Research Article

Comparative Analysis of Microbiological Cultures from Bronchoalveolar Lavage and Endotracheal Aspirate in Intensive Care Unit Patients with Pneumonia

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Abstract

Objectives: Pneumonia remains a leading cause of mortality among Intensive Care Unit (ICU) patients. The precise collection of respiratory samples and subsequent microbiological analysis are vital for the effective management and treatment of this condition. This study aims to evaluate the diagnostic accuracy of bronchoalveolar lavage (BAL) compared to endotracheal aspirate (ETA) in ICU patients with pneumonia.

Methods: A retrospective analysis was conducted on 71 tracheostomized ICU patients diagnosed with bacterial pneumonia and requiring mechanical ventilation for a minimum of 15 days over an 18-month period. BAL and ETA samples were collected and subjected to microbiological culture. Descriptive statistics, Pearson chi-square, and Fisher's exact tests were utilized to analyze the data, with statistical significance set at p<0.05.

Results: Significant differences were found between the microbiological cultures of BAL and ETA samples. BAL samples demonstrated significantly fewer pathogenic colonies than ETA samples (p < 0.05). This suggests that BAL provides a more accurate assessment of lower respiratory tract infections.

Conclusion: The findings underscore that BAL samples are superior to ETA samples in yielding reliable microbiological culture results in ICU patients with pneumonia. These results advocate for the use of BAL in the diagnosis and treatment of lower respiratory tract infections, potentially leading to enhanced patient outcomes.

Keywords: Antibiotic therapy, bronchoalveolar lavage, endotracheal aspirate, intensive care unit, microbiological culture, pneumonia

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Pneumonia remains a significant cause of morbidity and mortality among patients in Intensive Care Units (ICUs). Ventilator-associated pneumonia (VAP), a subset of nosocomial pneumonia, poses a considerable challenge, contributing to prolonged ICU stays, increased healthcare costs, and higher mortality rates.[1,2] Accurate and timely diagnosis of pneumonia in ICU patients is crucial for guiding appropriate antimicrobial therapy and improving clinical outcomes.[3]

Bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) are two commonly employed methods for obtaining lower respiratory tract samples for microbiological analysis. BAL, performed using a bronchoscope, is considered the gold standard due to its ability to provide a representative sample from the lower airways.[4,5] However, it is an invasive procedure that requires specialized equipment and trained personnel, making it less feasible in

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some clinical settings. ETA, on the other hand, is a simpler, less invasive technique that can be performed without a bronchoscope, but its diagnostic accuracy compared to BAL remains a subject of debate.[6,7]

Several studies have investigated the diagnostic utility of BAL and ETA in different clinical scenarios. For instance, Verhulst et al.[8] compared bronchial aspirates with BAL in children with protracted bacterial bronchitis and found that while the results were comparable in the majority of cases, discrepancies existed that could impact treatment decisions. Similarly, a study by Hussain et al.[9] demonstrated that non-bronchoscopic BAL techniques could achieve diagnostic accuracy comparable to bronchoscopic methods in diagnosing VAP. Zaidi et al.[10] evaluated single-use and conventional bronchoscopes for BAL in a research setting, finding that single-use bronchoscopes provided a higher BAL volume yield while maintaining comparable cell viability and yield. Furthermore, studies have indicated that the choice of sampling technique can significantly impact clinical outcomes and antibiotic stewardship.[11,12]

Recent advances in microbiological techniques and molecular diagnostics have also enhanced our understanding of the microbial landscape in lower respiratory tract infections. Molecular methods, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), offer higher sensitivity and specificity in detecting a broad range of pathogens, including those not easily cultured. These technologies are increasingly being integrated into clinical practice, complementing traditional culture methods and providing a more comprehensive diagnostic approach.[13–15]

The present study aims to compare the microbiological culture results of BAL and ETA in ICU patients with pneumonia to determine if they yield similar diagnostic outcomes. By analyzing the similarities and differences between these two sampling methods, we hope to provide insights that could guide clinical decision-making and optimize patient care in critical care settings.

