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Epidemiology of Lower Respiratory Tract Infections in Hospitalized Patients Using Multiplex PCR in Central Greece

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Abstract

Objective. This study investigated the epidemiology of LRTIs using multiplex Polymerase Chain Reaction (mPCR) at the General Hospital of Larissa. **Methods.** This retrospective observational study was conducted from January 2021 to June 2024 and included 210 patients with suspected LRTIs. Respiratory specimens, comprising bronchoalveolar lavage and sputum samples, were analyzed using the Biofire Filmarray Pneumonia Panel Plus (BFPP; Biofire, bioMérieux), which detects a wide spectrum of bacterial, viral, and atypical respiratory pathogens. **Results.** The most frequently detected pathogens were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, predominantly in patients admitted to the Intensive Care Unit. In contrast, specimens from the Department of Internal Medicine most often yielded *Haemophilus influenzae*, *Staphylococcus aureus*, and human rhinovirus/enterovirus. Overall, bacterial pathogens were more prevalent than viral pathogens. The use of mPCR enabled the rapid detection of respiratory pathogens and provided critical epidemiological insights into LRTI distribution across hospital departments. **Conclusions.** mPCR is a useful adjunctive tool for the detection of respiratory pathogens in patients with LRTIs and may support clinical decision-making if interpreted in the clinical context. The integration of molecular diagnostics into routine clinical practice may improve infection management and surveillance. Further multicenter studies and comparisons with conventional diagnostic methods are warranted to evaluate its impact on antimicrobial stewardship, clinical decision-making, and patient outcomes.

Key Words: Lower Respiratory Tract Infections ▪ Multiplex PCR ▪ Molecular Diagnostics.

Introduction

Lower respiratory tract infections (LRTIs) are a major global health concern and a leading cause of infectious disease-related morbidity and mortality (1). Anatomically, LRTIs are categorized into bronchitis and pneumonia, with pneumonia further classified by acquisition setting into community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP). This classification is essential, as etiological agents, diagnostic approaches, and therapeutic strategies differ. CAP occurs outside the healthcare system, whereas HAP develops ≥48 hours after admission, and VAP affects mechanically ventilated patients (2). Identifying the causative agent is challenging due to polymicrobial infections and substantial variability in microbial composition (3).

Immunocompromised individuals, including cancer patients, face an increased risk of LRTI, which frequently progresses to acute respiratory failure and sepsis. Diagnosing suspected LRTI infections in immunocompromised individuals is also difficult, given the extensive spectrum of potential viral, bacterial, and non-infectious causative conditions that can mimic one another (4). HAP and VAP are typically caused by Gram-negative bacilli (e.g., Enterobacteriaceae, *P. aeruginosa*, *A. baumannii*)

and *S. aureus* (including MRSA). Viral pathogens, such as RSV, influenza viruses, and adenoviruses, have also been implicated, particularly in pediatric and ventilated populations. In immunosuppressed hosts, fungal agents such as *Pneumocystis jirovecii*, *Aspergillus* spp., and *Cryptococcus* spp. pose additional risks (5).

Diagnosis relies on clinical presentation, radiographic findings, and microbiological confirmation via sputum and blood cultures, real-time PCR, and emerging technologies such as next-generation sequencing (NGS), which improve pathogen detection and resistance profiling (6, 7).

Treatment must be tailored to the clinical context, suspected pathogens, and resistance patterns. For CAP requiring hospitalization, first-line therapy includes β-lactam plus macrolide or respiratory fluoroquinolone monotherapy. For HAP/VAP, initial broad-spectrum empirical therapy—guided by the risk of multidrug-resistant (MDR) pathogens—is essential, with de-escalation once microbiology results are available. Preferred regimens for early-onset HAP or low-risk VAP include ceftriaxone, ampicillin-sulbactam, ertapenem, and fluoroquinolones. Late-onset or high-risk cases require antipseudomonal β-lactams (e.g., cefepime, meropenem, or piperacillin-tazobactam) plus antipseudomonal fluoroquinolone or aminoglycoside, with linezolid or vancomycin added if MRSA is suspected (8). The WHO and expert panels emphasize rational antimicrobial use to minimize resistance (9). Peralta et al. reported that inappropriate empirical therapy increased from 3% in susceptible infections to 35% in MDR cases, accompanied by higher mortality (10).

