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Performance Evaluation of the BioFire FilmArray Pneumonia Panel for Detection of Bacterial Pneumonia Causative Agents and Antimicrobial Resistance Genes in Bronchoalveolar Lavage Fluid

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Abstract

Background: Rapid detection of the causative agents is essential for determining the appropriate treatment for patients with lower respiratory tract infections. We evaluated the performance of the BioFire FilmArray pneumonia panel (FA-PE; BioFire Diagnostics, USA) in the identification of bacterial pathogens and antibiotic-resistance genes in endotracheal aspirate specimens. *Methods:* A total of 43 non-duplicated endotracheal aspirates were included in this study. The performance of the FA-PE was assessed using the routine culture method as the reference standard. *Results:* The FA-PE demonstrated a sensitivity of 92.9% and specificity of 79.3% for the identification of 15 bacterial targets compared to routine bacterial culture. Four antimicrobial resistance genes in 43 specimens were detected by the FA-PE. The most frequently detected resistance genes were *mecA/mecC* and SCC*mec* in three specimens, followed by CTX-M in one specimen. *Conclusion:* The FA-PE offers a rapid diagnostic method for lower respiratory tract infections. It may be useful at the early stage of pneumonia before routine culture and antimicrobial susceptibility results are available.

Keywords

Lower respiratory infections
Biofire FilmArray pneumonia panel

1. Introduction

Acute bacterial lower respiratory tract infections are a common cause of hospitalization worldwide and have a significant impact on patient morbidity and mortality [1]. Rapid pathogen detection in these lower respiratory tract infections can enable personalized treatment, thus improving prognosis and survival and preventing unnecessary antimicrobial use [2]. However, conventional diagnostic methods can take up to 48 hours or more, leading to empiric antimicrobial therapy

until results are available [3]. Therefore, rapid molecular diagnostic methods can be used to rapidly diagnose the cause of acute bacterial lower respiratory tract infections, allowing for shorter hospital stays and faster administration of appropriate antimicrobials [4,5].

BioFire FilmArray pneumonia panel (FA-PE; BioFire Diagnostics, Salt Lake City, UT, USA), which is approved by the US FDA and the Korean Ministry of Food and Drug Safety, is a test that simultaneously detects 33 pathogenic bacteria, atypical bacteria, respiratory viruses, and 7 antimicrobial resistance genes within about 1 hour using a fully automated multiplex nucleic acid method. In addition, it can help determine the presence of an infection by providing a semi-quantitative relative abundance of nucleic acids in bacteria.

This study was conducted to evaluate the diagnostic utility of the BioFire FilmArray pneumonia panel (hereinafter referred to as FA-PE) for the detection of bacterial pneumonia causative agents and antimicrobial resistance genes in bronchoalveolar lavage fluid compared to conventional diagnostic methods.

2. Materials and methods

2.1. Study population

Bronchoalveolar lavage fluid from patients admitted with pneumonia to Ajou University Hospital in Suwon from May to August 2022 was included in this study. Bronchoalveolar lavage fluid was collected from a total of 64 patients, and the FA-PE test was performed simultaneously with the conventional culture test. This study was approved by the Institutional Review Board of Ajou University Hospital and exempted from obtaining informed consent (IRB No. SMP-2022-078).

2.2. Culture methods

Specimen processing and culture were performed according to conventional methods [6]. The specimens were inoculated into blood agar, chocolate agar, and MacConkey's agar and incubated in a 35°C, 5% CO_2 incubator. The cultures were read at 18–24 and 48 hours and reported as negative if no bacterial growth was detected. If bacteria grew, they were identified using a VITEK MS system (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was performed using the VITEK 2 system (bioMerieux).

