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Original Article

Multiplex PCR-pneumonia panel: usefulness and cost-considerations in a post-transplant cohort in a lower middle-income country

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Abstract

Introduction: Post-transplant pneumonia is a clinical challenge. This study evaluated the clinical utility and cost-considerations of the multiplex polymerase chain reaction pneumonia panel (mPCR-PP) in a low-resource clinical setting.

Methodology: In this retrospective study, sputum or bronchoalveolar lavage (BAL) samples of 57 post-transplant adults with suspected lower respiratory tract infection were tested with mPCR-PP. The results were compared to standard cultures with the automated VITEK® system. The impact of mPCR testing on modulating antibiotic therapy was recorded. Cost implications of antibiotic changes were also estimated.

Results: A total of 33 (57.9%) patients with liver, 23 (40.4%) patients with renal, and 1 (1.8%) patient with hematopoietic stem cell transplantation were included. The sensitivity and specificity of multiplex PCR were 75% and 53.3%, respectively. The positive predictive value was 72% and the negative predictive value was 57%. Multi-pathogen detection was noted in 17 (43.6%) cases with mPCR and 6 (15.4%) cases with cultures. A total of 75 bacterial pathogens were identified: *Klebsiella pneumoniae* (n = 19; 25.3%), *Escherichia coli* (n = 13; 17.3%), and *Pseudomonas aeruginosa* (n = 11; 14.6%). Resistance markers including *CTX-M*, *NDM*, and *OXA-48-like* were identified through mPCR. Antibiotic regimens of 15 (26.3%) patients were subsequently modified leading to 11.1% reduction in antibiotic prescriptions. Cost assessment for patients undergoing meropenem de-escalation (n = 8) revealed that up to $232 USD could have been saved per patient with early mPCR implementation.

Conclusions: Multiplex PCR boosts pathogen detection and provides cost benefits which support its potential integration into management of post-transplant pneumonia.

Key words: multiplex PCR; immunocompromised; transplantation; pneumonia; antibiotics; antimicrobial stewardship.

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Introduction

The incidence of post-transplant nosocomial and community-acquired pneumonia continues to be a major challenge with the infectious etiologies markedly varying from immunocompetent populations. The overall incidence and etiology of lower respiratory tract infections (LRTI) may vary depending upon the timing and type of solid-organ transplantation (SOT). The risk of LRTI remains higher during early post-operative periods due to a multitude of factors including long-term intensive care unit (ICU) admission, poor mucociliary clearance due to ineffective cough mechanisms, and exposure to drug-resistant nosocomial agents [1]. Chronic immunosuppression can trigger acquisition of typical respiratory pathogens; as well as, less virulent, opportunistic organisms [2,3].

Solid organ transplants (SOT) carry a substantially high risk of post-transplant pneumonia where both donor and recipient-associated factors can precipitate LRTI [4,5]. A high incidence of pneumonia (43%) has been documented in liver transplantation cases [6]. Furthermore, LRTI can complicate up to 29% of renal transplants. Approximately 45% of post-renal transplant mortality can be attributed to pulmonary infections [7,8]. Given the intricate pathogenesis of pneumonia in post-transplant recipients, multiplex polymerase chain reaction pneumonia panel (mPCR-PP) testing represents an efficient diagnostic tool to accurately identify and treat multiple bacterial and viral pathologies. Previous literature has indicated the practical usefulness of mPCR in terms of generating an early diagnosis, as well as optimization of antimicrobial treatment. Multiplex PCR has been shown to cut down antimicrobial prescriptions by approximately 26% [9]. Furthermore, early implementation of mPCR-PP can help save approximately USD 7,587 by cutting down the overall cost of empirical antibiotic therapy [10]. Application of mPCR-PP can be particularly advantageous for developing countries such as Pakistan where early mPCR testing can avoid unsolicited in-hospital use of empiric antimicrobial agents.

This study highlights the efficacy and cost-

effectiveness of mPCR pneumonia testing in terms of identifying causative pathogens of LRTI amongst post-transplant patients within a low-to-middle income healthcare setting.

