Infection and Drug Resistance

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ORIGINAL RESEARCH

Molecular Diagnostics, Antimicrobial Resistance Patterns, and Clinical Outcomes in Hospitalized Pneumonia Patients: A Prospective Study from Jordan and a Call for National Guideline Integration

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Purpose: Pneumonia is still a leading cause of morbidity and death globally, with a significant percentage of cases having an unknown aetiology, and management becoming more difficult due to growing antimicrobial resistance (AMR). This study assessed clinical outcomes, antimicrobial susceptibility patterns, and pathogen detection using both conventional and molecular techniques in hospitalized pneumonia patients in Jordan.

Patients and Methods: 111 adults (≥18 years) who were admitted to a tertiary private hospital in Amman between May 2021 and January 2022 with either hospital-acquired (HAP) or community-acquired pneumonia (CAP) were included in this prospective study. Multiplex real-time PCR and conventional culture were performed on lower respiratory tract samples (FTD Respiratory Pathogens 33). Data on outcomes, microbiology, antimicrobial susceptibility, clinical, and demographics were gathered. McNemar's test was used to compare diagnostic performance, and logistic regression and chi-square analyses were used to evaluate the relationships between outcomes and adherence to guidelines.

Results: The average age was 64.0±20.6 years, and 58.6% of the population was male. 78.4% of cases were CAP. PCR detected pathogens in 74.8% of patients, whereas culture detected them in 57.7% ($p<0.001$). PCR showed a higher false-positive rate but a significantly higher sensitivity than culture (96.9% vs 86.3%, $p=0.039$). In 36.9% of cases, bacterial–viral co-infections were found. The overall death rate was 27.0%. Although not an independent predictor in logistic regression, non-guideline-concordant antibiotic therapy was substantially associated with mortality ($p=0.023$). High ampicillin resistance and notable trends in resistance to specific broad-spectrum agents were among the notable variations in AMR patterns observed.

Conclusion: Multiplex PCR reveals complex co-infection patterns in pneumonia and greatly enhances pathogen detection when compared to culture. Antimicrobial stewardship initiatives that incorporate molecular diagnostics may improve targeted treatment. To address changing AMR patterns in Jordan, national guidelines that include molecular testing are necessary.

Keywords: antimicrobial stewardship, antimicrobial susceptibility, molecular methods, outcomes, pathogen detection, middle east

Introduction

Inflammation of the lung parenchyma is the hallmark of pneumonia, which an infectious agent brings on. The majority of pneumonia cases can be categorised as community-acquired pneumonia (CAP) or hospital-acquired pneumonia (HAP).[1,2]

Lower respiratory tract infection (LRTI) (mainly pneumonia) leads to high rates of hospitalisation and causes substantial morbidity and mortality in adults worldwide.[3–5] Lower respiratory tract infections remain among the leading

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causes of years of life lost globally and account for approximately 2.6 million deaths annually, according to the Global Burden of Disease (GBD) 2019 study. Pneumonia still has a significant clinical and financial impact despite improvements in vaccination and antibiotic treatment, especially for older adults and patients with comorbid conditions. These updated statistics highlight the continued importance of developing better pneumonia diagnostic and treatment approaches on a global scale.[1]

Risk factors for CAP include advanced age, the presence of other medical conditions (such as asthma, COPD, bronchiectasis, cardiovascular disease, diabetes, immunosuppressive states, and stroke), a previous history of pneumonia, immunosuppressants use, viral respiratory infections, and lifestyle factors such as smoking, alcohol consumption, living in crowded areas, poor dental hygiene, and regular contact with children.[6,7]

The issue of antimicrobial resistance is most urgent in developing nations, where infectious diseases are highly prevalent. Factors contributing to this problem include excessive use of antibiotics, low quality of available antibiotics, and financial limitations that hinder the broad use of newer and more expensive treatments.[8,9]

An etiologic agent is not identified in 30% to 65% of patients with pneumonia.[10] Sputum or bronchoalveolar lavage culture is the mainstay of conventional microbiological diagnosis; however, its use is constrained by its lengthy turnaround time (48–72 hours), decreased sensitivity following previous antibiotic exposure, and failing to identify viral or specific pathogens. Multiplex real-time polymerase chain reaction (PCR), on the other hand, allows for the quick, simultaneous identification of several bacterial and viral pathogens in a matter of two hours, including those that are hard or impossible to cultivate. Molecular diagnostics could improve empirical therapy and lower diagnostic uncertainty by increasing pathogen identification rates and identifying mixed infections.

Rapid molecular diagnostics has major implications for antimicrobial stewardship (AMS) beyond pathogen detection. Early detection of the causing organisms can assist in maximizing targeted therapy, prevent unnecessary exposure to antibiotics in viral infections, and de-escalate the use of broad-spectrum empirical antibiotics. Rapid diagnostics integration into clinical decision-making processes may decrease inappropriate prescribing and enhance patient outcomes in environments where antimicrobial resistance is on the rise.

Approximately 17 to 41% of CAP cases in the US are thought to be due to *Streptococcus pneumoniae*.[11] It was frequently reported that CAP from *S. pneumoniae* is associated with high mortality, risk of shock, and the need for mechanical ventilation.[12]

Bacterial pneumonia is a frequently occurring condition, however, its causes differ depending on the geographical location.[13,14] Several studies have reported that the common causes of CAP are *S. pneumoniae, K. pneumoniae, E. coli, H. influenzae, S. aureus*, in addition to the atypical bacteria like *L. pneumophila, C. pneumoniae*, and *M. pneumonia* cases.[15–17] *S. pneumoniae* is the most common bacterial infection across all age groups, accounting for roughly 30% of pneumonia cases.[15,16]

Despite recent breakthroughs in microbiological techniques, the etiology of pneumonia is not entirely understood. The advancement of molecular techniques with enhanced sensitivity and specificity has facilitated the identification of new viruses, the detection of microorganisms that are challenging to cultivate, and the identification of pathogens at a later stage of the disease.[17–25] The majority of information on the etiology of pneumonia in Jordan and neighboring Middle Eastern countries comes from hospital-based culture studies, with multiplex molecular diagnostics incorporated into standard clinical practice only partially. As a result, thorough comparisons between real-time multiplex PCR and conventional culture in adult hospitalized pneumonia populations are still rare. To our knowledge, this is the first prospective study in Jordan to compare multiplex real-time PCR with traditional culture techniques in hospitalized adult patients with pneumonia that was acquired in the community and in the hospital. It also assesses clinical outcomes and patterns of antibiotic susceptibility. This study aims to address diagnostic gaps in the local hospital setting and to provide evidence to support national antimicrobial stewardship strategies by integrating molecular diagnostics with clinical and stewardship-related variables.

Material and Methods

Study Design and Patients

This study was conducted at the Islamic hospital in Amman, Jordan. The study followed a retro-prospective and cross-sectional design and was carried out from May 2021 to January 2022.

Waves of SARS-CoV-2 infection in Jordan occurred during the study period. To represent the complexity of real-world diagnosis during the pandemic, patients with confirmed COVID-19 were not excluded; rather, they were included in the larger pneumonia cohort. This method recognizes the possible influence of secondary bacterial pneumonia and viral co-infections as reported in COVID-19 literature.[26]

A total of 111 banked lower respiratory tract samples were collected from the hospital lab. The samples included hospitalised patients of adults aged ≥18 years who presented with pneumonia. Samples from patients who were under antibiotic treatment were excluded. Socio-demographic characteristics, clinical information, and other relevant variables were collected using the medical records.

The study was conducted as part of routine surveillance and was approved by the Institutional Review Board (IRB) committee (1053/2021/151). The IRB waived patient consent because there was no direct patient contact or intervention, and the study posed no risk to participants. Data were de-identified before analysis and were used solely for research purposes. This study complies with the Declaration of Helsinki. According to international ethical guidelines, informed consent can be waived when research involves low risk, does not adversely affect participants' rights and welfare, and cannot be practicably carried out otherwise. All data were anonymized before analysis.[27]

The definition of pneumonia was characterised by new pulmonary infiltrates on thoracic imaging and one or more of the following conditions: 1) novel or increased cough with or without sputum production and/or purulent respiratory secretions; 2) fever or hypothermia; 3) signs of systemic inflammation (leukocytosis >10,000 cells/cm^3, bandemia >10%, leukopaenia <4000 cells/cm^3), procalcitonin levels above the local upper limit of normal or increased C-reactive protein).[28]

The Infectious Diseases Society of America (IDSA) defines CAP as an acute infection of the pulmonary tissue accompanied by the presence of an acute infiltrate on a chest radiograph or auscultatory findings consistent with pneumonia in a patient who did not acquire it from a healthcare system or within the first 48 hours after hospitalisation.[29] In this study, we also included cases of HAP, which is defined as a type of pneumonia that occurs 48 hours or more after hospital admission and is not present at the time of admission.

In order to incorporate consecutively archived respiratory samples obtained during routine clinical care while prospectively conducting standardized molecular testing and statistical analysis, the study was designed as retro-prospective. This hybrid design ensured methodological consistency in data handling and molecular analysis while allowing evaluation of practical diagnostic procedures.

Sample Collection and Preparation

Specimens were collected from adult patients with pneumonia using a disposable, leak-proof, sterile, wide-mouthed container with a tight-fitting lid. During the sputum collection, each study participant was routinely instructed to breathe deeply and then cough deeply and vigorously to provide at least 2 mL of sputum specimen into the container provided. Soon after collection, they were transported to the bacteriology laboratory using an ice box and processed within 30 minutes of collection.

Respiratory samples were archived and stored at −80 °C before analysis. Nucleic acids were extracted from each sample using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. To prevent degradation, extracts were stored at 4 °C until manipulation was complete (1–3 days).