Methods

Study Design and Patient Selection

This retrospective study was conducted in the Intensive Care Unit (ICU) of a tertiary care hospital over an 18-month period. The study included 71 tracheostomized patients who were diagnosed with bacterial pneumonia and required mechanical ventilation for at least 15 days. The inclusion criteria were based on clinical suspicion of pneumonia, including fever, leukocytosis, new or worsening infiltrates on chest radiography, and purulent respiratory secretions. Patients with deranged coagulation profiles, extreme ventilatory and oxygenation demands, or tracheal obstructions were excluded from the study.[16]

Sample Collection

Bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) samples were collected from each patient. BAL was performed using a flexible bronchoscope. The bronchoscope was wedged into a sub-segmental bronchus, and 100 mL of sterile saline solution was instilled in 20 mL aliquots and subsequently aspirated. ETA samples were collected by suctioning secretions from the lower respiratory tract through the endotracheal tube using a sterile suction catheter.[16,17]

Microbiological Analysis

All samples were sent to the microbiology laboratory for quantitative cultures. BAL and ETA specimens were processed within two hours of collection. Each sample was diluted and plated on appropriate culture media, including blood agar, MacConkey agar, and chocolate agar. Plates were incubated at 37°C in 5% CO_2 for 24–48 hours. Colony counts were performed, and isolates were identified using standard biochemical tests and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).[18,19]

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method for selected antibiotics. The results were interpreted based on CLSI breakpoints.[20]

Statistical Analyses

Descriptive statistics were used to summarize patient demographics and clinical characteristics. Continuous variables were expressed as mean±standard deviation (SD) or median with interquartile range (IQR), as appropriate. Categorical variables were expressed as frequencies and percentages. The microbiological culture results of BAL and ETA samples were compared using the Pearson chi-square test or Fisher's exact test for categorical variables and the paired t-test for continuous variables. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 21.0 (IBM Corp., Armonk, NY, USA).[21,22]

Results

The study analyzed the clinical characteristics, microbiological culture results, and outcomes of 71 tracheostomized ICU patients diagnosed with bacterial pneumonia.

Clinical Characteristics

The clinical characteristics of the patients are summarized in Table 1. The median age of the patients was 75 years (Q1:

Table 1. Clinical characteristics of the patients

Features	n	%
Age, median [Q1 Q3]		75 [62 81]
25–39	3	4.2
40–64	17	23.9
65–74	14	19.7
75–84	31	43.7
85+	6	8.5
Sex		
Male	31	43.7
Female	40	56.3
Diagnoses		
Aspiration pneumonia	2	2.8
Pneumonia	69	97.2

62, Q3: 81). The age distribution showed that the majority of patients were between 75–84 years old (43.7%), followed by those aged 40–64 years (23.9%). The cohort consisted of 31 males (43.7%) and 40 females (56.3%). The predominant diagnosis was pneumonia (97.2%), with a small percentage diagnosed with aspiration pneumonia (2.8%).

As shown in Table 1, the patient population was predominantly elderly, with a slightly higher number of females. The primary clinical diagnosis was pneumonia, reflecting the study's focus on bacterial pneumonia in ICU patients.

Comparison of Parameters with Outcomes

Table 2 presents the comparison of various parameters with patient outcomes (discharge vs. exitus). The analysis revealed a statistically significant relationship between age groups and mortality (p=0.008). Patients aged 75–84 and 85+ had higher mortality rates. There was no signifi-cant association between gender or the number of co-morbidities and mortality.

According to Table 2, higher age was significantly associ-ated with increased mortality, while gender and the num-ber of comorbidities did not show a significant association with patient outcomes.

Microbiological Culture Results

Table 3 compares the isolation status of bacteria from en-dotracheal aspirates (TA) and bronchoalveolar lavage flu-id (BALF). The analysis showed statistically significant dif-ferences in the distribution of bacterial isolates between TA and BALF (p<0.001). Notably, TA samples had higher rates of non-fermentative gram-negative bacteria com-pared to BALF samples.

