



# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

# Diagnostic role of FilmArray Pneumonia Panel compared to routine microbiological methods to identify pneumonia pathogens

Maha A. Gad <sup>\*1</sup>, Amani A. El-Kholy <sup>1</sup>, Omnia M.A. Taher <sup>2</sup>

1- Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt.

2- Department of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University.

## ARTICLE INFO

### Article history:

Received 17 November 2022

Received in revised form 16 December 2022

Accepted 18 December 2022

### Keywords:

Lower respiratory tract pathogens  
Pneumonia  
Antimicrobial resistance  
FilmArray Pneumonia Panel  
Pneumonia pathogens

## ABSTRACT

**Background:** Lower respiratory tract infections can be caused by an array of pathogens. Rapid and accurate identification of these organisms is essential for selection of the appropriate antimicrobial regimen. **Objectives:** Identify microbiology of lower respiratory tract samples by the syndromic multiplex BioFire FilmArray Pneumonia Panel (FAPP) and correlate with the findings by routine microbiological methods. **Methods:** This study was conducted on 118 non-repetitive respiratory samples, collected through March 2020 to February 2021 for isolation and identification of respiratory pathogens. **Results:** The FAPP yielded an overall positivity rate of (85/118; 72%). Compared to routine culture; the FAPP were concordantly positive for 37/118 (31.4%) of specimens, and discordant for 39/118 specimens (33%) with 92.5% of positive percentage of agreement. The commonest bacterial pathogens detected by the two methods were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Of note, *Acinetobacter* and other bacterial pathogens were frequently detected by FAPP and not by culture, indicating colonization or contamination (false-positive results). False-negative FAPP results were *Candida* and bacterial pathogens not included in the FAPP panel, as *Morganella*. At the semi-quantification level, the concordance rate was 93.9% as out of forty-nine bacterial cultures with significant growth, 46 showed a higher semi-quantification of more than  $10^5$  copies of DNA/ml by FAPP and (27/46; 58.7%) showed total agreement with target pathogens isolated by the culture too. **Conclusion:** BioFire FilmArray Pneumonia Panel (FAPP) was sensitive and detected more potential pneumonia pathogens than culture methods, but it should be interpreted cautiously. Semi-quantification of FAPP helped to understand pathogen significance and correlate with true pathogen.

## Introduction

Lower respiratory tract infections (LRTI) can be caused by a wide array of bacterial, viral, or fungal pathogens but due to similarities in the clinical presentation and symptomatology, it is not possible to distinguish viral from bacterial infections

without the help of laboratory diagnostic techniques [1].

Rapid and accurate differentiation and identification of these pathogens is central to diagnostic clarity, targeted and timely therapy and implementation of infection control practices to limit transmission. However, current routine

diagnostic methods, as the microbiological culture and antimicrobial susceptibility testing (AST), do not produce results before 48–72 hours. Furthermore, culture methods sometimes fail to detect clinically important pathogens as atypical or fastidious bacteria due to prior empirical antibiotic treatment or stringent growth requirements [2].

Pneumonia guidelines now recommend that antibiotics be initiated as early as possible, based on evidence from several studies [3]. Some studies suggest that half of antibiotic prescriptions for acute respiratory conditions are unnecessary especially with viral infection. Rapid diagnostic testing for pneumonia has the potential to guide clinical decisions and reduce the use of antibiotics [4-6].

Molecular investigations have emerged as a diagnostic tool of choice for respiratory pathogens, particularly viruses due to its good sensitivity in detecting organisms that are difficult to isolate, less viable, or present in only small numbers [7]. However, prediction if the identified microorganisms by a molecular test are true pathogens or colonizing agents or contaminants in lower respiratory tract infection remains a challenge and needs more investigations [8].

