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Impact of Nested Multiplex Polymerase Chain Reaction Assay in the management of pediatric patients with acute respiratory tract infections: a single center experience

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SUMMARY

Purpose: Acute lower respiratory infection (ALRI) remains a global public health problem among children. Distinguishing the etiology of ALRI is challenging and rapid pathogen identification is critical for optimizing the diagnosis and treatment of infectious diseases. Multiplex polymerase chain reaction (PCR) is sensitive, simple, and rapid. Our objective was to evaluate the diagnostic yield and prognostic significance of the FilmArray test for identification of pathogens in pediatric patients with ALRI at a tertiary care center.
Methods: A prospective observational cross-sectional study involved 230 pediatric patients presented with acute lower respiratory tract (LRT) symptoms, for whom conventional bacterial culture and FilmArray testing was ordered to aid in the proper diagnosis of the implicated respiratory pathogens.
Results: FilmArray Respiratory panel (FARP) was positive in 201 patients (87.4%). The most common detected pathogens were *Respiratory syncytial virus (RSV), Human Rhinovirus/Enterovirus, Parainfluenza, Influenza A,* and *Klebsiella pneumoniae;* 45 (19.6%), 38 (16.5%), 11 (4.8%), 8 (3.5%) and 6 (2.6%) respectively. FilmArray enabled a change in antimicrobial therapy in 168 (73%) of the patients.
Conclusions: FilmArray enables to improve etiological diagnosis of ALRI and optimize the antimicrobial use of drugs in critical care pediatric patients. Clinical correlation is essential to interpret multiple pathogens and resistance genes.

Keywords: FilmArray, Syndromic testing, pediatric respiratory infections, PCR, Point of care testing, stewardship.

INTRODUCTION

Respiratory tract infections are frequent among different age groups and represent one of the leading causes of healthcare visits and contribute to morbidity and mortality, resulting in significant healthcare costs [1]. Symptoms of respiratory tract infections are very similar, while definitive diagnosis of the causative agents requires laboratory testing. Diagnosis of bacterial and fungal infections is usually performed through a culture which requires two to three days; thus, empirical therapy is often started, which increases the risk of antibiotic resistance [2]. Also, diagnosis of viral infections through direct fluorescent antibody tests or viral culture requires technical expertise and is time-consuming, while, antigen detection tests have low sensitivity [3].
Therefore, prompt identification of causative agents and initiation of proper antimicrobial therapy are urgently needed to improve the patient's

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prognosis. Nucleic acid detection tests have gained popularity because of the rapid turnaround as well as the wide panel of pathogens that can be detected [4, 5]. On this basis, syndromic testing has been implemented in most microbiology laboratories worldwide, where multiple agents are tested simultaneously through a single test. Syndromic testing offers multiple advantages including proper antimicrobial stewardship since the antimicrobial therapy is administrated in a timely appropriate way, and guards against undue use of antibiotics to treat viral infections, in addition to early isolation of specific cases to mitigate the risk of occurrence of outbreaks [6, 7]. In addition, rapid diagnosis of the causative agent and thus targeted management of respiratory infections aids in better patient cohorting, diminishing the length of hospital stay and reducing costs of unnecessary ancillary tests [8].

The BioFire FilmArray Respiratory Panel (FARP) is a nested multiplex molecular technique that detects targets; both viruses and bacteria through melting temperatures (Tm) using about 300 µl of the specimen [1]. BioFire offers multiple advantages; testing is carried on with minimal hands-on time, fully automated, and minimal turn-around time in about one hour [9]. The FARP has aided clinicians and microbiologists to identify respiratory pathogens especially those not detected with conventional methods, thus providing a significant impact on the care of patients with respiratory tract infections [6].

Aim: This study aims to identify organisms implicated in respiratory tract infections in children with using BioFire Film Array Respiratory Panel, to compare between Film Array results versus conventional bacterial culture and to determine impact of Film Array results on patient management.

PATIENTS AND METHODS

Study design

A prospective study was carried on between September 2022 to February 2023. We enrolled 230 pediatric patients with acute lower respiratory tract (LRT) symptoms aged between the neonatal period to 18 years old and presented to the Children's Hospital emergency room (ER), Faculty of Medicine, Ain Shams University, Cairo, Egypt. Diagnostic tests were performed according to the consultant assessment, *e.g.* Complete blood count (CBC), C-reactive protein (CRP), bacterial culture of an LRT specimen and FARP test. All specimens were collected at the Children's Hospital, then transported within two hours to the main laboratories at Ain Shams University Hospitals, Cairo, Egypt where, CBC, CRP, and lower respiratory tract (LRT) specimens were sent for culture and LRT specimens for FilmArray testing were sent to Hematology, Serology, Microbiology and Molecular Microbiology laboratories respectively. This study was approved by the ethical committee of the Faculty of Medicine, Ain Shams University.

Clinical diagnostic criteria

All patients with clinical suspicion of acute lower respiratory infection (ALRI) underwent thorough history-taking and clinical examination.

Participant inclusion criteria included:

1) age < 18 years;
2) clinical presentation compatible with acute respiratory disease (cough, difficult breathing, tachypnea) and/or signs and symptoms of infection (fever > 37.3°C or looking/feeling unwell) and
3) informed consent to participate obtained from parent or guardians.

Patients were excluded from the study if they had been hospitalized in the previous 14 days before the current episode.

Laboratory diagnosis

Sample processing

Complete blood count (CBC): was analyzed through XN-1000™ Hematology Analyzer (Sysmex, USA).

