1 (0.9%)	2 (1.9%)	0.09
Liver disease	21 (19.6%)	9 (8.4%)	6 (5.6%)	2 (1.9%)	4 (3.7%)	0.15
Pleural effusion	46 (43.0%)	21 (19.6%)	15 (14.0%)	10 (9.3%)	0 (0%)	0.04
Blood urea nitrogen ≥ 30 mg/dl	32 (29.9%)	18 (16.8%)	11 (10.3%)	2 (1.9%)	1 (0.9%)	0.32
Sodium < 130 mmol/litre	41 (38.3%)	22 (20.6%)	13 (12.1%)	5 (4.7%)	1 (0.9%)	0.03
Hematocrit < 30%	64 (59.8%)	45 (42.1%)	15 (14.0%)	4 (3.7%)	0 (0%)	0.02
Glucose ≥ 250 mg/dl	39 (36.4%)	20 (18.7%)	11 (10.3%)	3 (2.8%)	5 (4.7%)	0.03
30 days mortality	13 (12.1%)	4 (3.7%)	3 (2.8%)	5 (4.7%)	1 (0.9%)	0.65
In hospital mortality	19 (17.8%)	8 (7.5%)	4 (3.7%)	5 (4.7%)	2 (1.9%)	0.52

had virus infection alone (viral group) and 24 (22.4%) patients had bacterial-viral co-infections (mixed group) (Figure 1). Out of the 107 patients, 26 (24.3%), 15 (14.0%), 13 (12.1%) male patients and 19 (17.8%), 11 (10.3%), 11 (10.3%) female patients had bacterial infections, viral infections, bacterial-viral co-infections, respectively. There was no significant difference in the presence of any infection among male and female patients ($p > 0.05$). Twelve (11.2%; male: 5; female: 7) patient samples did not detect any of the tested viruses or bacteria (no etiology group). Among the included patients, bacterial infection was significantly higher in patients who had a history of smoking (17.8%; $p = 0.045$), patients with SAPS II score (25.2%; $p = 0.041$), patients with coronary diseases (11.2%; $p = 0.032$), patients with sodium level < 130 mmol/L (20.6%; $p = 0.031$), hematocrit of < 30% (42.1%; $p = 0.024$) and with a blood glucose level of ≥ 250 mg/dL (18.7%; $p = 0.039$). Viral infection was significantly higher in patients with antibiotics treatment prior to ICU admission (17.8%; $p = 0.017$). Thirteen (12.1%) patients died within 30 days of hospitalization, 4 (3.7%) out of them were from the bacterial group, 3 (2.8%) from viral group, 5 (4.7%) from mixed group and 1 (0.9%) from no etiology group. The overall in-hospital mortality rate was 17.8% (19 patients), among them 7.5% (8 patients) were from the bacterial group, 3.7% (4 patients) were from viral group, 4.7% (5 patients) were from mixed group and 1.9% (2 patients) were from no etiology group. There was no significant difference between the 30 days mortality and overall in-hospital mortality among the groups ($p > 0.05$) (Table 2).

Table 3. Distribution of microorganisms and resistance genes.

Bacteria	No. of Organisms (%)
Any bacteria	**69 (64.5%)**
Streptococcus pneumoniae	12 (11.2%)
Staphylococcus aureus	10 (9.3%)
Pseudomonas aeruginosa	9 (8.4%)
Acinetobacter calcoaceticus-baumannii complex	5 (4.7%)
Hemophilus influenzae	5 (4.7%)
Escherichia coli	5 (4.7%)
Mycoplasma pneumoniae	4 (3.7%)
Klebsiella aerogenes	4 (3.7%)
Klebsiella pneumoniae group	3 (2.8%)
Streptococcus pyogenes	3 (2.8%)
Chlamydia pneumoniae	2 (1.9%)
Klebsiella oxytoca	1 (0.9%)
Streptococcus agalactiae	1 (0.9%)
Legionella pneumophila	1 (0.9%)
Virus	
Any virus	**50 (46.7%)**
Influenza A	17 (15.9%)
Coronavirus	13 (12.1%)
Influenza B	8 (7.5%)
Human Rhinovirus/Enterovirus	5 (4.7%)
Adenovirus	3 (2.8%)
Parainfluenza virus	2 (1.9%)
Respiratory Syncytial virus	2 (1.9%)
Resistance Genes	
bla_{CTX-M}	23 (21.5%)
bla_{VIM}	17 (15.9%)
bla_{NDM}	16 (15.0%)
bla_{IMP}	13 (12.1%)
mecA/*mecC* and *MREJ*	3 (2.8%)
Oxa48-like	2 (1.9%)

Figure 1. Overall distribution of bacteria and viruses.



