Original Article

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Microbiology Section

Impact of BioFire FilmArray Multiplex PCR in the Detection of Microbial Agents causing Severe Acute Respiratory Infection in the COVID-19 Era: A Cross-sectional Study from a Tertiary Care Hospital in Central India

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ABSTRACT

Introduction: Many viral and bacterial respiratory tract infections can present with respiratory signs and progress to complicated pneumonia. In the recent Coronavirus Disease-2019 (COVID-19) pandemic, it is crucial to test all Severe Acute Respiratory Infection (SARI) patients for other microbial infections in addition to COVID-19, enabling timely diagnosis and treatment to reduce morbidity and mortality. The automated system, BioFire FilmArray, utilises multiplex Polymerase Chain Reaction (PCR) to rapidly detect and identify multiple respiratory pathogens, including selected Antimicrobial Resistance (AMR) genes, within an hour.

Aim: To detect bacterial and/or viral pathogens associated with hospitalised COVID-19-negative SARI patients using the BioFire FilmArray Pneumonia Panel (BFPP).

Materials and Methods: This laboratory-based cross-sectional study was conducted at All India Institute of Medical Sciences (AIIMS), Nagpur, Maharashtra, India, from June 2020 to February 2021. Respiratory samples, such as sputum, tracheal aspirate, Endotracheal (ET) secretions, and Bronchoalveolar Lavage (BAL), were collected from COVID-19-negative hospitalised SARI cases. A total of 81 patients were included in the study. The samples were tested using the BFPP (multiplex PCR) system and processed using conventional culture techniques. Patient characteristics, clinical and laboratory investigation data, and findings of respiratory viral and bacterial agents, as well as antibiotic resistance genes detected by BioFire FilmArray, were recorded using paper case reports. The data were collected and analysed using Statistical Package for Social Sciences (SPSS) software.

Results: Out of the 81 clinical samples processed, the BFPP detected 168 bacterial and 18 viral pathogens. Bacterial-viral co-detection was observed in 13 (16%) samples. Atypical bacteria were detected in 3% of cases. Among the bacterial pathogens, the AMR gene for New Delhi Metallo-beta-lactamases (NDM) was detected in 42 (25.9%) cases, followed by CTX-M beta-lactamases, VIM, and the oxacillinase group of β-lactamases.

Conclusion: The BFPP test is a valuable tool for the rapid detection of a wide range of pathogens, including associated AMR genes, with high sensitivity and specificity. This can greatly aid in treatment decisions.

Keywords: Antimicrobial drug resistance, Co-infection, Polymerase chain reaction

INTRODUCTION

The SARI, which primarily affects the respiratory tract, is one of the leading causes of morbidity and mortality worldwide. The World Health Organisation (WHO) defines SARI as an acute respiratory infections with a history of fever or measured fever >38°C and cough, with onset within the last 10 days and requiring hospitalisation [1]. SARI is a major cause of hospitalisation and death globally and can be caused by various viral and bacterial agents [2]. In the current COVID-19 pandemic era, it is crucial to test all SARI patients for other microbial infections in addition to COVID-19. Prompt treatment with antibiotics for bacterial infections can improve outcomes, and delays in effective antimicrobial therapy are associated with increased hospital stay and treatment costs.

Conversely, in cases of acute respiratory conditions caused by viral infections, the use of antibiotics is not recommended. Unnecessary antibiotic use contributes to the rise of antibiotic-resistant infections, leading to significant morbidity and mortality [3,4].

Rapid diagnostic testing for identifying the causative agents in SARI patients can facilitate proper management, discontinuation of unnecessary antibiotics, enhance supportive therapy, and reduce healthcare costs and hospital stay duration [5].

The aetiological diagnosis of respiratory tract infections is a challenging area in clinical microbiology due to the wide range of potential pathogens and the non sterile environment of the respiratory tract. Traditional diagnostic methods, such as Gram stain and culture, may have a low diagnostic yield [6-8].

The automated system, BioFire FilmArray, based on the principle of multiplex PCR, provides rapid detection and identification of multiple respiratory viral and bacterial pathogens, along with selected AMR genes, from respiratory samples such as sputum or BAL in individuals suspected of having Lower Respiratory Tract Infections (LRTI), within one hour [9]. Considering the ongoing COVID-19 pandemic, it was important to study the aetiology of cases with similar presentations to COVID-19 but tested negative by PCR. The BFPP was an ideal platform to simultaneously detect 27 different pathogens. The BFPP can detect 18 bacteria (11 Gram-negative, 4 Gram-positive, 3 atypical), seven antibiotic resistance markers, and nine viruses known to cause pneumonia and other LRTIs within one hour. It also provides a semi-quantitative measure of the organism load present in the sample [10].

