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A Comprehensive Examination and Meta-Analysis of Laboratory Methods for Ventilator-Associated Pneumonia Diagnosis

[1]Priya M, [2]Manjunathan K.S, [3]Nishanthini Sengeetha K.S, [4]Chukkala Venkatesh

[1] Assistant Professor, Pathology, Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

[2] Assistant Professor, Ophthalmology, Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

[3] Assistant Professor, Obstetrics & Gynecology, Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

[4] Assistant Professor, Biochemistry, Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

Corresponding Author: Govinda Raju B.T, Assistant Professor, Radiodiagnosis, Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

ABSTRACT

The risk of developing (VAP) is significantly increased for patients who are getting mechanical ventilation in hospitals. Whereas in the capacity of a result of this, it is absolutely necessary to obtain a precise diagnosis as quickly as possible in order to attain the most favorable possible outcomes within the treatment process. In the course of this investigation, a variety of laboratory procedures, were examined to determine the degree of precision with which they diagnose ventilator-associated pneumonia (VAP). The utilization of sophisticated statistical methods was utilized in both the analysis of studies and the evaluation of the diagnostic accuracy of laboratory data. Both of these processes were carried out. Multiplex polymerase chain reaction (PCR) was shown to be superior to culture methods and Gram's stain in terms of specificity (86%) and sensitivity (92%). In contrast, the Gram's stain showed that it had the lowest sensitivity (74.6%) and the highest specificity (78.9%). In contrast to the quantitative, semi-quantitative, enrichment culture results, the specificity was found to be lower (75.97%), while the sensitivity was found to be moderate (78.5%). According to the findings of a meta-analysis, the testing method that is based on multiplex PCR was discovered to be the most accurate diagnostic technique for VAP. Following closely behind were the methods of culture. A combination of multiplex PCR, culture, and Gram's stain, in addition to separate assays, was utilized in order to reach a higher level of sensitivity. When it comes to providing successful therapy, it is extremely necessary to make a diagnosis of VAP as quickly and accurately as possible. The multiplex polymerase chain reaction (PCR) continues to be the most accurate diagnostic procedure, despite the fact that there is the chance that it could be enhanced through combination diagnostic approaches. On the other hand, when it comes to improving and obtaining certification for VAP diagnostic instruments, extra study is required.

***Keywords*:** Infectious agents, microbiological culture, multiplex polymerase chain reaction, Gram's stain, and ventilator-associated pneumonia

INTRODUCTION

It has been found that there is a significant correlation between ventilator-associated pneumonia and death. One of the potential results of this illness is respiratory failure, which can lead to sepsis and the failure of numerous organs. Other potential outcomes include failure of the respiratory system. There are two factors that have the potential to influence the mortality rate among people who have ventilator-associated pneumonia (VAP). These factors are the severity of the illness and the existence of co morbidities. Despite the fact that early detection is still difficult to obtain due to the lack of sophisticated diagnostic methods, this mortality rate can range anywhere from 13% to 50%. A recent cost analysis carried out in the United States of America determined that the costs connected with VAP are $40,144 per specific instance. This is a major financial burden that is associated with VAP, which is associated with a significant financial burden. The cost of a VAP infection in India is estimated to be $5200 USD, with a confidence range ranging from $3245 to $7152. This estimate is based on what is known about the expense of the infection. This estimation is based on a probability that is estimated to be 95% accurate.

The probability of developing ventilator-associated pneumonia (VAP) might be increased by a variety of different circumstances. Patient characteristics, prolonged durations of artificial breathing and hospitalization, altered awareness, burns, pre-existing health issues, previous use of antibiotics, invasive procedures, and genetic polymorphisms are some of the variables that can contribute to the development of this condition. The clinical evaluation, which is also known as the examination of clinical symptoms, is a diagnostic tool that can be applied to diagnose (VAP). This is the nineteenth point on the list. Some of the symptoms that are connected with this disorder are purulent respiratory secretions, fever, leukocytosis, and increased oxygenation. Other symptoms include oxygenation of the blood.