C-reactive protein (CRP): was analyzed through Cobas c 311 (Hitachi High-Technologies, Japan).

Bacterial and fungal cultures of lower respiratory tract (LRT) samples; sputum, endotracheal aspirate (ETA) or bronchoalveolar lavage (BAL) were performed according to Ain Shams University Microbiology laboratory standard operating procedures (SOPs). As regard sputum samples, direct Gram staining was performed, Q -scoring was done and high-quality sputum specimens were accepted. Specimens were cultured on Blood agar, Chocolate agar, MacConkey agar and two Sabouraud dextrose agar (SDA) plates (Oxoid, UK) by semi-quantitative technique, plates were incubated aerobically for 24-48 hours at 37°C, one plate of

SDA was incubated at 28°C and the other at 37°C, both were examined each other day up to two weeks. Sputum, ETA and BAL cultures were considered positive if $\geq 10^6$, $\geq 10^5$ and $\geq 10^4$ CFU/mL were detected respectively. Presence of mixed growth of *Neisseria* spp., diphtheroids, alpha-hemolytic streptococci, or staphylococci was considered as normal flora. Positive bacterial cultures were identified through manual identification using colony morphology, Gram stain and biochemical reactions. Positive fungal cultures were identified through colony morphology, germ tube test, methylene blue stain using scotch tape technique and Gram-stained film. Antibiotic susceptibility testing was conducted on positive bacterial cultures by Kirby Bauer disc diffusion method (Oxoid, UK), and interpreted according to Clinical Laboratory Standard Institute [10].

FilmArray testing (BioFire Diagnostics, LLC, Salt Lake City, UT, USA)

Sample collection: ETA or BAL was collected from children who were intubated or underwent bronchoscopic examination. For other children, sputum samples were collected from children unless they were not able to expectorate, and a nasopharyngeal swab (NPS) was collected [11]. The NPS was immediately placed in viral transport media (VTM) (Disposable virus sampling swab kits, Bioteke corporation, Wuxi, Co., Ltd., China), sputum, ETA and BAL samples were collected in sterile containers.

Nasopharyngeal swabs were analyzed using BioFire® Respiratory Panel 2.1 plus (BRPP) which detects 17 targets.

Sputum, ETA and BAL were analyzed using BioFire Pneumonia panel plus (BPPP) which detects 34 targets.

Sample preparation, nucleic acid extraction, amplification, and analysis: FilmArray utilizes nested multiplex PCR (nmPCR) and melting analysis to identify nucleic acid targets from specimens. Hands-on time is about two minutes with overall turnaround time of about one hour, the specimen is loaded into a reagent pouch, which is placed into the instrument to start the run. The instrument interacts with the reagent pouch to extract nucleic acids (NA) from the sample and amplifies pathogen-specific NA sequences. Polymerase chain reaction (PCR) is the process of making billions of copies of DNA. Copies are made by melting the DNA into separate strands and using each strand as a template for the generation of a new strand. To identify specific pathogens using PCR, primers (short pieces of a specific DNA sequence) are included in the PCR reaction to target unique segments of the pathogen genome. If the organism of interest has an RNA genome, a process called reverse transcription (rt) is performed prior to PCR in order to convert the RNA template into a DNA template (rt-PCR). There are 3 steps to a PCR cycle: Step 1: Denaturation at 94°C. Step 2: Primer annealing at about 60°C. Step 3: Primer extension using Taq DNA polymerase. During the first-stage of nmPCR, outer primers perform multiplex PCR on the target sequences present in the specimen, followed by second-stage PCR in a singleplex format to amplify the NA copies generated during the first-stage.

LCGreen Plus binding dye is incorporated into the DNA copies during amplification. When the dye is bound to double-stranded DNA (dsDNA), fluorescence is detected by the instrument, as ds DNA reaches the melting temperature (Tm), the dye is released and fluorescence diminishes. PCR products rising from different targets have different sequences and, subsequent, different Tms. Since Tm of an amplicon is unique, PCR products can be identified and non-specific products with different Tms are excluded.

Statistical analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 20). Data were presented and suitable analysis was done according to the type of data obtained for each parameter, then the data was presented in Descriptive way or through the chi-square test to determine if the association between categorical variables.

RESULTS

Two-hundred thirty pediatric patients were enrolled in this study, including 133 (57.8%) males and 97 (42.2%) females, with a mean age of 3.7±4.2 years. Some patients presenting with acute respiratory symptoms were suffering from other comorbid conditions, *e.g.* leukemia 34 (14.8%), congenital heart disease 21 (9.1%), asthma 5 (2.2%), where leukemia was the most common comorbid condition. Among the 230 patients, total leucocyt-

ic count (TLC) was normal in 120 (52.2%) and 55 (23.9%) showed mild leukocytosis, 141 (61.3%) had a CRP level ≥ 6 mg/L. There were 118 (51.3%) LRT samples; 87 (37.8%) sputum, 13 (5.7%) ETA and 18 (7.8%) BAL samples, 97 (82.2%) showed growth of normal flora and 21 (17.8%) showed positive growth; where, *Klebsiella* species were the most commonly isolated (n=9, 7.6%), followed by *Acinetobacter* spp (n=5, 4.2%), *Candida* spp (n=3, 2.5%), *methicillin-resistant Staphylococcus aureus* (MRSA) as well as *Pseudomonas* spp (n=2, 1.7%) each. Most patients were diagnosed with pneumonia [129 (56.1%) and bronchitis 96 (41.7%)]. Table 1 shows the descriptive analysis for the comorbid conditions that were recognized, the different departments of admission of the studied patients, the general laboratory tests and diagnosis.

