

## Evaluation PCR panel of the FilmArray® pneumonia plus for pathogen detection of ventilator-associated pneumonia in children and its impact on therapeutic management

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### ABSTRACT

**Background and Objectives:** Ventilator-associated pneumonia (VAP) is the second most common nosocomial infection in pediatric intensive care units. The aim of this study was to evaluate the contribution of multiplex PCR in the diagnosis of VAP and its impact on the clinical and prognostic outcome of children in the ICU.

**Materials and Methods:** This is a prospective observational study from March to November 2021, including bronchial samples collected from 38 intubated children hospitalized in ICU. The detection of respiratory pathogens was performed by the FilmArray® Pneumonia Panel plus (FAPP).

**Results:** Multiplex PCR (mPCR) detected exclusively 46 potentially pathogenic bacteria, giving a sensitivity of 93%, specificity of 90%, negative predictive value of 100%, and positive predictive value of 23%. Overall, the sensitivity of mPCR was higher for Gram-negative bacteria (100%) than Gram-positive (92%). Bacterial etiology was the most frequent (69.3%), represented mainly by *Moraxella catarrhalis* (11.4%), followed by viral etiology (30.7%), with Rhinovirus/Enterovirus as the most prevalent virus. FAPP enabled a change in antibiotic therapy in 39.5% of the patients, with a 73.3% survival rate.

**Conclusion:** This study highlights the importance of mPCR in diagnosing VAP and improving antimicrobial therapy.

**Keywords:** Multiplex polymerase chain reaction; Ventilator-associated pneumonia; Children; Intensive care units; Antimicrobial stewardship

### INTRODUCTION

Lower respiratory tract infections are a global public health problem. Pneumonia is the leading cause of morbidity and mortality in young children

outside the neonatal period, particularly in low-to middle-income countries (1, 2). Ventilator-associated pneumonia (VAP) is the second most common nosocomial infection in the pediatric intensive care

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unit after bacteremia (3). It represents the main cause of death in the intensive care units and is associated with a long duration of ventilation, hospitalization, and increased costs of care (4). It's defined by the occurrence of pneumonia after a minimum of 48 hours after intubation. The management of nosocomial pneumonia in the ICU is a daily challenge for health care systems, especially in view of the impossibility of predicting the occurrence of VAP and of determining the causal pathogen, especially with prior antibiotic therapy. Conventional bacteriology techniques have long been a classic tool for diagnosing VAP. However, bacterial culture, although sensitive (90%), is hardly specific (5). It depends on several parameters, including the nature and quality of the sample and the use of prior antibiotic therapy. At best, results can only be obtained after 48 hours, during which time the patient is still undergoing probabilistic treatment, most often broad-spectrum antibiotics (4).

In severe conditions, especially for patients hospitalized in intensive care units (ICU), the detection of microbial agents responsible of VAP is urgent and crucial to starting a targeted and adapted antibiotic treatment. Probabilistic antibiotic therapy not only causes false-negative bacterial cultures but also is responsible for nearly half of all antibiotic consumption and increases the length of hospital stay as well as the morbidity and mortality rate (6). Faced with this problematic situation, new rapid diagnostic tools based on molecular biology techniques have emerged and have since become increasingly present on the world market (7). These new techniques are more and more requested and would constitute, in certain cases, an examination of choice for the search of bacterial and viral microbial agents present in small or significant quantities, which can be viable or in traces. They are less affected by the first antibiotic therapy and would also allow the detection of resistance genes to the antibiotics most involved in respiratory pathology. The BioFire® FilmArray® Pneumonia plus panel (FAPP) is a multiplex PCR technique based on a syndromic approach that was approved by the FDA (Food and Drug Administration) in 2018. It allows the detection and identification of 27 bacterial and viral respiratory pathogens and antibiotic resistance genes in lower respiratory tract samples.

The aim of this trial was to evaluate the contribution of multiplex PCR coupled with culture in the

diagnosis of these lower respiratory infections in patients presenting ventilator-associated pneumonia and its clinical and prognostic impact in children in the intensive care units.