Among molecular tests developed for the diagnosis of pneumonia caused by different respiratory pathogens the FilmArray Pneumonia and Pneumonia *plus* Panel (FAPP) which is a new multiplex molecular test for hospital-acquired pneumonia (HAP), which can rapidly within an hour detect 18 bacteria, 9 viruses, and 7 resistance genes (BioFire diagnostics LLC, Salt Lake City, UT, USA). Also, The Unyvero Hospitalised Pneumonia panel (Curetis GmbH, Holzgerlingen, Germany) another FDA approved and CE-marked test [9, 10].

#### **Aim of the study**

The aim of this study was to detect the lower respiratory tract pathogens by FAPP and identify the diagnostic role of the FAPP qualitative and semi- quantitative results, compared to routine microbiological methods.

#### **Materials and methods**

##### **Study design and setting**

This cross- sectional study was conducted at a tertiary care hospital in Egypt between March 2020 to February 2021. During the study period, an appropriate sample size was prospectively collected as we received 130 respiratory samples which

ordered by physician to be tested by FAPP and routine microbiological culture simultaneously. We enrolled a total of 118 non-repeat sputa or endotracheal aspirate (ETA) samples as twelve samples were excluded due to bad specimen quality as they were diluted with saliva. The samples were collected from patients who were admitted to medical or surgical intensive care units and had hospital acquired pneumonia or ventilator associated pneumonia according to U.S Centers for Disease Control and Prevention case definition for pneumonia [11]. No samples were involved from patients with chronic lung diseases as cystic fibrosis or bronchiectasis or from patients who were not willing to provide a lower respiratory tract sample (by either sputum induction or endotracheal aspiration). Also, we didn't involve respiratory samples when physician ordered either FAPP only or routine microbiological culture only from the provided respiratory sample.

##### **Sample collection of lower respiratory tract specimens**

Lower respiratory tract samples were collected from patients with suspected LRTI in intensive care unit (ICU), and immediately transported to the hospital laboratory. Morning samples with deep cough sputum into a sterile, leakproof, screw-cap container was preferred, antiseptic mouthwash was not recommended before obtaining the sample, and induction of cough by warmed aerosol saline was done especially when sputum was difficult to be obtained [12]. Sputa and ETA specimens were evaluated by Gram stain regarding the quality of specimen before culturing and testing by FAPP. Specimens with over 10 epithelial cells per low power field on conventional Gram stain were excluded and rejected [13, 14].

##### **Routine microbiological methods**

An accepted lower respiratory tract sample was processed immediately (within 2 hours) when it delivered to the microbiology lab and evaluated according to standard laboratory procedures to identify respiratory pathogens [15]. Briefly, a direct Gram stain provided us with information about sample quality. 5% sheep blood agar, chocolate agar and MacConkey agar (Oxoid, United Kingdom) were used to inoculate samples with good quality and streak for semi-quantification. All the agar plates were incubated at 37°C for 24 hours. The plates were incubated for another one day before being reported as negative for growth.

As **Webber et al.** respiratory samples were streaked for isolation using the quadrant streak method, examined and interpreted according to standard laboratory procedures. The results were reported semi-quantitatively as the following criteria: rare if 10 colonies or less in the first quadrant; few when greater than 10 colonies in the first quadrant; moderate if greater than 10 colonies and growth into the second quadrant; and heavy growth of colonies in the second quadrant up to growth into the third or fourth quadrant [5, 15].

Pathogens as *Haemophilus influenzae* (*H. influenzae*) and *Streptococcus pneumoniae* (*S. pneumoniae*), were reported only if they were present in “Moderate” to “Heavy” quantities as they are part of respiratory microbiota. Cultures were reported as “upper respiratory flora” if mixed normal respiratory microbiota with absence of a significant respiratory tract pathogen. The culture plates were examined, the isolated pathogens were identified by Vitek2 automated system (BioMérieux, Marcy l’Étoile, France) which also provided us with the susceptibility profile. The antimicrobial susceptibility data were interpreted according to CLSI 30th edition M-100 2020 breakpoints [16].

