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Research Article

Detection of viral and bacterial infections by means of Filmarray at a Tertiary Hospital in Greece

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Abstract

Introduction: Respiratory Viruses (RVs) and bacteria are the main cause of pulmonary infections that affect the community population. They consist major public health concern with high impact in the health care systems. Their seasonal circulation is of great importance for the public health sector.

Aim: The aim of the present study is to investigate the presence of RVs and bacteria in patients who visited the outpatients department, as well as in patients hospitalized at the GHNP "Agios Panteleimon" during 2024.

Materials and Methods: In the study 483 patients, 272 male (56.3%) and 271 female (43.7%) were included. Patient age ranged from 1 to 98 years (mean 69.46 y; SD 13.49 y). Samples were collected from January to December 2024. Identification of bacteria *(Bordetella pertussis, Mycoplasma pneumonia), and RVs (Adenovirus, Coronavirus 229E, Coronavirus HKU 1, Coronavirus NL 63, Coronavirus OCH3, Coronavirus OCK3, Human metapneumovirus, Influenza A, Influenza AH3, Influenza B, Parainfluenza 3 virus, Parainfluenza 1 virus, RSV and SARS-CoV-2)* was performed by the FILMARRAY Multiplex PCR System.

Results: From epidemiological aspect maximum peak occurred in March, following Christmas and winter vacation, as well as the effect of low temperature and elevated humidity levels during winter months. During spring and early summer we observed also a significant increase in infections, due to climate change in the country. Moreover, a significant elevation of infections was observed during August, September and October, following summer vacation, as well as the return to school and work, and the consequent close contact in classrooms and working areas. Statistical significance between the result positivity with patient gender or patient age was not observed.

Conclusions: Our study revealed seasonality of the infections. Early detection and surveillance of viral and bacterial infection can help in better organization, management and economic efficiency for the health care system.

Keywords: Filmarray, Multiplex PCR, viruses, bacteria, influenza, Corona virus, RSV

Introduction

Rapid and accurate identification of viral and bacterial pathogens in clinical specimens remains a central challenge in contemporary infectious disease diagnostics [1-3]. Traditional culture-based and immunoassay methods are often limited by long turnaround times, variable sensitivity, and dependence on specialist laboratory infrastructure, which can delay appropriate clinical management and antimicrobial stewardship decisions [4-7]. Molecular multiplex assays- also known as syndromic diagnostic tests- have emerged as a transformative approach in this context, enabling simultaneous detection of multiple targets with high analytical sensitivity and specificity directly from patient samples [8-15].

The FilmArray system (BioFire Diagnostics, bioMérieux) is a fully automated multiplex polymerase chain reaction (PCR) platform that integrates nucleic acid extraction, amplification, and detection within a closed pouch format. This design significantly reduces hands-on time and contamination risk while providing results in approximately one hour, facilitating syndromic pathogen identification across a range of clinical syndromes [16-20]. The FilmArray Respiratory Panel (RP and RP2.1) can detect an extensive panel of viral and bacterial respiratory pathogens, demonstrating robust performance in comparative studies and enabling identification of co-infections that might be missed by conventional methods *(e.g., adenoviruses, influenza viruses, common coronaviruses, Mycoplasma pneumoniae, Bordetella pertussis)* and support timely epidemiological insights [21-24]. The rapid turnaround and broad target range have also been eval-

uated in disease-specific contexts such as meningitis/encephalitis, where FilmArray's multiplex PCR panel for cerebrospinal fluid improved pathogen detection rates compared with standard diagnostics, although challenges remain in interpretation of false positives and negatives [25, 26].

In bloodstream infections, the Film Array Blood Culture Identification 2 (BCID2) panel has been shown to identify bacterial and fungal pathogens along with key antimicrobial resistance markers with high concordance to conventional methods, while substantially reducing time to result, which is critical for optimizing antimicrobial therapy [3, 27, 28]. Multiple studies in respiratory and bloodstream infection settings have confirmed that implementation of Film Array panels can influence clinical decision making, reduce time to appropriate therapy, and enhance diagnostic yield compared with traditional culture-based methods [29, 30].