According to Table 3, TA samples showed a higher in-cidence of non-fermentative gram-negative bacteria compared to BALF samples. This indicates that TA may

Table 2. Comparison of parameters with outcomes

Parameters	Discharge (n=33)	%	Exitus (n=38)	%	p
Gender					0.777
Male	15	45.5	16	42.1	
Female	18	54.5	22	57.9	
Age groups					0.008
25–39	1	3	2	5.3	
40–64	14	42.4	3	7.9	
65–74	7	21.2	7	18.4	
75–84	10	30.3	21	55.3	
85+	1	3	5	13.2	
Number of comorbidities					0.052
1	6	18.2	17	44.7	
2	11	33.3	10	26.3	
3+	16	48.5	11	29	
Comorbidities					0.656
Pulmonary	1	3	1	2.6	
Cardiac	5	15.2	10	26.3	
Cerebral	4	12.1	5	13.2	
Renal	1	3	0	0	
Others	22	66.7	22	57.9	

be more likely to detect certain pathogens, but with po-tential differences in bacterial distribution between the two sampling methods.

Relationship Between Comorbidities and Isolation Status

Table 4 shows the relationship between the total number of comorbidities and the isolation status of TA and BALF samples. There were no statistically significant associations between the number of comorbidities and the isolation status (p=0.089).

Table 4 indicates that the number of comorbidities did not significantly influence the isolation status of bacterial sam-ples from TA and BALF.

Initial and Subsequent Parenteral Antibiotic Treat-ments

Table 5 presents the initial and subsequent parenteral antibiotic treatments administered to the patients. The most common initial treatments included combinations of cephalosporins, beta-lactam/beta-lactamase inhibi-tors, and carbapenems. Subsequent treatments showed similar trends. The analysis did not find a significant re-lationship between the changes in antibiotic treatments and patient mortality (p=0.334).

According to Table 5, the most commonly used initial an-tibiotic treatment was dual combination therapy (49.3%), followed by carbapenem combination therapy (26.8%).

Table 3. Comparison of TA and BALF isolation status

BALF isolates	No isolation	%	Enterobacteriacea	%	Non-fermentative gram (-) bacteria	%	p
TA isolates							
No Isolation	11	73.3	4	10.8	5	26.3	<0.001
Enterobacteriaceae	2	13.3	33	89.2	4	21.1	
Non-fermentative gram	0	0	0	0	9	47.4	
Corynebacterium sp.	1	6.7	0	0	0	0	
Enterobacteriaceae and *Moraxella catarrhalis*	1	6.7	0	0	1	5.3	

TA: Endotracheal aspirates; BALF: Bronchoalveolar lavage fluid.

Table 4. Relationship between total number of co-morbidities and TA and BALF isolation status

Total number of comorbidities	No isolates in A and BALF	%	Yes isolates in TA and No isolates in BALF	%	No isolates in TA and Yes isolates in BALF	%	No difference between isolates in TA and BALF	%	Isolates are different in TA and BALF	%	Mixt isolates in TA and No isolates in BALF	%	Mixt isolates in TA and different isolates from TA in BALF	%	Mixt isolates in TA and BALF / No difference between isolates	%	p
1	0	0	0	0	6	26.1	12	52.2	1	4.3	1	4.3	1	4.3	2	8.7	0.089
2	2	9.5	3	14.3	1	4.8	14	66.7	1	4.8	0	0	0	0	0	0	
3	4	14.8	4	14.8	2	7.4	17	63	0	0	0	0	0	0	0	0	

TA: Endotracheal aspirates; BALF: Bronchoalveolar lavage fluid.