2.3. FilmArray FA-PE

The FA-PE test is performed in a closed pouch using the FilmArray 2.0 and FilmArray Torch instruments (BioFire Diagnostics, Salt Lake City, UT, USA) and includes multiplex nucleic acid amplification followed by endpoint melting curve analysis. The FA-PE test was performed according to the manufacturer's instructions. 200 μL of bronchoalveolar lavage fluid was added to an extraction tube containing extraction reagents, mixed, and injected into a vacuum-sealed BioFire FA-PE pouch, which was loaded into the instrument and tested using the software. The test took less than an hour. The FA-PE test can detect a total of 15 bacterial pathogens, including *Acinetobacter calcoaceticus baumannii* complex, *Enterobacter cloacae* complex, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumonia* group, *Moraxella catarrhalis*, *Proteus spp*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumonia*, *Streptococcus pyogenes*. In addition, seven resistance genes are available for testing and include methicillin resistance *mecA*/*mecC* and MREJ, extended-spectrum ß-lactamase (ESBL) CTX-M, carbapenemases NDM, IMP, OXA-48-like, KPC, and VIM (**Table 1**). Bacterial identification is reported semi-quantitatively using bins showing gene copy number (copies/mL) of bacterial nucleic acids of 10^4, 10^5, 10^6, or 10^7 or more per mL of specimen [7].

2.4. Confirmatory testing of discordant specimens

Specimens with discordant conventional culture and FA-PE results were subjected to 16SrRNA sequencing to identify the causative pathogen, and the primers

Table 1. BioFire FilmArray pneumonia panel detection targets pathogens and antibacterial resistance genes

Category	Target
Bacteria (Semi-quantitative)	*Acinetobacter calcoacticus-baumannii complex*
	Enterobacter cloacae complex
	Escherichia coli
	Haemophilus influenzae
	Klebsiella aerogenes
	Klebsiella oxytoca
	Klebsiella pneumoniae group
	Moraxella catarrhalis
	Proteus spp.
	Pseudomonas aeruginosa
	Serratia marcescens
	Staphylococcus aureus
	Streptococcus agalactiae
	Streptococcus pneumoniae
	Streptococcus pyogenes
Atypical bacteria	*Chlamydia pneumoniae*
	Legionella pneumophila
	Mycoplasma pneumoniae
Viruses	Adenovirus
	Coronavirus
	Human Metapneumovirus
	Human Rhinovirus/Enterovirus
	Influenza A
	Influenza B
	Parainfluenza Virus
	Respiratory Syncytial Virus
Antimicrobial resistance genes	IMP
- Carbapenemases	KPC
	NDM
	OXA-48-like
	VIM
- ESBL	CTX-M
- Methicillin resistance	*mecA/C* and MREJ (MRSA)

Abbreviations: ESBL, extended-spectrum ß-lactamase; MREJ, SCC*mec* right extremity junction; MRSA, methicillin-resistant *S. aureus*.

used to amplify the 16S rRNA gene were 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'. To confirm the *mecA* gene, a polymerase chain reaction (PCR) was performed. The *mecA* PCR method was as follows [8]. DNA was extracted from pure culture colonies isolated from the specimens using the QuickGene DNA Whole Blood Kit S (KURABO, Neyagawa, Osaka, Japan). The primers used were 5'-AAAATCGATGGTAAAGGTTGGC-3' and 5'-AGTTCTGCAGTACCGGATTTGC-3'. For PCR, 2 μL of DNA of the experimental strain and 1.5 μL of primers at a concentration of 1 pmol were added

to a 0.5 mL test tube containing the overall solution, lyophilized PCR premix (Bioneer, Daejeon, Korea: Taq polymerase 1 unit, dNTPs 250 μM, 10 mM Tris-HCl [pH 9.0], 30 mM KCl, 1.5 mM $MgCl_2$). Gene Amp PCR system 9600 (Perkin Elmer Co, Norwalk, CA, USA), predenaturation at 94°C for 1 min, followed by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, was repeated 40 times, with a final extension at 72°C for 5 min. The final PCR product was identified as an amplicon of 533 bp after electrophoresis on a 2.0% agarose gel. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as *mecA* gene negative control standard strains.

2.5. Data analysis

The results of traditional culture and FA-PE tests were compared for pathogen identification and antimicrobial resistance. The sensitivity and specificity of the FA-PE test results were calculated relative to traditional culture.

