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Diagnostic Performance and Impact on Antimicrobial Treatment of a Multiplex Polymerase Chain Reaction in Critically Ill Patients With Pneumonia: A Multicenter Observational Study (The MORICUP-PCR Study: Morocco ICU Pneumonia-PCR study)

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OBJECTIVES: Managing severe pneumonia remains a challenge. Rapid diagnostic tests, such as multiplex polymerase chain reaction (mPCR), facilitate quick microorganism identification and may enable timely and appropriate antimicrobial therapy. However, studies from low-income countries are scarce. This study aimed to evaluate the diagnostic characteristics of mPCR and its impact on antibiotic therapy and outcomes in critically ill patients with pneumonia.

DESIGN: Multicenter observational study.

SETTING: Twelve ICUs across Morocco.

PATIENTS: Adult patients with pneumonia requiring invasive mechanical ventilation, including community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP).

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: Respiratory samples were analyzed using both mPCR and conventional microbiological methods. The diagnostic performance of mPCR was evaluated, including its sensitivity and specificity. Additionally, the appropriateness of mPCR-induced modifications in empiric antibiotic therapy and their impact on patient outcomes were assessed. A total of 210 patients were included, with a median age of 50 years (range, 33–67 yr), of whom 66.2% were male. Pneumonia types were distributed as 30% CAP, 58% VAP, and 12% HAP. mPCR demonstrated a sensitivity of 96.9% (95% CI, 92.3–99.2%) and a specificity of 92% (95% CI, 91–93%). Following mPCR, antibiotic therapy modifications were observed in 58% of patients ($n = 122$), including de-escalation or cessation in 11% ($n = 23$), escalation in 26.5% ($n = 56$), adequacy adjustments in 7.5% ($n = 16$), and initiation of antibiotics in 13% ($n = 27$). The appropriateness of antibiotic therapy increased significantly from 38.7% ($n = 83$) to 67% ($n = 141$; difference, 27.5%; 95% CI, 18.3–36.7; $p < 0.0001$). Generalized mixed model analysis revealed that appropriate post-mPCR antibiotic therapy was associated with reduced mortality (adjusted odds ratio, 0.37; 95% CI, 0.15–0.93; $p = 0.038$).

CONCLUSIONS: Our findings suggest that the use of mPCR is associated with a significant improvement in the appropriateness of empiric antibiotic therapy and is also associated with a positive impact on the outcome of patients with pneumonia.

KEYWORDS: Africa; community-acquired pneumonia; hospital-acquired pneumonia; molecular diagnostics; rapid diagnostic test; ventilator-associated pneumonia

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KEY POINTS

Question: What is the diagnostic performance of multiplex polymerase chain reaction (mPCR) in critically ill patients with pneumonia in low- and middle-income settings? What is the impact of its implementation on antimicrobial therapy and patient outcomes?

Findings: In an observational study of 210 patients across 12 ICUs, including cases of community-acquired pneumonia, hospital-acquired pneumonia, and ventilator-associated pneumonia, mPCR demonstrated excellent diagnostic performance, with high sensitivity and specificity. Its use was associated with optimized antimicrobial management and improved patient outcomes.

Meaning: The implementation of mPCR in critically ill patients with pneumonia in low- and middle-income settings could enhance microbiological diagnostics, support antimicrobial stewardship, and potentially improve clinical outcomes.

Pneumonia is a leading cause of sepsis and ICU admissions, and it remains the most frequent hospital-acquired infection among critically ill patients (1, 2). Severe cases of community-acquired pneumonia (CAP) and healthcare-associated pneumonias—including ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP)—are associated with increased morbidity, mortality, and healthcare costs (3, 4). Additionally, pneumonia is the most common indication for antibiotic administration, accounting for approximately half of all antibiotic prescriptions in the ICU (5). Administering appropriate empirical antibiotic therapy is crucial for improving survival (6, 7). This emphasizes the importance of initiating timely and appropriate antimicrobial treatment. One of the main challenges is rapid microbiological documentation. Conventional techniques require at least 48–72 hours and fail to identify microorganisms in up to 30–70% of cultures (8, 9).