Early and accurate diagnosis, coupled with prompt administration of appropriate antimicrobial therapy, remains a cornerstone in the management of critically ill patients, playing a pivotal role in reducing both morbidity and mortality. Nevertheless, the escalating incidence of antimicrobial-resistant organisms in intensive care units represents a significant and evolving threat to optimal therapeutic outcomes (11, 12).

Rapid molecular diagnostic techniques have emerged as essential tools for the timely management of severe lower respiratory tract infections. The BioFire FilmArray Pneumonia Panel Plus (BFPP) is a notable example that offers expedited identification of a wide range of respiratory pathogens along with key antimicrobial resistance markers directly from clinical specimens. The BFPP pouch is a self-contained, single-use device that includes all the necessary components for nucleic acid extraction, purification, reverse transcription, and PCR. Once the pouch is prepared, it is placed into the instrument, which then automatically carries out the remaining procedures, including nucleic acid extraction and purification, nested multiplex PCR, and detection of each target in the panel (13).

Despite its diagnostic potential, the optimal timing of its use remains unclear. Furthermore, the available data regarding its role in supporting antimicrobial stewardship remain limited. However, given the critical need for early targeted therapy and the increasing prevalence of multidrug-resistant organisms, the incorporation of BFPP into routine clinical practice may provide significant benefits by improving diagnostic accuracy, enabling prompt pathogen-directed treatment, and reducing the unnecessary use of broad-spectrum antibiotics (14).

This study aimed to investigate the causes of lower respiratory tract infections and assess the frequency of pathogens potentially involved in the etiology of these infections using BFPP. Additionally, we compared the most prevalent pathogens between the ICU and internal medicine departments. Demographic data and medical histories were analyzed to assess the impact of comorbidities on these infections and their association with hospitalization outcomes.

Materials and Methods

This retrospective observational study was conducted at the General Hospital of Larissa, Greece, from January 2021 to June 2024, and a priori sample size calculation was not performed. All eligible patients during the study period were included, specifically only patients for whom multiplex PCR testing was requested by the treating clinician

based on their judgment. Therefore, the study population represents a selected subgroup of hospitalized patients with suspected LRTI. Lower respiratory tract samples were defined as sputum specimens confirmed by Gram staining or bronchoalveolar lavage (BAL) fluid. Sputum specimens with ≥10 squamous epithelial cells—specifically, counts equal to or exceeding 10 per low-power field (10× objective) were excluded, as this finding suggests contamination with oropharyngeal flora (15). When multiple samples originated from the same patient, duplicates were removed so that only one sample per individual was analyzed.

Statistical analyses of the clinical outcomes were performed at the patient level. Analyses of pathogen frequency were based on detection events. As multiple organisms were identified in several samples, these observations were not considered independent. Multiplex PCR was performed using the BioFire® FilmArray® Pneumonia Panel Plus, a rapid assay designed to detect pathogens associated with pneumonia and other lower respiratory tract infections. It can identify 34 targets, including 14 bacterial species, 3 atypical bacteria, 9 viruses, and 6 antimicrobial resistance genes, and provides results within approximately one hour (Table 1). Additionally, for 15 bacterial pathogens, the assay provides semi-quantitative outputs that help differentiate colonization from active infection. All BioFire FilmArray Pneumonia Panel (BFPP) tests were performed according to the manufacturer's guidelines, with an overall diagnostic turnaround time of approximately 60 minutes.