Molecular Testing

The FTD® Respiratory Pathogens 33 kit (Fast Track Diagnostics, Luxembourg) was used for molecular detection. This kit enables the detection of 33 pathogens, including viruses, bacteria, and fungi commonly associated with respiratory infections. The pathogens detected by this kit are available in Table 1. This is an open-platform multiplex real-time PCR assay that is meant to qualitatively detect respiratory pathogens. The FTD platform, on the other hand, needs separate

Table 1 List of Pathogens Available in FTD® Respiratory Pathogens 33 Multiplex Assay

Type of Pathogen	List of Pathogens
Viruses	1. Influenza A virus (IAV)
	2. Influenza B virus (IBV)
	3. Influenza C virus (ICV)
	4. Influenza A (H1N1) virus (swine lineage)
	5. Human parainfluenza virus 1 (HPIV-1)
	6. Human parainfluenza virus 2 (HPIV-2)
	7. Human parainfluenza virus 3 (HPIV-3)
	8. Human parainfluenza virus 4 (HPIV-4)
	9. Human coronavirus NL63 (hCoV NL63)
	10. Human coronavirus 229E (hCoV 229E)
	11. Human coronavirus OC43 (hCoV OC43)
	12. Human coronavirus HKU1 (hCoV HKU1)
	13. Human metapneumovirus A (HMPV A)
	14. Human metapneumovirus B (HMPV B)
	15. Human rhinovirus (HRV)
	16. Human respiratory syncytial virus A (HRSV A)
	17. Human respiratory syncytial virus B (HRSV B)
	18. Human adenovirus (HAdV)
	19. **Enterovirus (EV)**
	20. Enterovirus (EVHuman parechovirus (HPeV)
Bacteria	1. Mycoplasma pneumoniae
	2. Chlamydophila pneumoniae
	3. Streptococcus pneumoniae
	4. Haemophilus influenzae
	5. Staphylococcus aureus
	6. Moraxella catarrhalis
	7. Bordetella spp. (except Bordetella parapertussis)
	8. Klebsiella pneumoniae
	9. Legionella pneumophila
	10. Legionella longbeachae
	11. Salmonella spp.
	12. Haemophilus influenzae
Fungi	*Pneumocystis jirovecii*

nucleic acid extraction, reaction setup, and amplification on a regular real-time PCR instrument. This is different from closed, cartridge-based systems like the BioFire® FilmArray Pneumonia Panel (BioMérieux). The Prime Pro 48 real-time PCR system was used to amplify the samples in this study. Using controls provided by the manufacturer, standard curves were made so that cycle threshold (Ct) values could be used to make semi-quantitative estimates for microbial load.[30,31]

Bacterial loads (DNA copies/mL) were computed using the standard curves. By directly extrapolating PCR Ct values to the amount of DNA as read from the concentration versus the Ct standard curve, the amount of bacterial DNA contained in each sample was determined. Ct values greater than 30 were considered negative samples. Fumarate is the target of real-time quantitative PCR (qPCR).

Statistical Analysis

Statistical analyses were performed to evaluate the study participants' demographic characteristics, clinical outcomes, pathogen detection rates, and antibiotic susceptibility patterns. Continuous variables, such as age, and length of hospital stay, were summarised using means, standard deviations (SDs), and ranges. Or median (IQR) depending on the normality

test. Categorical variables, including gender, initial symptoms, comorbidities, pneumonia classification, and antibiotic resistance categories, were summarised using frequencies and percentages.

The Infectious Diseases Society of America (IDSA) guidelines for the empirical treatment of hap AND cap were used to define guideline adherence. Non-adherence was further divided into two categories: (1) overtreatment (using broader-spectrum antibiotics than recommended) or (2) undertreatment (using a narrower or delayed course of treatment than advised). Based on severity or resistance concerns, this distinction was made to distinguish between clinician-directed escalation and potentially harmful deviation.[32]

The Chi-square test or Fisher's exact test was used to compare categorical variables, as appropriate. The McNemar test was applied to assess the significance of the difference between the detection rates of pathogens using traditional culture methods and qPCR. Sensitivity for both culture and qPCR methods were calculated to compare their diagnostic performance, and the McNemar test was also employed to evaluate the statistical significance of the differences in sensitivity between the two methods.

To analyse the relationship between guideline adherence in antibiotic use and patient outcomes (improvement vs death), a Chi-square test was used to determine the association. Additionally, logistic regression was conducted to explore the predictive value of guideline adherence on patient outcomes, with model accuracy and p-values reported.

Given the limited number of mortality events, regression coefficients may be unstable and should be interpreted as exploratory rather than confirmatory findings.

Comparisons between the lengths of hospital stay in patients with different pneumonia classifications (CAP vs HAP) were conducted using the independent samples *t*-test or the Mann–Whitney *U*-test when the data did not meet the normality assumption. Antibiotic resistance patterns were visualised using bar charts and heatmaps, and monthly trends in antibiotic resistance were analysed using line plots.

All statistical analyses were performed using JASP (Jeffreys's Amazing Statistics Program) 0.18.3.0 software, and a p-value of <0.05 was considered statistically significant.

The number of eligible pneumonia cases with available archived respiratory samples during the study period (May 2021–January 2022) served as the basis for determining the sample size. With 80% power and $\alpha=0.05$, a minimum sample size of 92 was calculated to detect a 15% difference in detection rates between culture and PCR for diagnostic comparison using McNemar's test. This criterion for diagnostic yield analysis was surpassed by the final sample of 111 patients. However, given that regression modeling recommends at least 10 outcome events per predictor variable, the results of logistic regression should be interpreted cautiously because the sample size was not specifically powered for multivariable mortality prediction.[33]

Results

Demographic and Baseline Characteristics

The study included 111 participants with a mean age of 64.0 years (SD = 20.6, range = 18–91). Of these, 46 (41.4%) were female, and 65 (58.6%) were male (Table 2). The most common initial finding was shortness of breath, reported by 60 (54.1%) patients, followed by fever (15.3%), cough (14.4%), and pleuritic chest pain (12.6%).

Regarding comorbidities, hypertension was the most common (38.7%), followed by diabetes mellitus (30.6%), and cardiovascular disease (27.9%). The average number of previous hospitalisations was 2.2 (SD = 2.4, range = 0–14), and the average number of previous pneumonia episodes was 0.7 (SD = 1.1, range = 0–6) (Table 2).

Most samples were sputum, comprising 91.0% of the total 111 samples. Bronchoalveolar Lavage (BAL) accounted for 6.3% of the samples, while Endotracheal Aspirate comprised 2.7%.

Characteristics of Pneumonia

Among participants, 87 (78.4%) had CAP, and 24 (21.6%) had HAP, including 3 (2.7%) cases of ventilator-associated pneumonia (VAP) (Table 3). According to the CURB-65 severity score, most patients were classified as having mild-to-moderate pneumonia, with a score of 1 being the most common. The length of hospital stays (LOS) averaged 12.1 days (SD = 15.9, range = 1–83), and the length of ICU stay averaged 5.2 days (SD = 13.0, range = 0–83).

Table 2 Demographic and Baseline Characteristics of the Study Participants (N=111)

Characteristics	n (%)	Mean±SD and Range
Age (years)		64.0 ± 20.6, Range: (18–91)
Gender		
Female	46 (41.4)	
Male	65 (58.6)	
Initial finding		
Shortness of breath	60 (54.1)	
Fever	17 (15.3)	
Pleuritic chest pain	14 (12.6)	
Cough	16 (14.4)	
Sputum production	8 (7.2)	
Muscular or joint pain	4 (3.6)	
Abdominal pain or nausea	2 (1.8)	
Headache	2 (1.8)	
Hyperglycemia	1 (0.9)	
Comorbidities		
Hypertension	43 (38.7)	
Diabetes mellitus	34 (30.6)	
Cardiovascular disease	31 (27.9)	
Alzheimer's disease	17 (15.3)	
COPD	15 (13.5)	
Asthma	9 (8.1)	
Septic shock	7 (6.3)	
Dyslipidemia	5 (4.5)	
Hypotension	5 (4.5)	
GERD	5 (4.5)	
Hypoglycemia	5 (4.5)	
Asthma COPD overlap	4 (3.6)	
Skin disease	2 (1.8)	
No. of previous hospitalizations		2.2 ± 2.4, Range (0–14)
No. of previous pneumonia		0.7 ± 1.1, Range (0–6)

Abbreviation: COPD, Chronic obstructive pulmonary disease.

Table 3 Characteristics of Pneumonia in the Study Participants (N=111)

Characteristics	n (%)	Mean±SD and Range	Median (IQR)
Location			68.0 (27.5)
Outpatient	NA		
Ward	34 (30.6)		
ICU	77 (69.4)		
Pneumonia Classification			
CAP	87 (78.4)		
HAP (overall)	24 (21.6)		
VAP	3/111 (2.7), 3/24 (12.5)		
CURB-65 severity score			
0	4 (3.6)		
1	55 (49.5)		
2	32 (28.8)		
3	20 (18.0)		

(*Continued*)

Table 3 (Continued).

Characteristics	n (%)	Mean±SD and Range	Median (IQR)
4	0 (0.0)		
5	0 (0.0)		
PSI			68.0 (27.5)
Intubation and mechanical ventilation	4 (3.6)		
Aspiration	15 (13.5)		
Detection of microorganisms by traditional culture	64 (57.7)		
Detection of microorganism by PCR	83 (74.8)		
Current C0VID-19	24 (21.6)		
History of C0VID-19	14 (12.6)		
Outcome			
Improve	78 (70.3)		
Partialy improve	3 (2.7)		
Death	30 (27.0)		
Length of hospital stay (LOS)		12.1±15.9 Range: (1–83)	
Length of ICU stay		5.2±13.0 Range: (0–83)	

Abbreviations: CAP, Community-acquired pneumonia, ICU, Intensive care unit.

Pathogen Detection

PCR detected pathogens in 83 (74.8%) patients, while traditional culture methods detected pathogens in 64 (57.7%) patients. PCR detected multiple microorganisms, including *Bordetella pertussis*, *Chlamydophila pneumoniae*, and multiple viruses, not detected by culture. The presence of bacteria-virus co-infections was observed in 41 (36.9%) patients (Figure 1).

Bacterial Culture versus qPCR

Figure 2 shows the distribution of pathogens detected using the culture and molecular techniques. *Staphylococcus aureus* and *Escherichia coli* appearing as the most frequently detected pathogens. Other pathogens, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, are also detected, at lower frequencies.