In recent years, rapid molecular tests using multiplex polymerase chain reaction (mPCR) such as FilmArray Pneumonia Panel Plus (BioFire; Biomérieux, Marcy-l'Étoile, France) are being increasingly used. Timely identification of organisms with these tests could allow for targeted therapy, minimizing the use of broad-spectrum antibiotics, and thereby reducing the risk of the development of antibiotic resistance. Previous studies have shown good agreement between mPCR and conventional techniques (10–12). Furthermore, some studies suggested that mPCR could positively impact the appropriateness of empirical antibiotic therapy in critically ill patients with pneumonia, but its impact on outcomes is still unclear (9, 13–17). However, the majority of these findings are based on data from Western countries. Research from Africa, including low- and middle-income countries, with distinct epidemiological patterns, remains limited (18).

The objective of this study was to evaluate the diagnostic performance of mPCR in Moroccan critically ill patients with pneumonia requiring invasive mechanical ventilation and to assess its impact on appropriateness of empiric antibiotic therapy.

MATERIALS AND METHODS

Study Design—Ethics

This prospective, observational, multicenter study was conducted from February 2023 to February 2024 across 12 ICUs in Morocco. The study adhered to the principles of the amended Declaration of Helsinki and received approval from the Hospital-University Ethics Committee of Marrakech (reference: 02/2022, Pr. Ait Benali) on February 3, 2022. The study is titled "Diagnostic Performance and Impact on Antimicrobial Treatment of a Multiplex PCR in Critically Ill Patients with Pneumonia: A Multicenter Observational Study." Informed consent was obtained from the legal representatives of the patients. The study was registered on ClinicalTrials.gov under the identifier NCT05624684.

Patients Selection

Adult patients with pneumonia requiring invasive mechanical ventilation were considered.

Pneumonia Definition

The diagnostic criteria for pneumonia included the presence of a new lung radiological infiltrate (chest radiograph or CT scan) plus clinical evidence that the infiltrate is of infectious origin, such as the new onset of fever, purulent sputum, leukocytosis, and a decline

in oxygenation (19). HAP was defined as pneumonia not incubating at the time of hospital admission and occurring 48 hours or more after admission. VAP was defined as pneumonia occurring more than 48 hours after endotracheal intubation (19). CAP was defined as pneumonia acquired outside the hospital (20). Patients already included for a first episode of pneumonia were not repeatedly included.

Procedures and Microbiological Methods

Respiratory samples, including protected distal sampling (PDS), mini-bronchoalveolar lavage (mini-BAL), and endotracheal aspiration (ETA), were processed in local microbiology laboratories. Samples were obtained both before and after the initiation of antibiotic therapy. A direct Gram stain, classical microbiological quantitative culture (CMC), and antibiotic susceptibility testing (AST) were performed in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretative criteria (EUCAST clinical breakpoints). Microbial identification and AST were conducted using automated systems such as Vitek-2 (bioMérieux, Durham, NC) and BD Phoenix (BD, Sparks, MD). As per guidelines, the culture positivity thresholds were set at greater than or equal to 10^3 colony-forming units (CFUs)/mL for PDS, greater than or equal to 10^4 CFU/mL for mini-BAL, and greater than or equal to 10^5 CFU/mL for ETA. Bacterial cultures were considered negative if no bacteria were found or if only normal oropharyngeal flora were reported. In parallel, respiratory samples were analyzed using the FilmArray Pneumonia Plus Panel (BioFire; Biomérieux) following the manufacturer's instructions. Bacterial detection via the panel was considered negative if bacterial DNA was present at less than or equal to $10^{3.5}$ copies/mL.