Methodology

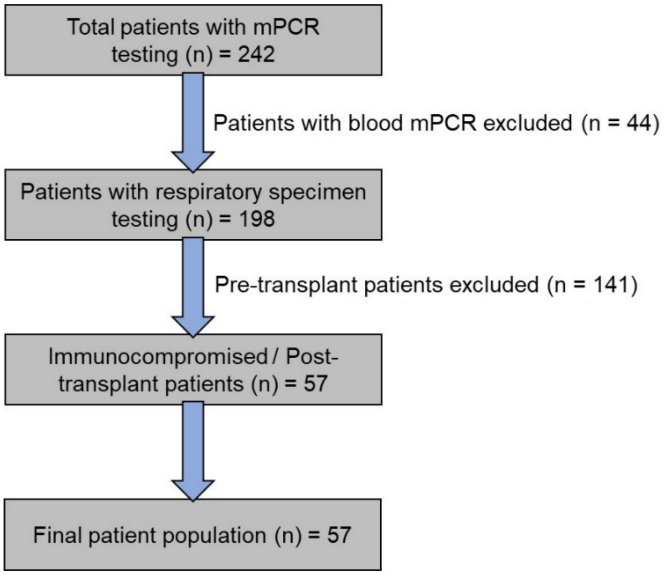
Study design and patient selection

This single-center, retrospective study was designed as a cross-sectional analysis. The study was conducted at the Pakistan Kidney and Liver Institute & Research Center (PKLI&RC). A total of 242 pre-transplant and post-transplant patients underwent mPCR pneumonia panel testing during 2022–2024. The mPCR-PP is validated for sputum, tracheal, and bronchoalveolar lavage (BAL) samples only; however, blood mPCR testing was also performed for n = 44 patients. All these patients were excluded from the study. Subsequently, data from the patients who had undergone mPCR testing on a respiratory specimen (n = 198) were included. The selected patient population had at least one or more of the following clinical findings on presentation: dry or productive cough, dyspnea, and/or compromised oxygen saturation at room air. The decision to perform mPCR-PP was undertaken by the treating physician in conjunction with the infectious diseases' consultant. Out of n = 198 patients, only post-transplant patients who had mPCR-PP test done on a respiratory specimen, were included in the final analysis (n = 57) (Figure 1).

Specimen collection

All mPCR samples are handled by laboratory technicians wearing standardized personal protective equipment. The specimens were labelled appropriately with patient biodata, specimen type, and date of collection. The preferable types of samples for this study were sputum (spontaneous or induced), BAL, and tracheal aspirate (for intubated patients). A minimal volume of 1–2 mL was considered as an adequate marker for all specimen types. All samples are analyzed within the first 2 hours of collection, as per hospital protocol.

Figure 1. Selection of patient population undergoing multiplex PCR (mPCR) pneumonia panel testing.