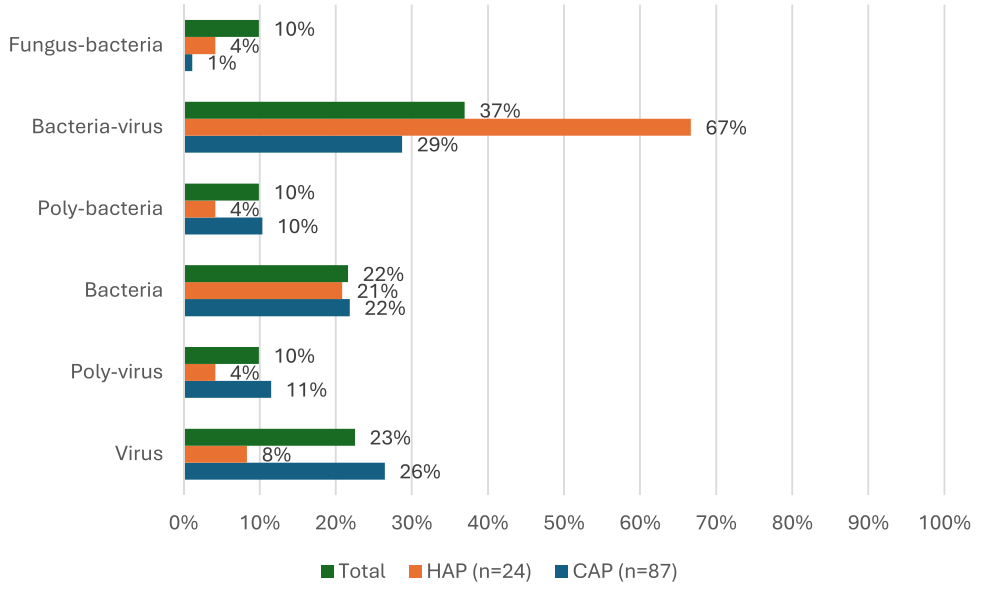

Figure 1 Distribution of detected microorganisms and co-infections among patients with CAP and HAP. Proportional distribution of microorganism types (virus, bacteria, poly-viral, poly-bacterial, bacteria–virus, and fungus–bacteria) in CAP (n = 87), HAP (n = 24), and the total study population.
Abbreviations: CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia.

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There is a statistically significant difference in detection rates between culture and PCR ($p < 0.001$) (Table 4).

While culture showed higher specificity (92.9%, 55.3%, respectively), PCR showed higher sensitivity (96.9%, 74.7%, respectively). Both methods had the same overall diagnostic accuracy (79.3%). While culture demonstrated strong rule-in performance (LR+ = 10.53), PCR demonstrated excellent rule-out performance (LR− = 0.06). Culture had a higher Youden's index (0.68; 95% CI: 0.54–0.81) than PCR (0.52; 95% CI: 0.37–0.67), suggesting a better overall discriminatory balance. PCR detects significantly more positive cases, as evidenced by the statistically significant difference in paired positivity rates ($\chi^2 = 14.09$, $p < 0.001$). Agreement between both techniques was moderate ($\kappa = 0.55$) (Table 5).

Clinical Outcome Analysis

Figure 3 shows the pathogen detection counts across different clinical outcomes. The relationship between the identified pathogen and the clinical outcome (death, improvement, or partial improvement) was investigated using a chi-square test of independence. A non-statistically significant, borderline association was found by the analysis ($\chi^2(30) = 43.25$, $p = 0.056$). Despite the marginal p-value, the effect size was moderate (Cramér's V = 0.36). Clinically, patients with *Bordetella pertussis* (8 deaths/14 cases, 57%), MRSA (3/3, 100%; very small sample), MSSA (8/15, 53%), *Pseudomonas* (7/18, 39%), and *Klebsiella pneumoniae* (5/11, 45%) had correspondingly higher mortality rates. On the other hand, viral pathogens like HCoV_229E (7/27, 26%), HCoV_OC43 (2/15, 13%), HPIV3 (7/21, 33%), and especially HRSV (0/9, 0%) and qPCR_HRV (0/2, 0%) were more often linked to clinical improvement and decreased observed mortality. Because fungal detections (eg, Aspergillus and Candida species) were rare, they were difficult to interpret accurately (Figure 3).

Given the small subgroup sizes and potential sparse-cell bias, interpretation should be undertaken with caution.

Patient outcomes and adherence to treatment guidelines are statistically significantly correlated ($\chi2 = 7.55$, $p = 0.023$) (Figure 4), with non-adherent patients having a higher mortality rate (64.3%) than adherent patients (35.7%). Nonetheless, there is no apparent variation in hospital stay duration ($U = 1245.0$, $p = 0.846$). With a model accuracy of 60.9%, logistic regression indicates that adherence is not a significant predictor of outcomes (coefficient = 0.126, $p = 0.764$). There is not a significant difference in length of stay between CAP and HAP patients, according to a *t*-test ($t = 0.534$, $p = 0.594$).

Antibiotic Susceptibility Patterns

The frequency/percentage of each antibiotic susceptibility category (sensitive, intermediate, and resistant) for each identified microbe is shown in Table 6. The recorded cases in the dataset exhibit different levels of antibiotic sensitivity and resistance. Levofloxacin is the antibiotic with the highest sensitivity rate. Ampicillin, on the other hand, has the highest rate of antibiotic resistance (Figure 5).

A heatmap illustrating the patterns of resistance to various antibiotics is presented in Figure 6. This displays the degree of antibiotic resistance in each sample. Resistance levels are shown as numerical values in the prepared susceptibility data:

0: Sensitive

0.5: Intermediate

1: Resistance

The monthly trends in antibiotic resistance for each antibiotic during the recorded period are displayed in the line plot in Figure 7. Any seasonal patterns or trends in the levels of antibiotic resistance can be found with the aid of this visualization.

Over time, patterns of antibiotic resistance reveal substantial patterns, with some antibiotics exhibiting steady resistance levels and others varying. It's interesting to note that resistance levels peak during specific months, suggesting potential outbreaks or seasonal variations. Meropenem and pipracillin-tazobactam resistance levels, for instance, show notable peaks in September and October, which may be connected to increased antibiotic use or higher infection rates during these months.

Resistance to Cefepime and Meropenem increases periodically, suggesting seasonal fluctuations or specific challenges. Cefepime resistance tends to increase as the year draws to a close, coinciding with the colder months.

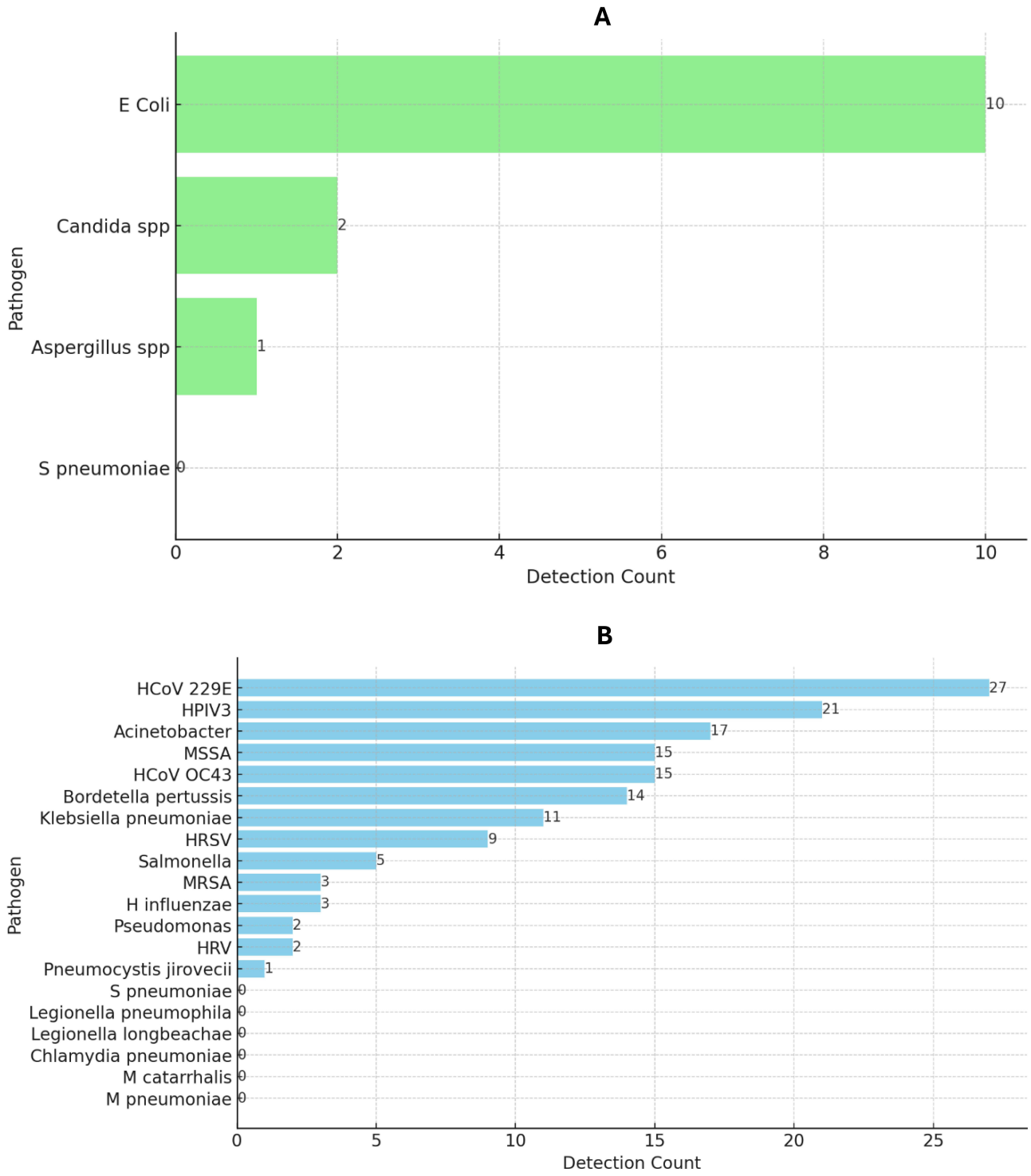

Figure 2 Distribution of pathogens detected using culture and molecular methods. (**A**) Using the culture method. (**B**) Using the qPCR. The frequency of microorganisms isolated from lower respiratory tract specimens of hospitalized pneumonia patients using standard microbiological culture is shown in a horizontal bar chart A. *Streptococcus pneumoniae* was not found by culture in this cohort, but *Escherichia coli* was the most commonly isolated pathogen (n = 10), followed by Candida spp. (n = 2) and Aspergillus spp. (n = 1). The frequency of respiratory pathogens determined by the FTD® Respiratory Pathogens 33 multiplex real-time PCR assay from lower respiratory tract specimens of hospitalized pneumonia patients is displayed in a horizontal bar chart B. PCR-positive cases per organism are represented by detection counts. A wider range of bacterial and viral pathogens was detected by qPCR as opposed to traditional culture.