#### **BioFire FilmArray Pneumonia Panel**

The FAPP (BioFire diagnostics LLC, Salt Lake City, UT, USA) is a syndrome-specific multiplex PCR test with closed, pouch-based system. It required about 200UL of specimen, all steps are performed and provided results within 75 minutes. The panel allows the detection of fifteen typical bacteria, three atypical bacterial pathogens, seven resistance genes (methicillin resistance (*mecA/C* and *MREJ*), carbapenemases (*blaKPC*, *blaNDM*, *blaOXA-48*-like, *blaVIM* and *blaIMP*) and extended-spectrum b-lactamases (ESBLs; *blaCTX-M*) and eight viruses adenovirus, coronavirus (except severe acute respiratory syndrome coronavirus 2), human metapneumovirus, human rhinovirus/enterovirus, influenza viruses A and B, parainfluenza virus and respiratory syncytial virus. Results for the typical bacteria are reported semi-quantitatively, providing estimates of the abundance of bacterial nucleic acids, with bins allowing the detection of approximately  $10^4$ ,  $10^5$ ,  $10^6$ , or  $\geq 10^7$  copies/ml. The results of the antimicrobial resistance genes are reported qualitatively if the potential microorganism of the gene is also detected. We should consider that the detected resistance markers cannot be linked to the detected microorganisms.

The FAPP panel testing method was done in accordance with the manufacturer’s instructions (BioFire diagnostics LLC, Salt Lake City, UT, USA) by transferring about 200 UL of the specimen to the sample injection vial. The sample mixed with the provided sample buffer. Then, this solution was loaded into a specific pouch, which in turn was loaded into the FilmArray instrument where nucleic acid extraction, multiplex PCR and postamplification analysis were performed. Each pouch included two process controls to be sure from the success of all steps before reporting the result.

#### **Data and statistical analysis**

Results from the routine microbiological analyses and FAPP were compared for the detection of typical respiratory bacterial pathogens and antibiotic resistance. The results were considered concordant, when both the FAPP and conventional methods were consistent regarding the detected pathogens. The discordant results were noticed if there were inconsistent between FAPP and the results of conventional methods.

According to **Mitton et al.** specimens that only grew normal respiratory microbiota were considered culture negative. A true positive (TP) result means both methods detected the target organism or resistance mechanism. A true negative (TN) result means neither method detected the target organism nor resistance mechanism. A false positive (FP) result means the FAPP panel detected an organism or resistance mechanism when conventional methods did not. A false negative (FN) result means the FAPP panel did not detect the organism or resistance mechanism while conventional method did [17].

The identification of bacterial targets by the FAPP panel was compared to the routine microbiological culture to calculate positive percentage of agreement (PPA) and negative percentage of agreement (NPA); the PPA was calculated as  $(TP/(TP + FN))$  and NPA as  $(TN/(TN + FP))$ . The positive predictive value (PPV) and the negative predictive value (NPV) were calculated as  $100*(TP/(TP + FP))$  and  $100*(TN/(TN + FN))$  respectively. The antibiotic resistance genes detected by the FAPP panel was compared to phenotypic methods as described above [2].

The FAPP results are expressed as DNA copies/mL, and the culture semi-quantitatively as mentioned above. Bacteria whose FAPP results were  $10^4$  DNA copies/mL, are only documented in the results, but

not compared with culture results, as usually they are not consistent with true pathogens [8, 18, 19].

## Results

### Pathogen detection

Of the 118 enrolled samples, culture and FAPP yielded positive results in 62 (52.5%) and 85 (72%), respectively. By culture, one pathogen was detected in 51 specimens, two pathogens were detected in eleven specimens and eleven samples showed growth of *Candida*.

BioFire FilmArray Pneumonia Panel (FAPP) yielded one pathogen in 55 specimens (10 of them were viral), two pathogens in 19 specimens, three pathogens in 7 specimens, and four or more pathogens in 4 specimens. Of these, (75/85; 88.2%) specimens were positive for typical bacterial targets. Atypical bacterium (*Legionella pneumophila*) was detected in only one sample. For 37 specimens with concordant positive findings, the most frequently detected pathogens were *K. pneumoniae* (15/37; 40.5%), *P. aeruginosa* in 6/37 results (16.2%). **Table 1** demonstrates distribution of detected organisms by FAPP and routine microbiological methods.