3. Results

3.1. Pathogen identification

Out of a total of 64 collected specimens, 54 samples underwent testing after excluding insufficient and duplicate specimens. Among the 54 samples, excluding 11 cases where *Candida* and *Enterococcus spp*. were cultured but not included in the FA-PE panel test, the final subjects included in the study were 43. Among these, the positive results were 14 from cultures (32.5%) and 19 from FA-PE panel tests (44.1%) (**Table 2**). Among these, there were 13 cases with simultaneous positive results in both culture and FA-PE panel tests, while 23 cases showed negative results. Additionally, 8 cases exhibited positive results solely in cases of a single cultured species, and 5 cases with positive results when two species were cultured. The most common bacteria were *Acinetobacter calcoaceticus-baumannii* complex, *S. pneumoniae*, methicillin-resistant *S. aureus* (MRSA), and *K. pneumoniae*, each appearing 3 times. Following these were 2 instances of *P. aeruginosa*, and 1 instance each of *E. coli*, *K. aerogenes*, *S. pyogenes*, and methicillin-susceptible *S. aureus*.

Based on the conventional culture method, the sensitivity of FA-PE was 92.9%, and the specificity was 79.3% (**Table 3**). There was only one case that showed positive results in culture alone, which was *K. pneumoniae* cultured in small quantities. However,

Table 2. Performance of the FilmArray pneumonia panel plus compared to the culture method

Pathogen	Culture (+)	Culture (+)	Culture (-)	Culture (-)
	FA-PE (+)	FA-PE (-)	FA-PE (+)	FA-PE (-)
Gram-positive bacteria				
Staphylococcus aureus	4	0	0	
Streptococcus pyogenes	1	0	0	
Streptococcus pneumoniae	3	0	0	
Enterobacterales				
Escherichia coli	1	0	1	
Klebsiella aerogenes	1	0	0	
Klebsiella pneumonia group	3	1	2	
Non-fermenter				
Acinetobacter calcoaceticus-baumannii complex	3	0	0	
Pseudomonas aeruginosa	2	0	0	
Other gram-negative bacteria				
Haemophilus influenza	0	0	4	
Total	18	1	7	23

Abbreviation: FA-PE, Biofire FilmArray pneumonia panel.

Table 3. Comparison of Biofire FilmArray pneumonia panel and culture in endotracheal aspirate specimens

Pathogen identification	Culture (+)	Culture (-)	Subtotal
FA-PE (+)	13	6	19
FA-PE (-)	1	23	24
Subtotal	14	29	43

Abbreviation: FA-PE, Biofire FilmArray pneumonia panel

in the FA-PE test, it yielded a negative result. There were 6 cases where only the FA-PE test showed positive results: 4 were *H. influenzae*, 1 was *E. coli*/*K. pneumoniae*, and 1 was *K. pneumoniae*. Upon sequencing analysis, only 1 case of *H. influenzae* was confirmed, while for the remaining cases, obtaining sequence results was difficult due to the challenges associated with the quality of respiratory specimen samples.

3.2. Detection of antimicrobial resistance genes

A total of four antimicrobial resistance genes were detected through FA-PE: three *mec*A/*mec*C with SCC*mec* right-extremity junction genes and one CTX-M. All four were MRSA and ESBL as determined by antimicrobial susceptibility testing. The MRSA were all positive for the *mecA* gene by PCR.

4. Discussion

The diagnosis of acute lower respiratory tract infections has traditionally relied on culture and antimicrobial susceptibility testing. However, these tests take at least 48 hours which is relatively long compared to the severity of the condition. Therefore, empirical antibiotic therapy will be administered during this period [9]. In view of this situation, various rapid tests have been developed, but they have rarely been used in clinical practice [5,10]. The FA-PE test, which was approved for use by the Ministry of Food and Drug Safety in 2021, is a molecular diagnostic test that rapidly detects bacteria, viruses, and resistance genes in lower respiratory tract specimens simultaneously, and can identify 33 of the most common pathogens that cause pneumonia in about 1 hour.