Pathogens detected using BioFire FilmArray

FilmArray samples included 112 (48.7%) NPS, 87 (37.8%) sputum, 13 (5.7%), ETA and 18 (7.8%) BAL samples. In comparison to conventional culture, FilmArray showed a greater diagnostic yield. There were 201 positive patients (87.4%); 178 (77.4%) with at least one viral agent, 22 (9.6%) with at least one bacterial agent and 1 (0.4%) showed mixed bacterial viral pathogens, where NPS showed 93 (46.2%) positive results, followed by sputum 81 (40.3%), BAL 14 (7%) and ETA 13 (6.5%).

The most frequent single targets detected by FilmArray were *Respiratory syncytial virus* (RSV), *Human Rhinovirus/Enterovirus* (HR/EV), *Parainfluenza, Influenza A* and *Klebsiella pneumoniae*; 45 (19.6%), 38 (16.5%), 11 (4.8%), 8 (3.5%) and 6 (2.6%) respectively as shown in Table 2. *Influenza A* subtypes were as follows: one sample showed isolation of *Influenza AH1 2005*, two samples showed isolation of each of *Influenza AH1 2009* and *Influenza AH3*, other isolates were reported as *Influenza A*. Co-infection, triple and quadruple infections were mainly recognized with NPS as shown in Table 3, where 66 (28.7%), 13 (5.7%) and two (0.9%) samples showed co-infections, triple and quadruple infection respectively. Due to financial constraints, triple and quadruple samples were not retested, the results were correlated with the patient clinical and medical status. The FilmArray panels showed 100% positive agreement (PPA) and 29.9% negative agreement (NPA) with conventional culture for bacterial targets.

Upon comparing BioFire target detection with bacterial culture results, it can be concluded that bacterial targets detected at a level of $\geq 10^7$ were also detected on culture plates, except for *S. pneumoniae* which may be attributed to the fastidious nature of the organism as shown in Table 3. Bacterial targets detected at a level $<10^7$ were not detected by culture.

As regard screening for antimicrobial resistance, the results of phenotypic antimicrobial resistance revealed 15 isolates (9 *Klebsiella* spp, 5 *Acinetobacter* spp and one *Pseudomonas* spp) phenotypically multidrug resistant including carbapenems resistance. All carbapenem resistant isolates by Kirby-Bauer disc diffusion testing had resistance genes detected by the FilmArray; $bla_{\text{CTX-M}}$ gene (n = 14), bla_{NDM} (n=14), $bla_{\text{OXA-48 like}}$ (n=10), bla_{VIM} (n=3), bla_{KPC} (n=3) and bla_{IMP} (n=1) genes. All methicillin resistant *S. aureus* cases were positive for the mecA/C and MERJ genes detected by FilmArray. It is worth mentioning that the turn-around time (TAT) by the FilmArray was 1.5 hours compared to 48-72 hours and two weeks by conventional bacterial and fungal culture respectively.

Among the nine *Klebsiella* spp isolates detected by culture two isolates were moderately sensitive to ampicillin-sulbactam (SAM) and seven isolates were resistant. All isolates were resistant to cefotaxime (CTX), ceftriaxone (CRO), ciprofloxacin (CIP) and amikacin (AK), four isolates were sensitive to gentamicin (CN) and imipenem (IPM), while five isolates were resistant to both antibiotics, three isolates were sensitive to meropenem (MEM), one was moderately sensitive and five isolates were resistant. All these isolates showed positive detection of $bla_{\text{CTX-M}}$ gene, eight were positive for bla_{NDM}, seven were positive for $bla_{\text{OXA-48 like}}$ and bla_{VIM} was positive in one isolate, also bla_{KPC} was positive once as mentioned in Table 4. As regard *Acinetobacter* spp isolates detected by culture, four isolates were extensively drug-resistant and one isolate only was sensitive to SAM, CTX, CRO, CIP, AK, CN, IPM and MEM. All these isolates showed positive detection of bla_{NDM} gene, four were positive for $bla_{\text{CTX-M}}$, one was positive for $bla_{\text{OXA-48 like}}$, also, one was positive for bla_{VIM}. Among the two *Pseudomonas* spp isolates detected by culture, one isolate was extensively drug-resistant and the other one was only sensitive to piperacillin-tazobactam (TZP), cefepime (FEP), IPM and MEM. Both isolates were positive for $bla_{\text{CTX-M}}$,

Table 1 - Descriptive data of the patients.

Age and Gender			
Gender	Females	97(42.2%)	
	Males	133 (57.8%)	
Age (years)	Mean±SD	3.68±4.18	
	Range	0.01-15	
Age groups	≤6 months	87 (37.8%)	
	6 months - 2 yrs	39 (17.0%)	
	2-8 yrs	53 (23.0%)	
	≥8 yrs	51 (22.2%)	
Comorbid conditions		*No.*	*%*
Normal		149	64.8%
Leukemia		35	15.2%
Congenital heart disease		21	9.1%
Asthma		5	2.2%
Convulsions		4	1.7%
Down syndrome		3	1.3%
Liver cell failure		2	0.9%
Hodgkin lymphoma		1	0.4%
Rhabdomyosarcoma		1	0.4%
Congenital adrenal hyperplasia		1	0.4%
Fanconi syndrome		1	0.4%
Systemic lupus erythromatosus		1	0.4%
Astrocytoma		1	0.4%
Neuroblastoma		1	0.4%
Tetralogy of Fallot		1	0.4%
Pneumothorax		1	0.4%
Ewings sarcoma		1	0.4%
Carditis		1	0.4%
Department of admission			
Pediatric ICU		95	41.3%
Emergency Room		48	20.9%
Hematology Unit		34	14.8%
Pediatric cartdiology unit		24	10.4%
NICU		16	7.0%
Hematopoietic stem cell transplantion unit		6	2.6%
Oncology Unit		4	1.7%
Hepatology Unit		2	0.9%
Immunology Unit		1	0.4%
Signs and symptoms			
Cough		219	95.2%
Difficulty in breathing		189	82.2%
Tachypnea		175	76.1%