To the best of the author's knowledge, this unique study is one of the first from Central India. Its aim was to detect bacterial and/or viral pathogens associated with hospitalised COVID-19-negative

SARI patients using the BFPP. The hypothesis of the study was to evaluate the utility of BioFire in the laboratory diagnosis of COVID-19-negative SARI cases, identifying multiple pathogens using the pneumonia panel with their antimicrobial-resistant genes, including viral and atypical bacteria that are difficult to diagnose using standard tests.

MATERIALS AND METHODS

This study was a laboratory-based cross-sectional study conducted at AIIMS, Nagpur, tertiary care hospital in Central India. The study was conducted from June 2020 to February 2021 in the Molecular Diagnostic Laboratory of the hospital. Ethical approval for the study was obtained from the Institute Ethical Committee with letter number IEC/Pharmac/2021/238.

The study included SARI patients who were tested negative for Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) using Real-Time Polymerase Chain Reaction (RT-PCR). These patients were hospitalised in various hospitals in the Vidarbha region of Maharashtra, which consists of 11 districts: Nagpur, Amravati, Akola, Chandrapur, Buldhana, Washim, Gondia, Yavatmal, Wardha, Bhandara, and Gadchiroli. Sputum, tracheal aspirate, ET secretions, and BAL samples were collected from these COVID-19-negative SARI patients and transported to the study centre while maintaining a cold chain temperature of 2-4°C.

Inclusion criteria:

1. Patients hospitalised with symptoms of SARI in various hospitals in the Vidarbha region of Maharashtra. SARI was defined according to the WHO criteria [1] as follows:
    a. Acute respiratory infections
    b. Fever or measured fever >38°C
    c. Cough, with onset within the last 10 days and requiring hospitalisation

Exclusion criteria:

1. Samples that tested positive for COVID-19.
2. Patients who did not fulfill the criteria for SARI definition.

During the study period, which spanned the first wave of COVID-19, a total of 81 samples were received from COVID-19-negative SARI patients who met the inclusion criteria and were included in the present study.

Methodology:

Sputum, tracheal aspirate, ET secretions, and BAL samples received from COVID-19-negative SARI cases during the study period were tested using the BFPP (multiplex PCR) system. All respiratory samples underwent gram stain and conventional culture on blood agar, MacConkey agar, and Chocolate agar as part of the Standard-Of-Care (SOC) testing. Samples were not processed if a score of 0 or 1 was detected according to the Murray Washington criteria [11] which are based on the number of squamous cells and neutrophils per low-power field on Gram's stain of sputum samples. Detailed demographic (age, sex, address), clinical (presenting symptoms, co-morbidity, days of intubation if present), and laboratory investigation details (C-reactive protein, d-Dimer, procalcitonin, X-ray findings) were collected from all the patients.

Methodology of BFPP testing:

- Samples were tested on the BFPP according to the manufacturer's instructions, which are as follows:
- Principle of the test:
    - The BioFire FilmArray disposable pouch is a freeze-dried multiplex PCR assay that contains all the necessary reagents for sample preparation, reverse transcription PCR, PCR, and detection.
    - During a test run, the system extracts and purifies all nucleic acids from the unprocessed sample. It then performs nested multiplex PCR in two stages. The first stage involves a single, large-volume, multiplexed reaction, while the second stage includes individual, single-plex reactions to detect the products from the first stage.
    - Using endpoint melting curve data, the BioFire System software automatically analyses the results for each target on the panel. At the end of the test run, the software reports whether each pathogen is detected in the sample or not. This information is printed in an automated response at the end of the test run.
- The BFPP targets 27 pathogens, including bacteria (such as *Acinetobacter calcoaceticus-baumannii* complex*, Enterobacter cloacae, Escherichia coli, Haemophilus influenzae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae* group, *Moraxella catarrhalis, Proteus* sp.*, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes),* Atypical bacteria (like *Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae*), and viruses {including Influenza A, Influenza B, Adenovirus, Coronavirus, Parainfluenza virus, Respiratory Syncytial Virus, Human Rhinovirus/Enterovirus, Human Metapneumovirus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV)}. It also detects seven AMR genes (ESBL-CTX-M; Carbapenemases-KPC, NDM, Oxa48-like, VIM, IMP; methicillin resistance *mecA/mecC* and MREJ), with an analysis time of about one hour, including sample preparation and DNA/RNA extraction.
    - Each test includes two internal controls: (1) to verify nucleic acid extraction; and (2) to verify PCR performance. The run is considered valid only if both controls pass.
    - Bacteria are reported semi-quantitatively at $\geq 10^7$, 10^6, 10^5, and 10^4 copies/mL of the specimen, where 10^4 copies/mL is the significant cut-off limit.
    - Viral and atypical bacterial agents are reported as not detected or detected.
    - AMR-genes are reported as positive only in the case of simultaneous detection of a compatible pathogen, i.e., *S. aureus* in combination with *mecA/mecC* and MREJ.
    - Values calculated below 10^4 copies/mL are reported as "not detected."

