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EVALUATION OF BIOFIRE FILMARRAY PNEUMONIA PANEL IN DIAGNOSIS OF PNEUMONIA IN PEDIATRIC PATIENTS IN INTENSIVE CARE UNIT IN COMPARISON TO VITEK 2 COMPACT SYSTEM AND ROUTINE CULTURE METHODS

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Lower respiratory tract infections can result from a variety of pathogens. The expeditious and precise identification of these microorganisms is crucial for determining the most suitable antimicrobial regimen. This study tried to identify microbiological causes of hospital-acquired and ventilator-associated pneumonia by the syndromic multiplex PCR "BioFire FilmArray Pneumonia Plus panel (FA-PP)" and to correlate the results with the findings obtained by routine culture methods. This study was conducted on 72 bronchoalveolar lavage (BAL) samples. The result revealed that bacterial and viral infections were common causes of nosocomial pneumonia among pediatric patients in the intensive care unit, the most commonly detected bacteria was Klebsiella pneumoniae, while the most commonly detected virus was rhinovirus. A high percentage of antibiotic resistance was reported, the most prevalent resistant genes in our study were CTX-M and NDM genes. There was substantial significant agreement between the two methods in the detection of bacteria and antibiotic resistance. FilmArray Pneumonia Plus panel presents a rapid and sensitive diagnostic approach for lower respiratory tract infections, it is recommended to establish clinical correlation for a comprehensive understanding of its significance, particularly in the interpretation of multiple pathogens and the detection of genes associated with antimicrobial resistance

Keywords: *Biofire, FilmArray, Pneumonia Panel, Pneumonia -Pediatric*

INTRODUCTION

Hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP), constitute a significant global public health challenge. Particularly in low- to middle-income countries, pneumonia stands as the foremost cause of morbidity and mortality among pediatric populations[1].

In severe conditions, particularly for patients admitted to intensive care units (ICU), the prompt identification of microbial agents causing hospital-acquired pneumonia (HAP) or ventilator-associated pneumonia (VAP) is imperative for initiating a tailored and appropriate antibiotic therapy[1].

Throughout history, the predominant laboratory diagnostic approach for lower respiratory tract infections has been the quantitative and qualitative bacterial cultures, these methods exhibit variability in recovering potential pathogens, this variability is attributed to factors such as prior antibiotic exposure, the fastidious growth characteristics of certain pathogens, or the proliferation of resident flora. Consequently, the sensitivity of cultures fluctuates, and the turnaround times extend to 48 hours or more. Additionally, the identification of atypical bacteria or viral pathogens necessitates supplementary specific culture or molecular tests, which clinicians may not routinely order. Collectively, these

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limitations undermine the efficacy of current standard-of-care methods[2].

Molecular diagnostics, including PCR-based assays, yield highly sensitive results within a few hours of specimen acquisition. These tests possess the capacity to abbreviate the duration of empirically administered broad-spectrum antibiotic therapy by expediently identifying pathogenic organisms or antibiotic resistance markers[3].

The FilmArray Pneumonia panel is an in vitro diagnostic test based on polymerase chain reaction (PCR) technology designed for sample-to-answer analysis. This test assesses untreated sputum, endotracheal aspirates, and bronchoalveolar lavage (BAL) specimens for the presence of bacteria, viruses, and genetic markers associated with antimicrobial resistance. The analysis is completed in approximately 75 min, requiring only 5 minutes of hands-on time[4].

The aim of this study was to detect pathogens causing pneumonia among pediatrics in ICU, as there was insufficient data about this age group in *Upper Egypt*, and to evaluate analytical performances of the FA-PP for the detection of bacteria and resistance genes by comparison with findings obtained by routine culture methods.

PATIENTS AND METHODS

Ethical statement

Informed consent was obtained from the patient's relatives. The study was conducted under the tenets of the Declaration of Helsinki and with approval from the Ethical Committee of the Faculty of Medicine (IRB no: 17200425), Assiut University, Egypt.

Study settings

This hospital-based descriptive cross-section study was performed at the microbiology unit of the clinical pathology department of Assiut University hospitals. This study was conducted on patients admitted to the pediatric ICU in Assiut University Pediatric Hospital from March 2021 to March 2022.

Selection criteria

Bronchoalveolar Lavage (BAL) samples were collected from pediatric patients (<18 years old) who were suspected to have hospital-acquired pneumonia (HAP) or ventilator-associated pneumonia (VAP) based on following clinical and laboratory data:

- Patients admitted to pediatric ICU or received mechanical ventilation for more than 48 hrs.
- A new or progressive lung lesion by chest radiography.
- Fever (>38 ◦C).
- Leukopenia or leukocytosis and elevated and C-reactive protein.
- New-onset purulent sputum increased respiratory secretions or a worsening gas-exchange profile.

For all patients, clinical data were collected and routine laboratory investigations were performed in the form of complete blood count and C-reactive protein.

Microbiological examination

Sample collection and processing

- **Bronchoalveolar lavage (BAL)-like specimens**

BAL and mini-BAL were collected according to the Standard Operating Protocols (SOPs) for sample collection. Endotracheal aspirates and sputum were excluded from the analysis.