Given the continued evolution of multiplex molecular diagnostics and their increasing integration into routine hospital workflows, it is essential to characterize the performance, clinical utility, and limitations of Film Array technology in the detection of viral and bacterial infections within specific healthcare settings. In this retrospective study, we aimed to characterize the spectrum of respiratory viral and bacterial pathogens identified by the Film Array system in a Greek tertiary hospital during 2024 and to highlight the potential role of syndromic testing in hospital-based surveillance.

Objective

The aim of the present retrospective study is to investigate the presence of RVs and bacteria in patients who visited the outpatients department, as well as in patients hospitalized at a tertiary hospital in Piraeus region, Greece, during 2024.

Material and methods

In the present retrospective study 483 patients, 272 male (56.3%) and 211 female (43.7%) were included. Patient age ranged from 1 to 98 years (mean 69.46 y; SD 13.49 y). Samples were collected from January to December as follows: January 18 samples, February 54, March 75, April 53, May 53, June 50, July 26, August 41, September 37, October 42, November 26 and December 8 samples. Among them 3 referrals were from the Cardiology department, 4 from the Neonatology, 353 from the Pulmonology, 3 from the Neurology, 105 from the Pathology, 3 from the Pediatrics, 1 from the Psychiatry, 1 from the Surgery, 1 from the ICU, 3 from the ED and 5 outpatients. The identification of bacteria and viruses was performed by means of FILM ARRAY Multiplex PCR System.

The study was conducted in accordance with the Declaration of Helsinki. All data were collected and processed in compliance with the General Data Protection Regulation (GDPR). Participant anonymity and confidentiality were strictly maintained, and no personally identifiable information was recorded. Informed consent was not required due to the retrospective nature of the study.

Results

Out of 483 patients, 272 were male (56.3%) and were 211 female (43.7%), Figure 1.

Single infections involving one pathogen were detected in 86 patients (Table 1), while mixed infections involving two or more pathogens were detected in 44 patients (Table 2). The annual distribution of the samples included in the study is presented in Table 3.

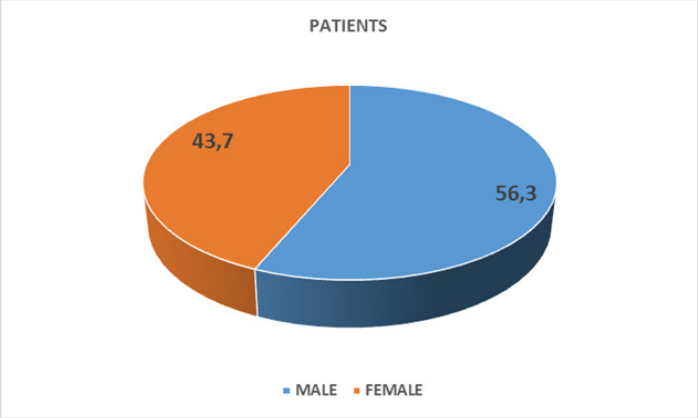

Figure 1. Gender distribution of the study population.

Table 1: Number of patients samples with single infections, involving only one pathogen.

SINGLE INFECTIONS	
Adenovirus	4
Bordetella pertussis	3
Corona virus 229E	2
Corona virus HKU1	1
Corona virus OCH3	1
Corona virus OCK3	2
Human metapneumovirus	11
Influenza A,	9
Influenza AH3	1
Influenza B	4
Parainfluenza 3 virus	9
Parainfluenza 1 virus	3
Mycoplasma pneumoniae	2
RSV	8
SARS-CoV-2	17
Infulenza A H1-2002	1
Corona virus NL 63	5
Corona virus OC43	3
TOTAL	**86**

Table 2: Number of patient samples with mixed infections, involving two or more pathogens.