## MATERIALS AND METHODS

**Patients.** This was a descriptive observational and prospective analysis carried out in the microbiology laboratory of the Mohamed VI University Hospital of Marrakech. This study covered a period of 9 months between March and November 2021 and included intubated children under 14 years old hospitalized in the pediatric intensive care unit of the Mohamed VI University Hospital of Marrakech, with the suspicion of VAP. Children over 14 years old or who are not intubated were excluded from this study.

Bronchial samples were collected, including 37 endotracheal aspirations (EA) and 8 protected specimen brush (PSB).

**Methods.** After receiving the samples, the culture was performed first with the cytobacteriological examination, followed by the search for microbial agents by the FilmArray® instrument (bioMérieux, France) with the FilmArray® Pneumonia plus panel (FAPP).

The cytobacteriological study of the bronchial samples was carried out according to the standard procedures of the French Society of Microbiology.

**BioFire® FilmArray® pneumonia panel plus test.** Approximately 200 µL of the received samples were used for the BioFire® FilmArray® Pneumonia plus panel (Biomérieux, Product references: RFIT-ASY-0142/ RFIT-ASY-0143) according to the manufacturer's recommendations. This multiplex real-time PCR assay consists of an automated nucleic acid assay based on acid extraction, reverse transcription, and nucleic acid amplification; with an average result turnaround time of 75 minutes per cycle and a handling time of less than 5 minutes. Transportation of samples from clinical services to the laboratory took about 20 minutes.

This PCR allows qualitative and semi-quantitative detection according to the pathogens: 15 bacteria (*Acinetobacter calcoaceticus-baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Kleb-*

*siella oxytoca*, *Klebsiella pneumoniae* group, *Moraxella catarrhalis*, *Proteus* spp, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*), 3 atypical bacteria (*Chlamydia pneumoniae*, *Legionella pneumophila* and *Mycoplasma pneumoniae*), 8 viruses (adenovirus, coronavirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza B, parainfluenza respiratory and syncytial viruses) and 7 antibiotic resistance genes (methicillin resistance [mecA/C and MREJ], carbapenemases [KPC, NDM, OXA-48-like, VIM, and IMP], and ESBL [CTX-M]). These resistance genes are not directly linked to the pathogen.

**Statistical analysis.** All statistical analyses were performed using SPSS software (version 23.0; SPSS, Inc, Chicago, IL, USA) and Microsoft Excel (Microsoft Corporation, Washington, USA). Statistical comparisons were performed using the Chi-square test. A probability value (p) of less than 0.05 was considered statistically significant.

**Ethics, authorization, and approval.** The study focuses on children. Hence, the anonymity of each patient was considered according to the recommendations of the Declaration of Helsinki. The study was approved by the ethics committee of the Faculty of Medicine and Pharmacy of Marrakech (N° 22/2021).

## RESULTS

**Patient demographics and clinical characteristics.** During the study period, 38 pediatric ICU patients received 45 FilmArray® Pneumonia plus panel PCR tests. Table 1 summarizes the clinical and demographic characteristics of the study population. The mean age of the patients was 3.06 years, with age extremes ranging from 3 days to 14 years, with male predominance (sex ratio M/F = 1.5). Acute respiratory distress was the most frequent reason for hospitalization, at 36.9%. Eleven (28.9%) patients had comorbidities, mainly congenital heart disease (n = 5, 18.4%). During this period, 86.8% of the patients were under probabilistic antibiotic therapy before the first PCR (started on average 5 days before the sampling). This prescription was mainly made of beta-lactams (81.5%).