Among 118 samples, FAPP identified both viral and bacterial pathogens from 19 samples. The most common viruses detected by FAPP were influenza A (13/118; 11 %), rhinovirus/enterovirus (12/118; 10.2%), and corona (5/118; 4.2%). FAPP identified only viral pathogens in 10 samples, influenza A was the most predominant of them followed by rhinovirus/enterovirus (60% and 50%, respectively). Also, seven samples showed 2 types of viruses.

When we compared the findings of FAPP with routine microbiological culture method; we found that 37 samples' findings were concordantly positive and 39 were concordantly negative with an overall accuracy (76/118; 64.4%) by FAPP among the tested samples. Some false-negative results were obtained with the FAPP. Three pathogens not included in the panel as *Morganella*, *Providencia* and *Achromobacter*. Moreover, *Staph aureus*, and

*P. aeruginosa* were not identified in 3 specimens although covered by the FAPP panel.

On the other side, FAPP identified false positive results in thirty-nine samples: *A. baumannii* in 15, *K. pneumoniae* in 11 and *Staph aureus* in 9 specimens. Fastidious organisms including *S. pneumoniae*, *H. influenza* and *Moraxella* represented (12/39, 30.8%) of the false positive results.

### Concordance between semi-quantification bin results and bacterial culture results

The concordance between FAPP semi-quantification results and positive bacterial culture results are shown in **table (2)**. Among 49 cultures that exhibited significant bacterial counts (heavy and moderate), 41 (83.7%) showed more than  $10^6$  copies/ml and 46 (94%) showed more than  $10^5$  copies/ of bacterial nucleic acids using FAPP. In contrast FAPP identified pathogens in  $10^6$  copies/ml or more while the results were deemed insignificant by culture in 4 samples (28.6%).

**Table 3** shows the correlations between negative culture or culture of normal respiratory microbiota, and FAPP results. Among culture negative samples, FAPP showed no detection in 10/17 (59%), while 3/17 (17.6%) were detected at  $10^6$  copies/ml by FAPP. Among 55 negative and insignificant bacterial cultures, 9 were detected in  $10^6$  copies/ml or more by FAPP. Moreover, culture detected significant growth of *Candida* in 11 samples that are not included in FAPP.

### Detection of antimicrobial resistance genes

The genes detected by FAPP were expressed phenotypically in 47 out of 70 samples (67.1 %). The overall positive agreement is 94.8% and the overall negative agreement is 32.3%. The most frequently detected resistance genes were *CTX-M* in 46 samples and *NDM* genes in 37 specimens followed by *OXA-48* in 27 specimens and *mecA/C* and *MREJ* among 13 specimens.

By FAPP false negative results were obtained in 2 samples. Twenty- one false positive results were obtained by FAPP.

**Table 1.** Bacteria identified using the FilmArray Pneumonia Panel and culture-based methods.

Bacteria isolates	No. of concordant bacteria isolates detected by the two methods*	No. of bacteria detected by FAPP only (false positive)	No. of bacteria detected by culture only (false negative)
<i>A. baumannii complex</i>	4	15	0
<i>E. cloacae</i>	3	2	0
<i>E. coli</i>	4	7	0
<i>H. influenza</i>	2	7	0
<i>K. pneumoniae</i>	15	11	0
<i>K. oxytoca</i>	0	1	0
<i>M. catarrhalis</i>	0	4	0
<i>Proteus spp.</i>	1	1	0
<i>P. aeruginosa</i>	6	9	1
<i>S. marcescens</i>	0	4	0
<i>S. aureus</i>	4	9	2
<i>S. agalactiae</i>	1	2	0
<i>S. pneumoniae</i>	5	7	0
<i>Providentia</i>	0	0	2
<i>Achromatobacter</i>	0	0	1
<i>Morganella</i>	0	0	2
<b><u>Atypical bacteria</u></b>			
<i>C. pneumoniae</i>	0	0	0
<i>L. pneumophila</i>	0	1	0
<i>M. pneumoniae</i>	0	0	0

\*Concordant results as isolates identified by both FAPP and routine microbiological culture method.