Impact of mPCR Results on Antibiotic Therapy

Empirical antibiotic therapy was prescribed by the attending physicians based on local ecology and the protocols of each ICU. The antibiotic therapy could be adjusted after receiving the mPCR results, with changes classified as discontinuation, de-escalation, escalation, and adequacy. De-escalation was defined as the replacement of broad-spectrum antimicrobials with agents of a narrower spectrum or lower ecological impact, or stopping components of an antimicrobial combination (21, 22). Escalation was defined as the introduction of a broader-spectrum antibiotic. Adequacy was defined by the introduction of an antibiotic to cover a microorganism that was not adequately treated initially (14). The ranking of antibiotics was determined based on their spectrum and ecological impact. β-lactams were classified into six groups according to the Weiss et al (23) classification. At the end of the study, an independent multidisciplinary team, comprising a microbiologist, an intensivist and infectious disease specialist reviewed the patient files to evaluate the appropriateness of mPCR-induced antibiotic therapy changes. The antibiotic therapy was deemed appropriate if it was effective against the causative pathogen, and it was considered optimal if it was appropriate and had the narrowest possible spectrum. The experts relied on culture and AST to determine the appropriateness of antibiotic therapy. In cases of negative culture, mPCR results and local ecology were used to assess antibiotic therapy appropriateness. When antibiotic changes could not be clearly categorized, they were labeled as undetermined. The expert panel also evaluated the concordance between the resistance genes detected by mPCR and the phenotypic resistance observed in AST.

The primary goal of the study was to assess the performance of mPCR in critically ill patients with pneumonia requiring invasive mechanical ventilation. Secondary goals were to evaluate its impact on the appropriateness of empiric antibiotic therapy and on patient outcomes, particularly ICU mortality.

Statistical Analysis

Quantitative data are reported as median (interquartile range [IQR]), whereas qualitative data are presented as frequency and percentage. For comparisons of categorical variables among multiple groups, the chi-square test was used. When significant differences were identified, post hoc pairwise comparisons with Bonferroni correction were performed to determine specific group differences. For continuous variables, the Mann-Whitney *U* test was employed for two-group comparisons, whereas the Kruskal-Wallis test was used for comparisons across multiple groups. We used the multivariate imputation by chained equations algorithm on the training data to impute missing data.

However, patients with missing outcome data were excluded from the analysis.

To assess the diagnostic performance of mPCR in detecting bacterial pathogens, CMC was considered the gold standard. A contingency table was constructed to classify the results. A result was classified as true positive (TP) or true negative (TN) if both mPCR and CMC results were concordant. Microorganisms detected by mPCR but not identified by CMC were classified as false positives (FPs), while pathogens identified by CMC but not detected by mPCR were considered false negatives (FNs). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios were calculated for each bacterial pathogen, as well as for the overall mPCR performance.

Changes in empirical antibiotic therapy induced by mPCR results were visualized using a Sankey flow diagram generated with SankeyMATIC.com. To evaluate the impact of appropriate antibiotic therapy on ICU mortality, a multivariable multilevel logistic random-intercept model was fitted, accounting for the hierarchical structure of the data (observations within study sites). This model adjusted for potential confounders such as gender, age, severity scores, and pneumonia categories. Fixed effects were used for these variables, while random effects accounted for variability between ICU units. Adjusted odds ratios (aORs) with 95% CIs were reported. The forest plot was created using R (R Foundation for Statistical Computing, Vienna, Austria), and all analyses were performed using Jamovi Version 2.3.28 (The jamovi project, Sydney, Australia) and SPSS Statistics for Macintosh, Version 26.0 (IBM Corp, Armonk, NY).

RESULTS

Demographics

During the study period, 228 patients were screened (**Fig. 1** and **Table 1**). After excluding patients for various reasons (e.g., mPCR not done, culture not done), 210 patients were included in the study. The distribution of pneumonia types was as follows: CAP (n = 63, 30%), HAP (n = 25, 12%), and VAP (n = 122, 58%).

The median age of the patients was 50 years (IQR, 33–57 yr), with a median Acute Physiology and Chronic Health Evaluation II (APACHE II) score of 15 (IQR, 11–19), and men accounted for 66% of the cohort. Patients in the CAP and HAP cohorts were older, had more comorbidities, higher APACHE II score, and lower Pao_2/Fio_2 ratios compared with those in the VAP cohort. Furthermore, ICU admissions were predominantly due to pneumonia in the CAP/HAP group, while patients in the VAP group were primarily admitted for trauma and neurologic disorders. The incidence of septic shock in the cohort was 35%, with a significantly higher occurrence observed in the HAP group.