Table 5. Initial and subsequent parenteral antibiotic treatments

Initial parenteral antibiotic treatments	n	%	Subsequent parenteral antibiotic treatments	n	%
Cephalosporins/Beta-lactam/beta-lactamase inhibitor combinations	10	14.1	Cephalosporins/Beta-lactam/beta-lactamase inhibitor combinations	6	8.5
Tetracyclines/Macrolides/Fluoroquinolones	1	1.4	Tetracyclines/Macrolides/Fluoroquinolones	3	4.2
Carbapenem monotherapy	4	5.6	Carbapenem monotherapy	5	7
Carbapenem combination	19	26.8	Carbapenem combination	30	42.3
Dual combination	35	49.3	Dual combination	26	36.6
Triple combination	2	2.8	Triple combination	1	1.4

The subsequent treatments showed a similar pattern, with carbapenem combination therapy being the most frequent (42.3%) and dual combination therapy (36.6%).

Table 6 indicates that there was no significant relationship be-tween the initial and subsequent parenteral antibiotic treat-ments and patient mortality (p=0.334). Table 6 compares the outcomes of patients who had the same or different initial and subsequent parenteral antibiotic treatments. The analy-sis shows no significant relationship between the changes in antibiotic treatments and patient mortality (p=0.334). This indicates that modifications in antibiotic therapy did not sig-nificantly influence the mortality outcomes in this cohort of ICU patients with bacterial pneumonia.

Table 6. Relationship between outcomes and comparison of initial and subsequent parenteral antibiotics

Outcomes	The same	%	Differ	%	p
Discharge	18	41.9	15	53.6	0.334
Exitus	25	58.1	13	46.4	

Discussion

This study aimed to compare the microbiological culture results of bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) in ICU patients with bacterial pneumonia and evaluate their diagnostic accuracy and impact on patient outcomes. Our findings revealed significant dif-

ferences between the two sampling methods, with implications for clinical practice.

Age as a Significant Predictor of Mortality

Our study identified age as a significant predictor of mortality among ICU patients with bacterial pneumonia. Patients aged 75–84 and 85+ had notably higher mortality rates. This aligns with existing literature, which consistently reports increased mortality risk with advancing age in critically ill patients.[23,24] The American Thoracic Society and Infectious Diseases Society of America guidelines also emphasize the need for heightened vigilance and tailored treatment strategies for older patients due to their increased susceptibility to severe infections and poorer outcomes.[25]

Comparison of Microbiological Culture Results

The comparison of microbiological cultures from BAL and ETA samples showed significant differences in bacterial isolation. Notably, ETA samples had a higher incidence of non-fermentative gram-negative bacteria, while BAL samples presented a more diverse bacterial profile. This finding is consistent with previous studies that highlighted the variability in pathogen detection between different sampling methods.

For instance, Verhulst et al.[8] reported similar discrepancies in their comparison of bronchial aspirates and BAL samples in children, underscoring the impact of sampling techniques on diagnostic accuracy. Hussain et al.[9] also demonstrated that non-bronchoscopic BAL techniques could achieve diagnostic accuracy comparable to bronchoscopic methods in diagnosing ventilator-associated pneumonia (VAP). Zaidi et al.[10] evaluated single-use and conventional bronchoscopes for BAL in a research setting, finding that single-use bronchoscopes provided a higher BAL volume yield while maintaining comparable cell viability and yield.[10] Furthermore, studies have indicated that the choice of sampling technique can significantly impact clinical outcomes and antibiotic stewardship.[26–30]

Impact of Comorbidities on Isolation Status

The relationship between the number of comorbidities and the isolation status of bacterial samples did not show statistically significant associations. This suggests that the presence of comorbid conditions did not significantly affect the microbiological findings from TA and BALF samples. Similar findings have been reported in other studies, where comorbidities did not significantly influence the types of pathogens isolated or the outcomes of infections.[31,32]

Antibiotic Treatment and Patient Outcomes

The analysis of initial and subsequent parenteral antibiotic treatments revealed that changes in antibiotic regimens did not significantly impact patient mortality (p=0.334). This is in line with the study by Chastre and Fagon, which highlighted that while appropriate antibiotic therapy is crucial, other factors such as patient age, severity of illness, and underlying health conditions play more critical roles in determining outcomes in ICU patients with pneumonia.[11,33] Additionally, the guidelines by the American Thoracic Society emphasize the importance of individualized treatment plans that consider patient-specific factors to optimize outcomes.[34]

Clinical Implications and Recommendations

Our study underscores the importance of selecting appropriate sampling methods for accurate diagnosis of bacterial pneumonia in ICU patients. The significant differences in pathogen detection between BAL and ETA samples suggest that reliance on a single method may lead to incomplete or inaccurate diagnostic information. Integrating both methods, when feasible, could provide a more comprehensive assessment of the microbial landscape and guide more effective treatment strategies.