**Table 2.** Concordance between FAPP semi-quantification and positive bacterial culture results.

Culture result	FilmArray Pneumonia panel copy/ml					
	Not detected	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	total
<b><u>Significant growth:</u></b>						
Moderate	1	2	3	10	18	34
Heavy	0	0	2	2	11	15
<b>Total significant</b>	1	2	5	12	29	49
<b><u>No Significant growth:</u></b>						
Rare	1	2	1	0	0	4
Few	0	3	3	1	3	10
<b>Total insignificant</b>	1	5	4	1	3	14

The numbers indicate the distribution of samples according to FAPP results for bacterial copies/ml in correlation with culture results.

**Table 3.** Correlation of FAPP semi-quantification and negative bacterial culture results.

Culture result	FilmArray Pneumonia panel copy/ml					
	Not detected	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	total
No growth	10	2	2	3	-	17
Mixed respiratory flora	14	3	4	1	5	27
Significant <i>Candida</i>	7	2	2	-	-	11
<b>Total</b>	31	7	8	4	5	55

The numbers indicate the distribution of samples according to FAPP results for bacterial copies/ml in correlation with culture results.

**Table 4.** Comparison between the FAPP and culture-based methods for detection of antimicrobial resistance.

Resistance by phenotypic method	No. of Sample				PPA	NPA
	FAPP+/CM + /CM -	FAPP+/CM -	FAPP-/CM +	FAPP- /CM -		
<b>MRSA (6)</b>	4	9	2	55	66.7%	85.9%
<b>ESBL Producer (6)</b>	6	8	0	56	100%	87.5%
<b>CR (27)</b>	27	4	0	39	100%	90.7%

FAPP: FilmArray Pneumonia Panel; CM: culture-based methods (culture methods and antimicrobial susceptibility testing); MRSA methicillin-resistant *Staphylococcus aureus*; ESBL: extended-spectrum  $\beta$ -lactamase; CR: carbapenem resistant; PPA: positive percent agreement; NPA: negative percent agreement.

MRSA was detected as *mecA/mecC* and MREJ genes using the FAPP.

ESBL producers were detected as *CTX-M* genes using the FAPP.

CR producers were detected as *blaKPC*, *blaNDM*, *blaOXA-48-like*, *blaVIM* and *blaIMP* genes using the FAPP.

## Discussion

To our knowledge, evaluation of the FAPP in Egypt was done in very limited studies as the recent study conducted by **Kamel et al.** [20]. We used sputa and ETA which are more readily available respiratory samples. In both studies, Gram negative bacteria dominated the pathogens, and viruses showed low prevalence. Our study showed discordance between FAPP and culture methods in detection of bacterial types and antimicrobial resistance.

*Klebsiella pneumoniae*, and *P. aeruginosa* were the most common organisms detected at a prevalence of 40.5%, and 16.2%, respectively by both culture and FAPP. The high prevalence of Gram-negative bacilli is in concordance with the reports from other studies either worldwide [21, 22] or from Egypt [23- 25].

The study revealed that FAPP rapidly and effectively detected a variety of pathogens in the lower respiratory tract specimens with more bacterial targets than the culture method [19, 26, 27]. The overall accuracy of FAPP was 64.4%. The low positive predictive value and low negative percentage of agreement (47.7% and 50% respectively) than other studies could be explained by the using sputa or ETA samples which contain higher bacterial load and diversity of microbiota [19].

Negative predictive value was 92.9% and positive percentage of agreement was 92.5%. These findings are generally in keeping with other studies that evaluated PPAs of the FAPP as 90.0-98.4% [2, 5, 8, 13, 19].