Sixty-two percent of patients received antibiotic therapy before respiratory sampling. This proportion was comparable across the three pneumonia groups (CAP: 64%; VAP: 58%; HAP: 77%; p = 0.201). The median delay between antibiotic administration and respiratory sampling was 48 hours (IQR, 24–96 hr). Prior antibiotic administration was significantly associated with an increased likelihood of a negative culture (odds ratio, 2.0; 95% CI, 1.1–3.7; p = 0.035).

Microbiological Results

The distribution of respiratory sample types was as follows: PDS (n = 124; 59%), ETA (n = 44; 21%), and mini-BAL (n = 42; 20%) (**Table 2**). The median turnaround time of the mPCR was 2 hours (IQR, 2–4 hr).

The rate of positive tests for bacterial microorganisms was 64% (135/210) for CMC vs. 82% (174/210) for mPCR ($p < 0.001$). The mPCR detected nearly twice as many bacterial pathogens as CMC, with the median number of pathogens isolated in CMC being 1 (IQR, 0–1) and the median number of pathogens detected in mPCR being 2 (IQR, 1–3; $p < 0.0001$). Viral targets were detected by mPCR in 29 patients, of whom 24 (83%) were co-infections. Multiple organisms were identified in 33 of 115 positive CMCs (27%) and in 125 of 174 positive mPCRs (72%).

The main microorganisms identified in CAP were *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus* (Table 2). Atypical bacteria were rarely detected, and influenza A was the most commonly detected virus. In VAP/HAP, the primary microorganisms were nonfermenting Gram-negative bacilli, particularly *Acinetobacter calcoaceticus-baumannii*, followed by *Enterobacterales* (*Klebsiella pneumoniae*, *Escherichia coli*) and *S. aureus*. Five off-panel bacteria were identified by CMC: *Stenotrophomonas maltophilia*, *Citrobacter koseri*,

TABLE 1.
Demographics and Clinical Characteristics of the Study Cohort

Variables	Community-Acquired Pneumonia ($n = 63$)	Ventilator-Associated Pneumonia ($n = 122$)	Hospital-Acquired Pneumonia ($n = 25$)	Cohort ($n = 210$)	p
Age, yr, median (IQR)	64 (45–70)	41 (28–63)	63 (40–71)	51 (33–67)	< 0.001
Female, *n* (%)	20 (31)	45 (36)	6 (24)	71 (34)	0.445
Charlson Comorbidity Index, median (IQR)	2 (0–4)	0 (1–3)	3 (1–5)	2 (0–3)	0.013
Acute Physiology and Chronic Health Evaluation II score, median (IQR)	17 (13–20)	13 (10–17)	15 (12–19)	15 (11–19)	0.002
Sequential Organ Failure Assessment score, median (IQR)	5.5 (3–8.75)	5 (3–7)	6 (4–8)	5 (3–8)	0.194
Reasons for ICU admission, *n* (%)					< 0.001
Pneumonia-related admissions	30 (48)	6 (5)	12 (48)	48 (23)	
Respiratory failure (nonpneumonia)	7 (11)	10 (8)	3 (12)	20 (10)	
Neurologic disorders	12 (19)	55 (45)	2 (8)	68 (32)	
Trauma	7 (11)	34 (28)	4 (16)	45 (21)	
Postoperative care	3 (5)	8 (6)	3 (12)	14 (7)	
Cardiovascular disorders	2 (3)	5 (4)	0	7 (3)	
Other causes	2 (3)	5 (4)	1 (4)	8 (4)	
Pao_2/Fio_2 ratio, mm Hg, median (IQR)	133 (195–283)	225 (158–301)	116 (75–210)	202 (123–297)	0.025
pH, median (IQR)	7.32 (7.15–7.4)	7.36 (7.29–7.44)	7.24 (7.15–7.37)	7.34 (7.23–7.43)	0.065
Lactates, mmol/L, median (IQR)	1.8 (1.05–3)	1.1 (1–1.6)	1.3 (1.1–3.4)	1.2 (1–2.3)	0.020
C-reactive protein, mg/L, median (IQR)	162 (59–288)	156 (82–236)	220 (68–295)	160 (75–257)	0.678
Procalcitonin, ng/L, median (IQR)	0.9 (0.3–13)	2.4 (0.7–12)	3.3 (0.6–8)	2.3 (0.5–11)	0.068
Septic shock, *n* (%)	21 (33)	37 (29)	16 (64)	74 (35)	0.004
ICU length of stay, d, median (IQR)	8 (5–12)	15 (10–29)	11 (7–20)	12 (8–24)	< 0.001
Survival, *n* (%)	34 (54)	61 (50)	8 (32)	103 (49)	0.407