MIXED INFECTIONS	
Influenza B/Bordetella pertussis	1
Adenovirus/Corona virus	1
Adenovirus/Corona virus 229 E	1
Corona virus OC43/Corona virus_NL¬_63	1
Rhinovirus/Enterovirus	31

Adenovirus/Rhinovirus/Entero-virus	1
Corona virusOC43/Rhinovirus/ Enterovirus	6
Corona virus 229E/Rhinovirus/ Enterovirus	1
Rhinovirus/Enterovirus/Parain-fluenza1	1
TOTAL	**44**

Table 3: Annual distribution of the samples included in the study.

NUMBER OF SAMPLES	
JANUARY	18
FEBRUARY	54
MARCH	75
APRIL	53
MAY	53
JUNE	50
JULY	26
AUGUST	41
SEPTEMBER	37
OCTOBER	42
NOVEMBER	26
DECEMBER	8
TOTAL	**483**

353 (73.1 %) samples were negative and 130 (26.9 %) were positive as follows: 4 Adenovirus cases, 3 Bordetella pertussis, 2 Corona virus 229E, 1 Corona virus HKU1, 1 Corona virus OCH3, 2 Corona virus OCK3, 11 Human metapneumovirus, 9 Influenza A, 1 Influenza AH3, 4 Influenza B, 9 Parainfluenza 3 virus, 3 Parainfluenza 1 virus, 2 Mycoplasma pneumoniae, 8 RSV, 17 SARS-COV-2, 1 Infulenza A H1-2002, 5 Corona virus NL 63 and 3 Corona virus OC43 cases.

Among the positive samples, 44 cases of mixed infection were detected as follows: 1 Influenza B/Bordetella pertussis, 1Adenovirus/Corona virus, 1 Adenovirus/Corona virus 229 E, 1 Corono virus OC43/Corona virus_ NL¬_63, 31 Rhinovirus/Enterovirus, 1 Adenovirus/Rhinovirus/Enterovirus, 6 Corono virusOC43/Rhinovirus/Enterovirus, 1 Corona virus229E/ Rhinovirus/Enterovirus and 1 Rhinovirus/ Enterovirus/ Parainfluenza1 case.

We did not detect any statistical significance between the result positivity with patient gender or patient age.

Discussion

Our study revealed seasonality of the infections. Maximum peak occurred in March, following Christmas and winter vacation, as well as the effect of low temperature and elevated humidity levels during winter months. During spring and early summer (April, May and June) we observe also a significant increase of the infections, due to climate change. Moreover, a significant elevation is observed during August, September and October, following summer vacation, as well as the return to school and work, and the consequent close contact in classrooms and working areas.

The present retrospective study provides important real-world evidence on the epidemiology of respiratory viral and atypical bacterial infections detected using the Film Array multiplex PCR platform in a Greek tertiary hospital during 2024. By characterizing pathogen distribution, co-infections, departmental burden, and seasonal trends, our findings contribute to the growing international literature supporting the role of syndromic molecular diagnostics in contemporary infectious disease practice.

Overall, approximately one quarter of analyzed respiratory specimens yielded a positive result, while the majority remained negative. This diagnostic yield is in line with international hospital-based evaluations of syndromic multiplex respiratory panels, which commonly report positivity rates between 20% and 40% depending on patient selection and seasonal circulation patterns [16, 21].

The high proportion of negative samples may reflect the multi factorial nature of respiratory syndromes in hospitalized elderly populations, the timing of sampling in relation to symptom onset, and the restricted target spectrum of the assay, underscoring the importance of integrating molecular results with clinical assessment and diagnostic stewardship principles [19].

A notable characteristic of our population was the advanced mean age (≈69 years) and the predominance of referrals from Pulmonology and Internal Medicine wards, highlighting the substantial burden of respiratory viral infections among older hospitalized adults. This finding aligns with international evidence showing that common respiratory viruses, including influenza, RSV, and seasonal corona viruses, remain major drivers of hospitalization and healthcare strain in elderly patients beyond the pandemic period [21]. Importantly, SARS-CoV-2 remained among the most frequently detected pathogens, supporting ongoing viral circulation and clinical relevance in the post–COVID-19 era.