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Table 4 Contingency Table of the Association Between PCR and Culture Results

		Culture		Total
		Positive	**Negative**	
PCR	Positive	62	21	83
	Negative	2	26	28
	Total	64	47	111

Notes: Chi-Square Statistic: 64.33. P-value: 1.05×10−151.05×10−15. Degrees of Freedom: 1. McNemar p-value < 0.001.

Table 5 Comprehensive Diagnostic Performance Comparison

Parameter	PCR (Culture Reference)	Culture (PCR Reference)
Sensitivity	96.9% (89.3–99.1)	74.7% (64.4–82.8)
Specificity	55.3% (41.2–68.6)	92.9% (77.4–98.0)
False Positive Rate (1–Specificity)	44.7% (31.4–58.8)	7.1% (2.0–22.6)
False Negative Rate (1–Sensitivity)	3.1% (0.9–10.7)	25.3% (17.2–35.6)
Positive Predictive Value	74.7% (64.4–82.8)	96.9% (89.3–99.1)
Negative Predictive Value	92.9% (77.4–98.0)	55.3% (41.2–68.6)
Accuracy	79.3% (70.8–85.8)	79.3% (70.8–85.8)
LR+	2.17 (1.60–2.95)	10.53
LR−	0.06 (0.02–0.23)	0.27
Youden's Index (J)	0.52 (0.37–0.67)	0.68 (0.54–0.81)
ROC-equivalent AUC	0.76	0.84
McNemar χ^2 (corrected)	14.09	—
McNemar p-value	0.00017	—
Cohen's κ	0.55 (0.39–0.70)	—

Notes: Data are presented as point estimates with 95% confidence intervals in parentheses.

The monthly resistance trends for the top five antibiotics with the highest levels of resistance are also shown in Figure 7. One of these antibiotics is represented by each line, illustrating the evolution of resistance.

During this research, ceftazidime and ampicillin exhibit high levels of resistance. Antibiotics like cefazolin and pipracillin-tazobactam, on the other hand, show apparent spikes in resistance at specific periods. Aztreonam exhibits fluctuations with sporadic spikes in resistance.

Antibiotic Usage

In targeted therapy and empirical settings, many antibiotics have been used in this study (Table 7). Ampicillin, azithromycin, clindamycin, amphotericin B, and imipenem-cilastatin were the antibiotics that were used the most. Meropenem, tigecycline, and cefepime all showed a clear move toward targeted therapy.

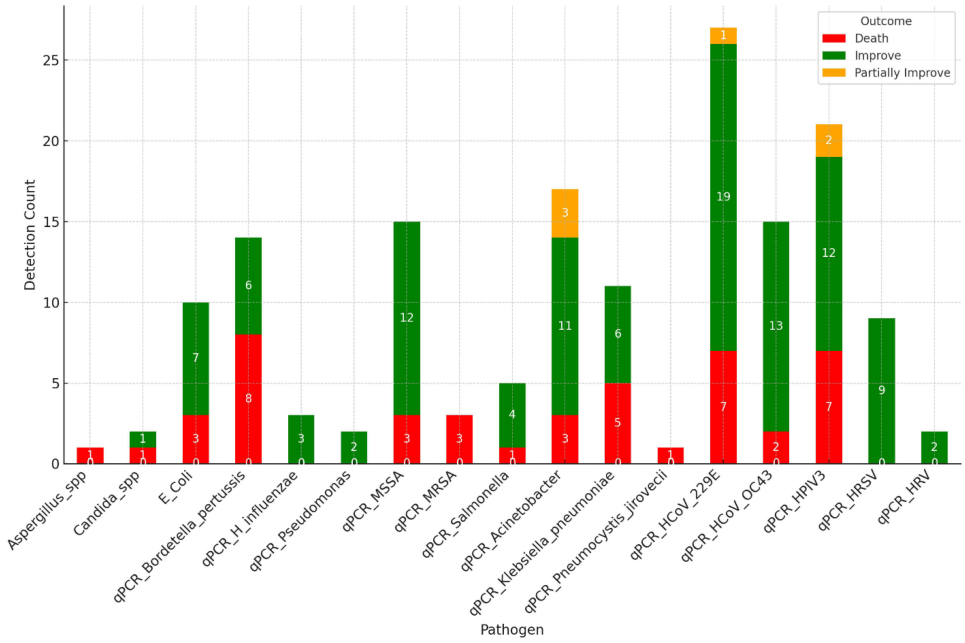

Figure 3 Visualisation of clinical outcomes and pathogen detection. ($\chi^2(30) = 43.25$, $p = 0.056$). The effect size was moderate (Cramér's V = 0.36). *Bordetella pertussis* (8 deaths/14 cases, 57%), MRSA (3/3, 100%; very small sample), MSSA (8/15, 53%), *Pseudomonas* (7/18, 39%), and *Klebsiella pneumoniae* (5/11, 45%) had correspondingly higher mortality rates. Viral pathogens such as HCoV_229E (7/27, 26%), HCoV_OC43 (2/15, 13%), HPIV3 (7/21, 33%), and, in particular, HRSV (0/9, 0%) and qPCR_HRV (0/2, 0%) were more frequently associated with clinical improvement and reduced observed mortality.

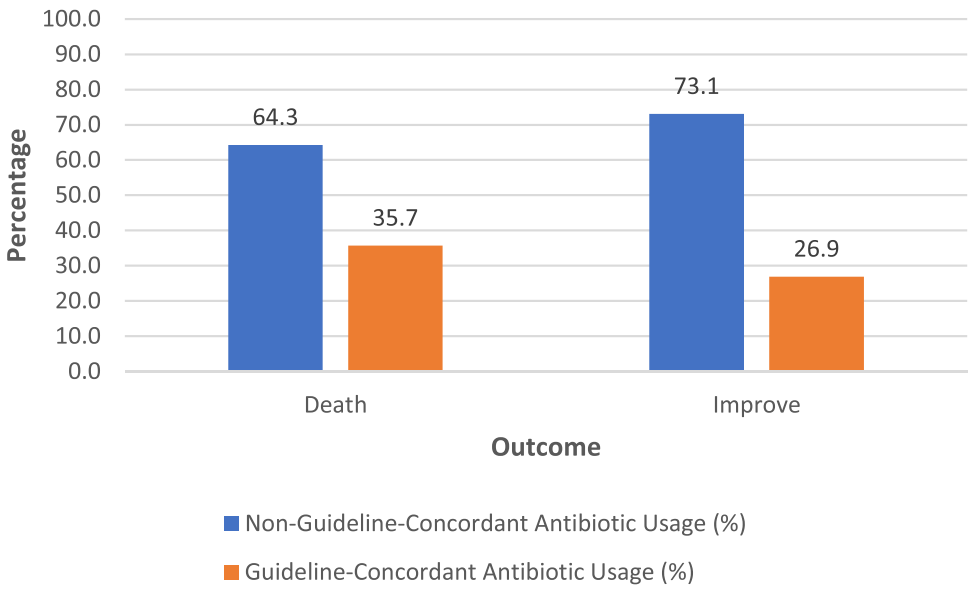

Figure 4 Guideline adherence proportions by outcome. The percentage distribution of antibiotic prescribing patterns, stratified by clinical outcome (death vs improvement), is shown in a bar chart. Orange bars indicate antibiotic use that is in line with guidelines, while blue bars indicate antibiotic use that is not. Compared to 35.7% of patients who received guideline-concordant therapy, 64.3% of patients who died received non-guideline-concordant therapy. Of the patients who showed improvement, 26.9% received guideline-concordant therapy and 73.1% received non-guideline-concordant therapy.

Discussion

In hospitalized adults with pneumonia, this study shows that multiplex real-time PCR has a significantly higher diagnostic yield than conventional culture (74.8% vs 57.7%, p<0.001). When dealing with fastidious organisms, viral pathogens, or prior antibiotic exposure, relying only on culture may underestimate the true microbial burden, as evidenced by the higher sensitivity of PCR (96.9% vs 86.3%, p=0.039). Importantly, bacterial–viral co-infections were

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Table 6 Antibiotic Susceptibility Profiles of Clinical Isolates by Microorganism

Microorganism	Antibiotic	Resistant (Frequency)	Resistant (%)	Intermediate (Frequency)	Intermediate (%)	Sensitive (Frequency)	Sensitive (%)
Acinetobacter spp	Imipenem	17	100	0	0	0	0
	Meropenem	17	100	0	0	0	0
	Ertapenem	17	100	0	0	0	0
	Pipracillin_tazobactam	17	100	0	0	0	0
	Cefepime	17	100	0	0	0	0
	Ceftriaxone	17	100	0	0	0	0
	Tigecycline	0	0	16	94	1	6
	Levofloxacin	16	94	1	6	0	0
	Amoxicilin_clav	14	82	0	0	3	18
	Ciproflxacin	17	100	0	0	0	0
	Ceftazidime	17	100	0	0	0	0
	Gentamicin	15	88	0	0	2	12
	Amikasin	15	88	2	12	0	0
	Aztreonam	17	100	0	0	0	0
	Colistin	0	0	0	0	17	100
	Cefotaxime	17	100	0	0	0	0
	Cefpodoxome	17	100	0	0	0	0
	Cefixim	17	100	0	0	0	0
	Cefuroxime	17	100	0	0	0	0
	Cefazolin	17	100	0	0	0	0
	Ampicillin	17	100	0	0	0	0
	TMP_SMX	17	100	0	0	0	0
Bordetella pertussis	Imipenem	5	36	0	0	9	64
	Meropenem	5	36	0	0	9	64
	Ertapenem	10	75	0	0	4	25
	Pipracillin_tazobactam	9	64	1	9	4	27
	Cefepime	6	45	3	18	5	36
	Ceftriaxone	10	75	0	0	4	25
	Tigecycline	0	0	14	100	0	0
	Levofloxacin	8	60	0	0	6	40
	Amoxicilin_clav	14	100	0	0	0	0
	Ciproflxacin	9	64	0	0	5	36
	Ceftazidime	9	64	0	0	5	36
	Gentamicin	5	36	0	0	9	64
	Amikasin	3	18	3	18	9	64
	Aztreonam	6	45	0	0	8	55
	Colistin	0	0	0	0	14	100
	Cefotaxime	10	75	0	0	4	25
	Cefpodoxome	10	75	0	0	4	25
	Cefixim	10	75	0	0	4	25
	Cefuroxime	14	100	0	0	0	0
	Cefazolin	14	100	0	0	0	0
	Ampicillin	14	100	0	0	0	0
	TMP_SMX	10	75	4	25	0	0
	Ceftazidime_Avibactam	0	0	0	0	14	100
E Coli	Imipenem	0	0	0	0	10	100
	Meropenem	0	0	0	0	10	100
	Ertapenem	0	0	0	0	10	100
	Pipracillin_tazobactam	3	30	1	10	6	60
	Cefepime	3	30	0	0	7	70
	Ceftriaxone	3	30	0	0	7	70
	Tigecycline	7	67	0	0	3	33
	Levofloxacin	2	17	0	0	8	83

(*Continued*)

Table 6 (Continued).