The review of risk factors, which may include, among other things, the presence of co-morbidities, the utilization of mechanical ventilation on a continuous basis, and a history of antibiotic utilization, among other things. Imaging methods, such as chest X-rays, are utilized with the purpose of determining whether or not there is consolidation or infiltration of the lungs. This is accomplished by the utilization of imaging techniques. Because it provides images of the lungs that are more precise than those obtained through other imaging technologies, a computed tomography (CT) scan can be useful in separating pneumonia from other lung conditions. Blood culture is one of the many laboratory methods used with the purpose of determining whether or not there are germs present in the blood, which is an indication of a systemic illness.

For the purpose of determining whether or not the blood contains germs, this procedure is carried out as well as through the culture of sputum, it is possible to determine the etiological agent that is responsible for the affected individual's condition. In the field of bronchoscope, a procedure that is utilized is called bronchoalveolar lavage, which is also referred to as BAL. In order to perform this method, a very small amount of sterile saline is injected into the lungs by the use of an appropriate needle. After that, collection of this saline is carried out for the goal of conducting additional analysis. As a consequence of this, the process of correctly identifying the specific pathogens that are accountable for the infection is simplified.This is done in order to determine

whether or not an infection is present.

When there is a higher count of colony-forming units (CFU), it indicates that there is a greater likelihood of (VAP). In the context of monitoring the presence of a wide variety of microorganisms and attempting to gain an understanding of the causes that contribute to chronic obstructive pulmonary disease (VAP), After an accurate diagnosis has been made, it is possible to put into action a treatment strategy that is tailored to the specific bacterial, viral, or fungal infections that are present. This is possible because of the progress that has been made in the diagnosis. All of the specimens that are cultured in a clinical microbiology laboratory are normally grown in an aerobic environment. On the other hand, routine investigations for rickettsial pathogens are carried out occasionally.

The second potential consequence of an incorrect diagnosis is that it may lead to the wrong prescription of antibiotics. This is due to the fact that various infections require different medications in order to be effective in treating patients. The process of delivering care to patients can become more difficult as a result of this, as there is a higher probability that the treatment will not be successful and that antibiotic resistance will develop. Patients are put at risk for new dangers, such as hospital-acquired infections, when they remain in the hospital for an extended period of time, which is caused by a delayed diagnosis. This not only results in an increase in the expenditures connected with medical care, but it also puts patients at risk for additional dangers. It is possible that a delayed or incorrect diagnosis of venous aortic thromboembolism (VAP) could result in an increase in the already high morbidity and mortality associated with the illness. This would have a negative impact on the outcomes for patients.

OBJECTIVE OF STUDY

When it comes to diagnosing VAP, there is a scarcity of research that examines the various laboratory procedures and their cumulative use.

MATERIALS AND METHODS

we were able to evaluate the studies' quality and potential for bias. This enabled us to make a well-informed choice. Additionally, we developed processes and utilized the resources at our disposal to generate and evaluate forest plots and summary receiving operating curves (SROC) for systematic and meta-analysis reviews using Review Manager Version 5.4.1. To ensure that we could appropriately assess the results of these reviews, this was done. The International Prospective Register of Systematic Reviews is where I registered the protocol I created. This happened after I had completed building the protocol.

RESULTS:

Figure 1 depicts the results of the search and selection process using the PRISMA flow chart. These findings are shown in the figure for your review. A combination of Pub Med and other sources yielded 154 papers, 83 of which underwent full-text screening. A total of 154 articles were discovered. Following this, 52 papers were examined and found to be appropriate for the systematic review, with 38 investigations included. Thirty of the 38 studies included research that reported on Gram's stain results (10 total), chest X-ray (2 total), quantitative/semi-quantitative/enrichment culture (6 total), and multiplex PCR (12 total). These studies were considered in the analysis. The meta-analysis contained thirty papers, however fifteen of them were excluded because they lacked a reference standard. The twenty-three papers that remained outstanding were included.

Study characteristics:

Table 1 summarizes the features of the papers that were included. The review included all 38 published publications.