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Signs and symptoms			
Fever > 37.3 °C		160	69.5%
Feeling/looking unwell		144	62.6%
SO2	Normal	179	77.8%
	On Mechanical ventilation	51	22.2%
General lab tests & Radiological findings		*No.*	*%*
TLC	Normal	120	52.2%
	Mild leukocytosis	55	23.9%
	Moderate leukocytosis	15	6.5%
	Mild leukopenia	19	8.3%
	Moderate leukopenia	4	1.7%
	Marked leukopenia	17	7.4%
CRP	Normal	89	38.7%
	Elevated	141	61.3%
Sputum culture	Negative	97	82.2%
	Klebsiella	9	7.6%
	Acinetobacter	5	4.2%
	Candida	3	2.5%
	S. aureus	2	1.7%
	Pseudomonas	2	1.7%
Diagnosis	Bronchiolitis	96	41.7%
	Pneumonia	129	56.1%
	Pleural effusion	2	0.9%
	Bronchial asthma	3	1.3%
Antimicrobial received	No	198	86.1%
	Cefotaxime	9	3.9%
	Ceftriaxone	8	3.5%
	Clarithromycin	2	0.9%
	Amoxacillin/clavulanic acid	2	0.9%
	Levofloxacin	1	0.4%
	Ampicillin/sulbactam	8	3.4%
	Gentamycin	4	1.7%
	Voriconazole	1	0.4%
	Polymyxin E	1	0.4%
	Ribavirin	2	0.9%
	Levofloxacin	1	0.4%
	Metronidazole	0	0.0%
	Ceftazidime	1	0.4%
	Meropenem	2	0.9%
	Clindamycin	6	2.6%
	Piperacillin-tazobactam	1	0.4%
	Ambizome /ciprofloxacin	1	0.4%

Notes: ICU: Intensive care unit, NICU: neonatal intensive care unit, SO2: oxygen saturation, TLC: total leucocytic count, CRP: c reactive protein.

bla_{NDM}, bla_{VIM} and $bla_{OXA\text{-}48\ like}$ genes. Regarding *S. aureus* isolates detected by culture, both isolates were sensitive to tetracycline (TE), trimethoprim-sulfamethoxazole (SXT) and vancomycin (VA), and resistant to penicillin (P) and cefoxitin (FOX). Only one isolate was resistant to erythromycin (E), clindamycin (DA), CN and CIP. Both isolates were positive for mecA, mecC and MERJ.

In this research, the results of BioFire enabled a change in antimicrobial therapy in 168 (73%) of the patients, where ribavirin antiviral therapy was started in 85 (36.9%) patients, oseltamivir was started in 33 (14.3%), antibacterial agents were started in 10 (4.3%), antibiotic de-escalation was performed in 12 (5.2%) patients and in 28 (12.2%) antibacterial therapy was stopped. Antimicrobial treatment protocol was not changed in 62 (26.9%) patients.

Table 2 - FilmArray test results in relation to the type of the specimens.

Results	*NPS*		*Sputum*	*ETA*	*BAL*	*No.*	*%*
Negative						29	12.6%
Positive						201	87.4%
Number of organisms detected per sample							
Single target detection	42		59	9	10	120	52.2%
RSV	26		13	5	1	45	19.6%
Human Rhinovirus/ Enterovirus	10		21	4	3	38	16.5%
Parainfluenza	1		8	–	2	11	4.8%
Influenza A	3		4	–	1	8	3.5%
Klebsiella pneumoniae	–		4	–	2	6	2.6%
Adenovirus	–		2	–	–	2	0.9%
Human metapneumovirus	–		2	–	–	2	0.9%
SARS-CoV-2	2		–	–	–	2	0.9%
Acinetobacter	–		2	–	–	2	0.9%
E. coli	–		1	–	–	1	0.4%
S. aureus	–		1	–	–	1	0.4%
P. aeruginosa	–		1	–	–	1	0.4%
St pneumoniae	–		–	–	1	1	0.4%
Double target detection	44		17	3	2	66	28.7%
RSV, Human Rhinovirus/ Enterovirus	21		5	2	1	29	12.6%
Adenovirus, Human Rhinovirus/ Enterovirus	4		2	1	–	7	3%
Influenza A, Human Rhinovirus/ Enterovirus	3		2	–	–	5	2.2%
Adenovirus, RSV	3		1	–	–	4	1.7%
Parainfluenza, Human Rhinovirus/ Enterovirus	2		1	–	–	3	1.3%
Coronavirus, RSV	2		1	–	–	3	1.3%
Adenovirus, Influenza A	3		–	–	–	3	1.3%
Parainfluenza, RSV	1		1	–	–	2	0.9%
Klebsiella pneumoniae, Acinetobacter	1		–	–	1	2	0.9%
Klebsiella pneumoniae, E.coli	–		2	–	–	2	0.9%
Parainfluenza, S. aureus	1		–	–	–	1	0.4%
RSV, Human metapneumovirus	1		–	–	–	1	0.4%