Methodology for culture Standard of Care (SOC) test:

- All specimens are cultured conventionally using blood agar, chocolate agar, and MacConkey agar plates and are incubated at 35°C in 5% CO_2. All plates are examined for growth at 18-24 hours and 48 hours of incubation (if no growth is observed at 18 to 24 hours).
- Any growth is subjected to identification by conventional methods, and antibiotic susceptibility testing is performed using the Kirby Bauer disc diffusion method on Mueller Hinton agar.

STATISTICAL ANALYSIS

Patient characteristics, clinical and laboratory investigation data, as well as data for all BioFire FilmArray findings of respiratory viral and bacterial agents, and antibiotic resistance genes, were collected. They were then entered into an Excel sheet and analysed using SPSS software version 28.0 to calculate percentages.

RESULTS

A total of 81 different samples from patients with non-COVID pneumonia were tested using the BFPP. The majority of samples were sputum (67, 82.7%), followed by BAL (08, 9.8%), and ET aspirate (06, 7.5%) [Table/Fig-1].

Among the 81 patients, 38 (47%) were female and 43 (53%) were male. The mean age of the patients was 47.8 years, with the minimum and maximum ages being 14 years and 82 years, respectively. The

Sample	Number (%)
Sputum	67 (82.70)
Bronchoalveolar Lavage (BAL)	8 (9.80)
Endotracheal (ET) aspirate	6 (7.50)
Total	**81 (100)**

[Table/Fig-1]: Distribution of samples processed using BioFire FilmArray Pneumonia Panel (BFPP) (n=81).

demographic details and infection marker test results are presented in [Table/Fig-2].

Patients presented with various clinical signs and symptoms. Bilateral pneumonia was the most common clinical diagnosis in 33 (40.7%) of the patients, followed by SARI in 25 (30.8%), and other respiratory manifestations such as LRTI, Cor-pulmonale, Acute Respiratory Distress Syndrome (ARDS), etc., in 11 (13.5%) of the patients [Table/Fig-3].

Characteristics	Number (%)
Gender	
Male	43 (53)
Female	38 (47)
Age (in years)	
Below 18	02 (2.4)
18-45	34 (42)
45-60	25 (30.9)
>60	20 (24.7)
Urban/Rural	
Urban	50 (61.7)
Rural	31 (38.3)
History of intubation*	
Yes	51 (63)
No	30 (37)
C-Reactive protein	
Raised	81 (100)
d-Dimer	
Raised	70 (86.4)
Normal	05 (6.2)
Not done	06 (7.4)
Procalcitonin	
Raised	49 (60.4)
Normal	11 (13.6)
Not done	21 (26)

[Table/Fig-2]: Demographic and details of infection marker tests in non COVID-19 SARI patients (n=81).
*History of intubation with ventilator support for >24 hour before BioFire testing

S. No.	Clinical diagnosis	n (%)
1	Bilateral Pneumonia/Bronchopneumonia	33 (40.7)
2	Severe Acute Respiratory Infection (SARI)	25 (30.8)
3	Other manifestations (like diarrhoea, septicaemia, meningitis, etc.,)	12 (15)
4	Other respiratory manifestations	11 (13.5)

[Table/Fig-3]: Clinical diagnosis of non-COVID SARI patients (n=81).

Out of the 81 samples processed, 63 (78%) samples showed the presence of only bacterial pathogens, and only viruses were detected in 5 (6.2%) cases. Two or more bacterial pathogens were detected in 58 (71.6%) samples, while only a single type of bacterium was detected in 5 (6.2%) samples. Bacterial-viral co-detection was observed in 13 (16%) of the samples [Table/Fig-4].

In the 81 samples tested, the BFPP detected a total of 185 bacterial and viral pathogens. Among them, 161 (87.02%) were

Pathogen detected	Number (%)
Single bacterial pathogens	5 (6.2)
Multiple bacterial pathogens	58 (71.6)
Bacterial-viral co-detection	13 (16)
Viral pathogens	5 (6.2)

[Table/Fig-4]: Showing the percentage of pathogens detected singly or with other pathogens (n=81).

bacterial agents, including 6 (3%) atypical bacteria. Among all bacterial pathogens, the highest number was 39 (24.2%) *Klebsiella pneumoniae*, followed by 33 (20.5%) *Acinetobacter calcoaceticus-baumannii complex*. The distribution of pathogens isolated from non COVID SARI patients by the BFPP is shown in [Table/Fig-5].