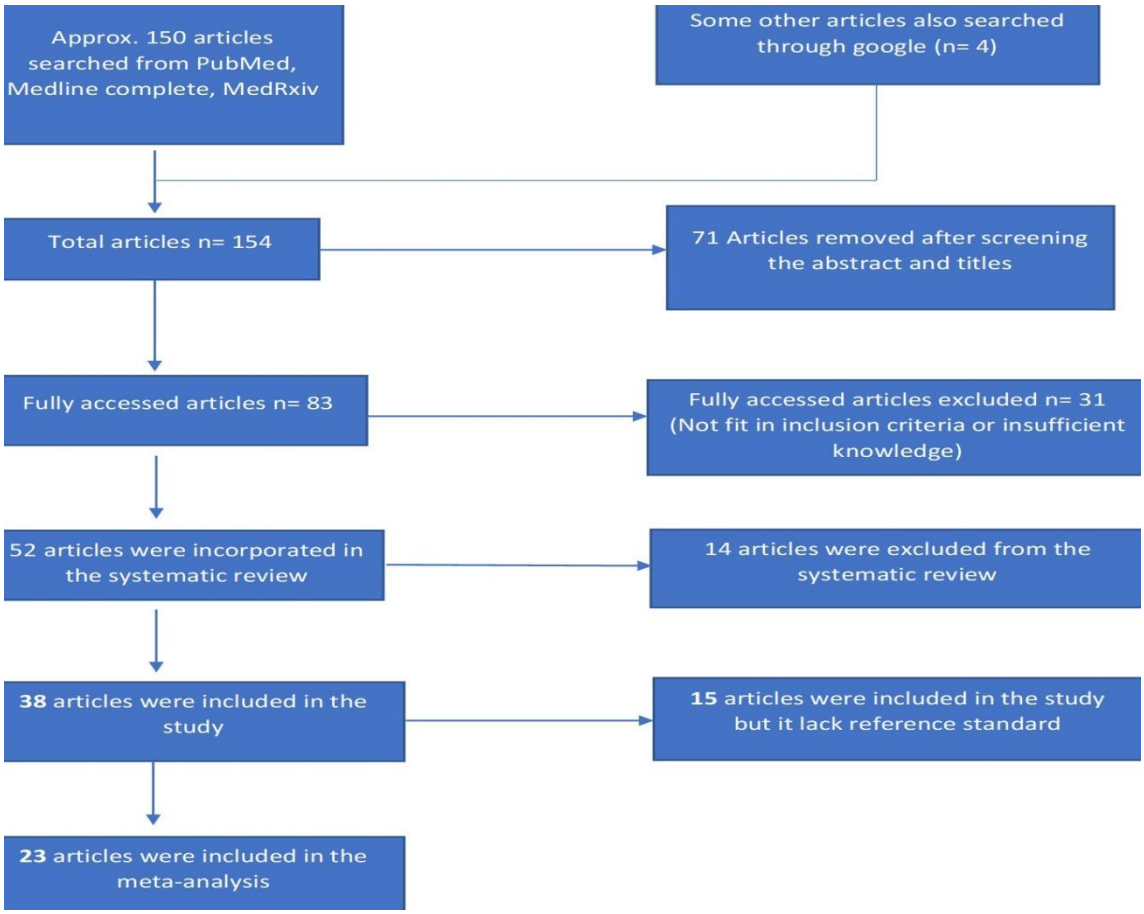

Figure 1. PRISMA flow chart for the search and selection of various research articles

Detailed findings of QUADAS-2 analysisQuality of included studies

The quality of the studies that were considered for inclusion in the analysis was evaluated. Supplementary information-1 (SI-1), SI-2, and SI-3 detailed the research that used classical culture, Gram's stain, and multiplex PCR. These studies raised questions about potential bias and their applicability. Separate presentations were made of the outcomes for the various methods. A small number of research were case-control investigations, which posed a significant risk of bias, in contrast to the majority of the studies that were cross-sectional.

Patient selection domain

Around 45% of the research were concerned about their findings' practicality, while 70% had a low risk of bias. Conversely, 45% of the studies showed a significant bias risk, while 10% showed an unclear bias risk. Concerns over the findings' potential relevance ranged from vague to extremely severe, with 10% expressing such fears. Just a small number of studies had issues with applicability and an unidentified danger of bias. There was a substantial possibility of bias in ten of the studies (45%) because they were case-control studies.