Upon completion, the BFPP reports either quantitative results, expressed as copies of bacterial genomic material per milliliter (copies/ml), or categorical (binned) values, such as "not detected", $<10^3$, 10^4, 10^5, 10^6, and $\geq 10^7$ copies/ml (16). High bacterial loads typically indicate active infection and assist clinicians in determining the most likely causative pathogen and providing targeted therapy, especially when multiple microbial targets are detected (17).

Ethics Statement

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Review Board of the Aristotle University of Thessaloniki (Approval No. 20/2024, approved on 1/12.11.2024).

Statistical Analyses

Demographic analyses of the samples (N=210) by year, sample type, gender, age, and clinical origin are presented in Table 2. An increasing trend was observed from 2021 to 2023, with the highest percentage of samples (30.5%) recorded in 2023. Although an increase in testing volume was observed over time, no formal time-trend statistical analyses were conducted. Therefore, conclusions regarding temporal changes should be considered descriptive. There were 110 (52.5%) BAL samples and 100 (47.4%) sputum samples. The distribution of samples by gender showed that males represented 64.3% of the

Table 1. PneumoPlus Panel Targets

Category	Organisms
Bacteria (semi-quantitative in copies/ml)	*Acinetobacter calcoaceticus-baumannii complex, Enterobacter cloacae complex, Escherichia coli, Haemophilus influenzae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae group, Moraxella catarrhalis, Proteus* spp., *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes*
Atypical bacteria (qualitative)	*Chlamydia pneumoniae, Legionella pneumophila, Mycoplasma pneumoniae*
Viruses	Adenovirus, Coronavirus, Human metapneumovirus, Human rhinovirus/enterovirus, Influenza A virus, Influenza B virus, Parainfluenza virus, Respiratory syncytial virus
Antimicrobial resistance genes	Carbapenemases: IMP, KPC, NDM, OXA-48-like, VIM; ESBL: CTX-M; Methicillin resistance: mecA/C and MREJ

IMP=*Active-on-imipenem;* KPC=*Klebsiella pneumoniae carbapenemase; NDM=New Delhi metallo-β-lactamase; OXA-48-like=Oxacillinase-48-like carbapenemases; VIM=Verona integron-encoded metallo-β-lactamase; ESBL=Extended-spectrum β-lactamase; CTX-M=Cefotaximase-Munich; Methicillin-resistance gene A/C=Methicillin-resistance gene C; MREJ=Methicillin-resistant Staphylococcus aureus element*

Table 2. Distribution of Samples by Year, Type, Gender, and Hospital Ward

Distribution		N (%)
Year	2021	36 (17.2)
	2022	53 (25.2)
	2023	64 (30.5)
	2024	57 (27.1)
Sample type	Sputum	100 (47.4)
	BAL	110 (52.6)
Gender	Male	135 (64.3)
	Female	75 (35.7)
Ward	Intensive care unit	145 (69.0)
	Pediatric surgery	1 (0.5)
	Pediatrics	12 (5.9)
	Internal medicine	45 (21.2)
	Cardiology	6 (2.9)
	General surgery	1 (0.5)

BAL=Bronchoalveolar lavage.

samples (135), whereas females accounted for 35.7% (75). The age of the individuals included in the study showed significant variation, with a minimum age of 2 years and a maximum age of 93 years. The mean age was 58.09 years, indicating that most participants were middle-aged or elderly. The standard deviation was 20.76 years, demonstrating considerable age dispersion, with ages ranging from very young to elderly. Many samples included in the study were from the ICU (145/210; 69.0%), followed by the internal medicine department (45/210; 21.2%) and pediatrics (12/210; 5.9%).