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Results	*NPS*		*Sputum*	*ETA*	*BAL*	*No.*	*%*
Coronavirus, Human Rhinovirus/ Enterovirus	1		–	–	–	1	0.4%
Influenza A, RSV	1		–	–	–	1	0.4%
Klebsiella pneumoniae, P. aeruginosa	–		1	–	–	1	0.4%
St pneumonia, Moraxella catarrhalis	–		1	–	–	1	0.4%
Triple target detection	5		5	1	2	13	5.7%
Adenovirus, RSV, Human Rhinovirus/ Enterovirus	2		1	1	1	5	2.2%
Parainfluenza, RSV, Human Rhinovirus/ Enterovirus	2		–	–	–	2	0.9%
Coronavirus, RSV, Human Rhinovirus/ Enterovirus	1		1	–	–	2	0.9%
Acinetobacter, Klebsiella pneumoniae, E.coli	–		1	–	1	2	0.9%
Acinetobacter, S. aureus, H. influenza	–		1	–	–	1	0.4%
Adenovirus, RSV, Parainfluenza	–		1	–	–	1	0.4%
Quadruple target detection	2		–	–	–	2	0.9%
Adenovirus, Coronavirus, Human Rhinovirus/ Enterovirus, Human metapneumovirus	1		–	–	–	1	0.4%
Chlamydia pneumonia, S. aureus, H. influenza, St pneumoniae	1		–	–	–	1	0.4%
Total	93		81	13	14	201	87.4%

Notes: NPS: nasopharyngeal swab, ETA: endotracheal aspirate, BAL: bronchoalveolar lavage, *RSV: Respiratory syncytial virus, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, St pneumoniae: Streptococcus pneumoniae H. influenza: Haemophilus influenza.*

Table 3 - Relation between diagnosis and FilmArray results.

Results	*Bronchiolitis*	*Pneumonia*	*Pleural effusion*	*Bronchial asthma*
	n=96	*n=129*	*n=2*	*n=3*
Negative	12 (12.5%)	17 (13.2%)	0 (0.0%)	0 (0.0%)
Positive	84 (87.5%)	112 (86.8%)	2 (100.0%)	3 (100.0%)
Number of organisms detected per sample				
Single target detection	51 (53.1%)	65 (50.4%)	2 (100.0%)	2 (66.7%)
RSV	34 (35.4%)	11 (8.5%)	–	–
Human Rhinovirus/ Enterovirus	10 (10.4%)	25 (19.4%)	1 (50%)	2 (66.7%)
Parainfluenza	2 (2.1%)	9 (7%)	–	–
Influenza A	3 (3.1%)	5 (3.9%)	–	–
*Klebsiella pneumonia*e	1 (1.04%)	5 (3.9%)	–	–
Adenovirus	–	2 (1.5%)	–	–
Human metapneumovirus	–	2 (1.5%)	–	–
SARS-CoV-2	–	2 (1.5%)	–	–
Acinetobacter	1 (1.04%)	1 (0.8%)	–	–
E. coli	–	1 (0.8%)	–	–
S. aureus	–	1 (0.8%)	–	–
P. aeruginosa	–	1 (0.8%)	–	–
St pneumoniae	–	–	1 (50%)	–

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Results	*Bronchiolitis*	*Pneumonia*	*Pleural effusion*	*Bronchial asthma*
	n=96	*n=129*	*n=2*	*n=3*
Double target detection	31 (32.3%)	34 (26.4%)	0 (0.0%)	1 (33.3%)
RSV, Human Rhinovirus/ Enterovirus	17 (17.7%)	12 (9.3%)	–	–
Adenovirus, Human Rhinovirus/ Enterovirus	5 (5.2%)	2 (1.5%)	–	–
Influenza A, Human Rhinovirus/ Enterovirus	1 (1.04%)	4 (3.2%)	–	–
Adenovirus, RSV	1 (1.04%)	3 (2.3%)	–	–
Parainfluenza, Human Rhinovirus/ Enterovirus	–	2 (1.5%)	–	1 (33.3%)
Coronavirus, RSV	–	3 (2.3%)	–	–
Adenovirus, Influenza A	–	3 (2.3%)	–	–
Parainfluenza, RSV	1 (1.04%)	1 (0.8%)	–	–
Klebsiella pneumoniae, Acinetobacter	2 (2.1%)	–	–	–
Klebsiella pneumoniae, E. coli	1 (1.04%)	1 (0.8%)	–	–
Parainfluenza, S. aureus	–	1 (0.8%)	–	–
RSV, Human metapneumovirus	1 (1.04%)	–	–	–
Coronavirus, Human Rhinovirus/ Enterovirus	1 (1.04%)	–	–	–
Influenza A, RSV	–	1 (0.8%)	–	–
Klebsiella pneumoniae, P. aeruginosa	1 (1.04%)	–	–	–
S. pneumonia, Moraxella catarrhalis	–	1 (0.8%)	–	–
Triple target detection	2 (2.1%)	11 (8.5%)	0 (0.0%)	0 (0.0%)
Adenovirus, RSV, Human Rhinovirus/ Enterovirus	2 (2.1%)	3 (2.3%)	–	–
Parainfluenza, RSV, Human Rhinovirus/ Enterovirus	–	2 (1.5%)	–	–
Coronavirus, RSV, Human Rhinovirus/ Enterovirus	–	2 (1.5%)	–	–
Acinetobacter, Klebsiella pneumoniae, E. coli	–	2 (1.5%)	–	–
Acinetobacter, S. aureus, H. influenzae	–	1 (0.8%)	–	–
Adenovirus, RSV, Parainfluenza	–	1 (0.8%)	–	–
Quadruple target detection	0 (0.0%)	2 (1.6%)	0 (0.0%)	0 (0.0%)
Adenovirus, Coronavirus, Human Rhinovirus/ Enterovirus, Human metapneumovirus	–	1 (0.8%)	–	–
Chlamydia pneumoniae, S. aureus, H. influenzae, St pneumoniae	–	1 (0.8%)	–	–