Index test domain

There was little to no worry about the research' relevance, and nearly half (almost 50%) had a low risk of bias. At the same time, there was substantial doubt over the studies' relevance, and over 30% of them had a high risk of bias. The potential for bias and applicability issues is unknown in very few studies. Because they used pre-specified threshold values and probably followed criteria for assessing test results while being blind to particular information, eleven of the studies had a minimum risk of bias. Standards in their interpretation of the index tests or failed to use pre-specified threshold values.

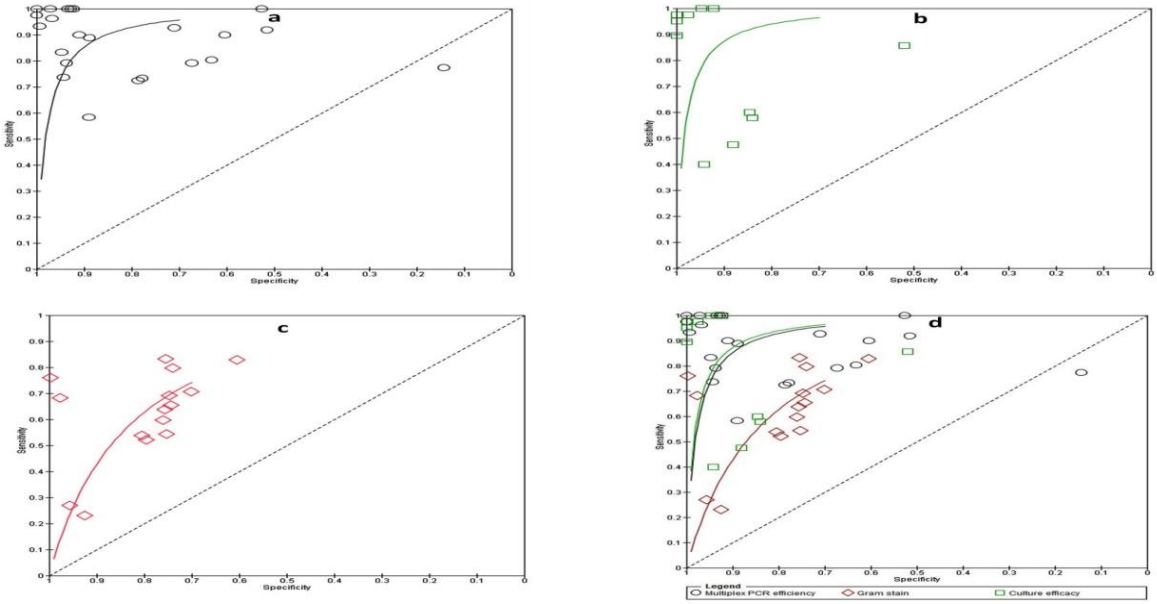

Figure 1. Summary ROC (Receiving operating curve) for a) Multiplex PCR efficiency, b) microbiological culture, c)Gram's stain, d) Multiple tests analysis (Multiplex PCR, Gram's stain & culture

Meta-analysis and quantification of the test

When compared to reference standards such as Gram's stain or multiplex PCR, the forest plot (SI-5) shows how semi-quantitative/quantitative/enrichment culture performed. Compared to earlier investigations, three findings (44, 29, 52) show a wide range of sensitivity for semi-quantitative/quantitative/enrichment cultures. According to the SI-5a forest plot, the probable sensitivity range is between 0.1852 and 1 [28]. Three studies in SI-5b found that semi-quantitative, quantitative, and enrichment cultures differed more in specificity than the others.45, 23, 52 With a specificity of 126 and a minimum of 0.444, the semi-quantitative/quantitative/enrichment culture was the most selective in the SI-5b. that the sensitivity estimates for the multiplex PCR tests, which included 12 samples, varied from 0.5819 to 119, 28, 40. The specificity levels were from 0.1415 and 1.26, 28. Figure shows that the majority of research had sensitivity > 0.80 and specificity > 0.88.