Routine conventional methods

a) Microscopic examination

Gram stain was done to all samples to assess the presence of bacteria, their morphology, single or multiple populations to guide through culture, Ziehl-Neelsen stain was done to exclude the presence of acid-fast bacilli[5].

b) Routine culture method

BAL specimens were subjected to culture examination, by streak plate technique, of blood agar, chocolate agar, MacConkey agar, and sabouraud dextrose agar using a 10-µL calibrated loop, all the agar plates were incubated at 37**°C** for 24 hrs, the plates were incubated for another day before being reported as negative for growth[5].

c) Identification of isolated pathogens by VITEK 2 compact system

The isolated pathogens were identified by VITEK 2 automated system (BioMérieux)

which also provided the antimicrobial susceptibility profile[5].

Molecular method

The BioFire®FilmArray® 2.0 using Pneumonia Plus panel (FA-PP, BioMérieux) was utilized in our institution for expedited molecular diagnosis of lower respiratory tract infections.

The Biofire®Filmarray® Pneumonia Plus panel, developed by Biomérieux, is a recently introduced diagnostic panel designed for Lower Respiratory Tract Infections (LRTI). It focuses on detecting 18 bacterial pathogens, 9 viruses, and 7 antibiotic resistance genes; methicillin resistance genes (*mecA/C and MREJ*), carbapenemases genes (*blaKPC, blaNDM, blaOXA-48-like, blaVIM, and blaIMP*) and extended-spectrum b-lactamases gene (*blaCTXM*). The panel delivers qualitative results "detected" or "not detected" for viral and atypical pneumonia-associated bacterial targets, as well as antibiotic resistance markers. Additionally, it provides semi-quantitative values for 15 bacterial targets[3].

Statistical analysis

Data analysis was performed using a statistical package for the social science (IBM-SPSS) version 26.0 software. Categorical data were represented in terms of frequencies and percentages. The Chi-square test was used to compare proportion between different groups. The degree of agreement is measured by Cohen's kappa (k) between routine culture methods with vitek2, and Biofire FilmArray Pneumonia Plus panel in the diagnosis of pneumonia among pediatric patients in the intensive care unit. Positive percent agreement, negative percent agreement, and accuracy of Biofire FilmArray Pneumonia Panel were calculated in comparison to culture methods, P value considered significant when <0.05.

RESULTS AND DISCUSSION

Results

Characteristics of the studied population (Table. 1)

Total BAL samples were 72, the median age of patients was 9 months and ranged from 1 month to 13 years, male patients were 42 (58%) and female patients were 30 (41%), out of the 72 patients 57 were mechanically ventilated, most cases were in Autumn (38.9%) then in Summer (27.8%), most cases admitted to ICU with GIT disorder (38.9%) mostly due to severe gastroenteritis or admitted with a neurological disorder (26.4%). CRP and WBCs were done as a part of routine lab investigations, median of CRP for patients was 20 and ranged from 0.4 to 210, while the median of WBCs was 16 and ranged from 4.0 to 35.

Table .1: Characteristics of studied pediatric patients in intensive care unit and clinical diagnosis on admission.

Variables	**N=72**	**%**
Age in years		
▪ Median (range)	**9 months (1 month-13-years)**	
Gender		
▪ Male	42	58.3%
▪ Female	30	41.7%
Season of infection		
▪ Autumn	28	38.9%
▪ Spring	18	25.0%
▪ Summer	20	27.8%
▪ Winter	6	8.3%
Mechanical ventilation		
▪ Ventilated	57	79.2%
▪ Non ventilated	15	20.8%
Diagnosis on admission		
GIT diseases	28	38.9%
Neurological diseases	19	26.4%
Renal diseases	10	13.9%
CVS diseases	8	11.1%
Respiratory diseases	7	9.7%
Investigation		
CRP		
▪ Median (range)	**20.0 (0.4-210.0)**	
WBCs		
▪ Median (range)	**16.0 (4.0-35.0)**	

Pathogens causing lower respiratory tract infection among studied patients identified by VITEK 2 and FilmArray Pneumonia Plus panel (FA-PP) (Table. 2)

By VITEK 2

Bacteria were detected in 46 samples; the most frequently detected bacteria were *Klebsiella pneumoniae* (36.1%), *Acinetobacter complex* (18.1%), and *Escherichia coli* (11%), other bacteria that detected less frequently were *Pseudomonas aeruginosa* (5.6%), *Streptococcus pneumoniae* (2.8%),

Staphylococcus aureus (1.4%), and *Enterobacter cloacae* (1.4%).

Fungi (Yeast) were detected in 29 samples, Candida spp. were detected in 28 samples; *Candida albicans* were the most frequently detected (15.3%) then *Candida tropicalis* (6.9%), other candida that detected less frequently were *Candida famata* (5.6%), *Candida ciferrii* (4.2%), *Candida lusitaniae* (2.8%), *Candida parapsilosis* (2.8%), and *Candida krusei* (1.4%) while *Cryptococcus laurentii* was detected in one sample (1.4%).