**Table 1.** Demographic and clinical characteristics of study participants (n=38)

Variable	Number (%)
Sex, male	23 (60.5)
Age (years), mean ± SD	3.06 ± 3.81
Reason for hospitalization	
Acute respiratory distress	14 (36.9)
Severe head trauma	7 (18.4)
Severe burns	4 (10.5)
Convulsive seizures	3 (7.9)
Esophageal atresia	1 (2.6)
Others	9 (23.7)
Comorbidities	11 (28.9)
Congenital heart disease	5 (13.2)
Prematurity (children < 2 years)	3 (7.9)
Chronic respiratory disease	1 (2.6)
Immunocompromised status	1 (2.6)
Other comorbidities	1 (2.6)
Biological data	
White blood cells (x10 <sup>3</sup> /mm <sup>3</sup> ), mean ± SD	14.08 ± 7.11
Hyperleukocytosis	14 (36.8)
Leukopenia	1 (2.6)
Lymphocytes (x10 <sup>3</sup> /mm <sup>3</sup> ), mean ± SD	2.78 ± 2.19
Lymphopenia	16 (42.1)
Platelets (x10 <sup>3</sup> /mm <sup>3</sup> ), mean ± SD	239.90 ± 157.91
Thrombocytopenia	7 (18.4)
Thrombocytosis	1 (2.6)
CRP (mg/L), mean ± SD	100.25 ± 105.18
CRP, high	31 (81.6)
Urea (g/L), mean ± SD	1.93 ± 7
Positive blood culture	10 (26.3)
Positive ECBU	4 (10.5)
Primary antibiotic therapy	33 (86.8)
Betalactams	33 (81.5)
Amoxicillin-clavulanic acid	13 (34.2)
Ceftriaxone	11 (28.9)
Carbapenems	7 (18.4)
Quinolones	2 (5.3)
Aminosides	3 (7.9)
Trimetroprime-Sulfamethoxazole	3 (7.9)
Glycopeptides	5 (13.2)
Colistin	2 (5.3)
Antifungals	5 (13.2)
Antivirals	2 (5.3)

**FilmArray® Pneumonia panel plus test results.** The FilmArray® Pneumonia Panel plus detected at least one pathogen in 37/45 tested samples, yielding a positivity rate of 82.2%. Table 2 summarizes all

**Table 2.** Distribution of pathogens detected by BioFire® FilmArray® Pneumonia plus panel (n=88)

Pathogens	Positive PCR N (%)
Bacteria	61 (69.3)
<i>Acinetobacter calcoaceticus-baumannii</i> -complex	5 (5.7)
<i>Enterobacter cloacae</i> complex	6 (6.8)
<i>Haemophilus influenzae</i>	9 (10.2)
<i>Klebsiella oxytoca</i>	1 (1.1)
<i>Klebsiella pneumoniae</i>	7 (8)
<i>Moraxella catarrhalis</i>	10 (11.4)
<i>Pseudomonas aeruginosa</i>	7 (8)
<i>Staphylococcus aureus</i>	4 (4.5)
<i>Streptococcus pneumoniae</i>	8 (9.1)
<i>Streptococcus pyogenes</i>	4 (4.5)
Virus	27 (30.7)
Adenovirus	4 (4.5)
Coronavirus	3 (3.5)
Rhinovirus/Enterovirus	11 (12.5)
Parainfluenza virus	4 (4.5)
Respiratory Syncytial Virus	5 (5.7)
Resistance genes	
Carbapenemases	15 (65.2)
NDM	7 (30.4)
OXA-48	6 (26.1)
VIM	2 (8.7)
ESBL: CTX-M	8 (34.8)

pathogens and resistance genes detected by FAPP. Endotracheal aspirations were the most common type of sampling (n=37, 82.2%), compared to only 8 (17.8%) PSBs. Bacterial etiology was the most frequent (69.3%), represented mainly by *Moraxella catarrhalis* (11.4%), *Haemophilus influenzae* (10.2%) and *Streptococcus pneumoniae* (9.1%); followed by viral etiology (30.7%), of which *Rhinovirus/Enterovirus* was the most prevalent among the identified viruses (12.5%). Viral and bacterial co-infections were detected in 23 patients (62.2%) with a positive PCR. The greatest number of pathogens detected in a single specimen was eight (*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Enterovirus/Rhinovirus* and *Adenovirus*). Simultaneous detection of viruses and bacteria was found in 27% (n=10) of all patients with positive PCR.

Patients with positive PCR had an elevated CRP (>5

mg/L) in 86.5%; leukopenia or hyperleukocytosis in 40.5%; lymphopenia in 40.5%. Associated bacteremia was found in 32.4% of patients. PCR results were obtained within 1 hour 30 minutes to 5 hours after sampling.