A. Bacterial	
Agent	**No. of isolates (%)**
Klebsiella pneumoniae	39 (24.2)
Acinetobacter calcoaceticus-baumannii complex	33 (20.5)
Pseudomonas aeruginosa	26 (16.2)
*Enterobacter cloacae**	19 (11.8)
Escherichia coli	14 (8.7)
Staphylococcus aureus	14 (8.7)
Haemophilus influenzae	05 (3.1)
Streptococcus pneumoniae	04 (2.5)
Serratia marcescens	04 (2.5)
Klebsiella aerogenes	01 (0.6)
Klebsiella oxytoca	01 (0.6)
Streptococcus agalactiae	01 (0.6)
Total	**161 (100)**
B. Viral	
Human rhinovirus/enterovirus	16 (89)
Adenovirus	02 (11)
Total	**18 (100)**
C. Atypical bacterial pathogens	
Mycoplasma pneumoniae	04 (66.67)
Legionella pneumophila	02 (33.33)
Total	**06 (100)**

[Table/Fig-5]: Distribution of pathogens isolated from non COVID SARI patients by BioFire FilmArray Pneumonia Panel (BFPP).
*One *Enterobacter cloacae* was isolated on culture but was not detected by BFPP

The BFPP detects pathogens using semi-quantitative log bin values. A pathogen was marked as detected only when it was detected at ≥10^4 copies/mL of the sample. [Table/Fig-6] depicts the numbers of bacterial pathogens with their corresponding log bin values as detected by the BFPP.

Among the 168 bacterial pathogens detected, 167 (99.4%) were detected by BFPP, and 152 (90.4%) were detected by the SOC test. The detection rate by BFPP and SOC was 100% for *Klebsiella pneumoniae* group, *Acinetobacter calcoaceticus-baumannii* complex, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella aerogenes*, and *Klebsiella oxytoca*. BFPP was negative in only one case of the on-panel bacterium, *Enterobacter cloacae* complex, which was detected by SOC but not by BFPP, and the authors could not provide justification for this discrepancy. In the case of *Streptococcus pneumoniae and Serratia marcescens*, 100% were detected by BFPP, but only 50% were detected by SOC tests. For the detection of *Streptococcus agalactiae* by the SOC test, it was considered insignificant as the growth was very scanty, but BFPP detected it with a log bin value of 10^4 copies/mL. Fastidious organisms like *H. influenzae and* atypical bacteria like *Mycoplasma pneumoniae* and *Legionella pneumophila* were detected only by BFPP, while SOC failed to detect them. This suggests that BFPP was a more sensitive

Pathogen detected	10^4 Copies/mL	10^5 Copies/mL	10^6 Copies/mL	10^7 Copies/mL
Klebsiella pneumoniae group (n=39)	9	17	5	8
A. baumannii complex (n=33)	13	4	8	8
Pseudomonas aeruginosa (n=26)	6	5	6	9
Enterobacter cloacae complex (n=19)	7	5	4	3
Escherichia coli (n=14)	5	3	3	3
Staphylococcus aureus (n=14)	3	6	4	1
Haemophilus influenzae (n=5)	3	2	0	0
Streptococcus pneumoniae (n=4)	2	2	0	0
Serratia marcescens (n=4)	2	2	0	0
Klebsiella aerogenes (n=1)	0	0	0	1
Klebsiella oxytoca (n=1)	1	0	0	0
Streptococcus agalactiae (n=1)	1	0	0	0

[Table/Fig-6]: Log bin values (copies/mL) of bacterial pathogens with numbers.

method for detecting fastidious and atypical organisms that could be missed by routine conventional culture techniques. The distribution of negative and positive results for all bacteria detected by BFPP and isolated by SOC is shown in [Table/Fig 7].

Isolates	No. SOC (+) BFPP (+)	No. SOC (-) BFPP (+)	No. SOC (+) BFPP (-)	Total BFPP (+)	Total SOC (+)	% Detection by BFPP	% Detection by SOC
Klebsiella pneumoniae group *(n=39)*	39	00	00	39	39	100	100
Acinetobacter calcoaceticus-baumannii complex *(n=33)*	33	00	00	33	33	100	100
Pseudomonas aeruginosa (n=26)	26	00	00	26	26	100	100
Enterobacter cloacae complex *(n=20)*	19	00	01	19	20	95	100
Escherichia coli (n=14)	14	00	00	14	14	100	100
Staphylococcus aureus (n=14)	14	00	00	14	14	100	100
Haemophilus influenzae (n=5)	00	05	00	05	00	100	0
Streptococcus pneumoniae (n=4)	02	02	00	04	02	100	50
Serratia marcescens (n=4)	02	02	00	04	02	100	50
Mycoplasma pneumoniae (n=4)	00	04	00	04	00	100	0
Legionella pneumophila (n=2)	00	02	00	02	00	100	0
Klebsiella aerogenes (n=1)	01	00	00	01	01	100	100
Klebsiella oxytoca (n=1)	01	00	00	01	01	100	100
Streptococcus agalactiae (n=1)	00	01	00	01	00*	100	0*
Total	**151**	**16**	**01**	**167**	**152**	-	-

[Table/Fig-7]: Summary of total BioFire FilmArray Pneumonia Panel (BFPP) and Standard Of Care (SOC) detections for all pathogens and for all specimen types (n=168).
*Growth on culture was considered insignificant as the growth was very scanty

A total of 162 AMR genes were detected from 161 bacterial pathogens using BFPP. Among the bacterial pathogens, the AMR gene for NDM was detected in 42 (25.9%) cases, followed by CTX-M beta-lactamases in 32 (19.8%) cases, VIM (Verona integron-encoded metallo-β-lactamase) in 29 (18%) cases, and Oxacillinase group of β-lactamases (OXA) in 27 (16.6%) cases. The detection of AMR genes was reported only if an applicable bacterium (i.e., potential carrier of AMR genes) was also detected in the sample. [Table/Fig-8] shows the distribution of AMR genes isolated from all bacterial pathogens detected by BFPP.