Table. 2 : Pathogens causing lower respiratory tract infection among studied patients.

Organisms	**Biofire(n=72)**	**Vitek (n=72)**
Typical bacteria	**56 (77.8%)**	**46 (63.9%)**
▪ *Klebsiella pneumoniae*	30 (41.7%)	26 (36.1%)
▪ *Acinetobacter*	21 (29.2%)	13 (18.1%)
▪ *Escherichia coli*	18 (25.0%)	11 (15.3%)
▪ *Pseudomonas aeruginosa*	13 (18.1%)	4 (5.6%)
▪ *Staphylococcus aureus*	8 (11.1%)	1 (1.4%)
▪ *Enterobacter cloacae*	4 (5.6%)	1 (1.4%)
▪ *Streptococcus pneumoniae*	4 (5.6%)	2 (2.8%)
▪ *Serratia marcescens*	1 (1.4%)	0 (0.0%)
▪ *Haemophilus influenzae*	10 (13.9%)	
▪ *Moraxella catarrhalis*	2 (2.8%)	
Atypical bacteria	**3 (4.2%)**	
▪ *Mycoplasma pneumoniae*	3 (4.2%)	
Viruses	**51 (70.8%)**	
▪ Rhinovirus	37 (51.4%)	
▪ Adenovirus	14 (19.4%)	
▪ HMP virus	5 (6.9%)	
▪ RSV	5 (6.9%)	
▪ Coronavirus	4 (5.6%)	
▪ Para influenza virus	3 (4.2%)	
▪ Influenza A virus	1 (1.4%)	
▪ MERS-cov	1 (1.4%)	
Fungi		**29 (40.3%)**
▪ *Candida spp.*		28 (38.9%)
▪ *Candida albicans*		11 (15.3%)
▪ *Candida tropicalis*		5 (6.9%)
▪ *Candida famata*		4 (5.6%)
▪ *Candida ciferrii*		3 (4.2%)
▪ *Candida lusitaniae*		2 (2.8%)
▪ *Candida parapsilosis*		2 (2.8%)
▪ *Candida krusei*		1 (1.4%)
▪ *Cryptococcus laurentii*		1 (1.4%)

By FA-PP; Typical bacteria were detected in 56 samples; the most frequently detected bacteria were *Klebsiella pneumoniae* (41.7%), *Acinetobacter calcoaceticus-baumannii* (29.2%), *Escherichia coli* (25%), and *Pseudomonas aeruginosa* (18.1%), other bacteria that detected less frequently were *Haemophilus influenzae* (13.9%), *Staphylococcus aureus* (11.1%), *Enterobacter cloacae* (5.6%), *Streptococcus pneumoniae* (5.6%), *Moraxella catarrhalis* (2.8%), and *Serratia marcescens* (1.4%). Regarding atypical bacteria; *Mycoplasma pneumoniae was detected in 3 patients* (4.2%).

Viruses were detected in 51 samples, the most frequently detected viruses were rhinovirus (51.4%), adenovirus (19.4%), other viruses that detected less frequently were human metapneumo virus (6.9%), respiratory syncytial virus (6.9%), coronavirus (5.6%), parainfluenza virus (4.2%), influenza A virus (1.4%), and middle east respiratory syndrome coronavirus (1.4%).

Distribution of detected respiratory pathogens by routine culture methods and Biofire FilmArray Pneumonia Panel among studied patients: (Fig.. 1**)**

Routine culture methods yielded an overall positivity rate of 76.4%. There were no detected pathogens (bacterial or fungal) in 17 (23.6%) cases (Negative), only one type of pathogen (bacterial or fungal) was detected in 35 (48.6%) cases, poly microbial infections (bacterial and fungal) were detected in 20 (27.8%) cases.

Regarding bacterial infection; there were no detected bacterial pathogens in 26 (36.1%) cases, a single bacterial pathogen was detected in 34 (47.2%) cases, two bacterial pathogens were detected in 12 (16.7%) cases and there were no cases with more than two detected bacterial pathogens. Fungal infection with a single fungus; was detected in 29 (40.3%) cases.