Notes: RSV: Respiratory syncytial virus, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, St pneumoniae: Streptococcus pneumoniae H. influenza: Haemophilus influenza.

DISCUSSION

This study enrolled 230 pediatric patients presenting with acute respiratory symptoms, some of whom were suffering from other comorbid conditions e.g. leukemia 34 (14.8%), congenital heart disease 21 (9.1%), and most patients were admitted to the pediatric ICU 95 (41.3%). Among the 230 patients, 120 (52.2%) and 55 (23.9%) showed normal TLC and mild leukocytosis respectively, 141 (61.3%) had a CRP level ≥6 mg/L. As regard bacterial culture, 21 (17.8%) showed positive growth, where, *Klebsiella* spp 9 (7.6%) were the most commonly isolated, followed by *Acinetobacter* spp 5 (4.2%), *Candida non-albicans* 3 (2.5%), MRSA as well as *Pseudomonas* spp 2 (1.7%) each.

This was concordant with Melebari et al who reported that normal flora was present in 56 (70%)

Table 4 - Antibiotic susceptibility testing of bacterial isolates.

Klebsiella	*Testing times*	*S*	*I*	*R*
SAM	9	0	2	7
CTX	9	0	0	9
CRO	9	0	0	9
CIP	9	0	0	9
AK	9	1	0	9
CN	9	4	0	5
IPM	9	4	0	5
MEM	9	3	1	5
Acinetobacter				
SAM	5	1	0	4
CTX	5	1	0	4
CRO	5	1	0	4
CIP	5	1	0	4
AK	5	1	0	4
CN	5	1	0	4
IPM	5	1	0	4
MEM	5	1	0	4
Pseudomonas				
TZP	2	1	0	1
CAZ	2	0	0	2
FEP	2	1	0	1
CIP	2	0	0	2
AK	2	0	0	2
CN	2	0	0	2
IPM	2	1	0	1
MEM	2	1	0	1
S. aureus				
P	2	0	0	2
FOX	2	0	0	2
CIP	2	1	0	1
CN	2	1	0	1
E	2	1	0	1
DA	2	1	0	1
TE	2	2	0	0
SXT	2	2	0	0
VA	2	2	0	0

Notes: S: sensitive, I: intermediate sensitive, R: resistant, SAM: ampicillin-sulbactam, CTX: cefotaxime, CRO: ceftriaxone, CIP: ciprofloxacin, AK: amikacin, CN: gentamicin, IPM: imipenem, MEM: meropenem, TZP: piperacillin-tazobactam, CAZ: ceftazidime, FEP: Cefepime, P: penicillin, FOX: cefoxitin, E: erythromycin, DA: clindamycin TE: tetracycline, SXT: trimethoprim-sulfamethoxazole, VA: vancomycin.

cases, no growth was detected in 6 (7.5%) and positive growth was detected in 18 (22.6%) cases among which *K. pneumoniae* was the most common 9 (11.3%), followed by MRSA 4 (5%) [12]. *Acinetobacter* and *Pseudomonas* spp were less than 2.5%. Unlikely, Ginocchio and his colleagues stated that the most prevalent isolates detected upon culture were *Pseudomonas aeruginosa* (12.8%), *S. aureus* (12.7%) and *Haemophilus influenzae* (8.7%) [13].

In our study, the FilmArray identified 201 pathogens (87.4%): 178 (77.4%) viral, 22 (9.6%) bacterial and 1 (0.4%) mixed bacterial/viral pathogens, while culture showed 97 (82.2%) growth of normal flora and 21 (17.8%) positive growth. This was concordant with Ginocchio et al who stated that most of FilmArray showed higher positive results (70.6%) compared to culture which detected (55.8%) positive growth [13]. Also, El-Nawawy et al reported that BioFire was associated with a 33.3% increase in positive specimens when compared to conventional methods [14]. Hayotte et al found that at least one target was identified by BPPP in (70%) of patients [15]. Positive BioFire results with negative bacterial culture may be related to multiple factors: low count of the isolate on culture plates compared to the cut-off which is therefore not reported, unavailability of culture media that support growth of fastidious bacteria, PCR may detect non-viable organisms, in addition to the antibiotic misuse which may inhibit growth on culture media, also the fact that the count released by BioFire is in the form of copies/ml which is greater when compared to the culture count in the form of CFU/ml [16]. This was discordant with Ginocchio et al who stated that the positive BioFire results (70.6%) were mainly bacterial (20.5%) rather than viral targets (2.9%) and Yen et al who stated that the three most common bacteria detected were *S. aureus*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* [11, 13]. Also, Debbagh et al found that bacterial etiology was the most common (69.3%), represented mainly by *M. catarrhalis* (11.4%), followed by viral etiology (30.7%), with HR/EV as the most prevalent [18]. This difference in epidemiological distribution may be related to the difference in the geographic distribution of pathogens as well as the studied populations.