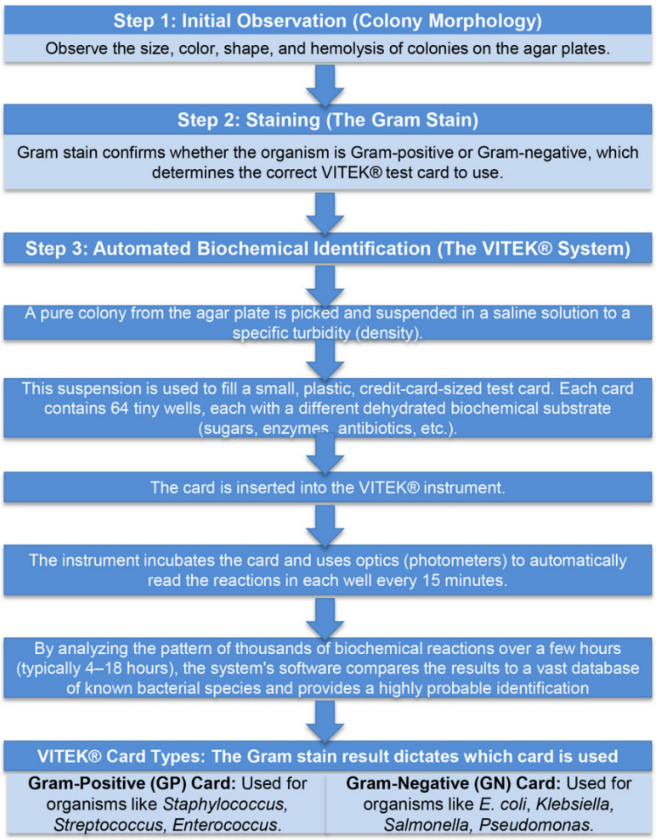
mPCR-PP pathogens

mPCR testing in the hospital was done by using the BioFire® FilmArray® (model no.2FA0727, Salt Lake City, Utah, USA). This model quantitatively assesses a total of 15 typical bacterial pathogens which are reported as the number of copies of DNA per milliliter (mL). The pneumonia panel fails to detect the organism if the number of copies of DNA are below $10^{3.5}$ or 3,162 (approximately) per mL. In addition, qualitative detection of atypical bacterial and viral pathogens was also performed.

Ethical Considerations

The institutional review board (IRB) at PKLI&RC provided ethical approval for the study (IRB-0130-R).

Figure 2. Study protocol for bacterial culture by using the automated VITEK® system.



Data parameters and statistical analysis

Patient age, gender, primary diagnosis, radiological findings, and antimicrobial treatment were recorded. Change in antibiotic therapy was defined as complete stoppage of the previous antibiotic regimen and initiation of a new antimicrobial agent based on the mPCR-PP results.

The microbiological findings obtained from sputum or tracheal aspirate culture and sensitivity reports were also recorded. Culture results are taken as a gold-standard measure and were assigned a comparator value for assessing parameters aimed at measuring the diagnostic accuracy of mPCR-PP. Figure 2 summarizes the overall procedure for bacterial Gram staining and accurate organism identification through an automated VITEK® system. The mPCR results were deemed as either true positive or true negative when they coincided with the positive or negative microbiological assays, respectively. The results were labelled as concordant when there was at least a single common organism isolated by both the mPCR and culture/sensitivity results of the same respiratory specimen.

Statistical Package for Social Sciences (SPSS) version 26.0 [11] was used to conduct data analysis, and an independent biostatistician reviewed the statistical tests applied. Accuracy indicators including sensitivity, specificity, and positive and negative predictive values were calculated for mPCR by constructing 2 x 2 tables in SPSS. kappa values were calculated to estimate concordance between culture/sensitivity and mPCR-PP results. A p value < 0.05 was considered significant.

Table 1. Chest imaging findings of participants (n = 55).

Imaging findings (n = 55)	Frequency (%)
Lung infiltrates/Patchy consolidation	14 (25.5%)
Unilateral pleural effusion	12 (21.8%)
Lobar consolidation	11 (20%)
Bilateral pleural effusion	9 (16.4%)
No acute finding	6 (10.9%)
Cavitary lesion	2 (3.6%)
Acute respiratory distress syndrome	1 (1.8%)

Table 2. Summary of multiplex PCR results for typical organisms (n = 75).

Multiplex PCR results	Frequency (%)
Klebsiella pneumoniae	19 (25.3%)
Escherichia coli	13 (17.3%)
Pseudomonas aeruginosa	11 (14.6%)
Acinetobacter	6 (8.0%)
Hemophilus influenzae	5 (6.7%)
Serratia marcescens	4 (5.3%)
Staphylococcus aureus	4 (5.3%)
Enterobacter	4 (5.3%)
Streptococcus pneumoniae	4 (5.3%)
Streptococcus pyogenes	2 (2.7%)
Moraxella catarrhalis	2 (2.7%)
Proteus	1 (1.3%)

Results

Baseline characteristics

A total of 57 post-transplant cases with LRTI were included in the study. A total of 42 (73.7%) participants were males. The mean age of the participants was 33.5 ± 20.7 years. A total of 33 (57.9%) patients underwent liver transplantation, whereas 23 (40.4%) had received a renal transplant. Only 1 patient had received a hematopoietic stem cell (HSC) transplant (n = 1; 1.8%).