Sixty-nine (64.5%) patient samples (bacterial and mixed groups) were positive for bacterial detection. Among these, *S. pneumonia* (12, 11.2%), *S. aureus* (10, 9.3%), *Pseudomonas aeruginosa* (9, 8.4%), *Acinetobacter calcoaceticus-baumannii* complex (5, 4.7%), *H. influenza* (5, 4.7%), and *E. coli* (5, 4.7%) were the predominantly identified bacterial species. *Moraxella catarrhalis* was not detected in any of the samples included in the panel. Fifty (46.7%) patient samples (viral and mixed groups) were positive for viral detection. Influenza A (17, 15.9%), Coronavirus (13, 12.1%), Influenza B (8, 7.5%), and Human Rhinovirus/Enterovirus (5, 4.7%) were the predominantly identified viruses. Other tested viruses and bacterial species were identified in lesser frequencies. Among the various antibiotic resistance genes detected, *CTX-M* (23, 21.5%) was the predominant resistance gene detected followed by *blaVIM* (17, 15.9%), *blaNDM* (16, 15.0%), *blaIMP* (13, 12.1%), *mecA*/*mecC* and *MREJ* (3, 2.8%) and *Oxa48*-like gene (2, 1.9%) (Table 3).

Among the 24 (22.4%) patients with bacterial-viral co-infection, *S. aureus*, along with Influenza A (4 patients) was the most common bacterial-viral combination identified. The second most common combination was *H. influenza* with coronavirus (3 patients). Other combinations include *E. coli* with HRV/Enterovirus (2 patients), *E. coli* with Adenovirus (2 patients) and *Mycoplasma pneumoniae* with

Table 4. Details on the concomitant bacterial-viral infection.

Microorganisms	Influenza A	Coronavirus	Influenza B	HRV/Entero	Adenovirus	Parainfluenza	RSV
Acinetobacter calcoaceticus-baumannii complex	0	**1**	0	0	0	0	0
Staphylococcus aureus	**4**	0	**1**	0	0	0	0
Escherichia coli	0	0	0	**2**	**2**	0	0
Streptococcus pneumoniae	**2**	**1**	0	0	0	0	0
Haemophilus influenzae	0	**3**	**1**	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	**1**	0
Mycoplasma pneumoniae	0	0	**2**	0	0	0	**1**
Klebsiella aerogenes	0	0	0	0	0	0	0
Klebsiella pneumoniae group	0	0	0	0	0	**1**	0
Streptococcus pyogenes	0	0	0	0	0	0	0
Chlamydia pneumoniae	0	0	0	0	**1**	0	0
Klebsiella oxytoca	0	0	0	0	0	0	0
Streptococcus agalactiae	0	0	0	0	0	0	0
Legionella pneumophila	0	0	0	**1**	0	0	0

Influenza B (2 patients). Bacteria, including *Klebsiella aerogenes*, *Streptococcus pyogenes*, *Klebsiella oxytoca*, and *Streptococcus agalactiae* were not identified in any of the samples that detected viruses (Table 4). Multivariate analysis revealed that coronary heart disease (Odds Ratio [OR], 4.15; 95% CI, 1.69-8.11; $p = 0.04$), pleural effusion (OR, 3.98; 95% CI, 1.42-4.11; $p = 0.03$), and length of ventilation for more than 10 days (OR, 4.23; 95% CI, 1.69-7.65; $p = 0.01$) were independent predictors of mixed infection (Table 5).

Discussion

Bacterial and viral pneumonia are well known health conditions. VAP typically affects critically ill patients who were in ICU and is the major source of increased illness and death [21]. The complex relationship between the endotracheal tube, presence of risk factors, virulence of the invading bacteria or virus and host immunity largely determine the development of VAP [9]. There is evidence that bacterial-viral co-infection may worsen patient outcomes, including the severity of disease and mortality [14,16,22].