Mixed infections accounted for a considerable proportion of positive cases, with *rhinovirus/enterovirus* co-detection being particularly prominent. Co-infections are increasingly recognized as a frequent finding in multiplex PCR testing, reflecting the ability of syndromic panels to identify pathogens that may be missed by conventional diagnostic workflows [15, 21]. However, interpretation of co-detections requires caution, as nucleic acid detection does not always indicate active causality and may occasionally represent prolonged shedding or incidental carriage, especially in older or hospitalized patients [17].

Finally, no statistically significant association was observed between test positivity and patient age or gender. Similar observations have been reported in other retrospective evaluations, suggesting that pathogen circulation is more strongly influenced by seasonal and epidemiological factors than by demographic variables alone within hospitalized cohorts [16]. Overall, these findings reinforce the value of Film Array-based syndromic testing as a rapid tool for pathogen surveillance and etiological clarification in routine hospital practice [8].

Importantly, negative results do not necessarily exclude infection, as syndromic panels are limited to predefined targets and may miss emerging pathogens, low viral load infections, or non-infectious causes of respiratory symptoms. Such interpretive challenges have been widely emphasized in diagnostic stewardship frameworks [19].

A major strength of our study lies in the detailed description of etiological agents circulating in the post COVID-19 period. SARS-CoV-2 remained among the most frequently detected pathogens, confirming its continued clinical impact beyond the pandemic peak. Concurrently, influenza viruses, RSV, human metapneumovirus, parainfluenza viruses, adenovirus, and seasonal corona viruses were also identified, demonstrating the re-establishment of the broader respiratory viral ecosystem. This pattern is consistent with international surveillance studies showing the resurgence of influenza and RSV following the relaxation of pandemic-related public health measures [21].

Notably, the detection of atypical bacterial pathogens such as *Mycoplasma pneumoniae* and *Bordetella pertussis*, although infrequent, highlights the diagnostic advantage of multiplex PCR assays over conventional culture-based workflows. These organisms are difficult to isolate with routine techniques, and delayed recognition may contribute to inappropriate antimicrobial exposure or missed opportunities for targeted therapy. Expert consensus documents increasingly recognize rapid multiplex syndromic testing as transformative, particularly in critically ill patients where early etiological clarification is essential [8, 15].

An important finding in our study population (patient sample) was the high proportion of mixed infections, with *rhinovirus/enterovirus* co-detection being particularly prominent. Co-infections have been repeatedly reported in international Film Array evaluations, especially among elderly and hospitalized populations, and represent a key benefit of broad multiplex testing compared with single-target PCR approaches [21]. However, co-detection also introduces interpretive complexity, since nucleic acid detection does not always imply active pathogenicity. Persistent shedding, colonization, or incidental viral carriage may complicate clinical decision making, reinforcing the need for careful correlation with symptoms and radiological findings [17].

International evidence supports that syndromic multiplex respiratory panels can provide earlier pathogen identification and higher diagnostic yield than conventional culture-based approaches, particularly in hospitalized patients [31, 32]. Rapid turnaround can facilitate earlier infection control interventions, improved cohorting of patients, and reduction of unnecessary antibiotic prescribing. Similarly, in bloodstream infections, Film Array BCID2 implementation has shown high concordance with standard methods while significantly reducing time to pathogen identification and resistance marker detection [27, 28, 33]. In stewardship-guided environments, rapid diagnostics such as the Film Array BCID2 panel have been shown to shorten time to actionable microbiological results and facilitate earlier antimicrobial optimization compared with conventional workflows [27, 28].

Although our study did not include outcome variables such as morbidity, mortality, or antimicrobial modifications, international evidence suggests that the principal value of Film Array testing lies in its ability to influence clinical decision making early in the diagnostic pathway. For example, respiratory panel testing has been linked to reductions in antibiotic days of therapy and more appropriate antiviral use in diverse clinical settings [34]. Therefore, the diagnostic patterns observed in our hospital likely carry meaningful implications for patient management even if direct outcome measures were not available.