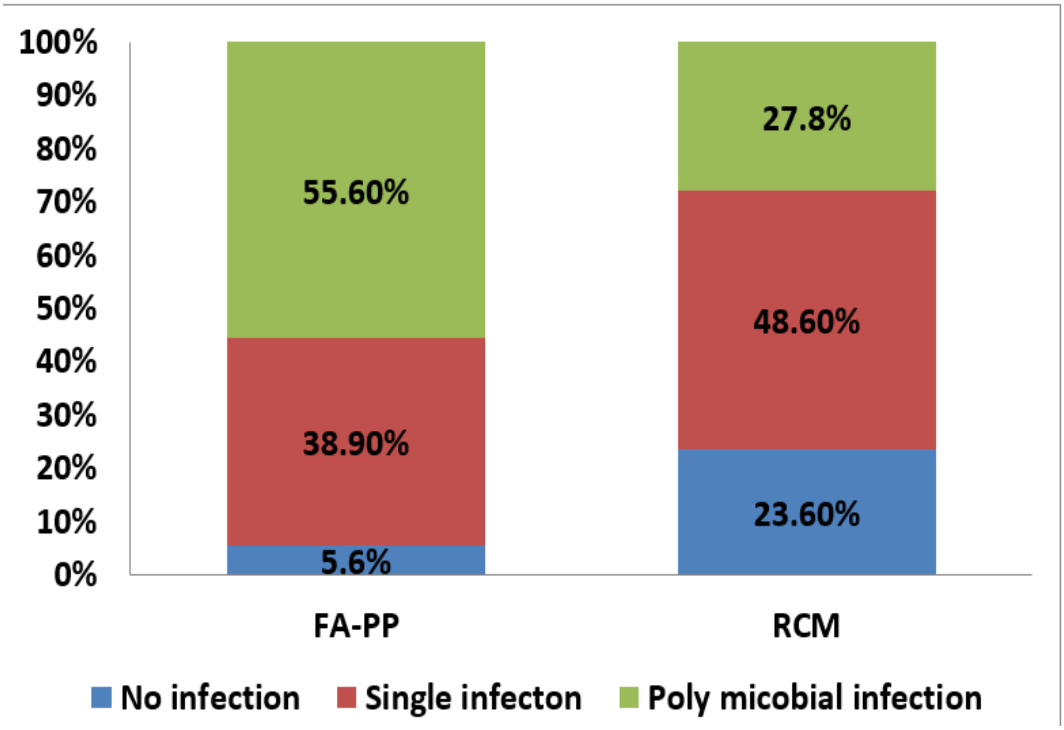

Fig. 1: Distribution of respiratory pathogens by Biofire Filmarray pneumonia panel and Routine culture method.

Biofire FilmArray Pneumonia Plus panel yielded an overall positivity rate of 94.4%. There were no detected pathogens (bacterial or viral) in 4 (5.6%) cases (Negative), only one type of pathogens (bacterial or viral) detected in 28 (38.9%) cases, poly microbial infections (bacterial and viral) were detected in 40 (55.6%) cases.

Regarding bacterial infection; there were no detected bacterial pathogens in 15 (20.8%) cases, a single bacterial pathogen was detected in 20 (27.8%) cases, two bacterial pathogens were detected in 22 (30.6%) cases, and more than two bacterial pathogens were detected in 15 (20.8%) cases. Regarding viral infections; there were no detected viruses in 21 (29.2%) cases, a single virus was detected in 35 (48.6%) cases, and more than two viruses were detected in 16 (22.2%) cases.

Performance of the FilmArray Pneumonia Plus panel (FA-PP) in the detection of bacterial targets

The performance of the FA-PP in the detection of bacterial targets was evaluated using the routine culture method and VITEK-2 compact system as a gold standard method.

There was **significant substantial agreement** between Biofire and Vitek2 in the diagnosis of *Klebsiella Pneumoniae*, *Acinetobacter*, E. coli, and *Streptococcus pneumoniae.* However, there was **significant moderate agreement** between the two methods in the detection of *pseudomonas aeruginosa*, and **significant fair agreement** in the detection of *Enterobacter cloacae and Staphylococcus aureus*. Positive percent agreement between the two methods ranged from 92.0% to 100.0%, Negative percent agreement ranged from 86.4% to 97.1% and the accuracy ranged from 87.5% to 97.2% **(Table. 3)**.

Totally, there was substantial significant agreement between the two methods, the FA-PP showed an overall PPA of 96.5%, NPA of 90.6%, and 91.3% accuracy in the detection of bacteria.

Table.3: Performance of the FilmArray pneumonia plus panel (FA-PP) in detection of bacterial target.

Bacterial target	No of specimens				performance				Agreement
	RCM+/ FA-PP+	RCM+/ FA-PP-	RCM-/ FA-PP+	RCM-/ FA-PP-	PPA	NPA	Accuracy	Cohen's kappa coefficient	P-Value
Klebsiella Pneumoniae	24	2	6	40	92.3%	87.0%	88.9%	0.767	**<0.001**
Acinetobacter	13	0	8	51	100.0%	86.4%	88.9%	0.697	**<0.001**
Escherichia coli	11	0	7	54	100.0%	88.5%	90.3%	0.702	**<0.001**
Pseudomonas aeruginosa	4	0	9	59	100.0%	86.8%	87.5%	0.421	**<0.001**
Streptococcus pneumoniae	2	0	2	68	100.0%	97.1%	97.2%	0.654	**<0.001**
Enterobacter cloacae	1	0	3	68	100.0%	95.8%	95.8%	0.386	**<0.001**
Staphylococcus aureus	1	0	7	64	100.0%	90.1%	90.3%	0.203	**0.004**
Total organism	**56**	**2**	**42**	**404**	**96.5%**	**90.6%**	**91.3%**	**0.670**	**<0.001**

RCM, routine conventional methods; FA-PP, FilmArray Pneumonia panel.,PPA, positive percent agreement; NPA, negative percent agreement.