IQR = interquartile range.

Pantoea species, *Streptococcus viridans*, and *Staphylococcus haemolyticus*. The ratio of bacterial pathogens identified by CMC to those detected by mPCR (CMC+/mPCR+) was significantly higher in HAP/VAP (0.43) compared with CAP (0.19; $p = 0.011$).

mPCR detected 88 resistance genes in the study cohort (Table 2). The proportion of resistance genes detected by mPCR was significantly lower in CAP samples (17/65; 26%) compared with VAP/HAP samples (71/145; 49%; $p < 0.0001$). Among Gram-negative bacteria, the most frequently encountered resistance genes were *blaCTX-M* ($n = 32$), followed by carbapenemases *blaNDM* ($n = 26$) and *blaOXA-48* ($n = 10$). No *blaKPC* genes were detected in this cohort. The *S. aureus* methicillin resistance gene *mecA/C-MREJ* was detected in 12 samples.

Diagnostic Performance of mPCR

The agreement between mPCR and CMC for bacterial pathogen identification showed the following

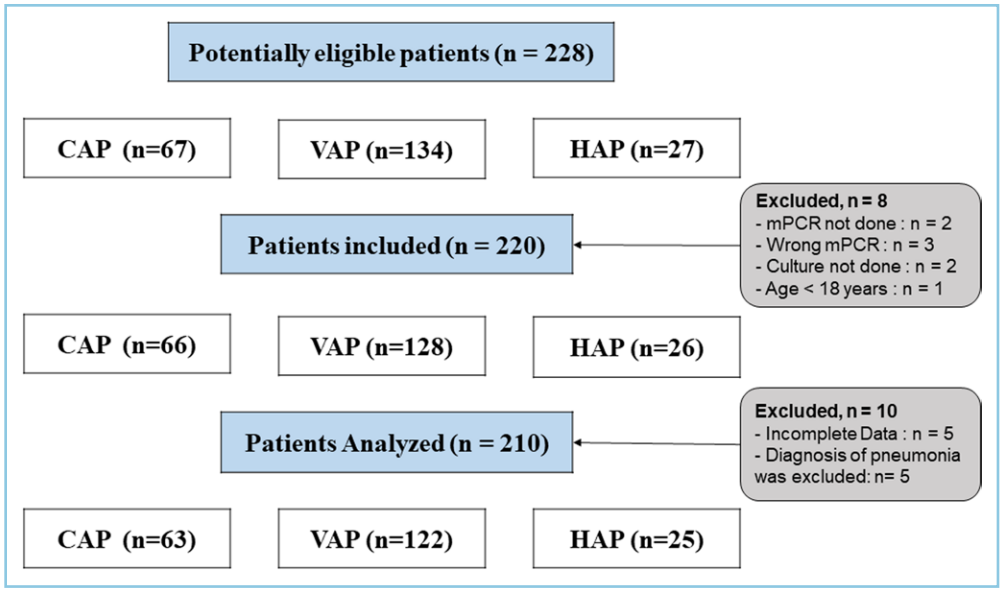

Figure 1. Flow chart of the study. CAP = community-acquired pneumonia, HAP = hospital-acquired pneumonia, mPCR = multiplex polymerase chain reaction, VAP = ventilator-associated pneumonia.