**Evaluation of the performance of BioFire® FilmArray® Pneumonia plus panel.** The culture of respiratory samples was systematically performed in parallel with the PCR test in the 38 patients included in this study. Sixteen bacteria were isolated from 15 patients, for a positivity rate of 33.3%. The most prevalent bacteria were *Pseudomonas aeruginosa* (n=4, 23.5%), followed by *Klebsiella pneumoniae* (n=3, 17.6%). Bacterial co-infection was found in 2 patients (11.8%).

Multiplex PCR, detected exclusively 46 potentially pathogenic bacteria, giving a sensitivity of 93%, specificity of 90%, negative predictive value of 100%, and positive predictive value of 23% (Table 3). The sensitivity of multiplex PCR was 100% for all bacteria except *Streptococcus* spp. However, the specificity of FAPP was very heterogeneous for the bacteria detected, ranging from 80% for *Moraxella catarrhalis*, to 86% for *Streptococcus pneumoniae*, to 93% for *Acinetobacter calcoaceticus-baumannii* complex, then to 100% for *Streptococcus* spp. Overall, the sensitivity of FA PP was higher for Gram-negative bacteria (100%) than Gram-positive (92%), which had a higher specificity (92%).

Concordance of FAPP PCR and bacterial culture results was found in 33 patients (73.3%). However, a discrepancy was noted with the detection of bacteria only by PCR in 11 patients (24.4%). In addition, the bacterial culture has a singly isolated *Streptococcus* spp, which was not detected among the agents found by the FAPP PCR performed on the same sample.

All patients with both positive samples (FAPP test and culture) had an elevated CRP (>5 mg/L) in 93.8%, lymphopenia in 43.8% and associated bacteremia in 50%. They had given probabilistic treatment to 87.5% of them.

**Impact on antimicrobial prescribing and ICU stay.** Thirty-three (86.8%) patients were under probabilistic antimicrobial therapy at the time of the respiratory sampling. In 60.5% (n=23) of patients hospitalized in the ICU, no change in treatment was made following the results of the FilmArray® Pneumonia

**Table 3.** Performance of the BioFire® FilmArray® Pneumonia plus Panel (n=62)

Bacteria N (%)	True positive (Culture+/PCR+)	False positive (PCR+/Culture-)	False negative (PCR-/Culture+)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
Gram-negative bacteria								
<i>Acinetobacter calcoaceticus- baumannii</i> complex	2	3	0	100%	93%	40%	100%	0.000
<i>Enterobacter cloacae</i> complex	0	6	0	-	87%	0%	100%	NA
<i>Haemophilus influenzae</i>	1	8	0	100%	82%	11%	100%	0.04
<i>Klebsiella oxytoca</i>	1	0	0	100%	100%	100%	100%	0.000
<i>Klebsiella pneumoniae</i>	3	4	0	100%	90%	43%	100%	0.000
<i>Moraxella catarrhalis</i>	1	9	0	100%	80%	10%	100%	0.055
<i>Pseudomonas aeruginosa</i>	4	3	0	100%	93%	57%	100%	0.000
Gram-positive bacteria								
<i>Staphylococcus aureus</i>	0	4	0	-	91%	0%	100%	NA
<i>Streptococcus pneumoniae</i>	2	6	0	100%	86%	25%	100%	0.002
<i>Streptococcus pyogenes</i>	0	4	0	-	91%	0%	100%	NA
<i>Streptococcus</i> spp.	0	0	1	0%	100%	-	98%	NA
Total	14	47	1	93%	90%	23%	100%	

PPV: Positive Predictive Value

NPV: Negative Predictive Value

plus panel PCR. The antibiotic spectrum was enlarged for 31.6% patients (n=12) and the introduction of an antiviral or antibiotic treatment concerned 3 patients (7.9%). No reduction in the spectrum of antibiotic therapy was noted.

These antibiotic therapy changes were based on multiplex PCR results in 69.2% of cases, compared to 30.8% for bacteremia. The most prescribed molecules following this change were carbapenems at 28.9%, aminoglycosides and colistin at 15.8% respectively, glycopeptides at 7.9% and quinolones and antifungals at 5.3% respectively.