Laboratory Results

Multiplex PCR results indicated that of the 210 samples, 158 were positive (75.7%), and the remaining 52 (24.3%) were negative. Of the 158 positive PCR results, 97 (61.4%) included more than one microorganism, whereas the remaining 61 (38.6%) identified only one. The distribution of microorganisms among the positive samples revealed that 227 of the total pathogens detected by PCR (46.9%) were Gram-negative bacteria. Moreover, 64 viruses (13% of detected pathogens) and 38 Gram-positive microorganisms (8% of detected pathogens) were identified. The detection of atypical microorganisms was minimal (3 of the detected pathogens; 0.6%) (Figure 1a).

Table 3. Total PCR Results of Study Samples

PCR target	Number of detections	% of all detected PCR targets*	% of positive samples†
Escherichia coli	7	1.4	4.4
Klebsiella pneumoniae	48	9.9	30.2
Klebsiella oxytoca	1	0.2	0.6
Acinetobacter baumannii	51	10.5	32.1
Enterobacter cloacae	5	1.0	3.1
Klebsiella aerogenes	2	0.4	1.3
Pseudomonas aeruginosa	56	11.5	35.2
Moraxella catarrhalis	9	1.9	5.7
Proteus mirabilis	10	2.1	6.3
Serratia marcescens	8	1.6	5.0
Haemophilus influenzae	30	6.2	18.9
Staphylococcus aureus	16	3.3	10.1
Streptococcus agalactiae	4	0.8	2.5
Streptococcus pneumoniae	15	3.1	9.4
Streptococcus pyogenes	4	0.8	2.5
Legionella pneumophila	3	0.6	1.9
Adenovirus	3	0.6	1.9
Coronavirus	6	1.2	3.8
HMPV virus	3	0.6	1.9
Rhinovirus/Enterovirus	26	5.4	16.4
Influenza A virus	10	2.1	6.3
Influenza B virus	1	0.2	0.6
Parainfluenza virus	5	1.0	3.1
RSV virus	9	1.9	5.7
CTX-M	30	6.2	18.9
KPC	34	7.0	21.4
NDM	38	7.8	23.9
VIM	47	9.7	29.6
mecA/C and MREJ	4	0.8	2.5

*Percentages in the third column use all detected PCR targets as the denominator (N=486); †Percentages in the fourth column use the number of samples with a positive PCR result as the denominator (N=158). CTX-M=*Cefotaximase-Munich; KPC=Klebsiella pneumoniae carbapenemase; NDM=New Delhi metallo-β-lactamase; mecA=Methicillin-resistance gene A; mecC=Methicillin-resistance gene C; MREJ=Methicillin-resistant Staphylococcus aureus element.*

Specifically, as shown in Table 3, the most frequently detected pathogen was *Pseudomonas*

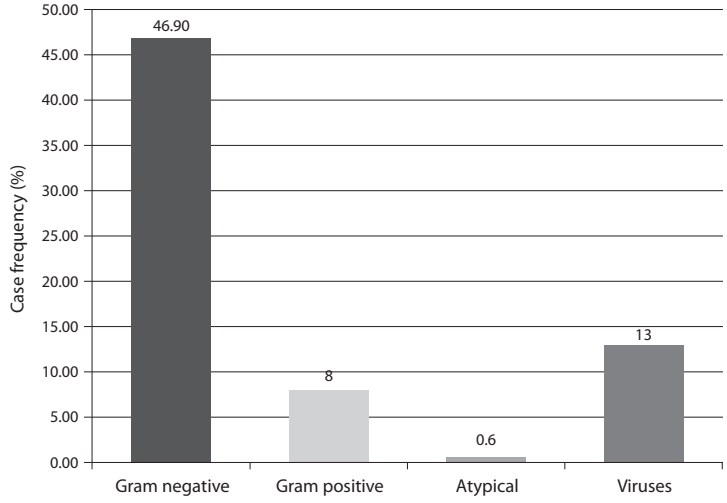

Figure 1a. Percentage distribution of pathogen categories detected by PCR. Percentages were calculated using all detected PCR targets as the denominator (N=333).