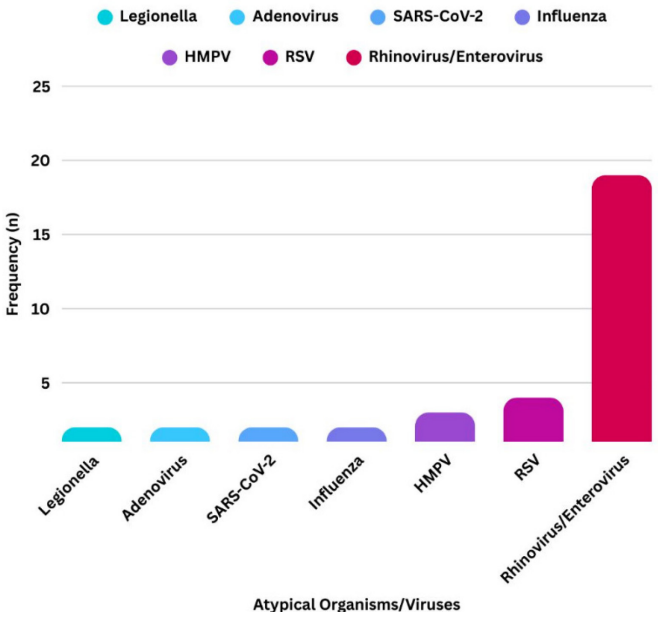
Table 1 summarizes the main findings from the chest imaging scans of the participants. Unilateral or bilateral pleural effusions were commonly reported in 21 (38.2%) cases. Fourteen (25.5%) individuals had lung infiltrates and/or patchy consolidation, whereas 11 (20%) had lobar pneumonia. Only 2 participants did not have any imaging scans available (Table 1).

Summary of mPCR results

A total of n = 75 typical organisms were isolated using mPCR (Table 2). *Klebsiella pneumoniae* (n = 19; 25.3%) was the most commonly isolated species, followed by *Escherichia coli* (n = 13; 17.3%) and *Pseudomonas aeruginosa* (n = 11; 14.6%). *Pseudomonas* infection was significantly more prevalent 1 year after organ transplant, compared to *K. pneumoniae* and *E. coli* (p = 0.045).

The number of atypical organisms/viruses detected was 34, and the Rhinovirus/Enterovirus group was the most isolated (19; 56%); as indicated in Figure 3.

Figure 3. Multiplex PCR results summary for atypical organisms and viruses (n = 34).



SARS-CoV-2: severe acute respiratory syndrome coronavirus; HMPV: human metapneumovirus; RSV: respiratory syncytial virus.

Table 3. Concordance of results between culture and multiplex PCR (n = 39).

Organisms	Culture results	Multiplex PCR results		Kappa value	*p* value
		Positive	Negative		
K. pneumoniae	Positive	9 (23.1%)	1 (2.6%)	0.805	< 0.001
	Negative	2 (5.1%)	27 (69.2%)		
E. coli	Positive	2 (5.1%)	1 (2.6%)	0.639	< 0.001
	Negative	1 (2.6%)	35 (89.7%)		
P. aeruginosa	Positive	5 (12.8%)	Nil	0.894	< 0.001
	Negative	1 (2.6%)	33 (84.6%)		

Comparison between mPCR and culture/sensitivity results

A total of n = 39 (68.4%) cases had culture and sensitivity results reported for their corresponding mPCR-PP results. Comparative analysis showed a sensitivity of 75% and a specificity of 53.3% for mPCR-PP. A positive predictive value of 72% and a negative predictive value of 57% were estimated. Multiple typical organisms were detected in 17 (43.6%) cases though mPCR, and in only 6 (15.4%) cases with microbiological culture. Overall, the results of 25 (64.1%) cases were found to be concordant between the two diagnostic modalities. Concordance was determined between multiplex PCR and culture results for the 3 most isolated typical organisms: *K. pneumoniae* (κ = 0.81), *E. coli* (κ = 0.64), and *P. aeruginosa* (κ = 0.89), as indicated in Table 3.