There was high false positive results by FAPP (39/118; 33.1%), which reduced the specificity and PPV of the assay. In accordance, **Faron et al.** who reported high false positive results

(52.9%) [28]. This could be explained by the high sensitivity of the assay compared to culture methods in detection of fastidious organisms, organisms present at low counts, and non-viable genomic material in respiratory specimens [17, 29, 30].

FAPP outperformed the routine culture in detection of fastidious organisms. In seven samples, fastidious pathogens were identified by both methods (6%). FAPP identified fastidious pathogens in (21/118; 17.8%) specimens, of which culture either identified other pathogens among 4 samples of 21 (19%), or revealed normal respiratory microbiota in 10 samples (47.6%). So, the distinction between the actual pathogens and respiratory microbiota is a challenge by FAPP only and correlation with culture results is mandatory.

Viruses are important LRTI pathogens. As ten samples yielded viral aetiology only (8.5%), while 19 (16.1%) samples co-detected virus/bacterial pathogen combinations. This is in concordance with other studies highlighting the role of viruses in LRTI [18, 19, 26, 27]. Results of FAPP should be interpreted in the context of clinical picture, culture results, biomarkers and procalcitonin to differentiate between colonizing and infectious agents [18, 19, 31].

Regarding the using of bin results of FAPP to detect pathogen; we found 49 cultures that exhibited significant growth (heavy and moderate) by routine culture, of which 41 (83.7%) showed more than  $10^6$  copies/ml of bacterial nucleic acids using FAPP as mentioned in **table (2)**. Pathogens missed by culture could be explained by their low counts or poor growth in routine culture, or false high quantitation by FAPP [8, 13, 18, 30].

FAPP identified the resistance genes with relatively good correlation with the culture-based antibiotic susceptibility in 67.1% of tested samples. The overall positive and negative percent agreement was 94.9% and 32.3%, respectively. So, it is important to obtain full susceptibilities of the identified target pathogens by culture and discriminate from nonviable, colonizers or normal respiratory microbiota.

Twenty- one samples showed discordance between antibiotic resistance genes identified by FAPP and negative by culture-based method. Among the samples with false positive resistance genes, mixed respiratory microbiota were detected in (14/21; 66.7%). In the remaining seven samples, the resistance genes could be genomic material of nonviable bacteria, or from bacteria in too low

numbers to grow in culture. Similar results were reported by **Gastli et al.** as Genes of *blaCTX-M*, and *mecA/C* and MREJ were detected as discordant results with routine antimicrobial susceptibility testing [2]. It was not unexpected to detect also false negative resistant results as MRSA; study of **Buchan et al.** observed similar findings due to divergent sequences within the MREJ region targeted by the FAPP [19].

### Limitation of the study

Limitations of this study include the lack of a “gold-standard” reference method to clarify the discrepant results between FAPP and conventional culture method. We used PPA and NPA to facilitate the findings comparison by both techniques. FAPP performance regarding virus detection could not be analyzed as no comparable methods in our laboratory were available. The presence of one of the resistance genes which included in the panel of FAPP not synonymous with resistance to the corresponding antibiotic and vice versa; so it is important to obtain full susceptibilities of the identified target pathogens by culture and discriminate from colonizers or normal respiratory microbiota.

### Conclusion

This study had the strength of evaluating the FAPP panel in a real world setting with good quality actual patients' respiratory specimens. FAPP has an advantage over conventional culture methods which is a significant improvement in the turnaround time. This will reflect on effective implications for antimicrobial stewardship and infection prevention and control. It is one of the syndromic panels that have been embraced by clinicians due to the broad number of targets, many of which not easy to be done routinely. But its implementation is not without challenges as the fastidious pathogens could represent colonization as opposed to infection. Also, it is difficult to link antibiotic resistance genes to a specific organism. Therefore, the FAPP results need to be used in conjunction with clinical correlation and culture results.

### Declarations and statements

#### Ethics statement

The study was approved by the Medical Research Ethics Committee, Faculty of medicine, Cairo University (N61-18). The specimens used in the study were part of the routine patient laboratory testing, without any additional sampling. We used

anonymized data to compare test results. No informed consent was needed.