levels of agreement

- Kappa < 0: No agreement
- Kappa between 0.00 and 0.20: Slight agreement
- Kappa between 0.21 and 0.40: Fair agreement
- Kappa between 0.41 and 0.60: Moderate agreement
- Kappa between 0.61 and 0.80: Substantial agreement
- Kappa between 0.81 and 1.00: Almost perfect agreement

Semi-quantitative values of bacteria measured by FilmArray Pneumonia Plus panel in culture positive and culture negative samples

The BioFire Filmarray Pneumonia Panel offers a semi-quantitative bin result, indicating the presence of specific bacterial genomes in the specimen at varying levels ($10^{\wedge}4$ copies/mL, $10^{\wedge}5$ copies/mL, $10^{\wedge}6$ copies/mL, or $\geq 10^{\wedge}7$ copies/mL). For assays with a value less than $10^{\wedge}3.5$ copies/mL, the result is considered negative.

Comparison between the two methods was applied to bacteria detected commonly by routine culture methods e.g. (*Serratia, Haemophilus influenzae,* and *Moraxella*) not included in the comparison. The FA-PP has detected 51 bacterial targets of $\geq 10^7$copies/mL, out of these targets were 39 bacteria detected by the culture method (positive cultures), additionally, the FA-PP has detected 24 bacterial targets of 10^6 copies/mL, 16 of them were detected by culture **(Table. 4).**

On the other hand, the FA-PP has detected 16 bacterial targets of 10^5 copies/mL, and only one of them was detected in the culture, while there were 7 bacterial targets of 10^4 copies/mL detected by the FA-PP, all of them were not detected in the culture (negative cultures) **(Table. 4).**

Antibiotic resistance genes detected by FilmArray pneumonia panel

A total of 138 antibiotic resistance genes were detected by FA-PP. The most frequently detected genes were carbapenemase genes (62.3%), and the most detected carbapenemase gene was *NDM* (26 %). *CTX-M* gene which codes for ESBL resistance was frequently detected (34.8%) bacteria. *MecA/C-MREJ* genes which code for MRSA resistance were the least detected (2.9%) **(Table. 5)**

NB; Out of 8 *Staphylococcus aureus* targets detected by FA-PP there were 4(50%) bacteria have the *MecA/C-MREJ* resistance genes.

Performance of the FilmArray pneumonia panel (FA-PP) in the detection of antibiotic resistance

The performance of the FA-PP in the detection of antibiotic resistance was evaluated using the minimum inhibitory concentration (MIC) provided by VITEK-2 compact system.

The antibiotic resistance pattern was detected using vitek2 MIC breakpoints, Gram-negative bacteria that showed resistance to any of the tested carbapenems (meropenem, imipenem, and ertapenem) by VITEK-2 recorded as carbapenems resistance, Gram-negative isolates that showed resistance to 3rd-generation cephalosporins (ceftriaxone, cefotaxime, and ceftazidime) or the ESBL test was positive by VITEK-2 recorded as ESBL resistance. *Staphylococcus aureus* bacteria that showed positive cefoxitin screen test by VITEK-2 recorded as MRSA.

There was **substantial significant agreement** in the detection of carbapenems resistance and ESBL resistance between the FA-PP and VITEK-2 methods the Kappa coefficient values were 0.608 and 0.640 respectively with p-value < 0.001, regarding the detection of MRSA there was a **substantial fair agreement** between the two methods, the Kappa coefficient value was 0.386 with p-value < 0.001. Positive percent agreement between the two methods ranged from 94.6% to 100.0%, Negative percent agreement ranged from 65.7% to 95.8% and the accuracy ranged from 80.6% to 95.8 % **(Table. 6).**

Totally, there was a substantial significant agreement between the two methods, and the FA-PP showed overall PPA of 92.7%, NPA of 82.8%, and 86.6% accuracy in the detection of antibiotic resistance.

Table 4: Semi quantitative values of bacteria measured by FilmArray pneumonia plus panel in culture positive and culture negative samples.

FA-PP panel(copies/mL)	FA-PP Total Positive Targets	Culture method	
		Positive	Negative
▪ $\geq 10^7$	**51**	39	12
▪ 10^6	**24**	16	8
▪ 10^5	**16**	1	15
▪ 10^4	**7**	0	7
Total	**98**	**56**	**42**

Table 5 : Antibiotic resistance genes detected by FilmArray pneumonia plus panel.

	Total detected gene n=138	%
Carbapenemase genes	**86**	**62.3%**
NDM	36	26.1%
VIM	23	16.7%
OXA-48	19	13.8%
KPC	7	5.1%
IMP	1	0.7%
ESBL gene	**48**	**34.8%**
CTX	48	34.8%
MRSA genes	**4**	**2.9%**
MecA/C* and *MREJ	4	2.9%

Table 6 : Performance of the FilmArray pneumonia plus panel in detection of antibiotic resistance.