S. No.	Detection of AMR genes	Number (%)
1	NDM	42 (25.9)
2	CTX-M	32 (19.8)
3	VIM	29 (18)
4	OXA	27 (16.6)
5	IMP	13 (8)
6	mec-A/C and MREJ	13 (8)
7	KPC	6 (3.7)
Total		162 (100)

[Table/Fig-8]: Antimicrobial Resistance (AMR) genes associated with pathogens detected by BioFire FilmArray Pneumonia Panel (BFPP).

DISCUSSION

The COVID-19, first recognised in Wuhan, China, in December 2019, has rapidly spread across the globe and become a public health crisis. The emergence and geographical spread of COVID-19 coincided with the influenza season in many temperate regions [12,13]. The typical clinical presentation of SARS-CoV-2 is an acute respiratory illness or viral pneumonia with rapid respiratory deterioration [14]. The COVID-19 pandemic has also affected bacterial co-pathogens, which are commonly identified in respiratory tract infections and are an important cause of morbidity and mortality, necessitating timely diagnosis and antibacterial therapy [15].

Many systemic bacterial infections may present with respiratory signs or progress to complicated pneumonia. Empiric therapy for SARI often involves broad-spectrum antibiotics to cover both gram-positive and gram-negative bacteria due to the risk of infection with multidrug-resistant pathogens [16]. However, the broad use of empiric antibiotic treatment for undiagnosed infections has been associated with increased antibiotic resistance globally [17]. Therefore, identifying the specific pathogen(s) is crucial for prompt initiation of targeted and effective therapy.

Rapid detection of the causative agent of respiratory infections, coupled with the detection of prominent markers of antibiotic resistance, can help reduce unnecessary broad-spectrum antimicrobial treatment [18].

This study is one of the first from Central India aimed at detecting other bacterial and viral agents in COVID-19-negative SARI patients using BFPP and guiding clinicians about appropriate antimicrobial therapy during the COVID-19 pandemic. The BioFire FilmArray is a rapid method for the detection and identification of common bacterial pathogens and resistance markers in suspected SARI patients. It also identifies viral agents and atypical bacteria that are challenging to isolate using conventional culture techniques.

A total of 81 samples from SARI patients were tested in this study, of which 18 (22%) samples were positive for viral targets, including 13 (16%) specimens with bacterial-viral co-detection. Bacterial co-detection was identified in 58 (71.6%) cases. In a study by Webber DM et al., bacterial-viral co-detection was noted in 4.6% and 2.9% of sputum and BAL samples, respectively, while only virus detection was observed in 8.5% and 12.9% of samples [19]. In another similar study by Yoo IY et al., co-detection of two pathogens was found in 23% of samples, three pathogens in 12% of samples, and four or more pathogens in 9% of specimens, although the authors did not provide details about the specific types of co-detection [20].

Among the total of 18 viral agents detected in the present study, 16 (89%) were Human Enterovirus/Rhinovirus, and 2 (11%) were Adenovirus. In a study by Kyriazopoulou E et al., [21], which evaluated BFPP for cases of LRTI, bacterial-viral co-infections were observed in 25.6%, higher than in this study, and bacterial co-detection was seen in 17.8%, much lower compared to this study. In another similar study, bacteria and virus co-detection were found in 14.78%, and bacterial co-detection was seen in 26.27% [22].

The most commonly detected organism in this study was *Klebsiella pneumoniae*, with 39 (24.2%) cases, followed by 33 (20.5%) cases of *Acinetobacter calcoaceticus-baumannii* complex*, and* 26 (16.2%) cases of *Pseudomonas aeruginosa*. Fastidious organisms like *Haemophilus influenzae* and *Streptococcus pneumoniae* were detected in 3.1% and 2.5% of cases, respectively. In a study by Kosai K et al., that aimed to detect pathogens of LRTI using BFPP, the most commonly detected organisms were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both with 17 (20%) cases, followed by *Klebsiella pneumoniae* in 10 (12%) of the detected organisms. *Haemophilus influenzae* 06 (7%) and *Streptococcus pneumoniae* 04 (4.7%) were also detected in the same study [23]. These findings are almost comparable to the findings of this study.