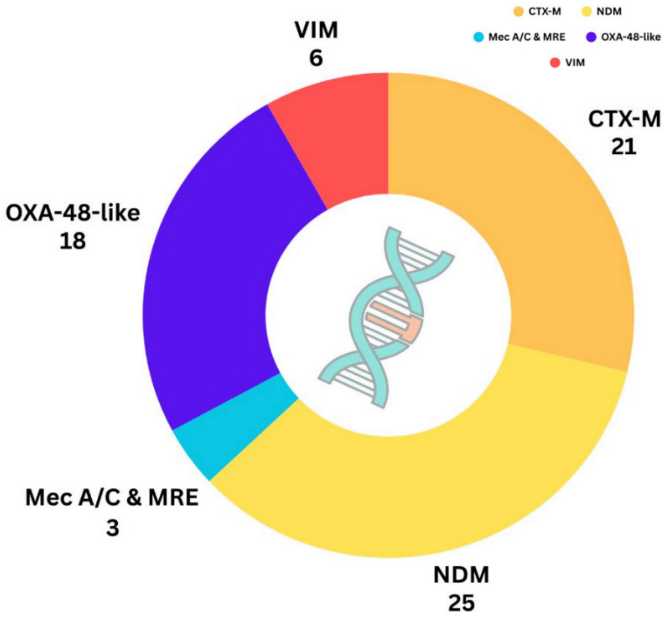
Antimicrobial resistance in mPCR

Figure 4 summarizes the antimicrobial resistance patterns in the n = 57 mPCR case results. The most prevalent mutations included *NDM* (n = 25; 43.9%), *CTX-M* (n = 21; 36.8%), and *OXA-48-like* (n = 18; 31.6%).

Antibiotic treatment and mPCR

The patients received empirical antibiotic therapy prior to implementation of mPCR-PP and this commonly included agents such as meropenem (n = 42; 24.6%), colistin (n = 30; 17.5%), and vancomycin (n = 19; 11.1%). This empirical regimen was typically administered for a mean duration of 7.5 ± 8.2 days before mPCR results became available. The antibiotic

Figure 4. Multiplex PCR prevalence of antimicrobial resistance genes (Total cases with multiplex PCR = 57).



CTX-M: cefotaxime-Munich; IMP: Imipenemase; NDM: New Delhi metallo-β-lactamase; OXA-48: oxacillinase-48-like β-lactamase; VIM: Verona integron-encoded metallo-beta-lactamase; MecA/C: methicillin-resistance; MRE: macrolide-resistance.

Table 4. Implementation of antibiotic changes following multiplex PCR (n = 15).

No.	Antibiotics before mPCR			Duration before mPCR (days)	Antibiotics after mPCR		
	1st	2nd	3rd		1st	2nd	3rd
1	Pip-Taz	Linezolid	Azithromycin	1	Cephalosporin	None	None
2	Meropenem	Vancomycin	None	4	Imipenem	None	None
3	Imipenem	Cotrimoxazole	None	14	Stopped	Stopped	Stopped
4	Meropenem	Linezolid	None	1	Cephalosporin	None	None
5	Cephalosporin	Teicoplanin	None	20	Imipenem	Linezolid	Colistin
6	Meropenem	Vancomycin	Colistin	7	Cephalosporin	None	None
7	Meropenem	Vancomycin	Colistin	7	Cephalosporin	None	None
8	Meropenem	Vancomycin	Linezolid	1	Cephalosporin	None	None
9	Pip-Taz	None	None	3	Meropenem	Vancomycin	None
10	Meropenem	Vancomycin	Tigecycline	15	Levofloxacin	Colistin	Cephalosporin
11	Meropenem	None	None	4	Cephalosporin	None	None
12	Meropenem	Colistin	None	30	Cephalosporin	None	None
13	Meropenem	Colistin	Vancomycin	1	Imipenem	Amikacin	Cotrimoxazole
14	Meropenem	Vancomycin	Doxy	5	Cotrimoxazole	Levofloxacin	None
15	Pip-Taz	None	None	2	Meropenem	None	None

Pip-Taz, piperacillin-tazobactam; cotrimoxazole, sulfamethoxazole-trimethoprim.

management was modified in 15 (26.3%) cases following the availability of the mPCR-PP results, indicating a substantial influence of molecular diagnostics on clinical decision-making. A notable reduction in overall antibiotic prescriptions by 11.1% was observed during the post-mPCR phase, reflecting a trend toward more targeted antimicrobial use. Additionally, when mPCR testing was performed ≤ 5 days after initiation of empirical therapy, a higher frequency (n = 9; 60%) of antimicrobial switching was noted, although this trend was not statistically significant ($p = 0.51$).