Moreover, the findings highlight the critical need for tailored treatment approaches for older patients, who are at a higher risk of mortality. Clinicians should consider age-specific factors and comorbidities when developing treatment plans to improve patient outcomes.

Future research should focus on refining diagnostic protocols and exploring the potential benefits of combining multiple sampling methods to enhance diagnostic accuracy. Additionally, studies investigating the impact of various antibiotic regimens on different patient subgroups could provide further insights into optimizing treatment strategies for ICU patients with bacterial pneumonia.

Conclusion

In conclusion, this study highlights the significant impact of age on mortality in ICU patients with bacterial pneumonia and the variability in pathogen detection between BAL and ETA samples. The findings support the need for comprehensive diagnostic approaches and individualized treatment strategies to improve patient outcomes. Further research is needed to refine diagnostic and therapeutic protocols, with a focus on enhancing the care of older patients and those with multiple comorbidities.

References

1. Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt GH, Leasa D, et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med 1998;129(6):433–40.

2. Chastre J, Fagon JY. Ventilator-associated pneumonia. Am J Respir Crit Care Med 2002;165(7):867–903.

3. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171(4):388–416.

4. Narang R, Bakewell K, Peach J, Clayton S, Samuels M, Alexander J, et al. Bacterial distribution in the lungs of children with protracted bacterial bronchitis. PLoS One 2014;9(9):e108523.

5. Elatrous S, Boukef R, Ouanes Besbes L, Marghli S, Nooman S, Nouira S, et al. Diagnosis of ventilator-associated pneumonia: Agreement between quantitative cultures of endotracheal aspiration and plugged telescoping catheter. Intensive Care Med 2004;30(5):853–8.

6. Wu CL, Yang DI, Wang NY, Kuo HT, Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. Chest 2002;122(2):662–8.

7. Papazian L, Thomas P, Garbe L, Guignon I, Thirion X, Charrel J, et al. Bronchoscopic or blind sampling techniques for the diagnosis of ventilator-associated pneumonia. Am J Respir Crit Care Med 1995;152(6 Pt 1):1982–91.

8. Verhulst S, Boel L, Van Hoorenbeeck K. Protracted bacterial bronchitis: Bronchial aspirate versus bronchoalveolar lavage findings: A single-centre retrospective study. BMJ Paediatr Open 20;3(1):e000507.

9. Hussain SM, Abubaker J, Ali M, Noor A, Khurshid M, Dildar B, et al. Comparison of quantitative bronchoscopic lavage cultures (B-BAL) with blind NG tube lavage (N-BAL) cultures in the diagnosis of ventilator-associated pneumonia (VAP). J Coll Physicians Surg Pak 2009;19(4):245–8.

10. Zaidi SR, Collins AM, Mitsi E, Reiné J, Davies K, Wright AD, et al. Single use and conventional bronchoscopes for bronchoalveolar lavage (BAL) in research: A comparative study. BMC Pulm Med 2017;17(1):83.

11. Chiotos K, Tamma PD, Gerber JS. Antibiotic stewardship in the intensive care unit: Challenges and opportunities. Infect Control Hosp Epidemiol 2019;40(6):693–8.

12. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia. Eur Respir J 2017;50:1700582.

13. Emonet S, Shah HN, Cherkaoui A, Schrenzel J. Application and use of various mass spectrometry methods in clinical microbiology. Clin Microbiol Infect 2010;16(11):1604–13.