Summary ROC

Using SROC (summary receiving operating curve), we analyzed previous studies that used a number of procedures; including Gram's stain, microbiological culture, and multiplex PCR-based molecular testing. The tests' effectiveness was shown by the SROC curve, which placed the most effective tests in the top left corner of the graph. The closeness of the

curve to the top left corner was considered while assessing the level of diagnostic accuracy. Because of how close they are, we may say that the curve is very sensitive and particular. A more precise diagnosis was achieved by extending the curve's distance from the top left corner.

The SROC curve is one kind of graph that shows how a diagnostic test's sensitivity and specificity relate to one another. How likely it is that a test will indicate that a person has a given illness is what the term "sensitivity" refers to. The specificity of a test is defined as the likelihood that it will provide a negative result in the absence of the disease of interest. An indicator of a test's capacity to distinguish between disease-afflicted and non-afflicted individuals is the area under the receiver operating characteristic curve (AUC). Generally speaking, the discrimination strength is proportional to the area under the curve (AUC). Figure 3 shows that the area under the curve (AUC) for multiplex PCR is 0.95, which is greater than the AUC for Gramme stain (0.87) and microbiological culture (0.89). As a result, multiplex PCR proves to be a superior tool for infection diagnosis than microbiological culture and Gramme stain. A more sensitive method than Gram stain or microbiological culture is the multiplex polymerase chain reaction (PCR). This indicates that the chances of accurately identifying infected individuals are higher. When compared to microbiological culture, Gramme stain isn't as specific, but multiplex PCR is. This provides more evidence that the possibility of falsely diagnosing healthy individuals as sick is lower.

CONCLUSION

The multiplex PCR test is the most accurate diagnostic test for VAP, according to our data, followed by culture techniques as the second most reliable diagnostic test. In addition, it appears that combining the Gram's stain, culture, and multiplex PCR testing results in a higher level of sensitivity when compared to utilising any of these tests on its own, independent of the method. Nevertheless, the Gram's staining technique that is now being used has a low level of precision, which indicates that there is a need for future research to develop ways that are both more quick and more targeted. These methods should be suitable for individual patients and should be able to be utilized in large-scale prevalence investigations of VAP.

REFERENCES

1. Centers for Disease Control and Prevention. (2021). Pneumonia (Ventilator-associated VAP and non- ventilator-associated Pneumonia PNEU.) Event Table of Contents. 2013:1-19. Https://Www.Cdc.Gov/Nhsn/Pdfs/Pscmanual/4psc_clabscurrent.Pdf (Accessed 27 Jan 2021).
2. Arthur LE, Kizor RS, Selim AG, van Driel ML, Seoane L. Antibiotics for ventilator associated pneumonia. *Cochrane Database of Syst Rev*. 2016(10):CD004267. doi: 10.1002/14651858.CD004267.pub4
3. Papazian L, Klompas M, Luyt CE. Ventilator-associated pneumonia in adults: a narrative review. *Intensive care Med.* 2020;46(5):888-906. doi: 10.1007/s00134-020-05980-0
4. Behera AK, Guruprasad H, Reddy M, et al. Incidence, Risk Factors and Microbiological Profile of Ventilator Associated Pneumonia Patients in ICU in Tertiary Care Hospital. *J Adv Med Pharm Sci*. 2024;26(3):37-44. doi: 10.9734/jamps/2024/v26i3675
5. Kharel S, Bist A, Mishra SK. Ventilator-associated pneumonia among ICU patients in WHO Southeast Asian region: A systematic

review. *PloS ONE*. 2021;16(3):e0247832. doi: 10.1371/journal. pone.0247832

6. Ferrer M, Torres A. Epidemiology of ICU-acquired pneumonia. *Curr Opin Crit Care*. 2018;24(5):325-331. doi: 10.1097/MCC.0000000000000536

7. Mathai AS, Phillips A, Kaur P, Isaac R. Incidence and attributable costs of ventilator-associated pneumonia(VAP) in a tertiary-level intensive care unit (ICU) in northern India. *J Infect Public Health*. 2015;8(2):127-135. doi: 10.1016/j.jiph.2014.07.005

8. Gautam A, Ganu SS, Tegg OJ, Andresen DN, Wilkins BH, Schell DN. Ventilator-associated pneumonia in a tertiary paediatric intensive care unit: a 1-year prospective observational study. *Crit Care Resusc*. 2012;14(4):283- 289. doi: 10.1016/S1441-2772(23)01769-6