Cost implications of using mPCR-PP

Antibiotic de-escalation was witnessed in n = 10 (17.5%) out of 57 cases. Out of these cases, meropenem was switched to a 3rd or 4th generation cephalosporin in 7 (70%) patients, and to levofloxacin in 1 (10%) patient (Table 4). Meropenem is a single intravenous injection, and de-escalating meropenem helped lower the cost of antibiotic treatment by approximately $109.12 USD for 8 patients. In an ideal scenario, this is approximately equivalent to $1,088.5 USD for a total of 5 days of empirical therapy with twice daily meropenem regimen for 8 patients. Additionally, vancomycin was also switched to either cephalosporin (n = 4; 40%) or levofloxacin (n = 1; 10%), saving up to USD 9.2 per IV injection (Table 5). In contrast, meropenem was administered for an average of 8.8 days prior to de-escalation in a subset of patients with no sputum production or no tracheal secretions (n = 8) (Table 4), likely incurring an additional cost of approximately $231.8 USD per patient during the pre-mPCR-PP implementation phase.

Discussion

Broad detection of typical and atypical bacteria and viruses is particularly valuable in immunocompromised hosts. In this study, 57 post-transplant patients with suspected pneumonia were included and the mPCR-PP had a better diagnostic yield than conventional culture techniques for detecting difficult-to-culture microbes. mPCR detected 75 typical bacterial and 34 viral/atypical pathogens. Moreover, mPCR identified multiple pathogens in 17 (43.6%) cases. mPCR-PP had a sensitivity of ~75% and specificity of ~53%, while yielding a concordance of 64% compared to culture. Importantly, the mPCR-PP results prompted targeted antibiotic changes for 15 (26.3%) patients. In addition, an overall 11% reduction in antimicrobial use indicated a meaningful stewardship impact.

The mPCR-PP results largely corroborate recent studies demonstrating a broader pathogen detection than standard tests. Lee *et al.* assessed the role of mPCR in identifying respiratory pathogens in a total of 51 patients admitted to the ICU. A positive percent agreement of 90% and a negative percent agreement of 97.4% was reported for the FilmArray Pneumonia Panel. Moreover, this study showed concordance rates of 53.6% for culture-positive specimens [12]. This is comparable to an overall concordance of 64% found in the present study. In another study, by Cojuc-Konigsberg *et al.* mPCR was 95% sensitive and 60% specific for detecting co-infections among coronavirus disease 2019 (COVID-19) patients with ventilator-associated pneumonia [13]. Studies have consistently noted that mPCR often detects pathogens in specimens that are culture-negative. For instance, Ferrer *et al.* found that 23.9% of ICU respiratory specimens were positive by mPCR-PP, but negative by culture [10]. In the present cohort, 43.6% of cases showed multiple organisms on the PCR panel, versus only 15.4% on culture; suggesting that culture may likely miss co-infections with key pathogens. Cojuc-Konigsberg *et al.* found that raising the panel's threshold up to > 10^5 copies/mL can improve specificity from 60% to 86% by using a higher cutoff for typical pathogens [13]. Therefore, while mPCR enhances detection, results must be integrated with clinical context and other diagnostics to avoid overtreatment of colonization [14].

The panel's impact has been documented from an antibiotic stewardship perspective. In the present study, the antibiotic regimen of 10 (17.5%) patients was de-escalated after mPCR, while post-mPCR antibiotic use also declined by 11%. Other studies have reported comparable effects. For instance, Lee *et al*. suggested mPCR-guided changes could alter therapy in ~40% cases [12]. The present study also observed that when mPCR was performed earlier (≤ 5 days after empiric therapy), more antimicrobial changes (60%) were made

Table 5. Intravenous antibiotics that were de-escalated and corresponding injection costs (n = 10).