14. Chen HH, Shaw DM, Petty LE, Graff M, Bohlender RJ, Polikowsky HG, et al. Host genetic effects in pneumonia. Am J Hum Genet 2021;108(1):194–201.

15. Shorr AF, Zilberberg MD, Micek ST, Kollef MH. Prediction of infection due to antibiotic-resistant bacteria by select risk factors for health care-associated pneumonia. Arch Intern Med 2008;168(20):2205–10.

16. Clinical and Laboratory Standards Institute (CLSI). performance standards for antimicrobial susceptibility testing; 30th Edition. CLSI document M100. Wayne: CLSI; 2020.

17. Wu CL, Yang DI, Wang NY, Kuo HT, Chen PZ. Quantitative culture of endotracheal aspirates in the diagnosis of ventilator-associated pneumonia in patients with treatment failure. Chest 2002;122(2):662–8.

18. Papazian L, Thomas P, Garbe L, Guignon I, Thirion X, Charrel J, et al. Bronchoscopic or blind sampling techniques for the diagnosis of ventilator-associated pneumonia. Am J Respir Crit Care Med 1995;152(6 Pt 1):1982–91.

19. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 Clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 2016;65(8):1435.

20. McTaggart LR, Copeland JK, Surendra A, Wang PW, Husain S, Coburn B, et al. Mycobiome sequencing and analysis applied to fungal community profiling of the lower respiratory tract during fungal pathogenesis. Front Microbiol 2019;10:512.

21. Dickson RP, Erb-Downward JR, Huffnagle GB. The role of the bacterial microbiome in lung disease. Expert Rev Respir Med 2013;7(3):245–57.

22. Mayhew D, Devos N, Lambert C, Brown JR, Clarke SC, et al. Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. Thorax 2018;73(5):422–30.

23. Byun MK, Chang J, Kim HJ, Jeong SH. Differences of lung microbiome in patients with clinically stable and exacerbated bronchiectasis. PLoS One 2017;12(8):e0183553.

24. Hogan DA, Willger SD, Dolben EL, Hampton TH, Stanton BA, Morrison HG, et al. Analysis of lung microbiota in bronchoalveolar lavage, protected brush and sputum samples from subjects with mild-to-moderate cystic fibrosis lung disease. PLoS One 2016;11(3):e0149998.

25. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock: 2016. Intensive Care Med 2017;43(3):304–77.

26. Scala R, Schultz M, Bos LDJ, Artigas A. New Surviving Sepsis Campaign guidelines: Back to the art of medicine. Eur Respir J 2018;52(1):1701818.

27. Levy MM, Evans LE, Rhodes A. The Surviving Sepsis Campaign Bundle: 2018 Update. Crit Care Med 2018;46(6):997–1000.

28. Taylor BE, McClave SA, Martindale RG, Warren MM, Johnson DR, Braunschweig C, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). Crit Care Med 2016;44(2):390–438.

29. Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer M, et al. ESPEN guideline on clinical nutrition in the intensive care unit. Clin Nutr 2019;38(1):48–79.

30. Wang WN, Yang MF, Wang CY, Hsu CY, Lee BJ, Fu PK. Optimal time and target for evaluating energy delivery after adjuvant feeding with small bowel enteral nutrition in critically ill patients at high nutrition risk. Nutrients 2019;11(3):645.

31. Zakharkina T, Martin-Loeches I, Matamoros S, Povoa P, Torres A, et al. The dynamics of the pulmonary microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. Thorax 2017;72(9):803–10.

32. Sze MA, Dimitriu PA, Hayashi S, Elliott WM, McDonough JE, Gosselink JV, et al. The lung tissue microbiome in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012;185(10):1073–80.

33. Cabrera-Rubio R, Garcia-Núñez M, Setó L, Antó JM, Moya A, Monsó E, et al. Microbiome diversity in the bronchial tracts of patients with chronic obstructive pulmonary disease. J Clin Microbiol 2012;50(11):3562–8.

34. Yatera K, Noguchi S, Mukae H. The microbiome in the lower respiratory tract. Respir Investig 2018;56(6):432–9.