Pattern of resistance	No.of specimens				Performance			Agreement	
	RCM+/ FA-PP+	RCM+/ FA-PP-	RCM-/ FA-PP+	RCM-/ FA-PP-	PPA	NPA	Accuracy	Cohen's kappa coefficient	P-Value
	35	2	12	23	94.6%	65.7%	80.6%	0.608	**<0.001**
	40	4	8	20	90.9%	71.4%	83.3%	0.640	**<0.001**
	1	0	3	68	100.0%	95.8%	95.8%	0.386	**<0.001**
	76	**6**	**23**	**111**	**92.7**	**82.8**	**86.6**	**0.726**	**<0.001**

RCM, routine conventional methods; FA-PP, Film Array Pneumonia panel.PPA, positive percent agreement; NPA, negative percent agreement.

Discussion

In the context of Hospital-Acquired Pneumonia (HAP) and Ventilator-Associated Pneumonia (VAP), empirical therapeutic approaches frequently involve the administration of broad-spectrum antibiotics targeting both Gram-positive and Gram-negative bacterial strains. This is necessitated by the potential susceptibility to infections caused by multidrug-resistant pathogens. However, it is noteworthy that the utilization of broad-spectrum antimicrobial therapy has been recognized as a risk factor, contributing to elevated mortality rates and heightened complications in affected individuals[6].

Conventional diagnostic approaches for Lower Respiratory Tract Infections (LRTI) presently exhibit limitations in terms of both speed and sensitivity, thereby impeding timely clinical decision-making regarding the selection of antimicrobial therapy. This is primarily attributable to the prolonged duration required for microbiological culture and antimicrobial susceptibility testing (AST), with results often becoming available only after 48–72 hrs. Moreover, these culture methods sometimes fail to detect clinically significant

pathogens, particularly atypical or fastidious bacteria, owing to factors such as prior empirical antibiotic treatment or stringent growth requirements[7].

The potential of rapid molecular testing lies in its ability to decrease reliance on broad-spectrum empirical treatment for Lower Respiratory Tract Infections (LRTI). It has become the preferred diagnostic tool for respiratory pathogens, especially viruses, owing to its high sensitivity in detecting organisms that are challenging to isolate, less viable, or present in limited numbers[5].

This study was conducted on 72 patients who were admitted to the pediatric ICU in Assiut University Pediatric Hospital from March 2021 to March 2022, the patients were admitted to the ICU for 48 hrs or more, with clinical suspicion of HAP or VAP.

Bronchoalveolar lavage samples were obtained as unfortunately, non-invasive sample types are more susceptible to contamination by commensals or colonizing microorganisms from the upper respiratory tract. This risk is particularly heightened in patients with chronic tracheostomies, where the tracheostomy tube is often colonized[8]. Theoretically, bronchoalveolar lavage (BAL) has the potential to yield a 'superior' quality result by its site-directed collection approach, which limits contamination. This implies that organisms cultured from these samples are more likely to accurately reflect the true pathogen causing lower respiratory tract infections. Consequently, this facilitates a simplified interpretation of the laboratory report[9]**.**

The median age of patients was 9 months and ranged from 1 month to 13 years, males were 42 (58%) and females were 30 (41%), out of the 72 patients 57 were on mechanical ventilators. most cases admitted to the ICU with GIT disorder mostly due to severe gastroenteritis (38.9%) or Neurological disorder (26.4%), the high male-to-female ratio was also reported by another Egyptian study conducted on 50 HAP patients' sputum samples and 50 VAP patients (25 endotracheal aspirates and 25 bronchoalveolar lavages)[10]**.**

The most frequently detected bacteria in this study, by both FA-PP and routine culture methods, were *K. pneumoniae* group (41.7% and 36.1%), *A. baumannii* (29.2% and 18.1%), the high prevalence of these Gram-negative bacteria is in concordance with the reports from other studies from Egypt which reported a nearly similar prevalence with *K. pneumoniae* group and *A. baumannii*[5,11,12,13].

In the current study, we also reported a high percentage of viral infection among hospitalized pediatrics; at least a single virus was detected in 70.8% of patients, with the predominance of rhinovirus (51.4%) and adenovirus (19.4%) which also reported by **Bozan et al.** and **Edin et al.** as the most common detected viruses in nosocomial pneumonia[14,15]**.**

The high rate of infection with adenoviruses may contribute to the high percentage of gastroenteritis cases in the ICU as adenoviruses can also affect the gastrointestinal tract causing gastroenteritis. diarrhea, nausea, and vomiting[16].

Candida spp. are commonly found in respiratory secretions of mechanically ventilated patients, either as a result of hematogenous dissemination or aspiration of gastric contents and may represent colonization of the tracheobronchial tree[17].

In the present study, yeasts were detected in 29 specimens; Candida spp. were detected in 28 specimens (38.9%), with a predominance of *Candida albicans*, A previous study conducted by **Ginocchio et al.** has reported a similar prevalence of Candida spp. (43%) in respiratory samples obtained from patients with HAP/VAP[18]**.**

In the recent study, we demonstrated that the FA-PP detected more pathogens than culture methods and has a superior role in the detection of mixed bacterial infections and poly microbial infections than the culture method, as the FA-PP has detected poly microbial infections (bacterial and viral) in 55.6% of patients and detected two bacterial pathogens or more in 51.4% of patients with overall positivity rate 94.4%, while culture method has detected poly microbial infections in 27.8% of patients and detected two bacterial pathogens in 16.7% of samples with overall positivity rate 76.4%. This high rate of detection of single and multiple pathogens by FA-PP was also reported by other studies[4,13,18,19,20]**.**

Also, we reported that the co-detection of mixed bacterial infections and polymicrobial infections was obvious in the patients on mechanical ventilators. **Bozan et al.** has also

reported a high percentage of infections with multiple pathogens among patients diagnosed with VAP, considering mechanical ventilation as a risk factor for the poly microbial infections[14].