Nosocomial infection was the main complication occurring in patients (n=12; 31.6%), followed by acute respiratory distress syndrome (ARDS) (n=9; 23.7%) and shock (n=5; 13.2%). The overall survival rate for these patients was 68.4% (n=26).

The average ICU stay for the study population was 12 days with a median stay of less than one week for 42.1% (n=16) of patients. However, the average hospital stay was 20 days with a stay of more than two weeks for more than half of the patients (n=22; 57.9%). Survival in patients who received a change in treatment was 73.3% (n=11). Table 4 summarizes the impact of FilmArray® Pneumonia plus panel PCR on mortality, treatment, and length of stay in the ICU.

## DISCUSSION

The BioFire® FilmArray® Pneumonia panel plus is a multiplex PCR test for the rapid identification of 27 pathogens (15 bacteria, 3 atypical bacteria and 8 viruses) most involved in lower respiratory tract infections and 7 antibiotic resistance markers. This panel provides information on the relative abundance of 15 bacteria via semi-quantitative analysis, to facilitate the distinction between normal and potentially pathogenic flora. To our knowledge, this prospective study is the first to evaluate the interest of FilmArray® Pneumonia plus panel (FAPP) multiplex PCR in the detection of ventilator-associated pneumonia and its impact on antibiotic prescription and morbidity in the pediatric ICU population in Morocco. The sensitivity of detection of bacteria by multiplex PCR was 93% and its specificity 90%. It allowed us to modify the antibiotic treatment in 15 patients, with a survival rate of 73% (n=11/15).

Performance studies of the different techniques of multiplex respiratory PCR had very heterogeneous results. The present analysis had the highest sensitivity (90%) and lowest specificity (90%), broadly similar to the study by Ozongwu et al. (8) (sensitivity= 88%; specificity= 94%), but differs from the data of Nathan et al. (4) (sensitivity= 80%; specificity= 99%). The FAPP allowed the detection of the main bacteria incriminated in VAP. In line with our results, Gram-negative bacteria were the most detected



**Table 4.** Impact of FilmArray PP PCR on mortality, treatment and length of stay in the ICU (n=38)

	Survivors (n; %)	Deceased (n; %)	Total (n; %)
Modification of antibiotic therapy			
No change	15 (39.5)	8 (21.1)	23 (60.5)
Introduction of antibiotics	1 (2.6)	2 (5.3)	3 (7.9)
Escalation	10 (26.3)	2 (26.3)	12 (31.6)
Length of stay in ICU			
≤ 7 days	11 (28.9)	5 (13.2)	16 (42.1)
≤ 14 days	8 (21.1)	3 (7.9)	11 (28.9)
≤ 21 days	5 (13.2)	2 (5.3)	7 (18.4)
≤ 30 days	1 (2.6)	1 (2.6)	2 (5.3)
> 1 month	1 (2.6)	1 (2.6)	2 (5.3)
Total	26 (68.4)	12 (31.6)	38 (100)

by multiplex PCR (4, 9). However, the distribution of these bacteria varied from one study to another: *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Enterobacteriaceae* are always isolated at variable rates. *Moraxella catarrhalis*, the first microbial agent in this work, was absent in Lee et al. and only slightly found in Monard et al. (9, 10). *Staphylococcus aureus* and *Streptococcus pneumoniae* are commonly the most isolated Gram-positive bacteria, with proportions varying according to the authors. In this study, the only false negative found was the isolation of *Streptococcus* spp. by culture only. This agent is not one of the targets of FAPP, hence the importance of interpreting any multiplex PCR result with the presence of a complementary bacterial culture. Incriminating pathogens detected by multiplex PCR is difficult due to the presence of commensal bacterial flora that varies according to the site and the nature of the sample, including mainly four genera: *Haemophilus*, *Streptococcus*, *Staphylococcus* and *Pseudomonas*. Thus, protected bronchoscopic samples are to be preferred to sputum and endotracheal aspirations, since they are performed in a way to reduce contamination by this oral and respiratory microbiota (11). The detection of bacteria at semi-quantitative thresholds made it easier to distinguish between their commensal or pathogenic state. The higher the threshold, the greater the probability that the bacterium is implicated in a lower respiratory tract infection. Since pathogens can persist in vivo as nucleic acid independently of their viability, their detection by multiplex PCR does not clearly incriminate them as pathogens (9). As observed in previous studies, viral etiology was found in second place after Gram-negative bacteria, at an estimated rate of