results: TP = 126, FP = 241, FN = 4, and TN = 2779 (**Table 1 Supplementary Material**, http://links.lww.com/CCX/B471). This yielded an overall sensitivity of 96.9% (95% CI, 92.4–98.8%) and a specificity of 92% (95% CI, 91–92.9%). The sensitivity and specificity were consistent across CAP and HAP/VAP patients, as well as among different respiratory sampling methods (DPS, mini-BAL, or ETA). PPV was 34.3% (95% CI, 30–39.3%) and NPV was 99.9% (95% CI, 99.6–99.9%). PPV was lower in CAP compared with HAP/VAP patients: 20.2% (95% CI, 17.7–24.3%) vs. 38.2% (95% CI, 34.8–41.6%), respectively (**Tables 2 and 3 Supplementary Material**). Standard diagnostic tests were not performed for viral or atypical pathogens, so diagnostic performance for these targets could not be evaluated. For resistance gene detection, three FPs were identified for the NDM and mecA/C genes, with an additional three FNs noted for mecA/C. In 30 cases, resistance concordance could not be fully assessed due to the absence of bacterial growth in culture.

Impact of mPCR on Empirical Antibiotic Therapy

The expert panel judged that among the cohort, empiric antibiotic therapy was appropriate in 39.5% (n = 83) and inappropriate in 38% (n = 80). In 11% of patients (n = 23), antibiotic therapy was not prescribed before polymerase chain reaction results. The appropriateness of antibiotic therapy was significantly higher in CAP patients (62%, n = 39) compared with VAP (29.5%, n = 36) and HAP patients (32%, n = 8; $p < 0.001$). mPCR results led to changes in antibiotic therapy in 122 patients (58%): de-escalation or antibiotic cessation in 11% (n = 23), escalation in 26.5% (n = 56), adequacy in 7.5% (n = 16), and initiation of antibiotic therapy in 13% (n = 27) of patients (**Fig. 2*A***). The mPCR results led to a significant increase in antibiotic therapy appropriateness from 38.7% (n = 83) to 67% (n = 141; difference, 27.5%; 95% CI, 18.3–36.7; $p < 0.0001$). However, post-mPCR antibiotic therapy remained inappropriate in 21% of patients (n = 45). The rate of appropriate post-mPCR antibiotic therapy was not significantly different between CAP, VAP, or HAP: 76% (n = 48), 61% (n = 75), and 72% (n = 18), respectively (p = 0.277). The experts could not determine the appropriateness of antibiotic therapy in 12% of patients (n = 24).

After the performance of mPCR, experts suggested that 35% (n = 73) of treatments could still be optimized. These potential optimizations included de-escalation or cessation of antibiotic therapy in 26% of cases (n = 54), escalation in 2% of cases (n = 4), adequacy in 3% of cases (n = 7), and initiation of antibiotic therapy in 4% of cases (n = 8). The comparison between actual post-mPCR antibiotic therapy changes (Fig. 2*A*) and optimal changes (**Fig. 2*B***) is represented in the Sankey diagram. Finally, mPCR led to inappropriate antibiotic therapy in two cases (approximately 1% of patients). In one case, *Acinetobacter baumannii* was not detected by mPCR, and in the other, the mecA/mREJ gene was not identified in a methicillin-resistant *S. aureus* isolate.

Impact of Appropriate Antibiotic Therapy on Outcome

In the overall cohort, an appropriate post-mPCR antibiotic therapy was associated with a statistically

TABLE 2.
Comparison of Microorganisms Identified by Multiplex Polymerase Chain Reaction and Classical Microbiological Culture Among ICU Patients With Pneumonia