Microorganism	Antibiotic	Resistant (Frequency)	Resistant (%)	Intermediate (Frequency)	Intermediate (%)	Sensitive (Frequency)	Sensitive (%)
	Amoxicilin_clav	8	80	1	10	1	10
	Ciproflxacin	3	29	1	14	6	57
	Ceftazidime	3	30	0	0	7	70
	Gentamicin	0	0	0	0	10	100
	Amikasin	0	0	0	0	10	100
	Aztreonam	3	30	0	0	7	70
	Cefotaxime	3	30	0	0	7	70
	Cefpodoxome	5	50	0	0	5	50
	Cefixim	7	70	0	0	3	30
	Cefuroxime	8	80	0	0	2	20
	Cefazolin	8	80	0	0	2	20
	Ampicillin	10	100	0	0	0	0
	TMP_SMX	0	0	2	20	8	80
Klebsiella pneumoniae	Imipenem	2	18	0	0	9	82
	Meropenem	2	18	0	0	9	82
	Ertapenem	3	27	0	0	8	73
	Pipracillin_tazobactam	4	36	2	18	5	45
	Cefepime	5	45	0	0	6	55
	Ceftriaxone	5	45	0	0	6	55
	Tigecycline	4	40	4	40	2	20
	Levofloxacin	2	20	0	0	9	80
	Amoxicilin_clav	7	64	2	18	2	18
	Ciproflxacin	2	20	0	0	9	80
	Ceftazidime	5	45	0	0	6	55
	Gentamicin	2	18	0	0	9	82
	Amikasin	2	18	0	0	9	82
	Aztreonam	5	45	0	0	6	55
	Colistin	0	0	0	0	11	100
	Cefotaxime	5	45	0	0	6	55
	Cefpodoxome	5	45	0	0	6	55
	Cefixim	8	73	0	0	3	27
	Cefuroxime	7	64	0	0	4	36
	Cefazolin	8	73	0	0	3	27
	Ampicillin	10	91	0	0	1	9
	TMP_SMX	5	45	0	0	6	55
	Ceftazidime_Avibactam	0	0	0	0	11	100
MRSA	Macrolide	2	67	0	0	1	33
	Tigecycline	0	0	0	0	3	100
	Vancomycin	0	0	0	0	3	100
	Levofloxacin	0	0	0	0	3	100
	Clindamycin	2	67	0	0	1	33
	Ciproflxacin	0	0	0	0	3	100
	Gentamicin	0	0	0	0	3	100
	Amikasin	0	0	0	0	3	100
	TMP_SMX	0	0	0	0	3	100
	Methicillin	1	33	0	0	2	67
	Teicoplanin	0	0	0	0	3	100
	Doxycyclin	0	0	0	0	3	100
	Rifampin	0	0	0	0	3	100
MSSA	Imipenem	8	50	0	0	8	50
	Meropenem	8	50	0	0	8	50
	Ertapenem	10	60	0	0	7	40
	Pipracillin_tazobactam	8	50	3	20	5	30

(*Continued*)

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Table 6 (Continued).

Microorganism	Antibiotic	Resistant (Frequency)	Resistant (%)	Intermediate (Frequency)	Intermediate (%)	Sensitive (Frequency)	Sensitive (%)
	Cefepime	8	50	0	0	8	50
	Ceftriaxone	10	60	0	0	7	40
	Tigecycline	0	0	17	100	0	0
	Levofloxacin	11	63	0	0	6	38
	Amoxicilin_clav	7	40	0	0	10	60
	Ciproflxacin	12	70	0	0	5	30
	Ceftazidime	8	50	0	0	8	50
	Gentamicin	8	50	0	0	8	50
	Amikasin	5	30	3	20	8	50
	Aztreonam	8	50	0	0	8	50
	Colistin	0	0	0	0	17	100
	Cefotaxime	10	60	0	0	7	40
	Cefpodoxome	10	60	0	0	7	40
	Cefixim	10	60	0	0	7	40
	Cefuroxime	17	100	0	0	0	0
	Cefazolin	17	100	0	0	0	0
	Ampicillin	17	100	0	0	0	0
	TMP_SMX	10	60	7	40	0	0
	Ceftazidime_Avibactam	0	0	0	0	17	100
Nontypeable H influenzae	Imipenem	0	0	0	0	4	100
	Meropenem	0	0	0	0	4	100
	Ertapenem	0	0	0	0	4	100
	Pipracillin_tazobactam	0	0	0	0	4	100
	Cefepime	0	0	0	0	4	100
	Ceftriaxone	0	0	0	0	4	100
	Macrolide	0	0	4	100	0	0
	Tigecycline	0	0	0	0	4	100
	Levofloxacin	0	0	0	0	4	100
	Amoxicilin_clav	0	0	0	0	4	100
	Ciproflxacin	0	0	0	0	4	100
	Ceftazidime	0	0	0	0	4	100
	Aztreonam	0	0	0	0	4	100
	Cefotaxime	0	0	0	0	4	100
	Cefpodoxome	0	0	0	0	4	100
	Cefixim	0	0	0	0	4	100
	Cefuroxime	0	0	0	0	4	100
	Cefazolin	0	0	0	0	4	100
	Ampicillin	0	0	0	0	4	100
	TMP_SMX	4	100	0	0	0	0
Pseudomonas aeruginosa	Imipenem	5	29	0	0	12	71
	Meropenem	5	29	0	0	12	71
	Ertapenem	0	0	0	0	17	100
	Pipracillin_tazobactam	10	59	0	0	7	41
	Cefepime	2	12	8	47	7	41
	Levofloxacin	9	53	0	0	8	47
	Ciproflxacin	7	41	0	0	10	59
	Ceftazidime	10	59	0	0	7	41
	Gentamicin	5	29	0	0	12	71
	Amikasin	0	0	2	12	15	88
	Aztreonam	5	29	0	0	12	71
	Colistin	3	18	0	0	14	82
	Ceftazidime_Avibactam	4	25	0	0	13	75

(*Continued*)

Table 6 (Continued).

Microorganism	Antibiotic	Resistant (Frequency)	Resistant (%)	Intermediate (Frequency)	Intermediate (%)	Sensitive (Frequency)	Sensitive (%)
Salmonella	Imipenem	0	0	0	0	5	100
	Meropenem	0	0	0	0	5	100
	Pipracillin_tazobactam	0	0	0	0	5	100
	Cefepime	0	0	0	0	5	100
	Levofloxacin	0	0	0	0	5	100
	Ciproflxacin	0	0	0	0	5	100
	Ceftazidime	0	0	0	0	5	100
	Gentamicin	0	0	0	0	5	100
	Amikasin	0	0	0	0	5	100
	Aztreonam	0	0	0	0	5	100
	Colistin	0	0	0	0	5	100
	Ceftazidime_Avibactam	0	0	0	0	5	100

present in 36.9% of patients, indicating the complexity of pneumonia etiology in the population studied. The study's primary objective of evaluating diagnostic performance is directly supported by these findings, which also suggest that a significant number of etiologies might be missed by the culture-based workflows currently employed in routine hospital practice.

This discrepancy in pathogen detection is in line with earlier studies that highlighted PCR's higher sensitivity and ability to identify specific organisms, like viruses and some bacteria, that might not grow in culture.[34–36] For example, FilmArray PP found respiratory pathogens with a 90% positive agreement rate and changed the antibiotic prescriptions for 40.7% of patients.[37] The Real-timePCR has consistently showed higher sensitivity than culture technique for detecting microorganisms.[25,38] For instance, in a study involving COPD patients, real-timePCR identified significantly more bacterial microorganisms than culture (P<0.001), with common pathogens like *S. pneumoniae* and *P. aeruginosa* being more frequently detected.[34]

One of the best aspects regarding qPCR compared to culture is how quickly it operates. Results from traditional culture methods can take up to 72 hours, but results from qPCR can be ready in a matter of hours. For instance, a multiplex qPCR test for finding CAP-related microorganisms had a turnaround time of less than one working day, which is much faster than culture methods.[39] This rapid pathogen identification is criticalfor timely clinical decision-making and appropriate antibiotic selection.

A previous study used qPCR and culture for detecting respiratory microorganisms in pneumonia patients indicated higher positivity rates for *H. influenzae* and *M. catarrhalis* than culture.[35] Moreover, the qPCR allows the detection of additional pathogens that were not identified by culture, indicating qPCR's superior sensitivity.[36]

One major benefit of qPCR assays over culture methods is their ability to detect multiple pathogens and antibiotic resistance genes at the same time. This provides thorough diagnostic information that culture methods cannot match.[36] This multiplex ability is very helpful for treating pneumonia patients, who often have co-infections and patterns of resistance.