In the present study we reported strong performances of FA-PP in the detection of bacterial pathogens, similar to those reported by multicentric evaluation studies for other Biofire panels; meningitis panel (PPA 85.7%; NPA 99.5%[21], blood culture identification panel (PPA 96.5%; NPA 99.7%)[22], respiratory panel (PPA 93.7%; NPA 94.1%)[23] and gastrointestinal panel (PPA 76.6% %; NPA 99.8%)[24].

In the present study, the overall performance of FA-PP in the detection of bacterial targets compared to the routine culture method was accurate with a total PPA of 96.5% and NPA of 90.6%. The false-negative results with the FA-PP were low in our study (n =2) and reported only with *Klebsiella pneumoniae* which might be explained by point mutations of the bacteria so couldn't detected by the PCR, some false-negative results have been reported elsewhere for *Klebsiella spp.* and *Pseudomonas aeruginosa* in previous studies;[7,19]and[25]**.**

However 42 false positive bacterial targets were obtained by FAPP which slightly reduced the negative percent agreement to 90.6%, A similar NPA (90%) has been reported by **Kamel et al.** which 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[13].

In **Yoo et al.** study the overall sensitivity and specificity for organism detection using FA-PP were 98.5% and 76.5%, respectively[25]. Also, **Gastli et al.** has reported PPA and NPA values of 94.4% and 96.0% respectively when compared with culture[7].

Another advantage of the BioFire®FilmArray®Pneumonia Panel is that it can semi-quantify bacterial targets, semi-quantitative bin (copies/mL) results generated by the FA-PP are not equivalent to CFU/mL and do not consistently correlate with the quantity of bacterial analytes compared to CFU/mL, as mentioned in FA-PP instructions[3]**.**

In the current study, we reported that the bin $\geq 10^6$ (10^7 or 10^6) values were considered significant while 10^4or 10^5 copies/ml were considered not significant as out of 56 positive bacteria detected by the routine culture method, there were 39 bacteria detected with $\geq 10^7$ copies/ml by FA-PP, 16 bacteria detected with 10^6 copies/ml and only one bacteria detected with 10^5 copies/ml, while all bacterial targets that detected with 10^4 copies/ml by the FA-PP were not detected by the culture.

The bin $\geq 10^6$ values were considered significant by previous studies when compared with culture[3,9,18]. Additionally, 90.1% and 88.2% of bacteria considered as significant by culture were also reported with a bin $\geq 10^6$ or $\geq 10^7$ by **Gastli et al.** and **Yoo et al.** respectively[7,25]**.**

The overestimation of bacterial load by FA-PP is likely due to the detection of non-cultivable viable or dead bacteria. Additionally, exposure to antibiotic therapy can significantly diminish the recovery of potential pathogens through culture-based methods. It is noteworthy that up to 80% of cases show positive PCR results but negative culture outcomes may be linked to recent exposure to empirical antibiotics[26]**.**

The choice of empiric antibiotics during stays in the intensive care unit is influenced by the patient's prior infections and the specific infectious agent. Empiric antibiotic treatment strategies are adjusted when there is a recent infection with multidrug-resistant (MDR) organisms within the past 90 days. Ensuring the appropriateness of antibiotic therapy is crucial in all settings, and globally, preventing the dissemination of antibiotic-resistant bacteria is a top priority[27].

The Biofire pneumonia panel can provide a preliminary indication of potential antimicrobial susceptibility data for some commonly encountered pathogens via the detection of selected AMR genes. In the current study we demonstrated that the identification of resistance genes by using the FA-PP revealed a significantly high prevalence of carbapenemases (62.3%) and ESBLs (34.8%), **Debbagh et al.** has reported a similar prevalence of carbapenemases (65.2%) and ESBLs (34.8%) using the FA-PP[1].

The most prevalent resistant genes in our study were *CTX-M* and *NDM* which also had

been reported by other Egyptian studies as **Kamel et al.** has reported similar prevalence[13], Another Egyptian study was carried out in a pediatric intensive care unit has reported that the most prevalent-resistant genes detected in *K. pneumoniae* were (*NDM*) gene and (*CTX-M*) gene[28].