In a study by Faron ML et al., BFPP identified 67% more total bacterial targets in BAL samples and 100% more targets in sputum samples compared to culture. Additionally, it identified multiple potential pathogens (up to 4) in 14% of BAL samples and 34% of sputum specimens, compared with 7% and 16% of routine cultures, respectively [24]. Results from the FilmArray may aid in the earlier identification of respiratory pathogens and optimisation of antibiotic therapy. Respiratory bacterial pathogens impact public health by affecting healthy and immunocompromised individuals, causing postviral infections in both community and hospital settings [25].

The worldwide problem of AMR particularly hampers developing countries due to high infectious disease exposure, antibiotic overconsumption, and poor quality of antibiotics [26]. Several studies have reported the emergence of Multi-Drug Resistant (MDR) bacterial pathogens from different sources, including humans, birds, and cattle, which highlights the need for routine application of antimicrobial susceptibility testing methods to screen and detect emerging MDR strains [27-30].

The present study also aimed to detect the aetiological agents and associated AMR genes (ARGs) early, so that prompt treatment could be initiated with the appropriate drug to inhibit the emergence of drug resistance. NDM production was found in 42 (25.9%) of the bacterial pathogens. Since the discovery of NDM-producing bacteria in 2008, NDM carbapenemases have been reported globally [31]. NDM enzymes confer resistance to almost all β-lactam drugs (except aztreonam), including carbapenems, which are often considered the drugs of last resort for the treatment of serious infections [31]. Treatment options for infections caused by NDM-producing bacteria are very limited. In a study, out of 356 clinical isolates, 160 showed carbapenem resistance, and among them, 131 displayed Metallo β-Lactamases (MBLs) production. PCR amplification confirmed 31 (23.6%) isolates carrying the blaNDM-1 gene in MBLs-producing organisms [32]. This finding was comparable to the results of the present study. Apart from a significant number of isolates producing NDM, the authors in this study also found CTX-M, VIM, OXA, and IMP in 32 (19.8%), 29 (18%), 27 (16.6%), and 13 (8%) isolates, respectively. The expression of these genes limits treatment options and often leads to poor prognosis. Additionally, the sensitivity for the detection of these genes is lower than conventional methods.

Klebsiella pneumoniae Carbapenemase (KPC) has been the most frequently encountered class A carbapenemase since its first description in the eastern USA in 1996 [31,33]. KPC is often associated with hospital and nosocomial infections, resulting in high morbidity and mortality [34]. In the present study, 6 (3.7%) KPC was detected out of 162 ARGs. In a study by Awoke T et al., the overall prevalence of carbapenemase production among total *K. pneumoniae* isolates was 28/132 (21.2%) using the modified Carbapenem Inactivation Method (mCIM) [35]. Despite a high disease burden, reports from India on the prevalence of resistance mechanisms in MDR *K. pneumoniae* isolates are limited [36].

Among the 14 *S. aureus* isolates detected in this study, 13 showed the presence of *mecA/mecC* or *MREJ* gene. These genes encode a Penicillin-Binding Protein (PBP2a) that leads to low affinity for beta-lactam antibiotics and are carried on a chromosomally integrated mobile genetic element known as the Staphylococcal Cassette Chromosome mec (SCCmec). To distinguish between Methicillin-Resistant *S. aureus* (MRSA) or co-detection of Methicillin-Sensitive *S. aureus* (MSSA) and another *Staphylococcus* spp. carrying the SCCmec cassette and mecA/mecC, the BFPP contains an additional assay that detects the SCCmec Right-Extremity Junction (MREJ), which links the SCCmec cassette to the *S. aureus* genome and indicates MRSA. In a study by Buchan BW et al., among 18 isolates with routine susceptibility results available, the BFPP demonstrated 81.8% (9/11) sensitivity and 85.7% (6/7) specificity for the identification of MRSA (based on the detection of both *mecA* and *mecC* and the SCCMREJ) [37].

As the world has witnessed the devastating waves of COVID-19, healthcare facilities were overcrowded even in developed countries [38]. The overwhelming number of cases increased the burden on frontline Healthcare Workers (HCWs) in patient-facing roles [39]. Although there is no documentation stating breaches in infection control practices in such overcrowded and overburdened healthcare facilities during the COVID-19 pandemic, the combination of increased workload and reduced numbers of HCWs is likely to strain the capacity to maintain essential healthcare services [40]. In such situations, the chances of nosocomial cross-contamination with MDR superbugs from one patient to another increase. In a study, it was observed that patients with Carbapenem-Resistant Enterobacteriaceae (CRE) infections were three times more likely to receive inappropriate empiric treatment than non CRE infections. In turn, inappropriate empiric treatment was associated with an adjusted increase in mortality of 12% (95% confidence interval 3% to 23%) and an excess of 5.2 days [41]. To combat this, early detection of aetiological agents and associated ARGs plays an important role.

With technically low demanding preprocessing of samples and a shorter turnaround time for detection, the BFPP may provide actionable information on antimicrobial susceptibility and ARGs for key pathogens causing pneumonia.