30.7% in this work. The incriminated viral species differed according to the epidemiology of the locations of each analysis. Rhinovirus/Enterovirus, was in our case, the most isolated species, since it is the most frequent viral agent in our context (12). It is important to recognize that the interpretation of FAPP results is delicate, especially when it comes to the detection of co-infections (viral and bacterial, or multiple bacteria isolated). In this study, co-infection was present in 62.2% of patients with multiplex FAPP and in 2 patients with a positive bacterial culture. The presence of a significant rate of co-infections raises the issue of lower respiratory tract infection, that is polymicrobial for more than one third of the cases. The results of this study concerning the detection of resistance genes found a high rate of carbapenemases (65.2%) and ESBLs (34.8%), which could be explained by the use of prior antibiotic therapy, often broad spectrum, in severely ill patients hospitalized in the ICU. This prescription was found in 86.8% of the patients, mainly made of beta-lactams (81.5%) and administered on average five days before the FAPP test. This could also be the reason for the low PPV calculated (23%) and the discrepancies observed between the results of the multiplex PCR and those of the conventional bacterial culture.

In this analysis, the syndromic approach by FAPP multiplex PCR allowed the change of antibiotic therapy in 39.5% of the patients. This change mainly concerned the broadening of the spectrum of action (31.6%) rather than the introduction of antibiotics (7.9%). No reduction in spectrum was noted. Carbapenems were the most prescribed molecules (28.9%) following this replacement, followed by quinolones and colistin (15.8%). This prescription for severe ill

patients hospitalized in the intensive care unit with suspected VAP, would be at the origin of the difference in our results compared to Monard et al. (10) who evaluated FAPP in less severe patients suspected of having pneumonia. VAP represents a real therapeutic challenge. However, there are international recommendations for empirical antibiotic therapy for Gram-negative VAP with the prescription of broad-spectrum antibiotics including carbapenems essentially in the case of prior antibiotic therapy, in patients colonized by multi-resistant bacteria, or in the case of any late VAP (more than 5 days after the start of mechanical ventilation) (4, 13). This undocumented prescription exposes to the risk of selection of resistant mutants in the patients' intestinal microbiota (11) and the consequent development of multi-resistant bacteria responsible for high morbidity and mortality (14). In this study, the survival rate for patients who received a change in antibiotic therapy following FAPP multiplex PCR was 73.3%. This rate is encouraging to perform more and more multiplex PCR for patients with suspected FAPP, in order to allow an optimized and rationalized management.

It is important for health professionals and biologists to be aware of the major limitations of multiplex PCR. Although FAPP allows the detection of a large number of pathogenic targets, it does not include in its panel some bacteria of major medical interest, such as *Morganella* spp, *Citrobacter* spp, *Hafnia alvei*, *Stenotrophomonas maltophilia* or *Pneumocystis jirovecii*. In presence of severe ill patients hospitalized in intensive care units, some of whom may be immunocompromised, a negative multiplex PCR result does not necessarily rule out an evolving infection. Furthermore, the detection of antibiotic resistance genes does not allow direct linkage to the pathogen or prediction of phenotypic sensitivity (10). It should be noted that even with semi-quantitative quantification of bacterial targets provided by the BioFire® FilmArray® Pneumonia Panel, distinguishing between a simple colonization and a true infection is difficult. In addition, it does not allow monitoring of therapeutic efficacy as long as bacterial resistance genes to antibiotics can be detected despite adequate treatment (10, 13). As a result, clinicians and biologists should think of the FilmArray® Pneumonia Plus panel as a microbiological test that necessitates careful and sensitive analysis and interpretation of all clinical and biological data, as well as the results of conventional microbiological tests.