Enhanced sensitivity and rapid real-time PCR can help healthcare providers obtain better results by facilitating accurate, timely diagnoses. For example, real-time PCR for detecting *S. aureus* in endotracheal aspirates was more sensitive and specific than the traditional technique.[40] Moreover, real-time PCR's ability to identify microorganisms in samples where culture fails, such as in patients pre-treated with antibiotics, underscores its clinical utility.[41]

UUnlike culture techniques, real-time PCR provides a molecular approach to pathogen detection, identifying and quantifying nucleic acids from microorganism present in samples.[17–19,21,23,42,43] Compared to culture methods, this study shows a wider variety of pathogens detected, including both cultivable and non-cultivable species. This technique is sensitive enough to detect uncommon bacteria that might not grow well in culture, such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. A deeper understanding of the infectious landscape is made possible by the broader detection

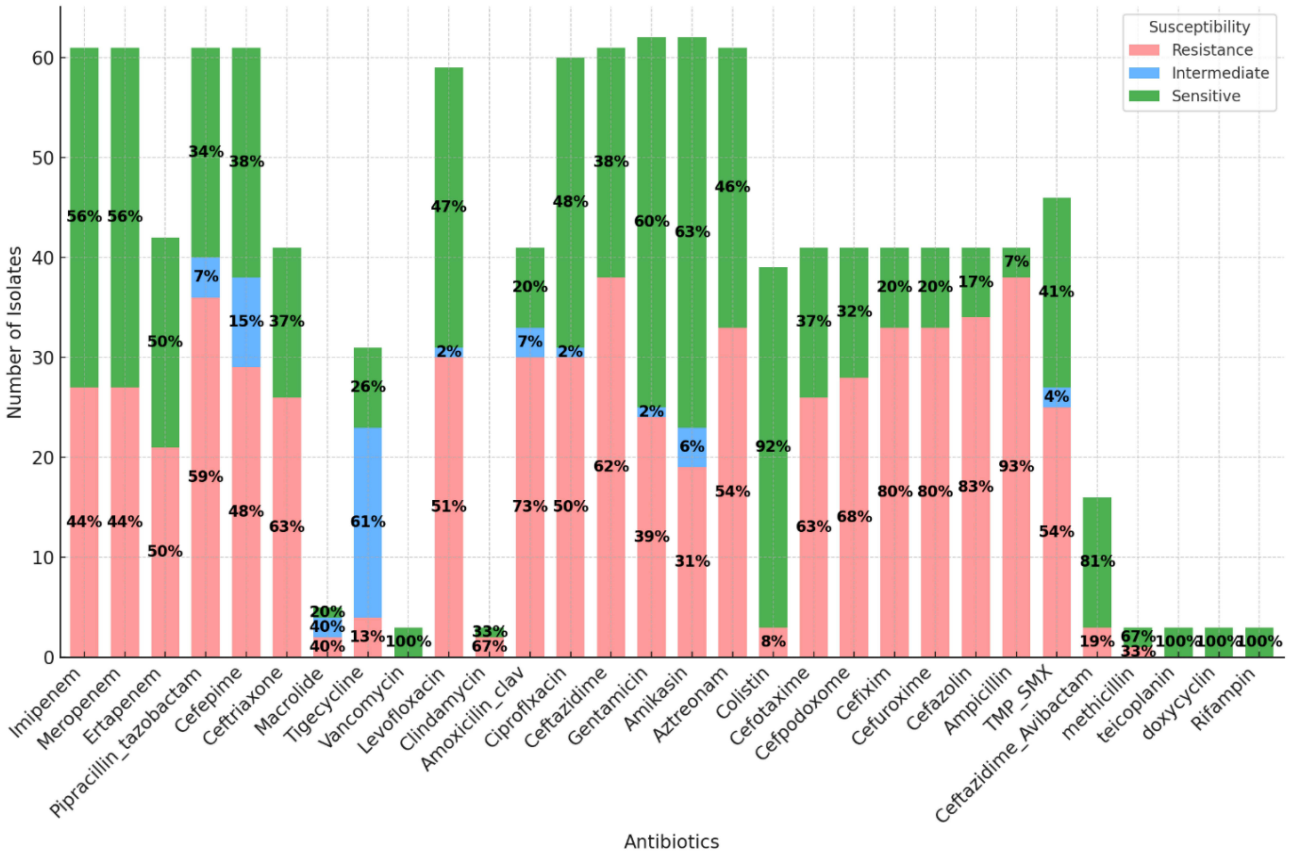

Figure 5 Visualisation of antibiotic resistance patterns. The bar chart displays only the antibiotics with non-zero counts.

range, which facilitates the development of focused treatment strategies. Real-time PCR can quantify the amount of microbial DNA or RNA in a sample. This can help figure out how bad an infection is and how well treatment is working.

Multiplex PCR has some drawbacks in spite of its outstanding diagnostic yield. Molecular platforms are more expensive directly, need specialized lab equipment, and might not be accessible everywhere in environments with limited resources. Furthermore, without quantitative thresholds or clinical correlation, PCR cannot accurately differentiate between colonization and active infection because it detects nucleic acid rather than living organisms. Therefore, conventional microbiology and clinical judgment should be complemented rather than replaced by molecular diagnostics.

In this study, the VITEK® 2 automated system was used for conventional bacterial identification. Despite being widely used, VITEK-2 is unable to detect fastidious, atypical, or non-cultivable organisms and may misidentify some non-fermenting Gram-negative bacilli. Additionally, culture-based techniques may produce false-negative results after previous antibiotic exposure and rely on viable organisms. The lower detection rate seen with culture was probably caused in part by these intrinsic methodological limitations.

The various pathogens detected by PCR in our study, including *Bordetella pertussis, Chlamydophila pneumoniae*, and several viral agents, illustrate the complexity of pneumonia. The high prevalence of bacterial-virus co-infections (36.9%) is particularly noteworthy, as previous studies indicate that polymicrobial infections can complicate clinical outcomes and require personalized antimicrobial treatments.[17,21,44] This complication highlights the drawbacks of depending solely on culture methods, which might miss significant co-pathogens.

The prevalence of *S. aureus* and *E. coli* as dominant bacterial species aligns with previous studies that state these pathogens as leading causes of pneumonia.[45,46] Nevertheless, detecting a various range of microorganisms through real-time PCR suggests that a more comprehensive diagnostic approach could improve empirical treatment plans and patient outcomes.

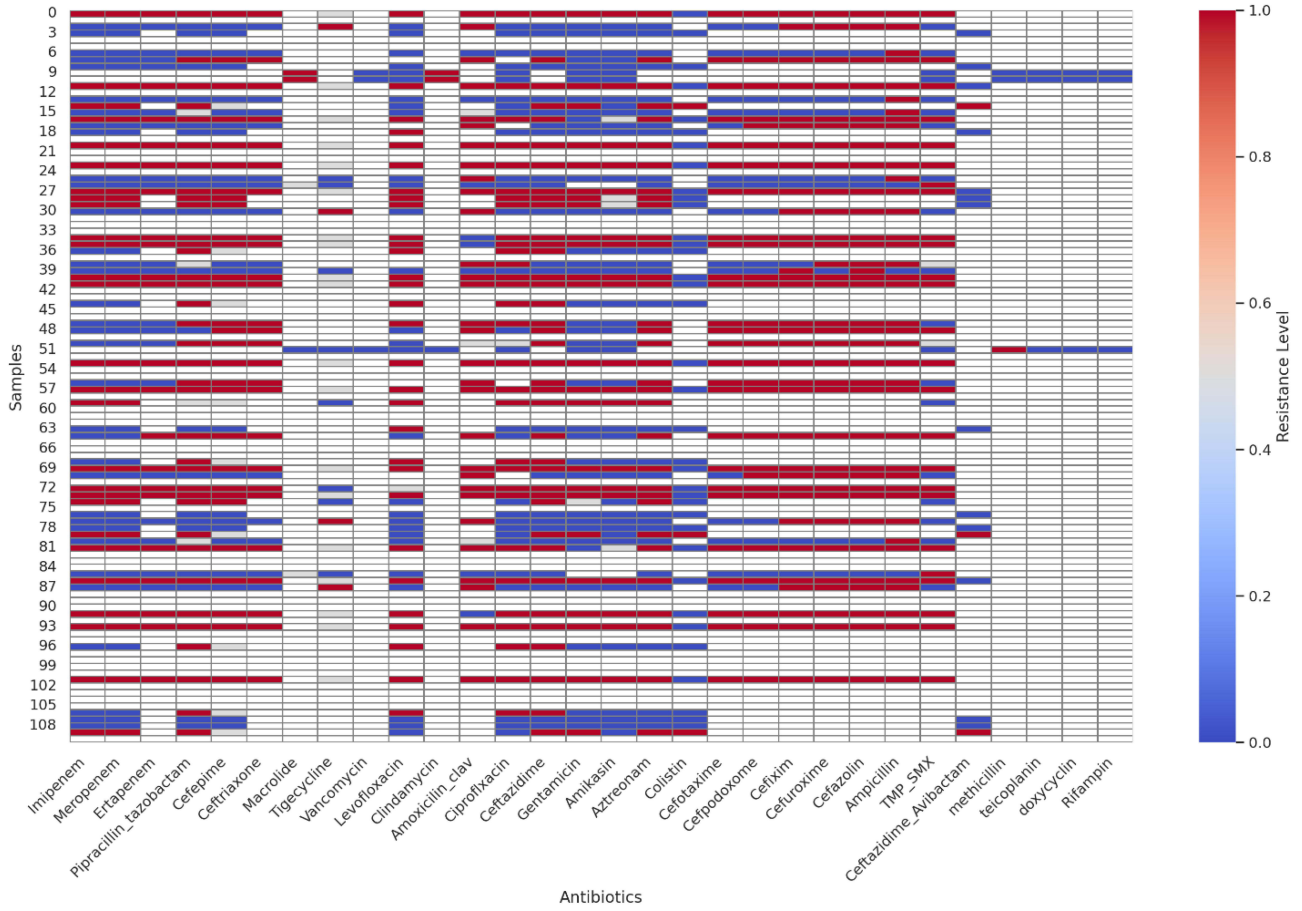

Figure 6 Heatmap of Antibiotic Resistance Patterns. The heatmap visualises the number of resistant isolates for each antibiotic. The intensity of the color represents the resistance level, with darker shades indicating higher resistance.