This multiplex PCR-based test has raised the expectations of clinical microbiology laboratories and clinicians regarding the possibilities for

rapid and accurate diagnosis of infective aetiology in respiratory tract infections. The upcoming years should see a continued increase in options for rapid, sensitive, and simple-to-perform molecular assays for infectious diseases.

However, despite the wide array of detection and many advantages of BFPP, there are a few limitations when using BFPP for respiratory samples:

1. The respiratory tract, being an unsterile site, has a lot of commensals, non pathogenic, or colonised flora, and sample collection is prone to oropharyngeal contamination. The challenge of interpretation from the pneumonia panel is determining if the organisms detected are clinically significant.
2. Determining whether the AMR genes that are detected are actually being expressed in the pathogen or not poses a challenge when considering treatment changes.
3. The cost of BioFire FilmArray is high compared to traditional methods and other commercially available molecular diagnostic tools.
4. Finally, the impact of new and emerging infections cannot be denied. In recent years, COVID-19 or the monkeypox virus reminded us of how quickly a new and emerging virus can appear and spread. The challenge for manufacturers is to regularly and timely accommodate potential emerging pathogens in the detection panel while maintaining cost-effectiveness.

Limitation(s)

The samples were processed only from hospitalised patients. Additionally, the sample size was low. The follow-up of the escalation or de-escalation of antibiotic therapy after the report of BFPP could not be tracked.

CONCLUSION(S)

Respiratory tract infections are caused by a wide array of pathogens, including viruses, typical bacteria, and atypical bacteria. In the treatment of these infections, drug resistance among gram negative pathogens poses a risk factor for inappropriate empiric treatment, subsequently increasing the risk of mortality. The BFPP tests for a wide range of pathogens, along with their associated AMR genes, promptly and with high sensitivity and specificity. This study has an impact on the timely detection of aetiological agents and associated antimicrobial-resistant genes that cause SARI in non COVID patients through the use of BFPP.

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REFERENCES

[1] WHO surveillance case definitions for ILI and SARI. https://cdn.who.int/media/docs/defaultsource/influenza/who_ili_sari_case_definitions_2014.pdf. Date of access: 14/05/2023.
[2] Aggarwal A, Shrivastava A, Kumar A, Ali A. Clinical and epidemiological features of SARS-CoV-2 patients in SARI ward of a tertiary care centre in New Delhi. J Assoc Physicians India. 2020;68(7):19-26.
[3] Houck PM, Bratzler DW, Nsa W, Ma A, Bartlett JG. Timing of antibiotic administration and outcomes for Medicare patients hospitalised with community-acquired pneumonia. Arch Int Med. 2004;164(6):637-44.
[4] O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. Davos Declaration, Declaration by the Pharmaceutical, Biotechnology and Diagnostics Industries on Combating Antimicrobial Resistance. 2016. [Online], Available at: http://amr-review.org/industry-declaration. page- 01-80.
[5] Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2019. US Department of Health and Human Services, Centers for Disease Control and Prevention; 2019. 01-140.
[6] Loeffelholz M, Chonmaitree T. Advances in diagnosis of respiratory virus infections. Int J Microbiol. 2010;2010:126049.
[7] Bartlett JG. Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis. 2011;52(suppl_4):S296-304.
[8] Feikin DR, Hammitt LL, Murdoch DR, O'Brien KL, Scott JA. The enduring challenge of determining pneumonia etiology in children: Considerations for future research priorities. Clin Infect Dis. 2017;64(suppl_3):S188-96.
[9] https://www.biofiredx.com/syndromictesting/#:~:text=The%20BioFire%20System%20utilises%20multiplex,cause%20overlapping%20signs%20and%20symptoms. Date of access: 15/09/2023.
[10] https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/. Date of access: 15/09/2023.
[11] Murray PR, Washington JA. Microscopic and bacteriologic analysis of expectorated sputum. In Mayo Clinic Proceedings. 1975;50(6):339-44.
[12] Zhu H, Wei L, Niu P. The novel coronavirus outbreak in Wuhan, China. Glob Health Res Policy. 2020;5:6.
[13] Baral SD, Rucinski KB, Twahirwa Rwema JO, Rao A, Prata Menezes N, Diouf D, et al. The relationship between the global burden of influenza from 2017 to 2019 and COVID-19: Descriptive epidemiological assessment. JMIR Public Health Surveill. 2021;7(3):24696.
[14] Machhi J, Herskovitz J, Senan AM, Dutta D, Nath B, Oleynikov MD, et al., The natural history, pathobiology, and clinical manifestations of SARS-CoV-2 infections. J Neuroimmune Pharmacol. 2020;15(3):359-86.
[15] Mirzaei R, Goodarzi P, Asadi M, Soltani A, Aljanabi HA, Jeda AS, et al., Bacterial co-infections with SARS-CoV-2. IUBMB life. 2020;72(10):2097-111.
[16] Guidelines on Clinical management of severe acute respiratory illness (SARI) in suspect/confirmed novel coronavirus (nCoV) cases. https://main.mohfw.gov.in/sites/default/files/Guidelines%20on%20Clinical%20management%20of%20severe%20acute%20respiratory%20illness.pdf. Date of access: 15/09/2023.
[17] Ueda T, Takesue Y, Nakajima K, Ichiki K, Ishikawa K, Yamada K, et al. Correlation between antimicrobial resistance and the hospital-wide diverse use of broad-spectrum antibiotics by the antimicrobial stewardship program in Japan. Pharmaceutics. 2023;15(2):518.
[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):00128-20.
[19] 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 centre. J Clin Microbiol. 2020;58(7):e00343-20.
[20] 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. IJID. 2020;95:326-31.
[21] Kyriazopoulou E, Karageorgos A, Liaskou-Antoniou L, Koufargyris P, Safarika A, Damoraki G, et al. BioFire® FilmArray® Pneumonia Panel for severe lower respiratory tract infections: Subgroup analysis of a randomized clinical trial. Infect Dis Ther. 2021;10(3):1437-49.
[22] Ginocchio CC, Garcia-Mondragon C, Mauerhofer B, Rindlisbacher C. Multinational evaluation of the BioFire® FilmArray® Pneumonia plus Panel as compared to standard of care testing. Eur J Clin Microbiol Infect Dis. 2021;40(8):1609-22.
[23] 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.
[24] 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. InClinical Virology Symposium 2017. [https://www.biofiredx.com/wp-content/uploads/2018/08/Faron-M-et-al.-CVS.-2017.pdf].
[25] Assefa M, Tigabu A, Belachew T, Tessema B. Bacterial profile, antimicrobial susceptibility patterns, and associated factors of community-acquired pneumonia among adult patients in Gondar, Northwest Ethiopia: A cross-sectional study. PloS one. 2022;17(2):0262956.
[26] Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob Resist Infect Control. 2017;6:47.
[27] Makharita RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections. Infect Drug Resist. 2020:3991-4002.
[28] Chinnnusamy N, Vedachalam D, Arumugam V. A study on bacteriological profile and antimicrobial susceptibility pattern of sputum samples in patients with lower respiratory tract infections a tertiary care hospital. Indian J Microbiol Res. 2016;3(1):27-30.
[29] Algammal AM, Hetta HF, Batiha GE, Hozzein WN, El Kazzaz WM, Hashem HR, et al. Virulence-determinants and antibiotic-resistance genes of MDR-E. coli isolated from secondary infections following FMD-outbreak in cattle. Scientific Reports. 2020;10(1):19779.
[30] Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:249856.
[31] Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother. 2009;53(12):5046-54.
[32] Nahid F, Khan AA, Rehman S, Zahra R. Prevalence of metallo-β-lactamase NDM-1-producing multi-drug resistant bacteria at two Pakistani hospitals and implications for public health. J Infect Public Health. 2013;6(6):487-93.