Our study investigated the impact of concomitant bacterial-viral pneumonia among the ICU patients who were under mechanical respiratory support. All previous studies which discussed bacterial-viral co-infections were on community-acquired pneumonia (CAP); therefore a direct comparison of our result mimicking our clinical settings was not feasible [13,15-17]. Hence, we compared our results with studies on CAP and other clinical settings. The mean length of ventilation in our study was found to be 22.8 ± 14.3 days, which was higher than that reported from China, [17] France [15] and comparable to another study from France [12]. Patients with viral infection alone (85.7 ± 27.6 days) and viral-bacterial co-infection (70.8 ± 42.7 days) had longer hospital stay compared to patients with bacterial infection alone (63.5 ± 36.3 days). Similarly, the mean length of mechanical ventilation in patients with viral infections alone (24.1 ± 8.9 days) and viral-bacterial co-infection (27 ± 14.3 days) was longer compared to those with bacterial infection alone (23.7 ± 13.4days). The longer ICU stay and longer duration of mechanical ventilator use could reveal a possible association between patients with viral infection alone and viral-bacterial co-infection. In our study, there was no significant difference in the presence of any infection among male and female patients ($p > 0.05$), which was similar to that reported by Voiriot *et al.* [15]. Ko *et al*. reported that concomitant pneumonia and COPD were the 5th leading causes of death [23]. Among the 68 patients with COPD, 23 patients had bacterial infection alone, 25 patients had viral infection alone and 14 patients had bacterial-viral co-infection. Although not significant, viral infection among patients with COPD was higher than bacteria alone and bacterial-viral co-infection. In our study, bacterial

Table 5. Multivariate analysis of risk factors for concomitant bacterial-viral infection.

Risk Factors	OR	95% CI	*p* value
Smoking	0.61	0.32-2.32	0.36
SAPS II score	2.65	1.36-6.54	0.06
Length of hospital stay (> 15 days)	0.43	0.40-3.65	0.2
Coronary heart disease	4.15	1.69-8.11	0.04
Pleural effusion	3.98	1.42-4.11	0.03
Length of ventilation (> 10 days)	4.23	1.69-7.65	0.01

infection was significantly higher (p = 0.04) among patients with SAPS II score.

S. pneumonia, *H. influenza*, and *S. aureus* were the most common bacterial species associated with ventilator-associated bacterial pneumonia [4,10]. In our study, 64.5% of patient samples (bacterial and mixed groups) were positive for bacterial detection. Out of these, *S. pneumonia* (11.2%) was the predominant bacterial species identified. A study from France on the viral-bacterial co-infection among CAP, reported that *S. pneumonia* (23%) as the most common bacteria isolated from patients, however, with a higher percentage than that reported in our study [15]. The study reported *H. influenza* (7.5%) as the second most common bacteria detected and *S. aureus* (6.9%) as the third most common bacteria detected [15]. In our study, *S. aureus* (9.3%) was the second most common bacteria and *H. influenza* (4.7%) was detected in lesser frequency. Another study which investigated the impact of viral-bacterial co-infection in hospitalized children with *M. pneumoniae pneumonia*, reported *S. aureus* as the most common bacteria isolated, which is similar to our findings; however, the study reported a much higher rate (43/107, 40.2%) of occurence [17]. The incidence of respiratory tract infection caused by *S. pneumoniae* and *H. influenzae* could be reduced following the implementation of vaccination programs among the elderly and youngsters. In our study, none of the samples detected *M. catarrhalis*, which is similar to that reported by Lee *et al.* [24].

Among the ventilator-associated viral pneumonia patients, herpes simplex virus, cytomegalovirus, influenza virus and enterovirus were the commonly reported viral pathogens [11,12]. In our study, 50 (46.7%) patient samples (viral and mixed groups) were positive for viral detection. Influenza A (15.9%) was the most common virus detected among our samples, which is in corroboration with that reported by other studies [15,24]. Among these, Voiriot *et al*. reported a higher rate of Influenza A (18.4%) than that reported in our study [15]. Lee *et al*. reported a much lower rate (8.5%) compared to our results [24]. The higher rate of Influenza A virus among our population might be due to low influenza vaccination. However, the non-availability of vaccination history prevents us from drawing any conclusion on this aspect. Coronavirus (12.1%) was the second most common virus detected among our samples; however, it was detected in lesser frequencies in other studies [15,24]. In our study, viral infection was significantly higher (17.8%) in patients with antibiotics treatment prior to ICU admission.

In our study, 22.4% of the patients had bacterial-viral co-infection, which is higher than that reported in Korea [25]. *S. aureus*, along with Influenza A (4 patients) was the most common bacterial-viral combination identified. A study from China reported that *S. pneumonia*, along with Influenza A (3/107 cases) and respiratory syncytial virus (3/107 cases) was the most common combination among children [17]. However, in our study *S. pneumoniae*, along with Influenza A virus, was detected in 2/107 cases. The second most common combination in our study was *H. influenza* with coronavirus (3 patients). In addition, 29.0% of our patients were immunocompromised, 15.0% of the patients had cancer and 4.7% underwent organ transplantation. It is likely that the presence of these conditions may also have contributed to the higher concomitant bacterial-viral co-infections in the patients. The reason for the difference in the bacterial-viral combination might be due to local factors, difference in the clinical settings and differences in the included population.