9. Wu D, Wu C, Zhang S, Zhong Y. Risk factors of ventilator-associated pneumonia in critically III patients. *Front pharmacol.* 2019;10:482. doi: 10.3389/ fphar.2019.00482

10. Abayasekara LM, Perera J, Chandrasekharan V, et al. Detection of bacterial pathogens from clinical specimens using conventional microbial culture and 16S metagenomics: a comparative study. *BMC Infec Dis.* 2017;17:1-1. doi: 10.1186/s12879-017-2727-8

11. Zaragoza R, Peman J, Salavert M, et al. Multidisciplinary approach to the treatment of invasive fungal infections in adult patients. Prophylaxis, empirical, preemptive or targeted therapy, which is the best in the different hosts? *Ther Clin Risk Manag.* 2008;4(6):1261-1280. doi: 10.2147/tcrm.s3994

12. Monteiro-Neto V, Lima-Neto LG, Abreu AG, Monteiro CRAV. Microbiology of Ventilator-Associated Pneumonia. doi: 10.5772/intechopen.69430

13. Thakur HK, Tarai B, Bhargava A, et al. Pathogenesis, Diagnosis and Therapeutic Strategies for Ventilator- associated Pneumonia. *J Pure Appl Microbiol.* 2024;18(2):772-796. doi: 10.22207/JPAM.18.2.10

14. Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis.* 2004;4(6):337-348. doi: 10.1016/S1473- 3099(04)01044-8

15. Buchan BW, Windham S, Balada-Llasat JM, et al. Practical comparison of the BioFire FilmArray pneumonia panel to routine diagnostic methods and potential impact on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. *J Clin Microbiol.* 2020;58(7):10-128. doi: 10.1128/jcm.00135-20

16. McInnes MD, Moher D, Thombs BD, et al. Preferred reporting items for a systematic review and meta- analysis of diagnostic test accuracy studies: the PRISMA-DTA statement. *JAMA.* 2018;319(4):388-396. doi: 10.1001/jama.2017.19163

17. Cumpston M, Li T, Page MJ, et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database Syst Rev.* 2019;10(10):ed000142. doi: 10.1002/14651858. ED000142

18. Luyt CE, Hekimian G, Bonnet I, et al. Usefulness of point-of-care multiplex PCR to rapidly identify pathogens responsible for

ventilator-associated pneumonia and their resistance to antibiotics: an observational study. *Crit Care.* 2020;24:378. doi: 10.1186/s13054-020-03102-2

19. Clavel M, Barraud O, Moucadel V, et al. Molecular quantification of bacteria from respiratory samples in patients with suspected ventilator-associated pneumonia. *Clin Microbiol Infect.* 2016;22(9):812. e1-812.e7. doi: 10.1016/j.cmi.2016.06.013

20. Whiting PF, Rutjes AWS, Westwood ME, et al. Quadas-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med.* 2011;155:529-536.

21. Pickens CO, Gao CA, Cuttica MJ, et al. Bacterial superinfection pneumonia in patients mechanically ventilated for COVID-19 pneumonia. *Am J Respir Crit Care Med.* 2021;204(8):921-932. doi: 10.1164/ rccm.202106-1354OC

22. Hou D, Ju M, Wang Y, et al. PCR coupled to electrospray ionization mass spectrometry for microbiological diagnosis and surveillance of ventilator associated pneumonia. *Exp Ther Med.* 2020;20(4):3587-3594. doi: 10.3892/etm.2020.9103

23. Nusrat T, Akter N, Haque M, et al. Comparative study of CDST & multiplex PCR to detect MBL producing gram-negative bacilli among VAP patients admitted in a public medical college hospital of Bangladesh. *Pathogens.* 2019;8(3):151. doi: 10.3390/ pathogens8030151

24. Morris AC, Gadsby N, McKenna JP, et al. 16S pan-bacterial PCR can accurately identify patients with ventilator- associated pneumonia. *Thorax.* 2017;72(11):1046- 1048. doi: 10.1136/thoraxjnl-2016-209065