S. aureus, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), has been increasingly recognised as a significant microorganism in different infections, including CAP and HAP.[47]

S. aureus-associated pneumonia often presents with severe symptoms such as hemoptysis, multilobar infiltrates, and neutropenia, especially in community-acquired MRSA.[45] *S. aureus*-associated pneumonia has a high fatality rate, especially in young, healthy people and those who also have viral infections.[48]

The rise of antibiotic-resistant *S. aureus* strains, both MSSA and MRSA, complicates treatment, necessitating alternative more aggressive antibiotics.[45,49]

Escherichia coli is a common cause of urinary tract infections and gastrointestinal diseases. However, its role in CAP has been increasingly recognised, prompting investigations into its epidemiology, clinical features, and outcomes. Some studies suggest *E. coli* is an essential and severe cause of CAP with high mortality rates, while other studies indicate it is an infrequent cause but still associated with significant mortality and complications.[50,51]

E. coli is an under-recognised but important cause of CAP, with studies showing it accounts for a small but significant percentage of pneumonia cases.[51,52] Patients with *E. coli* CAP tend to be older, more severely ill, and have higher in-hospital and 90-day mortality rates compared to those with pneumococcal pneumonia.[51,52] *E. coli* CAP is associated with higher rates of ICU admission, mechanical ventilation, and vasopressor use compared to pneumococcal pneumonia.[52]

E. coli pneumonia is frequently associated with bacteremia, with many cases showing positive blood cultures.[50,52] High resistance rates to fluoroquinolones and ceftriaxone have been reported, necessitating cautious use of these antibiotics in empirical treatment.[51,52]

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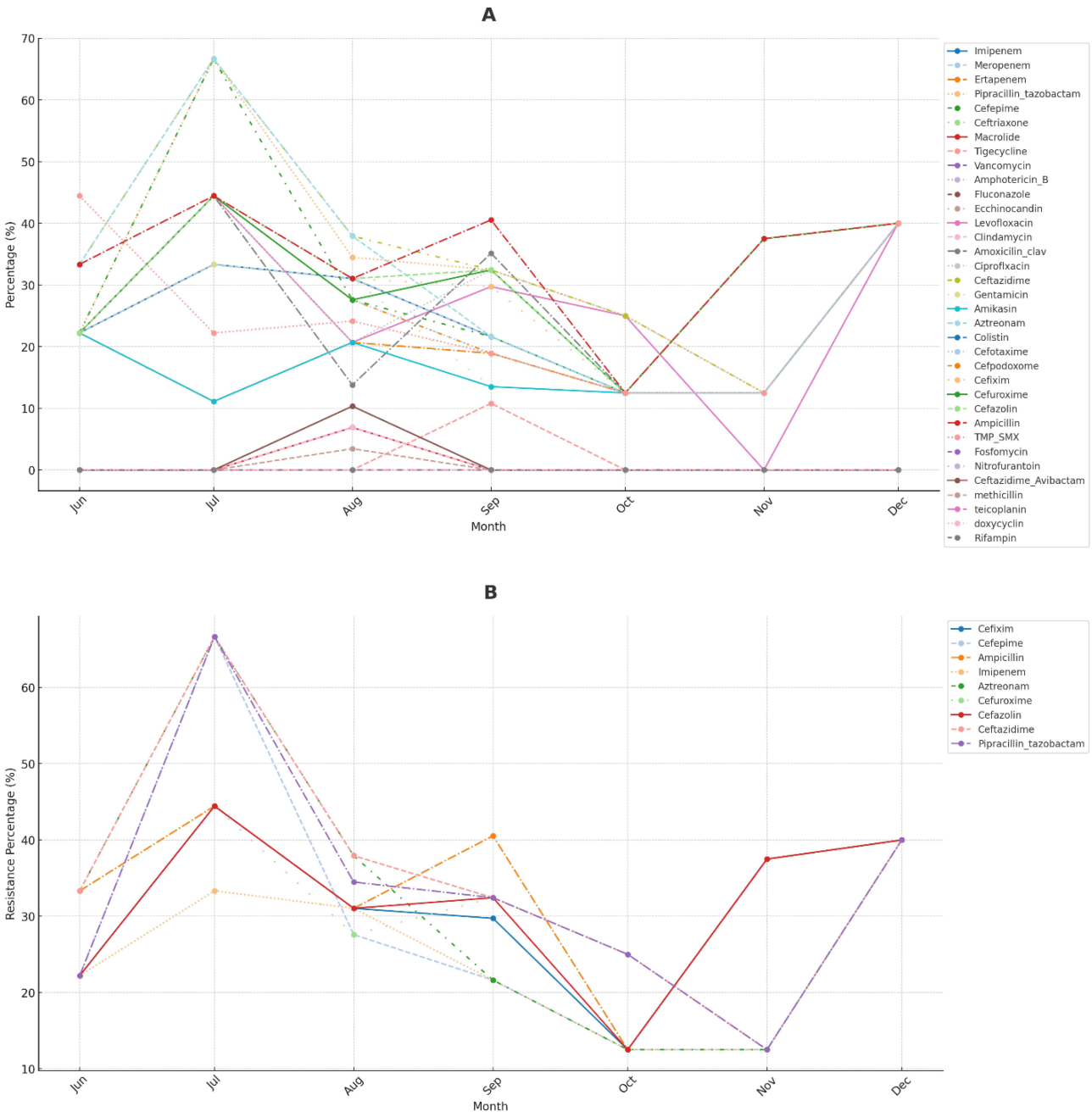

Figure 7 Monthly antibiotic resistance rends of all antibiotics (**A**) and the top 7 antibiotics with the highest fluctuation trend (**B**). The x-axis represents time in year-month format, showing the timeline of the study period. The y-axis indicates the number of resistant isolates identified each month for each antibiotic. Each line represents one antibiotic, and its position on the y-axis reflects the number of resistant isolates detected in that month. Different colors distinguish the antibiotics, with a legend provided to the right for clarity.

E. coli CAP patients are often older, more likely to be female, and frequently come from nursing homes. They also present with severe illness and confusion.[53] The infection may originate from an occult gastrointestinal source, even without abdominal or urinary symptoms.[50]

This study highlights the importance of precise pathogen identification in directing treatment choices and enhancing patient outcomes by offering insightful information about the clinical implications of various bacterial infections. Healthcare professionals can more effectively customize their therapeutic approaches to address the particular difficulties of different infections by associating the presence of pathogens with particular outcomes, which will ultimately improve patient care.

Table 7 Antibiotic Usage Among Participants

Antibiotic	Category	Frequency	Percentage
Azithromycin	Empiric	14	12.6%
	Targeted	3	2.7%
Amphotericin_B	Empiric	11	9.9%
	Targeted	3	2.7%
Ampicilin	Empiric	11	9.9%
	Targeted	4	3.6%
Clindamycin	Empiric	10	9.0%
	Targeted	5	4.5%
Imipenem_cilastatin	Empiric	10	9.0%
	Targeted	5	4.5%
Ciprofloxacin	Empiric	9	8.1%
	Targeted	7	6.3%
Metronidazol	Empiric	9	8.1%
	Targeted	3	2.7%
Ecchinocandin	Empiric	8	7.2%
	Targeted	2	1.8%
Pipracillin_tazobactam	Empiric	8	7.2%
	Targeted	10	9.0%
Doxycycline	Empiric	5	4.5%
	Targeted	6	5.4%
Ertapenem	Empiric	5	4.5%
	Targeted	5	4.5%
Amoxicilin_clavulinic acid	Empiric	7	6.3%
	Targeted	15	13.5%
Vancomycin	Empiric	6	5.4%
	Targeted	6	5.4%
Fluconazole	Empiric	0	0.0%
	Targeted	8	7.2%
Meropenem	Empiric	0	0.0%
	Targeted	23	20.7%
Colistin	Empiric	0	0.0%
	Targeted	9	8.1%
Tigecycline	Empiric	0	0.0%
	Targeted	18	16.2%
Levofloxacin	Empiric	0	0.0%
	Targeted	13	11.7%
Cefepime	Empiric	0	0.0%
	Targeted	17	15.3%
Ceftriaxone	Empiric	4	3.6%
	Targeted	7	6.3%
Fluconazol	Empiric	0	0.0%
	Targeted	8	7.2%
Amikacin	Empiric	0	0.0%
	Targeted	5	4.5%

Notes: The table is sorted based on the percentage of the "Empiric" category, from highest to lowest.

TThe average length of hospital stay (12.1 days) in our study exceeds the findings of comparable research (Median = 9 days),[54–56] which highlights the substantial healthcare burden of pneumonia and the need for improved management strategies. However, the ICU stay (Mean = 5.2 days) in our study is less than that of other studies (Mean = 12 days).[57]

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The study's mortality rate of 27% is higher than what has been reported in other studies. This suggests that better ways to diagnose and treat patients could lead to higher survival rates. For instance, the use of molecular diagnostics has made it easier to find pathogens early on, which has led to more targeted antimicrobial therapy and may have lowered the death rate. The death rate for pneumonia patients after 30 days is between 11.1% and 13%.[58,59] Intra-hospital mortality rates for CAP can be as high as 20.4%.[60]

The study demonstrated that qPCR displayed greater sensitivity and more extensive detection capabilities compared to conventional culture techniques ($p < 0.001$). This finding supports earlier research that highlights the enhanced sensitivity of qPCR in identifying fastidious and non-culturable pathogens, which are frequently overlooked by culture methods.[61–65]

The qPCR is still effective at identifying pathogens even after antibiotic treatment, which is a major advantage over culture methods that often fail in these situations.[66]

The increased prevalence of PCR-positive/culture-negative cases should not be considered as conclusive "false positives." Because traditional culture has known flaws, such as lower sensitivity after antibiotic exposure and the inability to find non-cultivable organisms, these conflicting results probably show real infections that culture did not find. Thus, the observed difference is more likely due to culture-related underdiagnosis than to molecular overdiagnosis. It remains important to carefully compare clinical data to distinguish colonization from true infection.