	Community-Acquired Pneumonia ($n = 63$)		Hospital-Acquired Pneumonia/Ventilator-Associated Pneumonia ($n = 147$)	
Microorganisms	**mPCR+**	**CMC+**	**mPCR+**	**CMC+**
Bacteria				
Acinetobacter calcoaceticus-baumannii			72	49
Enterobacter cloacae complex	5		15	4
Escherichia coli	3	1	17	11
Haemophilus influenzae	20	1	44	6
Klebsiella aerogenes			2	
Klebsiella oxytoca	1		1	1
Klebsiella pneumoniae	6	1	24	13
Moraxella catarrhalis	5		6	
Proteus species			7	
Pseudomonas aeruginosa	4	1	26	7
Serratia marcescens	1		6	7
Staphylococcus aureus	20	6	48	23
Streptococcus agalactiae	5		4	1
Streptococcus pneumoniae	15	4	15	3
Streptococcus pyogenes		1		
Atypical microorganisms				
Chlamydophila pneumoniae		N/A	1	N/A
Legionella pneumophila		N/A		N/A
Mycoplasma pneumoniae	1	N/A	1	N/A
Virus				
Adenovirus		N/A	1	N/A
Coronaviruses OD43, NL63, HKU1, and 229E	4	N/A	4	N/A
Human metapneumovirus		N/A	1	N/A
Human rhinovirus/enterovirus	1	N/A	5	N/A
Influenza A	5	N/A	3	N/A
Influenza B	1	N/A		N/A
Parainfluenza virus	1	N/A	1	N/A
Respiratory syncytial virus		N/A	1	N/A
MERS coronavirus	1	N/A	1	N/A

(*Continued*)

TABLE 2. (*Continued*)

Comparison of Microorganisms Identified by Multiplex Polymerase Chain Reaction and Classical Microbiological Culture Among ICU Patients With Pneumonia

	Community-Acquired Pneumonia ($n = 63$)		Hospital-Acquired Pneumonia/Ventilator-Associated Pneumonia ($n = 147$)	
Microorganisms	**mPCR+**	**CMC+**	**mPCR+**	**CMC+**
Resistance genes				
KPC	0		0	
NDM	2		24	
OXA-48	1		9	
VIM	2		5	
IMP	0		1	
CTX-M	8		24	
mecA/C and MREJ	4			

229E = Human coronavirus 229E, CMC = classical microbiological culture, CTX-M = Cefotaximase-Munich, HKU1 = human coronavirus HKU1, IMP = Imipenemase metallo-β-lactamase, KPC = *Klebsiella pneumoniae* carbapenemase, mecA/C = genes encoding penicillin-binding protein 2a [PBP2a], MERS = Middle East Respiratory Syndrome coronavirus, mPCR = multiplex polymerase chain reaction, MREJ = mec-associated direct repeat unit junction, NDM = New Delhi metallo-β-lactamase, NL63 = human coronavirus NL63, OD43 = human coronavirus OC43, OXA-48 = Oxacillinase-48, VIM = Verona integron-encoded metallo-β-lactamase.