The high rate of antibiotic resistance can be attributed to the administration of previous, frequently broad-spectrum, antibiotic treatment among critically ill patients admitted to the intensive care unit, this increased selective pressure has allowed for the emergence of new resistant phenotypes[13]**.**

Regarding the performance of the FA-PP in the detection of the resistance gene markers we reported that the FA-PP showed relatively high agreement with VITEK-2 MIC breakpoints, with 92.7% PPA and 82.8 % NPA. In another study conducted by **Lee et al.** the PPA and NPA were 97% and 95% respectively when FA-PP results were compared to standard antibiotic sensitivity testing[19]**. Webber et al.** also reported 100% PPA between the FA-PP and the standard of care testing in the detection of antimicrobial resistance, suggesting high assay sensitivity[20].

However, the detection of a genetic marker for antimicrobial resistance cannot be conclusively associated with the detected microorganism(s) due to the potential presence of multiple organisms in the same sample. The challenge of linking a resistance gene with a specific pathogen is particularly evident, especially for CTX-M and carbapenemase genes. This limitation becomes apparent when FA-pp identifies two or more pathogens in a single sample, each potentially containing the resistance markers. Consequently, it is essential to complement FA-pp results with culture findings to accurately determine susceptibility or resistance[25]**.**

Also, we have reported that 6 bacterial isolates were resistant using the VITEK-2 breakpoints while the FA-PP didn't detect resistant genes, this may be explained by that the antimicrobial susceptibility can be decreased through other resistance mechanisms that were not implemented in the FilmArray Pneumonia Plus panel like changes in membrane permeability to antibiotics or presence of efflux pumps[29], Also ESBL resistant could be caused by another gene rather than CTX gene[30].

Conclusion

The BioFire® FilmArray Pneumonia Plus panel (FA-PP) holds promise in providing prompt identification of microorganisms and detection of antibiotic resistance genes, as it showed high NPA, PPA, and high accuracy in correlation with routine culture methods. This rapid molecular diagnostic pneumonia panel presents numerous advantages that include reducing unnecessary empirical antibiotic use, particularly in pediatrics where viral infections are prevalent. Furthermore, the FA-PP can aid in the implementation of antimicrobial stewardship programs, as rapid identification of microorganisms and detection of antibiotic resistance genes have a further positive impact on applying antimicrobial stewardship programs through the de-escalation and escalation of antimicrobial agents.

Further updates in the FA-PP are recommended to include other clinically important bacteria e.g. *Citrobacter spp*., *Stenotrophomonas maltophilia,* and *Achromobacter spp*., which are potential causes of nosocomial pneumonia, even though they weren't detected by culture methods in our study, Also the panel should include markers for other antibiotic resistance genes like colistin resistance which has emerged as a significant threat worldwide, especially in hospital-acquired infections (HAIs).

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تقييم لوحة الالتهاب الرئوي الخاصه بجهاز البيوفير في تشخيص الالتهاب الرئوي لدى مرضى الأطفال في وحدة العناية المركزة بالمقارنة مع جهاز الفيتك وطرق الزراعه الروتينية

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يمكن أن تنجم التهابات الجهاز التنفسي السفلي عن مجموعة متنوعة من الميكروبات. يعد التشخيص السريع والدقيق لهذه الكائنات الحية الدقيقة أمرًا بالغ الأهمية لتحديد نظام مضادات الميكروبات الأكثر ملاءمة.حاولت هذه الدراسة التعرف على انواع الميكروبات المسببة لعدوى الجهاز التنفسي السفلي بين المرضى الاطفال الذين تمت دراستهم ومعدلات حدوث هذه العدوي والكشف عن مقاومه المضادات الحيوية وذلك باستخدام تفاعل البوليمراز المتسلسل المتعدد الخاص بجهاز البيوفير وربط النتائج بالموجودات التي تم الحصول عليها بواسطة طرق زراعة البكتريا الروتينية. تم إجراء هذه الدراسة على ٧٢ عينة من غسيل القصبات الهوائية.

كشفت النتائج أن العدوى البكتيرية والفيروسية كانت الأسباب الشائعة للالتهاب الرئوي بين المرضى الأطفال في وحدة العناية المركزة، و كانت أكثر بكتيريا شيوعًا هي الكلبسيلا الرئوية ، بينما كان الفيروس الانفي هو الاكثر شيوعا بين الفيروسات. تم الإبلاغ عن نسبة عالية من مقاومة للمضادات الحيوية فى هذه الدراسه. كان الأداء العام للوحة الالتهاب الرئوي الخاصه بجهاز البيوفير في الكشف عن البكتريا مقارنة بطرق الزراعة الروتينية دقيقًا حيث كانت نسبه الاتفاق التنبؤي الايجابي الكليه ٩٦,٥% وكانت نسبه الاتفاق التنبؤي السلبي الكليه ٩٠,٦%، وكان هناك اتفاقا مرتفعا نسبيا بين لوحة الالتهاب الرئوي وجهاز الفيتك في الكشف عن مقاومة المضادات الحيويه حيث كانت نسبه الاتفاق التنبؤي الايجابي الكليه ٩٢,٧% وكانت نسبه الاتفاق التنبؤي السلبي الكليه ٨٢,٨%، وقد توصلت الدراسة الى ان استخدام لوحة الالتهاب الرئوي الخاصه بجهازالبيوفير توفر طريقة تشخيص سريعة وحساسة لعدوى الجهاز التنفسي السفلي.