In 2019, Lee *et al.* conducted a study comparing the FA-PE test with the conventional culture method on 59 samples and reported positive and negative concordance rates of 90% and 97.4%, respectively [11], and Yu *et al.* reported sensitivity and specificity of 98.5% and 76.5%, respectively in 2020 [12]. In this study, the sensitivity and specificity were found to be 92.9% and 79.3%, respectively, which were similar to the results of Yu *et al.* The duration of the test was around 1 hour on average for FA-PE and 48 hours on average for conventional culture.

Except for one case, when both culture and FA-PE yielded positive results, the bacterial counts were sufficient, ranging from 10^5 to 10^7. In one instance, *S. pneumoniae* was isolated with a bacterial count of 10^4, and in this case, the same bacterium was cultured in a few quantities as well.

In this study, 7 samples exhibited discordant results between the conventional culture method and FA-PE. Among the 7 cases where only FA-PE yielded positive results, 6 cases were identified: 4 cases of *H. influenzae*, and 1 case with simultaneous *E. coli*/*K. pneumoniae* positivity, and 1 case of *K. pneumoniae* positivity. Among these, *H. influenzae* was the most frequent, showing positive results in FA-PE despite not being cultured using the conventional method. This phenomenon could potentially be explained by the inhibitory effect of antimicrobial agents used before sample collection, leading to suppressed growth of *H. influenzae* or the detection of nucleic acids from non-viable bacteria [4]. These findings align with previous

research [12]. It is more suggestive that these results are due to insufficient bacterial counts for culture or issues related to bacterial viability rather than false positives. The remaining 2 cases involved 1 case with simultaneous positive results for *E. coli* and *K. pneumoniae*, and 1 case with *K. pneumoniae* as the only positive result. All cases where only FA-PE showed positive results had bacterial counts of 10^4, indicating that in cases of low bacterial count, cultures might not yield positive results. However, in cases of discordance, the inability to perform analysis due to sample quality issues was evident from the 16S rRNA sequencing test conducted. This limitation was overlooked in the study design and should be considered in future research.

In cases where growth was observed in culture but FA-PE yielded negative results, a specific instance involved *K. pneumoniae* being cultured in small quantities. For this case, a reanalysis was conducted through the manufacturer. The results from the manufacturer indicated that *K. pneumoniae* had shown delayed amplification in both tests and a melting curve was formed as well. However, the manufacturer reported that the quantity of *K. pneumoniae* nucleic acid was below $10^{3.5}$ copies/mL (the detection limit of FA-PE). As a result, despite the presence of amplification and a melting curve, the test results were reported as negative due to the nucleic acid concentration falling below the detection threshold. As mentioned above, when the number of bacteria is low, it may be positive only by FA-PE or culture, so it is believed that FA-PE cannot replace the existing culture test and both tests should be used complementarily.

In this study, all MRSA cases were positive for the *mecA* gene on FA-PE, which showed a 100% concordance rate with culture and antimicrobial susceptibility testing, which is thought to be helpful for rapid treatment of patients. In such cases, a rapid change of chemotherapy from empiric antimicrobial therapy to vancomycin is expected to improve the prognosis of patients.

The FA-PE test cannot completely replace conventional culture and antimicrobial susceptibility testing, as the most important thing in the diagnosis of acute lower respiratory tract infections is information for drug selection. The presence of pathogens or resistance genes not included in the panel should also be considered. Antimicrobial resistance emerges rapidly and mutates rapidly, making it difficult to test for specific genes. This is why molecular biological diagnostics cannot replace traditional culture tests. The problem of detecting dead bacteria, which is a limitation of molecular biological diagnostics, should also be considered. However, the rapidity of the FA-PE test is likely to be of great benefit in the diagnosis of most lower respiratory tract infections, especially in the case of MRSA. Therefore, rapid diagnosis of acute lower respiratory tract infections using FA-PE is expected to reduce unnecessary antimicrobial use and shorten hospital stays.

Based on the above results, it is believed that the FA-PE test can not only provide quick results within 1 hour but also help diagnose pathogens because some cases with low bacterial counts are positive only by FA-PE instead of the culture method. Although the number of cases in this study is small at 43, the outcome can still be considered significant although larger studies would be needed in the future.

Disclosure statement

The author declares no conflict of interest.

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