This study has some limitations. Multiplex PCR is a new molecular biology technique that is supposed to have better sensitivity than conventional culture. The use of the latter as a reference method to evaluate the results of the FAPP is the main limitation of this type of study (9) and leads to inadequate consideration of some significant results as false positives. Also, the lack of evaluation of the semi-quantitative results obtained by FAPP, couldn't decide on the involvement of the isolated bacteria in VAP. Moreover, this study did not allow the evaluation of the FAPP results concerning the detected viruses to decide on their pathogenicity, hence the interest of new studies conducted in this perspective. Finally, the socio-economic impact of this costly technique (150 -200 euros per test) has not been determined, and the absence of sufficient material resources during this work did not allow the evaluation of FAPP results according to the sampling types. It would be interesting to complete this work with other studies in order to make recommendations on the conditions and protocols for using this new diagnostic technique.

## CONCLUSION

Ventilator-associated pneumonia is a daily concern for the medical staff in intensive care units. Their microbiological diagnosis requires an update, which should integrate new diagnostic tools, in particular multiplex PCR. The BioFire® FilmArray® Pneumonia plus panel is a real technological innovation in the field of microbiology. It allows the detection of the microbial etiology among its targeted agents and helps guide the clinician's therapeutic choice by searching for the most frequent resistance genes. However, its routine use is not systematic, given its high cost and lack of specificity. This tool is in fact a diagnostic complement whose results must be interpreted in the presence of other clinical, radiological, and biological arguments.

## REFERENCES

1. Bhutta ZA, Das JK, Walker N, Rizvi A, Campbell H, Rudan I, et al. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *Lancet* 2013; 381: 1417-1429.

2. GBD 2015 LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis* 2017; 17: 1133-1161.
3. Foglia E, Meier MD, Elward A. Ventilator-associated pneumonia in neonatal and pediatric intensive care unit patients. *Clin Microbiol Rev* 2007; 20: 409-425.
4. Peiffer-Smadja N, Bouadma L, Mathy V, Allouche K, Patrier J, Reboul M, 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: 366.
5. Chang I, Schibler A. Ventilator associated Pneumonia in children. *Paediatr Respir Rev* 2016; 20: 10-16.
6. Vincent J-L, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; 302: 2323-2329.
7. Bhat N, O'Brien KL, Karron RA, Driscoll AJ, Murdoch DR, Pneumonia Methods Working Group. Use and evaluation of molecular diagnostics for Pneumonia etiology studies. *Clin Infect Dis* 2012; 54 Suppl 2(Suppl\_2): S153-8.
8. Ozongwu C, Personne Y, Platt G, Jeanes C, Aydin S, Kozato N, et al. The Unyvero P55 'sample-in, answer-out' pneumonia assay: A performance evaluation. *Biomol Detect Quantif* 2017; 13: 1-6.
9. Lee SH, Ruan S-Y, Pan S-C, Lee T-F, Chien J-Y, Hsueh P-R. Performance of a multiplex PCR pneumonia panel for the identification of respiratory pathogens and the main determinants of resistance from the lower respiratory tract specimens of adult patients in intensive care units. *J Microbiol Immunol Infect* 2019; 52: 920-928.
10. Monard C, Pehlivan J, Auger G, Alviset S, Tran Dinh A, Duquaire P, 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: 434.
11. Armand-Lefèvre L, Angebault C, Barbier F, Hamelet E, Defrance G, Ruppé E, et al. Emergence of imipenem-resistant Gram-negative Bacilli in intestinal flora of intensive care patients. *Antimicrob Agents Chemother* 2013; 57: 1488-1495.
12. Lamrani Hanchi A, Guennouni M, Rachidi M, Benhoumich T, Bennani H, Bourrous M, et al. Epidemiology of respiratory pathogens in children with severe acute respiratory infection and impact of the multiplex PCR film array respiratory panel: A 2-year study. *Int J Microbiol* 2021; 2021: 2276261.
13. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, 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; 63(5): e61-e111.
14. Bassetti M, Righi E, Vena A, Graziano E, Russo A, Peghin M. Risk stratification and treatment of ICU-acquired pneumonia caused by multidrug-resistant/ extensively drug-resistant/pandrug-resistant bacteria. *Curr Opin Crit Care* 2018; 24: 385-393.