The increasing drug resistance towards various antibiotics leads to severe life-threatening conditions and are a challenge during the treatment of bacterial infections. Thus, it is important to screen for antibiotic susceptibility for effective treatment. In our study, *blaCTX-M* (21.5%) was the predominant resistance gene detected. Similarly, Lee *et al*. reported that *blaCTX-M* was the predominant gene detected among their samples but with less (8.5%) frequency [24]. In our study, *blaVIM* (17, 15.9%) was the second most predominant gene detected followed by *blaNDM* (16, 15.0%), *blaIMP* (13, 12.1%), *mecA*/*mecC* and *MREJ* (3, 2.8%), and *Oxa48*-like gene (2, 1.9%). Lee *et al*. who has used Biofire® FilmArray® Pneumonia Panel plus (bioMérieux SA, France) in similar clinical settings reported the presence of *blaVIM*, *blaNDM* and *blaIMP*, but not *mecA*/*mecC* and *MREJ* and *Oxa48*-like genes [24]. Several bacteria exhibit resistance towards multiple antibiotics leading to the development of multidrug-resistant (MDR) strains. The increase in MDR strains is a global problem [26]. Generally, early VAP is caused by pathogens that are sensitive to antibiotics, whereas late-onset VAP is caused by MDR and harder to treat bacteria. A group of experts from the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention defined MDR as "acquired non-susceptibility to at least one agent in three or more antimicrobial categories" [27]. Although we have not tested any isolates for the resistance genes, 5 (4.7%) samples have amplified more than two resistance genes. This may indicate that there

could be an MDR strain present in those samples. Several reports suggested that bacterial-viral co-infection might worsen patient outcomes and increase disease severity and mortality [14,16,22]. In our study the overall in-hospital mortality was 17.8% while the mortality rate in patients with bacterial-viral co-infections was 4.7%. Voiriot *et al*. reported a much higher in-hospital mortality rate (28.9%), compared to that reported (17.2%) in our study [15]. In our study, there was no significant difference (p = 0.52) in the mortality rates between different groups, which is similar to that reported by Voiriot *et al*. (p = 0.10) [15]. Multivariate analysis revealed that coronary heart, pleural effusion and length of ventilation for more than 10 days were independent predictors of mixed infection. Our results have clinical relevance and may be used to predict bacterial-viral co-infection, which may help in developing an appropriate treatment strategy and reduce disease severity and mortality.

The limitations of this study include the single center observational design; the small number of observed VAP may limit the interpretation and the clinical relevance of our data. FilmArray® Pneumonia Panel Assay is useful in detecting the bacterial/viral pathogens; however, the mere detection of resistance genes may not necessarily reflect the true resistance of bacteria. There is a possibility that a strain may possess a resistance gene but may not express the same. The antibiotic resistance results from FilmArray® Pneumonia Panel Assay test can be used to start an empirical treatment, however, isolation of the bacterial species and performing susceptibility testing is highly recommended while using the FilmArray® Pneumonia Panel Assay.

Conclusions

The predominantly detected bacterial and viral pathogens were *S. pneumonia* and Influenza A virus respectively. Concomitant bacterial-viral co-infection was higher compared to previously published studies and was associated with increased disease severity. The duration of mechanical ventilation is strongly associated with the development of VAP. Therefore, strategies aimed toward reducing the duration of tracheal intubation may reduce the incidence of VAP. Further studies with a larger population and the same clinical settings are required to validate our findings.

Funding

The project is supported by the project of Baoshan District Science and Technology Committee of Shanghai (NO.18-E-4) and the special fund of senior respiratory crisis of Dachang hospital.

Authors' Contributions

Study concept and design: Wenming Cheng, Encai Liu; Acquisition of data: Xiaoyun Shen, Wenming Cheng, Bo Feng; Analysis and interpretation of data: Xiaoyun Shen, Bo Feng, Encai Liu; Drafting of the manuscript: Xiaoyun Shen, Weiyi Shi, Bo Feng; Critical revision of the manuscript for important intellectual content: Tiefeng Zhang; Statistical analysis: Weiyi Shi; Study supervision: Tiefeng Zhang.

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Conflict of interests: No conflict of interests is declared.