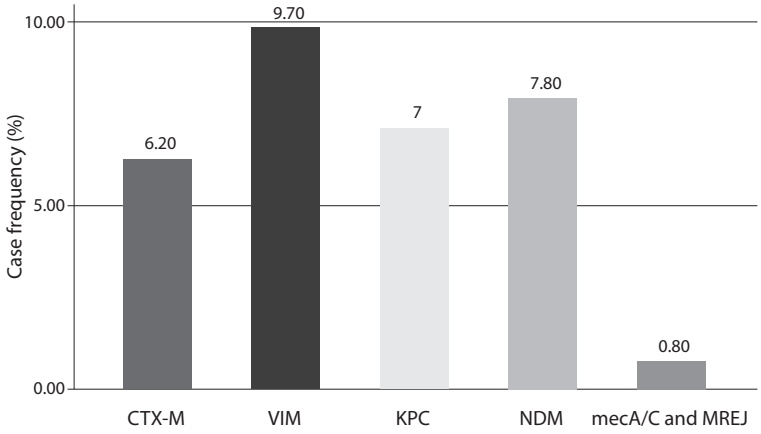

Figure 1b. Percentage distribution of resistance genes detected by PCR. Percentages were calculated using all resistance-gene targets as the denominator (N=153).

aeruginosa, identified in 56 samples, followed by *Acinetobacter baumannii* in 51 samples, and *Klebsiella pneumoniae* in 48 samples. In addition, an exceptional observation was that *Haemophilus influenzae* was identified in 30 samples. The most frequent viral target was human rhinovirus/enterovirus, which was detected in 26 samples. Antimicrobial resistance genes were detected in 66 (31.5 %) samples. bla_{VIM} was detected in 47 samples. NDM was present in 38 samples, KPC in 34 samples, and CTX-M in 30 samples (Figure 1b).

Statistical Analysis of the ICU and Internal Medicine Department Data

Since most patients included in the study were hospitalized in the ICU and internal medicine department, further analysis of the respective results and hospitalization outcomes of these two departments was performed. In the ICU, 36 of 145 patients (24.8%) had negative PCR results, whereas 109 (75.2%) had positive results. In the internal medicine department, the respective percentages were 35.6% negative (16 of 45 patients) and 64.4% positive (29 of 45 patients). The Chi-square test revealed no significant difference between the positivity rates of the two departments (P=0.133). As shown in Figure 2, the predominant pathogens detected in the ICU were *P. aeruginosa* (44 detections), *A. baumannii* (43 detections), and *K. pneumoniae* (37 detections), whereas VIM carbapenemase was the most frequently detected resistance gene (39 detections). In the internal medicine department, the most frequently detected targets were *S. aureus* (12 detections), the viral target human rhinovirus/enterovirus (12 detections), *H. influenzae* (10 detections), and *K. pneumoniae* (8 detections).

A significantly higher rate of in-hospital mortality was observed among ICU patients than among those hospitalized in the Internal Medicine Department. Specifically, in the ICU, 40 out of 145 patients (27.6%) recovered and were discharged, 100 of 145 patients (69.0%) died during hospitalization, and 5 of 145 (3.4%) were transferred to another healthcare facility. In contrast, in the internal medicine department, 38 patients were discharged (84.4%), and 7 of 45 patients died (15.6%). This finding likely reflects differences in baseline

disease severity, mechanical ventilation requirements, and comorbidity burden rather than an independent effect of the hospitalization setting.

Patient History and Overall Hospitalization Outcomes

Of the 210 patients included in the study, 110 (52.4%) had documented medical histories, whereas 100 (47.6%) did not. The underlying medical conditions documented in the patient cohort included COVID-19, tracheotomy, gastrostomy, endotracheal intubation, diabetes mellitus, bronchial asthma, lung carcinoma, pulmonary fibrosis, cystic fibrosis, chronic immobility (bedridden status), interstitial lung disease, chronic obstructive pulmonary disease (COPD), and hematological disorders. Of the 110 patients with documented medical histories, 27 had more than one comorbidity. Among these patients, the most frequent conditions were diabetes mellitus (29 patients), COVID-19, and prior intubation (25 patients each).