A further contribution of this work is the demonstration of clear seasonal variation [35].Peaks during late winter and early spring were observed, consistent with established respiratory virus circulation dynamics in temperate climates, where low temperature, indoor crowding, and humidity changes promote viral transmission. Additional increases during late summer and autumn may reflect population mixing after vacation periods and school reopening, trends also documented in international epidemiological studies [36, 37].

Continuous multiplex surveillance may therefore support hospital preparedness, staffing allocation, and infection prevention planning [38-40]. Departmental distribution of testing revealed that the majority of specimens originated from Pulmonology and Internal Medicine wards, reflecting the substantial burden of respiratory infections among hospitalized adults and elderly patients. Given the advanced mean age of thepatient sample (study population), these findings align with global data demonstrating that respiratory viruses remain a major cause of hospitalization and healthcare strain in older populations even outside pandemic conditions [39-42].

Clinical implications for Greece

The implementation of rapid syndromic molecular diagnostics such as Film Array has particular relevance for the Greek healthcare system [43-45].

Greece, like many Southern European countries, faces seasonal surges of respiratory infections, high antibiotic consumption rates, and increasing antimicrobial resistance pressures [46-51].

Rapid multiplex testing may support earlier differentiation between viral and bacterial etiologies, enabling improved antimicrobial stewardship and reducing unnecessary antibiotic exposure, a critical priority for national public health [52]. Moreover, hospital-based molecular surveillance can provide valuable epidemiological intelligence for regional preparedness, especially in the context of tourism-driven population mobility and climate-related shifts in respiratory virus seasonality [45, 53]. Expanding access to multiplex diagnostics across Greek hospitals could therefore strengthen both patient-level management and national infectious disease surveillance capacity [54].

Limitations

Several limitations and potential sources of bias should be acknowledged in this study. First, the retrospective single-center design restricts the generalizability of our findings and prevents causal inference regarding the clinical impact of Film Array testing. In addition, selection and referral bias may be present, as multiplex PCR was primarily requested for hospitalized patients particularly from Pulmonology and Internal Medicine wards, therefore not fully representing the broader community population or other hospital departments. Second, the absence of detailed clinical outcome data, including antimicrobial therapy modifications, length of hospitalization, morbidity, or mortality, limits the ability to evaluate patient-centered benefits or the direct influence of syndromic testing on clinical decision making. Third, multiplex PCR assays detect microbial nucleic acid rather than viable organisms; consequently, positive results may not always reflect active infection, and co-detections may occasionally represent prolonged viral shedding or incidental carriage rather than true etiological relevance. Furthermore, the restricted target range of the assay may contribute to negative results, as pathogens outside the panel remain undetectable. Finally, the uneven distribution of samples across months may have influenced the observed seasonal patterns, underscoring the importance of interpreting epidemiological trends within the context of testing practices and diagnostic stewardship [19].

Conclusions

Despite these limitations, our retrospective study provides valuable post-pandemic real-world evidence on the etiological spectrum, co-infection burden, and seasonal dynamics of respiratory pathogens detected by Film Array technology in a Greek tertiary hospital during 2024. The diversity of identified viruses, the detection of atypical bacteria, and the frequency of mixed infections underline the diagnostic utility of syndromic multiplex PCR not only for individual patient evaluation but also for hospital epidemiological surveillance. Future prospective multicenter studies incorporating antimicrobial stewardship metrics and clinical outcomes are warranted to further define the full impact of multiplex diagnostics in Greece and internationally.

Author contributions

Antonia Mourtzikou wrote the initial and final versions of the manuscript. Christina Seitopoulou conducted the laboratory analysis.Georgia Kalliora performed the literature search.Marilena Stamouli provided the statistical analysis.

All authors contributed equally to the discussion of the content and to

writing, reviewing, and editing the manuscript prior to submission.

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Conflict of Interest

The authors declared no conflict of interest.

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