BioFire samples included 112 (48.7%), 87 (37.8%), 13 (5.7%) and 18 (7.8%) NPS, sputum, ETA and BAL samples respectively, where NPS showed 93

(46.2%) positive results, followed by sputum 81 (40.3%), BAL 14 (7%) and ETA 13 (6.5%). The nasopharynx (NP) microbiome at the time of upper respiratory viral infections during infancy are a significant determinant of risk for the spread of infection to the lower airways and for the resultant expression of inflammatory symptoms marked by fever [19]. Yen and his team study showed that the concordance rate of pathogens detected in the upper and lower respiratory tract specimens was 96% and therefore, recommended that LRT specimens are more preferred to upper respiratory tract (URT) specimens, however, since obtaining high-quality LRT specimens from children is difficult [11]. Therefore, URT specimens associated with molecular testing techniques could be a potential solution.

In the present study, we found that the most commonly detected pathogens were RSV, HR/EV, *Parainfluenza, Influenza A, and K. pneumoniae;* 45 (19.6%), 38 (16.5%), 11 (4.8%), 8 (3.5%) and 6 (2.6%) respectively. This was in agreement with Melebari et al who stated that RSV was the most prevalent 64 (80%), followed by HR/EV 13 (16.3%) [12]. However, Ginocchio and his co-workers in a multicenter study that involved 52 laboratories from 14 countries reported that the most commonly detected bacteria using FilmArray were *S. aureus* (21.2%), *H. influenzae* (19.7%) and *P. aeruginosa* (15.6%) [13]. Similarly, El-Nawawy et al found that HR/EV was the most frequently detected virus (n=21, 29.2%) followed by RSV (n=19, 26.4), *Influenza A* (n=8;11.1) and *Adenovirus* (n=7, 9.7%) [14]. Also, Hayotte and his colleagues reported that *Human Rhinovirus* (n=215,56%) and RSV(n=106, 28%) were the two predominant targets identified by BioFire followed by *Adenovirus* (n=55, 14%), *Parainfluenza* (n=48, 13%), *Human metapneumovirus* (n=25, 7%), Influenza (n=26, 7%) and Coronavirus (n=24, 6%) [15]. This can be due to the decreased RSV infections owing to Palivizumab prophylaxis [20]. Concordantly, Leli et al who conducted a study on pediatric, adults and elderly patients reported that the most frequently encountered viral pathogens were RV/EV (n=89, 15.5%), RSV (n=54, 9.4%), *Influenza* virus (n=51, 8.9%) and *Adenovirus*(n=31, 5.4%) [16].

Our study revealed that positive FilmArray results [201 (87.4%)] were mainly single targets: n=120 (52.2%). Co-infections, triple and quadruple infections represented 66 (28.7%), 13 (5.7%) and two (0.9%) samples respectively. This was in agreement with Lee et al who reported that BPPP showed (42.3%) double targets [21]. Similarly, Webber et al reported (25%), Murphy et al found (29.5%) and Ginocchio et al (41.9%) [13, 22, 23]. Hayotte and his colleagues stated that bacterial co-infection was present in 25% of cases [15]. Molecular detection of more than one target requires correlation with the patient clinical status, Gram stain, specimen type; where sputum samples are more susceptible to oropharyngeal contamination compared to ETA or BAL, in addition to target detection level, other laboratory test; CRP, TLC and radiological findings to differentiate between infection versus colonization, since, depending on the FilmArray results solely could lead to antimicrobial overuse. Microbiology laboratories should set reporting guidelines and provide advisory services for physicians to ensure proper result interpretation. It is also essential to mention that among the limitations of BioFire, is that its panel does not include some pathogens as *Morganella* spp, *Citrobacter* spp, *Hafnia alvei*, *Stenotrophomonas maltophilia* or *Pneumocystis jirovecii*.

The overall percent agreement between the FilmArray panels and conventional bacterial culture in our study was 100%, with 100% PPA and 29.9% NPA. This was concordant with Iannello et al who reported 89.0% PPA and 95.9% NPA with bacterial culture [24]. Edin and his team demonstrated 100% PPA and 73.2% NPA [25]. Yoo and his team detected that 86% of bacteria that showed significant growth yielded BPPP results of $\geq 10^7$, also, Gastli et al found that 90.1% of targets with a BPPP result $\geq 10^6$ showed significant growth on culture plates [26, 27]. Ginocchio et al found that PPA for the single BPPP bacterial pathogens ranged from 50.0 - 100% for BAL specimens and 81.0 - 100% for sputum specimens. NPA for the single bacterial pathogens ranged from 90.2 - 99.4% for BAL and 83.9 - 99.2% for sputum, Westhuyzen et al reported 80% correlation between culture and BPPP results with $\geq 10^7$ copies/ml and Yen et al. demonstrated 89% PPA and 98% NPA for bacterial targets [11, 13, 28]. This difference in percent agreement (PA) can be attributed to the difference in the lower limit of reporting for bacteria by standard culture compared to BioFire, where, the PA increase as the count detected by BPPP increase to 10^6 - $\geq 10^7$.

This was discordant with Webber and his coworkers who reported an overall percent agreement

(99.2%) between the FARP and culture [22]. Similarly, Buchan and his team, reported 96.2% PPA and 98.1% NPA [17]. Also, El-Nawawy et al reported that the overall percent agreement between FilmArray and culture was 100%, with 100% PPA and 95.6% NPA [14].