Regarding patient outcomes, 110 of the 210 hospitalized patients included in the study (52.2%) died during hospitalization, 81 (38.7%) were discharged, and 19 (9.1%) were transferred to other healthcare facilities. Among the 110 patients who died, the cause of death was recorded in the medical records of 81 patients. In 10 of these cases, the cause of death was attributed to more than one factor. The most frequent cause of death was multi-organ failure, which accounted for 48 deaths. Septic shock was reported in 21 patients, respiratory and renal failure in 3 patients each, and cardiac arrest was the cause of death in 2 patients. Hepatic failure and post-cardiac arrest syndrome were the least common causes (1 patient each, representing 1.2% of deceased patients). Among those who died, 74/110 (67.3%) were male, and 36/110 (32.7%) were female. On the other hand, among the discharged patients, 54/81 were male (66.2%), and 27/81 (33.8%) were female. The chi-square test revealed no statistically significant association between patient sex and clinical outcomes (P=0.881). In contrast, a statistically significant relationship was observed between age and outcome using the Mann–Whitney U test (P=0.003), indicating that patients who died were significantly older than those who survived. Further analysis of the individual medical history and hospitalization outcomes identified two statistically significant associations. Using the Chi-square test, significant relationships were found between mortality and history of COVID-19 (P=0.015) and between mortality and prior intubation (P=0.034). No other medical conditions were statistically significantly associated with hospitalization outcomes.

Discussion

Multiplex PCR succeeded in rapid and comprehensive detection of respiratory pathogens and antimicrobial resistance genes in lower respiratory tract samples, with Gram-negative bacteria predominating (46.3% of cases) among the identified pathogens. *P. aeruginosa* was the most frequently identified pathogen, followed by *A. baumannii* and *K. pneumoniae*. Resistance genes were detected in 31.5% of the samples tested in the present study, with VIM being the most prevalent.

Most patients on mechanical ventilation are managed in intensive care units, where the use of antibiotics is more frequent, and the selective pressure for antimicrobial resistance is higher than that in other hospital settings (18). Consequently, these environments exhibit a higher prevalence of resistant pathogens. In the United Kingdom, VAP occurs in approximately 9% to 27% of patients undergoing mechanical ventilation, with an incidence of 5 cases per 1,000 ventilator days (19). Therefore, this study also focused on outcomes from the intensive care unit, with particular emphasis on the incidence and characteristics of VAP in mechanically ventilated patients. Among the samples obtained from the ICU, the most commonly detected pathogens were *A. baumannii* (13.03%), *K. pneumoniae* (11.21%), and *P. aeruginosa* (13.33%). Similar distributions were reported in a 2019 study using the FilmArray® Pneumonia Panel (BioFire Diagnostics) on 59

BAL and endotracheal aspirate samples, which identified *K. pneumoniae* (21.3%), *P. aeruginosa* (14.9%), *A. baumannii* (12.8%), and *S. aureus* (12.8%) as the most frequent organisms (20).

Based on these considerations, a retrospective study was conducted at a tertiary referral hospital in Taiwan, involving adult ICU patients diagnosed with HAP or VAP who underwent BioFire FilmArray Pneumonia Panel (BFPP) testing between November 2019 and October 2022. A total of 592 respiratory specimens were analyzed. The most frequently detected pathogens were *A. baumannii* (N=168), *P. aeruginosa* (N=139), and *K. pneumoniae* (N=130). Antimicrobial resistance genes were identified in 157 specimens, accounting for 26.5% of the total. Among these, CTX-M was detected alone in 17.1% of bacterial isolates, carbapenemase genes alone in 11.8%, and both CTX-M and carbapenemase genes were co-detected in 25.1% of resistant isolates (14).