Injectable antibiotics stopped and de-escalated	Average duration before mPCR (days)	Number of patients (n)	Cost per injection (USD)
Meropenem	8.8	8	$13.6
Vancomycin	7	5	$9.2
Pip-Taz	1	1	$5.3
Colistin	30	1	$3.5
Imipenem	14	1	$5.4

Pip-Taz: piperacillin-tazobactam.

to targeted therapy. This is in line with the findings by Li-Geng *et al*. who reported that mPCR results led to antibiotic de-escalation a median of 3 days earlier (IQR 2–4 days) than would have been possible with culture alone [15]. Moreover, Miller *et al*. observed a trend towards shorter durations of empirical therapy after mPCR implementation, with a median 7-hour decrease in anti-staphylococcal agent duration and a median 36-hour reduction for anti-pseudomonal therapy [16]. Comparably, another trial also noted a significantly reduced usage of beta-lactam and macrolide prescriptions among patients tested with a pneumonia panel [9]. These reductions can help mitigate drug toxicity and unprecedented antibiotic resistance [17]. Furthermore, point-of-care availability of mPCR results can allow earlier implementation of transmission-based precautions. Concomitantly, a multi-center randomized trial with 545 participants found that 24 hours after diagnosis, a significantly higher proportion of patients receiving mPCR-guided care (i.e., 76.5%) were on adequate antibiotics versus only 55.9% patients with culture-based care [18].

While literature has increasingly recognized the practical utility of mPCR-PP, its cost-effectiveness, especially in low- and middle-income countries, is not well-defined. The overall running cost of the BioFire® pneumonia panel, including test cartridges, may require a significant upfront investment with prices averaging approximately USD 159.6 per test [19]. However, data from the current study indicates that for individuals who had antibiotic de-escalation, an early optimization of empirical antibiotic therapy could have helped offset up to USD 231.8 per patient in terms of meropenem use only. Studies also suggest that the savings from reduced antibiotic use can counterbalance the cumulative cost of running mPCR-PP. In line with this, results from a Spanish ICU study found that appropriate adjustments guided by the pneumonia panel could yield a net saving of ~USD 7591.76 [10]. Early accurate microbial detection in resource-limited settings like Pakistan could prevent indiscriminate use of expensive injectable antibiotics while also lowering the risk of adverse events. Nevertheless, a formal economic evaluation is needed while taking into account local test costs, antibiotic expenses, hospital stay fees, and patient outcomes. Careful stewardship and selective use of mPCR may be prudent while awaiting more robust cost-effectiveness data.

The results of the present study should be interpreted in light of a few potential limitations. Firstly, this was a single-center retrospective analysis of only 57 post-transplant cases, which limits generalizability and statistical power of the findings. Secondly, selection bias is possible since only those patients for whom clinicians ordered an mPCR panel were included without establishing any standardized criteria. Thirdly, a few patients might already have been treated with empiric antibiotics prior to mPCR, which could have suppressed bacterial growth in culture testing. Furthermore, the authors could not include clinical outcome endpoints (e.g., mortality rate or duration of hospitalization) due to heterogeneity of patient records. Lastly, given the relatively low specificity, mPCR-PP detected pathogens may have represented colonization or DNA from non-viable organisms. Future trials involving prospective, controlled studies with larger cohorts, detailed outcome tracking, and formal economic modeling are warranted to overcome this.

Conclusions

The findings of this study provide supporting evidence for the integration of mPCR-PP into the clinical management of post-transplant pneumonia. Use of mPCR-PP significantly improves the detection of respiratory pathogens, enabling more accurate and timely identification of causative organisms. This enhanced diagnostic capability may facilitate more individualized and targeted antimicrobial therapy, thereby optimizing treatment strategies in this high-risk, immune-compromised patient population.

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Conflict of interest

No conflict of interest is declared.

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