#### Competing interests

Authors declare that they have no competing interests.

**Funding:** Not applicable.

#### References

- 1-Trotter AJ, Aydin A, Strinden MJ, O'grady J. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. *Curr Opin Microbiol* 2019;51:39–45.
- 2-Gastli N, Loubinoux J, Daragon M, Lavigne JP, Saint-Sardos P, Pailhoriès H, et al. French FA-PP study group. Multicentric evaluation of BioFire FilmArray Pneumonia Panel for rapid bacteriological documentation of pneumonia. *Clin Microbiol Infect* 2020; 1:S1198-743X(20)30710-2.
- 3-Kao CC, Chiang HT, Chen CY, Hung CT, Chen YC, Su LH, et al. National bundle care program implementation to reduce ventilator-associated pneumonia in intensive care units in Taiwan. *J Microbiol Immunol Infect* 2019; 52:592–597.
- 4-Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am J Respir Crit Care Med* 2019; 200(7):e45-e67.
- 5-Webber DM, Wallace MA, Burnham CA, Anderson NW. Evaluation of the BioFire FilmArray Pneumonia Panel for detection of viral and bacterial pathogens in lower respiratory tract specimens in the setting of a tertiary care academic medical center. *J Clin Microbiol* 2020; 58(7):e00343-20.
- 6-Novello S, Huang DB. The basics and the advancements in diagnosis of bacterial lower respiratory tract infections. *Diagnostics (Basel)* 2019;9(2):37.
- 7-Bhat N, O'Brien KL, Karron RA, Driscoll AJ, Murdoch DR. Use and evaluation of molecular diagnostics for pneumonia etiology studies. *Clin Infect Dis* 2012;54(Suppl 2):S153e8
- 8-Lee SH, Ruan SY, Pan SC, Lee TF, Chien JY, Hsueh PR. 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(6):920-928.
- 9-Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. *Clin Microbiol Rev* 2017;31(1):e00024-17.
- 10-Poole S, Clark TW. Rapid syndromic molecular testing in pneumonia: the current landscape and future potential. *J Infect* 2020;80(1):1-7.
- 11-Centers for Disease Control and Prevention. Pneumonia (Ventilator-associated [VAP] and non-ventilator associated Pneumonia [PNEU]) Event: Available at: [https://www.cdc.gov/nhsn/pdfs/pscmanual/6psc\\_vapcurrent.pdf](https://www.cdc.gov/nhsn/pdfs/pscmanual/6psc_vapcurrent.pdf).
- 12-Tille, Patricia M., Bailey & Scott's Diagnostic Microbiology. St. Louis, Missouri .Elsevier, 2014.
- 13-Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFire FilmArray Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal



- aspirate specimens. *Int J Infect Dis* 2020; 95:326-331.
- 14-**Rosón B, Carratalà J, Verdaguier R, Dorca J, Manresa F, Gudiol F.** Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. *Clin Infect Dis* 2000; 31(4):869-874.
- 15-**Leber AL.** Respiratory Tract Cultures. In *Clinical Microbiology Procedures Handbook*; ASM Press: Washington, DC, USA, 2016. Available at: <https://www.worldcat.org/title/clinical-microbiology-procedures-handbook/oclc/943710632>
- 16-**Weinstein MP, Limbago B, Patel JB, Mathers AJ, Campeau S, Mazzulli T, et al.** M100 Performance Standards for Antimicrobial Susceptibility Testing. CLSI 2020. Wayne, PA.
- 17-**Mitton B, Rule R, Said M.** Laboratory evaluation of the BioFire FilmArray Pneumonia plus panel compared to conventional methods for the identification of bacteria in lower respiratory tract specimens: a prospective cross-sectional study from South Africa. *Diagn Microbiol Infect Dis* 2021;99(2):115236.
- 18-**Murphy CN, Fowler R, Balada-Llasat JM, Carroll A, Stone H, Akerele O, et al.** Multicenter evaluation of the BioFire FilmArray Pneumonia/Pneumonia Plus Panel for detection and quantification of agents of lower respiratory tract infection. *J Clin Microbiol* 2020;58(7):e00128-20.
- 19-**Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, 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:e00135-20.
- 20-**Kamel NA, Alshahrani MY, Aboshanab KM, El Borhamy MI.** Evaluation of the BioFire FilmArray Pneumonia Panel Plus to the conventional diagnostic methods in determining the microbiological etiology of hospital-acquired pneumonia. *Biology (Basel)* 2022;11(3):377.
- 21-**Jones RN.** Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator associated bacterial pneumonia. *Clin Infect Dis* 2010;51(Suppl1): S81–87.
- 22-**Herkel T, Uvizl R, Doubravska L, Adamus M, Gabrhelik T, Htoutou Sedlakova M, et al.** Epidemiology of hospital-acquired pneumonia: results of a central european multicenter, prospective, observational study compared with data from the european region. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2016;160(3):448-455.
- 23-**Farag AM, Tawfick MM, Abozeed MY, Shaban EA, Abo-Shadi MA.** Microbiological profile of ventilator-associated pneumonia among intensive care unit patients in tertiary Egyptian hospitals. *J Infect Dev Ctries* 2020;14(2):153-161.
- 24-**Galal YS, Youssef MR, Ibrahim SK.** Ventilator-associated pneumonia: incidence, risk factors and outcome in pediatric Intensive Care Units at Cairo University Hospital. *J Clin Diagn Res* 2016;10(6):SC06-SC11.
- 25-**See I, Lessa FC, ElAta OA, Hafez S, Samy K, El-Kholy A, et al.** Incidence and pathogen distribution of healthcare-associated infections in pilot hospitals in Egypt. *Infect Control Hosp Epidemiol* 2013;34(12):1281-1288.
- 26-**Kosai K, Akamatsu N, Ota K, Mitsumoto-Kaseida F, Sakamoto K, Hasegawa H, et al.** BioFire FilmArray Pneumonia Panel enhances

- detection of pathogens and antimicrobial resistance in lower respiratory tract specimens. *Ann Clin Microbiol Antimicrob* 2022;21(1):24.
- 27-**Rand KH, Beal SG, Cherabuddi K, Couturier B, Lingenfelter B, Rindlisbacher C, et al.** Performance of a semiquantitative multiplex bacterial and viral PCR panel compared with standard microbiological laboratory results: 396 patients studied with the BioFire Pneumonia Panel. *Open Forum Infect Dis* 2020;8(1):ofaa560.
- 28-**Faron ML, Mahmutoglu D, Huang A, Balada-Llasat JM, Relich RF, Humphries R, et al.** Clinical evaluation of a semi-quantitative multiplex molecular assay for the identification of bacteria, viruses and fungi in lower respiratory specimens. In: *Proceedings of the European Society of Clinical Microbiology and Infectious Diseases Meeting 2017*. Basel, Switzerland.
- 29-**Driscoll AJ, Deloria Knoll M, Hammitt LL, Baggett HC, Brooks WA, Feikin DR, et al.** PERCH Study Group. The effect of antibiotic exposure and specimen volume on the detection of bacterial pathogens in children with pneumonia. *Clin Infect Dis* 2017; 64(Suppl 3):S368 –S377.
- 30-**Rand KH, Beal SG, Rivera K, Allen B, Payton T, Lipori GP.** Hourly effect of pretreatment with IV antibiotics on blood culture positivity rate in emergency department patients. *Open Forum Infect Dis* 2019;6(5):ofz179.
- 31-**Gilbert DN, Leggett JE, Wang L, Ferdosian S, Gelfer GD, Johnston ML, et al.** Enhanced detection of community-acquired pneumonia pathogens with the BioFire Pneumonia FilmArray Panel. *Diagn Microbiol Infect Dis* 2021;99(3):115246.