The study lacked the power to determine pathogen-specific mortality risk using adjusted multivariable modeling, despite descriptive visualization suggesting higher mortality among patients in whom specific pathogens were detected. Consequently, rather than being interpreted as causal relationships, these observations should be viewed as exploratory signals. To ascertain independent pathogen-related mortality risks, larger, sufficiently powered studies are needed. In agreement with our findings, previous studies suggest that *Bordetella pertussis* is associated with higher pneumonia mortality rates.[67–73] *Bordetella pertussis* was associated with pneumonia in children.[71]

Rather than reflecting superiority of non-adherence, the apparent paradox that non-guideline-concordant therapy was associated with higher improvement rates likely reflects a "guideline–severity mismatch." Clinicians may have appropriately escalated to broader-spectrum therapy beyond first-line recommendations in cases of severe pneumonia or suspected multidrug-resistant infections. In these situations, deviating from static guidelines might not be an indication of inappropriate prescribing, but rather a clinically justified escalation. The finding emphasizes the necessity of dynamic stewardship frameworks that incorporate fast molecular diagnostics and local resistance patterns. Furthermore, data limitations may also play a role: the dataset used may not capture all relevant factors, such as comorbidities or illness severity, which could influence adherence and outcomes.

Some studies suggest that adherence to community-acquired and ventilator-associated pneumonia guidelines is associated with better outcomes. In contrast, other studies indicate no significant improvement in outcomes for hospital-acquired pneumonia or with specific feedback interventions. Implementing guidelines for community-acquired pneumonia (CAP) led to improvements in the care process, such as increased adherence to recommended antibiotic treatments and reduced CAP-related mortality, although not all results were statistically significant.[74] Real-time electronic clinical decision support tools in emergency departments improved adherence to guidelines and were associated with lower mortality in patients with CAP.[75] A quality improvement project for CAP showed that guideline adherence reduced the hospital LOS and improved other process indicators.[74] Feedback with blinded peer comparison significantly improved physician adherence to guidelines for pneumonia and sepsis, leading to better compliance with recommended treatments.[76] Guidelines for VAP emphasise the importance of timely and appropriate antibiotic therapy, which is associated with improved survival rates.[77]

Understanding that treatment guidelines are general recommendations for average cases is essential. They may not always apply to the unique needs of individual patients. Making decisions in clinical practice can be difficult, and when a clinician realizes that a patient would benefit more from alternative treatments, they may decide to not follow through.

When analyzing the data, it's important to recognize the difference between association and causation. It has been shown that improvement and non-adherence are linked, but this does not mean that non-adherence causes improvement. Exploratory analysis is essential to understand the underlying causes and potential effects, as evidenced by the significant

rate of non-adherence among individuals who showed improvement. More research is needed to figure out why individuals fail to adhere to the guidelines and how that affects their health.

This analysis indicates that patient outcomes in this dataset are not significantly influenced by adherence to treatment recommendations. This indicates that the present sample size or data quality may be inadequate to detect a significant effect, or that alternative factors may be more critical in influencing outcomes. Further research encompassing additional variables or an expanded sample size may yield more substantial insights into the predictors of patient outcomes.

To fully understand how adherence to guidelines affects patient outcomes, we need to conduct further research. Subsequent research ought to investigate the factors influencing adherence, such as comorbidities, illness severity, and specific treatments administered. Moreover, it is essential to recognize the significance of clinical judgment in treatment decisions and the potential necessity for individualized strategies that extend beyond established standards. These interpretations underscore the complexity of clinical outcomes and the necessity for complex analyses that extend beyond simple adherence metrics.

The study's analysis of antibiotic resistance patterns found that some antibiotics, especially imipenem, piperacillin-tazobactam, and cefepime, were very resistant. The heatmap displayed varying resistance levels: piperacillin-tazobactam demonstrated elevated resistance rates, while imipenem exhibited a balanced distribution between resistance and sensitivity. These findings verify previous studies demonstrating elevated resistance rates to piperacillin-tazobactam, imipenem, and cefepime, specifically 33.9%, 38.6%, and 35.6%, respectively.[78–80]

Research shows high resistance rates to non-carbapenem beta-lactams, including piperacillin-tazobactam and Cefepime, in Turkey, India, China, and Spain.[78,79] For instance, resistance rates for piperacillin-tazobactam and Cefepime in *P. aeruginosa* were reported to be 33.9% and 35.6% in Turkey.[78]

In a randomised trial, imipenem/cilastatin/relebactam was found to be noninferior to piperacillin/tazobactam in treating HAP or VAP, with similar safety profiles.[80]

The resistance to Imipenem, piperacillin-tazobactam, and Cefepime is notably high in certain countries and has been increasing over time, particularly in ICU settings. Imipenem-cilastatin generally achieves higher treatment success and lower mortality rates than piperacillin-tazobactam and Cefepime, although it is associated with more adverse events. Piperacillin-tazobactam remains viable in settings with lower resistance profiles but is less effective in high-resistance regions.[78–80] Local resistance patterns and patient-specific factors should guide the choice of antibiotics to optimise treatment outcomes.

The line plot analysis of monthly trends in antibiotic resistance revealed seasonal peaks in resistance levels, particularly for Imipenem and piperacillin-tazobactam, around September and October. This may reflect increased infection rates and antibiotic use during these months.

Some antibiotics fluctuate according to seasonal patterns, especially in September and October. During the study-months, imipenem exhibits apparent resistance peaks, indicating seasonal variations associated with elevated antibiotic use or infection rates. This trend could lead to a rise in hospitalizations for respiratory diseases in the fall and a greater use of broad-spectrum antibiotics like imipenem. Resistance to pipracillin/tazobactam spikes in September and October, coinciding with an increase in infectious diseases during the fall. More antibiotic prescriptions could result in resistant strains of the drug. Cefepime resistance trends rise toward the end of the year, which corresponds with the colder months, because doctors may use it to treat severe infections, especially when other antibiotics are developing resistance. According to the data, antibiotic resistance for some medications rises in the fall, which may be related to an increase in prescription rates brought on by seasonal illnesses. Understanding these trends can help guide antibiotic stewardship initiatives, ensuring safe consumption during periods of high demand. Hospitals and clinics may implement targeted interventions in the fall to reduce down on unnecessary antibiotic use and prevent resistance. This analysis emphasizes how seasonal trends contribute to antibiotic resistance and how crucial it is to modify public health initiatives appropriately. Despite the fact that resistance fluctuated over time, the study design precludes drawing firm conclusions about seasonal patterns. The observed variability is more likely to be due to dynamic antibiotic use and local epidemiology. It would take several years of continuous surveillance to identify true seasonal resistance trends. Previous studies have not provided conclusive evidence that resistance rates to imipenem and piperacillin-tazobactam are higher in September and

October.[78,80–82] The studies pay less attention to particular seasonal patterns and more attention to broad trends and regional variances.[78,80]

By adapting treatments to particular microbial profiles and susceptibility patterns, qPCR combined with culture techniques improves pathogen detection and advances personalized medicine.

Based on these findings, there is a compelling reason for Jordan to develop national guidelines that combine molecular and traditional diagnostic methods to support personalized medicine approaches. Such guidelines would allow for more accurate diagnosis and tailored treatment plans that improve patient outcomes and reduce hospital stays. Additionally, the high prevalence of viral infection among our patients highlights the importance of viral screening in the management of pneumonia.

Overall, the findings provide strong support for the inclusion of multiplex molecular diagnostics in routine evaluations of pneumonia in Jordanian healthcare facilities. The demonstrated superiority of PCR in pathogen detection, particularly for viral and mixed infections, provides compelling justification for updating national diagnostic algorithms. When combined with local resistance surveillance and antimicrobial stewardship programs, molecular testing may enhance targeted therapy, reduce needless use of broad-spectrum antibiotics, and improve regional responses to antimicrobial resistance.

Study Limitations

This study has some limitations. First, because it was conducted at a single tertiary care facility, the generalizability of these findings may be limited. Second, there could be selection bias when archived samples are included. Third, the sample size was adequate for comparing diagnoses but was not large enough to support robust multivariable modeling of mortality. Fourth, the COVID-19 waves that occurred across the study period may have impacted the distribution of pathogens. Fifth, the direct impact of stewardship was less clear because molecular findings were not consistently associated with treatment modification or time-to-de-escalation outcomes. Future multicenter studies involving larger cohorts and prospective integration of outcomes are required.

Conclusion

This study shows that multiplex real-time PCR is better than traditional culture at finding pathogens in adults with pneumonia who are in the hospital. Significantly, PCR detected a considerable fraction of viral and mixed infections that routine culture would not have identified. Results that are PCR-positive but culture-negative may not reflect not “false positives.” They likely indicate that PCR is more sensitive for detecting infections than culture-based methods. The apparent link between not following recommendations and clinical improvement probably means that severe or multi-drug-resistant cases should be treated more aggressively, not that ignoring recommendations is better. This suggests that static empirical guidelines may not accurately represent local antimicrobial resistance patterns or disease severity, underscoring the need for adaptive stewardship frameworks guided by rapid diagnostics. From a medical perspective, a “personalized approach” should be defined as immediate antimicrobial optimization, encompassing the early de-escalation of ineffective broad-spectrum therapy, guided by fast molecular identification of pathogens and co-infections. This type of integration may assist with antimicrobial stewardship and lower the use of antibiotics when they are not necessary.

Nevertheless, the cost, laboratory infrastructure, and resource availability of systematic molecular testing must be taken into account, especially in middle-income countries like Jordan. While universal adoption may not be immediately feasible, targeted use in severe cases, or with patients who are at high risk for multidrug-resistant organisms may be a practical and cost-effective approach.

This study, which was done at only one center and had a small sample size and exploratory outcome modeling, mostly demonstrates that multiplex PCR is better for diagnosing than for predicting clinical outcomes. However, as far as we know, this is the first prospective study in Jordan to systematically compare multiplex PCR with conventional culture in adult pneumonia while also taking into account issues related to antimicrobial stewardship. These results provide us information that is useful in this region and can help with future multicenter studies and possible changes to national diagnostic and stewardship policies.

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This paper has been uploaded to Preprint.org as a preprint: https://eur02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.preprints.org%2Fmanuscript%2F202506.1035&data=05%7C02%7Ca_alsayed%40asu.edu.jo%7C7d79f33b23364d9539a608de39e46183%7Ca6bdeb1e77244165b796640034f507ba%7C0%7C0%7C639011850212757232%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=qWSUe4s4XxapmCIbBP%2Fc7XM0pZa2QLGq9SCdL1GgDoM%3D&reserved=0.

Disclosure

The authors report no conflicts of interest in this work.

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