25. Peiffer-Smadja N, Bouadma L, Mathy V, et al.Performance and impact of a multiplex PCR in ICU patients with ventilator-associated pneumonia or ventilated hospital-acquired pneumonia. *Crit Care.* 2020;24(1):366. doi: 10.1186/s13054-020-03067-2

26. van der Schalk TE, Coppens J, Timbermont L, et al. Evaluation of GeneXpert PA assay compared to genomic and (semi-) quantitative culture methods for direct detection of *Pseudomonas aeruginosa* in endotracheal aspirates. *Antimicrob Resist Infect Control.* 2021;10(1):110. doi: 10.1186/s13756-021-00978-9

27. Bianco A, Quirino A, Giordano M, et al. Control of carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit of a teaching hospital in Southern Italy. *BMC Infect Dis.* 2016;16(1):747. doi:10.1186/s12879-016-2036-7

28. Coppens J, Van Heirstraeten L, Ruzin A, et al. Comparison of GeneXpert MRSA/SA ETA assay with semi-quantitative and quantitative cultures and *nuc* gene-based qPCR for detection of *Staphylococcus aureus* in endotracheal aspirate samples. *Antimicrob Resist Infect Control.* 2019;8:4. doi: 10.1186/s13756-018-0460-8

29. Enne VI, Aydin A, Baldan R, et al. Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study. *Thorax.* 2022;77(12):1220-1228. doi: 10.1136/ thoraxjnl-2021-216990

30. Hughes S, Troise O, Donaldson H, Mughal N,

Moore LS. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clin Microbiol Infect.* 2020;26(10):1395-1399. doi: 10.1016/j. cmi.2020.06.025

31. Karolyi M, Pawelka E, Hind J, et al. Detection of bacteria via multiplex PCR in respiratory samples of critically ill COVID-19 patients with suspected HAP/VAP in the ICU. *Wien Klin Wochenschr*. 2022;134(9-10):385-390. doi: 10.1007/s00508-021-01990-0

32. Khosroshahi ND, Farivar TN, Johari P. Identification of *Legionella pneumophila* in intubated patients with TaqMan real time PCR. *Jundishapur J Microbiol.* 2015;8(3):e15094. doi: 10.5812/jjm.15094

33. Krishnamurthy V, Vijaykumar GS, Kumar S, Prashanth HV, Prakash R, Nagaraj ER. Phenotypic and genotypic methods for detection of extended spectrum

34. lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from ventilator associated pneumonia. *J Clin Diagn Res.* 2013;7(9):1975-1978. doi: 10.7860/JCDR/2013/6544.3376

35. Loughlin L, Hellyer TP, White PL, et al. Pulmonary aspergillosis in patients with suspected ventilator- associated pneumonia in UK ICUs. *Am J Respir Crit Care Med.* 2020;202(8):1125-1132. doi: 10.1164/ rccm.202002-0355OC

36. Nolan TJ, Gadsby NJ, Hellyer TP, et al. Low-pathogenicity *Mycoplasma* spp. alter human monocyte and macrophage function and are highly prevalent among patients with ventilator-acquired pneumonia. *Thorax*. 2016 ; 71 (7):594 -600 . doi: 10 .1136 /thoraxjnl-2015-208050

37. Monard C, Pehlivan J, Auger G, et al. Multicenter evaluation of a syndromic rapid multiplex PCR test for early adaptation of antimicrobial therapy in adult patients with pneumonia. *Crit Care*. 2020;24(1):434. doi: 10.1186/s13054-020-03114-y

38. Nowak J, Zander E, Stefanik D, et al. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *J Antimicrob Chemother*. 2017;72(12):3277-3282. doi: 10.1093/jac/dkx322

39. Razazi K, Delamaire F, Fihman V, et al. Potential of multiplex polymerase chain reaction performed on protected telescope catheter samples for early adaptation of antimicrobial therapy in ARDS patients. *J Clin Med.* 2022;11(15):4366. doi: 10.3390/ jcm11154366

40. Rouze A, Martin-Loeches I, Povoa P, et al. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: a European multicenter cohort study. *Intensive Care Med.* 2021;47(2):188-198. doi: 10.1007/s00134-020-06323-9