[33] Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: Epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016;7:895.
[34] Ghaith DM, Zafer MM, Said HM, Elanwary S, Elsaban S, Al-Agamy MH, et al. Genetic diversity of carbapenem-resistant Klebsiella Pneumoniae causing neonatal sepsis in intensive care unit, Cairo, Egypt. Eur J Clin Microbiol Infect Dis. 2020;39(3):583-91.
[35] Awoke T, Teka B, Aseffa A, Sebre S, Seman A, Yeshitela B, et al. Detection of bla KPC and bla NDM carbapenemase genes among Klebsiella pneumoniae isolates in Addis Ababa, Ethiopia: Dominance of bla NDM. Plos one. 2022;17(4):0267657.
[36] Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathogens and Global Health. 2017;111(5):240-46.
[37] 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 hospitalised patients with lower respiratory tract infections. J Clin Microbiol. 2020;58(7):e00135-20.
[38] Sandhu P, Shah AB, Ahmad FB, Kerr J, Demeke HB, Graeden E, et al. Emergency department and intensive care unit overcrowding and ventilator shortages in US hospitals during the COVID-19 pandemic, 2020-2021. Public Health Reports. 2022;137(4):796-802.
[39] Nguyen LH, Drew DA, Graham MS, Joshi AD, Guo CG, Ma W, et al. Risk of COVID-19 among front-line health-care workers and the general community: A prospective cohort study. The Lancet Public Health. 2020;5(9):e475-83.
[40] Mersha A, Shibiru S, Girma M, Ayele G, Bante A, Kassa M, et al. Perceived barriers to the practice of preventive measures for COVID-19 pandemic among health professionals in public health facilities of the Gamo zone, southern Ethiopia: A phenomenological study. BMC Public Health. 2021;21(1):199.
[41] Zilberberg MD, Nathanson BH, Sulham K, Fan W, Shorr AF. Carbapenem resistance, inappropriate empiric treatment and outcomes among patients hospitalised with Enterobacteriaceae urinary tract infection, pneumonia and sepsis. BMC Infect Dis. 2017;17(1):01-03.

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