Identifying the genes of antimicrobial resistance by BioFire allows early contact isolation, patient cohorting, implementing antimicrobial stewardship programs, better patient outcomes, reduced hospital stay and fatality rate [29]. As regard the screening for antimicrobial resistance, our results of phenotypic antimicrobial resistance revealed 15 Gram-negative isolates (9 *Klebsiella*, 5 *Acinetobacter* and one *Pseudomonas* spp) phenotypically multidrug resistant including carbapenems and two *S. aureus* isolates were methicillin-resistant. All carbapenem-resistant Gram-negative isolates by Kirby-Bauer disc diffusion testing had resistance genes by the FilmArray. The detected resistance genes included; $bla_{\text{CTX-M}}$ gene (n = 14), bla_{NDM} (n=14), $bla_{\text{OXA-48 like}}$ (n=10), bla_{VIM} (n=3), bla_{KPC} (n=3) and bla_{IMP} (n=1) genes. Methicillin-resistant *S. aureus* cases were positive for the mecA/C and MERJ genes by FilmArray. It is worth mentioning that the turn-around time (TAT) by the FilmArray was 1.5 hours compared to 48-72 hours and two weeks by conventional bacterial and fungal culture respectively. This was concordant with Ginocchio et al where BPPP detected Gram-negative bacteria in 1537 specimens, of these bacteria 185 (12.04%) isolates had a total of 229 resistance genes ($bla_{\text{CTX-M}}$ (n = 133), bla_{KPC} (n = 67), bla_{IMP} (n = 1) [13]. Also, Debbagh et al reported a high rate of carbapenemases (65.2%) and Extended-spectrum beta-lactamases (ESBLs) (34.8%) resistance genes among Gram-negative targets, this may be attributed to prior antibiotic therapy, especially in severe cases [18]. However, Ginocchio et al., found that among 531 specimens contained *S. aureus*, two specimens reported as MRSA by standard culture were reported as methicillin-sensitive *S. aureus* (MSSA) by BPPP [13]. However, it is important to mention that the presence of resistance genes does not confirm resistance, as these genes may be not expressed. Also, we cannot confirm that BioFire has no false negative results as regards the detection of resistance genes as resistance can be mediated by a variety of enzymes, other than those available in the panel and are still capable of expressing resistant phenotype [1].

In the present research, the results of BioFire enabled a change in antimicrobial therapy in 168 (73%) of the patients, where Ribavirin antiviral therapy was started in 85 (36.9%) patients, oseltamivir was started in 33 (14.3%). Antibacterial agent was started in 10 patients (4.3%). In additional 12 (5.2%) patients, narrowing antibiotic spectrum was performed and in 28 (12.2%) antibacterial therapy was stopped. Antimicrobial treatment protocol was not changed in 62 (26.9%) patients. This was concordant with El-Nawawy et al. who reported that FilmArray results had an impact on the antimicrobial therapy, where, 12 (12.2%) cases started antiviral therapy, in 26 (26.5%) cases, the antimicrobial was replaced with broad spectrum agents [14]. Treatment was stopped in about 47 (48%) patients; 38 (38.8%) stopped antibiotics, 7 (7.1%) stopped antifungal and 2 (2%) stopped antiviral, no change in the antimicrobial protocols was needed in 37 (37.8%) patients. Except for the antiviral therapy, these impacts could have been implemented through the bacterial culture, however, BioFire provided early results for intervention, which definitely affect the clinical outcome. Also, Debbagh et al stated that an antimicrobial agent was introduced in 3 (7.9%), antibiotic spectrum was broadened in 12 (31.6%) cases and no change in therapy was made in 23 (60.5%) [18].

The limitation of this study is that it was performed in a tertiary care hospital with several immunocompromised patients, it was conducted during a limited period (from September 2022 to February 2023), and therefore a number of other seasonal pathogens may have not been represented. Also, the lack of confirmatory testing through PCRs or sequencing for pathogens which are only detected by BFPP should represent a limitation. However, considering the rapid high sensitivity and specificity of BPPP as reported by the FDA, in addition to the detection of both Gram-positive and Gram-negative resistance genes, BPPP may help to optimize therapy, through de-escalation, escalation or discontinuation through case by case thorough evaluation, since, lack of 24/7 available diagnostics for viruses and atypical bacteria in multiple healthcare settings and time taken for routine bacterial culture result to be released lead to empiric antimicrobial treatment using broad-spectrum antibiotics which may increase the antimicrobial resistance and other adverse events as pseudomembranous colitis.

In conclusion, FilmArray is a rapid and simple diagnostic tool with an extremely shortened turnaround time, which aids in starting the appropriate effective antimicrobial agent in a timely efficient manner. Although viruses are the major cause of ARTI in the pediatric population, antibiotics are often prescribed which augment antibiotic resistance crisis. The diagnostic performance of the FARP assay is better than conventional methods and allows the diagnosis of viral as well as atypical bacterial infections. Detection of viral and bacterial targets together with bacterial resistance genes have great impact on starting the proper antimicrobial therapy, applying antimicrobial stewardship policies and proper infection preventive measures. However, the results should be interpreted based on correlation with clinical evaluation as well as other laboratory and radiological investigations.

Ethical approval
This research was approved by Ethical Research committee, faculty of Medicine, Ain Shams university, No. FMASU R246/2022.

Consent to participate
Informed consent to participate obtained from parents or guardians.

Consent for publication
The authors affirm that human research participants provided informed consent for publication.

Conflict of interest
The authors have no relevant financial or non-financial interests to disclose.

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