In contrast, a study from Lyon using BFPP on tracheal aspirates and BAL samples collected on average 7.5 days after ICU admission revealed a different pathogen profile. The most frequently identified bacteria included *S. aureus* (21.7%), *K. pneumoniae* (20%), *H. influenzae* (15%), *E. coli* (8.3%), and *S. pneumoniae* (8.3%) (21). These findings are similar to those observed in samples from the Internal Medicine department, where a considerable rate of viral co-detections was also noted, particularly human rhinovirus/enterovirus (7.89%).

It is essential to acknowledge several limitations when interpreting these findings. First, this was a single-site analysis based on data from the Regional Secondary Care Hospital of Larissa, without the inclusion of other hospitals in the region, which may limit the generalizability of the results. Additionally, patient inclusion was restricted to individuals who underwent mPCR testing at the discretion of the treating physician. This approach may have introduced selection bias and further limited the applicability of the findings to a broader population of hospitalized patients with LRTIs.

Another limitation concerns the absence of a priori sample size calculation; consequently, the statistical power may have been insufficient to detect certain clinically relevant associations. Furthermore, mortality analyses were solely based on univariable comparisons without multivariable adjustment; therefore, residual confounding cannot be excluded. Future studies incorporating adjusted analyses are warranted to better clarify independent predictors of mortality. Multiple statistical comparisons were also conducted without formal correction for multiple testing, which increases the risk of Type I error; therefore, the findings should be considered exploratory rather than confirmatory evidence. Incomplete documentation of patients' medical histories in the electronic database restricted the assessment of comorbidities or risk factors influencing pathogen distribution and antimicrobial resistance. Finally, semi-quantitative bacterial load data were not incorporated into the analysis, which may have limited a more nuanced interpretation of the microbiological findings.

Nevertheless, this study has notable advantages. To date, no comparable data have been published from Greece. This study offers essential baseline epidemiological information to assist not only future multicenter investigations but also local clinical and public health strategies.

Conclusion

The findings of this study provide an overview of the respiratory pathogens and antimicrobial resistance genes detected among hospitalized patients with suspected lower tract infections. Gram-negative bacteria, particularly *P. aeruginosa*, *A. baumannii, and K. pneumoniae,* were frequently identified, especially among patients admitted to the ICU, whereas viral targets were more commonly detected among patients in the Internal Medicine Department. The frequent detection of multiple microbial targets and antimicrobial resistance genes highlights the complexity of the microbiological landscape of hospitalized patients with suspected LRTIs. Further prospective multicenter studies are required to evaluate the clinical utility of multiplex PCR and its potential impact on antimicrobial management and patient outcomes.

What Is Already Known on This Topic:

LRTIs are a leading cause of hospitalization and mortality worldwide, with Gram-negative bacteria increasingly implicated, particularly in hospitalized and critically ill patients. Molecular diagnostic methods, including Multiplex PCR assays, enable the rapid and sensitive detection of respiratory pathogens and resistance genes, thereby improving etiological diagnosis and antimicrobial management. However, molecular epidemiological data remain limited in several regions, including Greece.

What This Study Adds:

This study contributes to the field of molecular epidemiology of respiratory pathogens and antimicrobial resistance. To date, comparable data from Greece are lacking; therefore, our findings provide novel national baseline epidemiological data. These data may serve as a reference for future multicenter studies and support improvements in diagnostic strategies, antimicrobial stewardship, and public health policies at both local and national levels.

CRediT Authorship Contribution Statement: Conception and design: IA and GM; Acquisition, analysis and interpretation of data: IA and SA; Drafting the article: IA and GM; Revising it critically for important intellectual content: GM, EP and MK; Approved final version of the manuscript: IA, SA, GM, EP and MK.

Conflict of Interest: The authors declare that they have no conflict of interest.

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