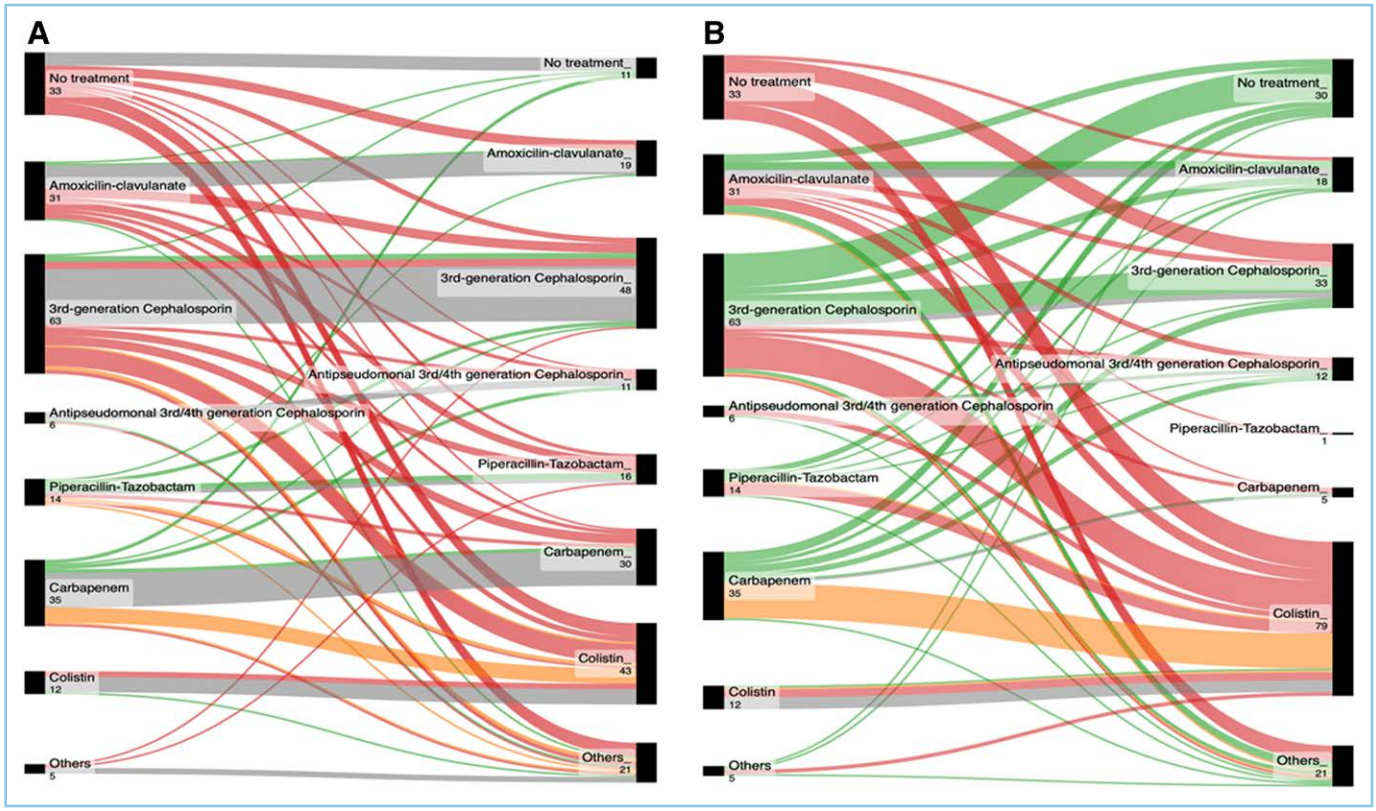

Figure 2. Sankey diagram illustrating the flow of antibiotic therapy changes in critically ill patients with pneumonia following multiplex polymerase chain reaction (mPCR) testing. The diaphragm compare actual mPCR-induced antibiotic therapy changes implemented by intensivists (**A**) vs. optimal changes recommended by experts (**B**). *Gray flows*: Continuation of antibiotics. *Red flows*: Initiation or escalation of antibiotics. *Green flows*: De-escalation or withdrawal of antibiotics. *Orange flows*: Adequacy of antibiotics. The *thickness* of *each line* represents the frequency of changes, with *thicker lines* indicating a higher number of changes.

significant reduction in ICU mortality, with an aOR of 0.37 (95% CI, 0.15–0.93; $p = 0.038$) (**Fig. 3**). Subgroup analyses revealed that the protective effect was consistent across most subgroups, with the exception of patients with CAP. In contrast, appropriate empiric antibiotic therapy was not associated with a reduction in mortality in either univariate or multivariate analyses.

DISCUSSION

To our knowledge, this is the first large multicenter observational study investigating the use of mPCR for managing pneumonia in critically ill patients within an African context. Our findings suggest that mPCR has excellent diagnostic performance for bacterial pathogen identification, marked by high sensitivity, specificity, and NPV, although its PPV was relatively low. mPCR was associated with modifications in antibiotic management for 58% of the patients, with escalation being the most frequent change. However, antibiotic therapy could still be further optimized in 34.7% of patients, largely due to missed opportunities for de-escalation or discontinuation. We found a low rate of appropriate empiric antibiotic therapy, which significantly improved following mPCR, increasing from 38.7% to 67% ($p < 0.0001$). Finally, our study suggested that appropriate post-mPCR antibiotic therapy was associated with improved ICU survival.

Several studies have evaluated the diagnostic performance of mPCR assays, particularly the FilmArray Pneumonia Panel, and found a high accuracy (10, 18, 24–27). A recent meta-analysis of 30 observational studies, most of which involved ICU patients, analyzed 8968 samples and reported a global sensitivity

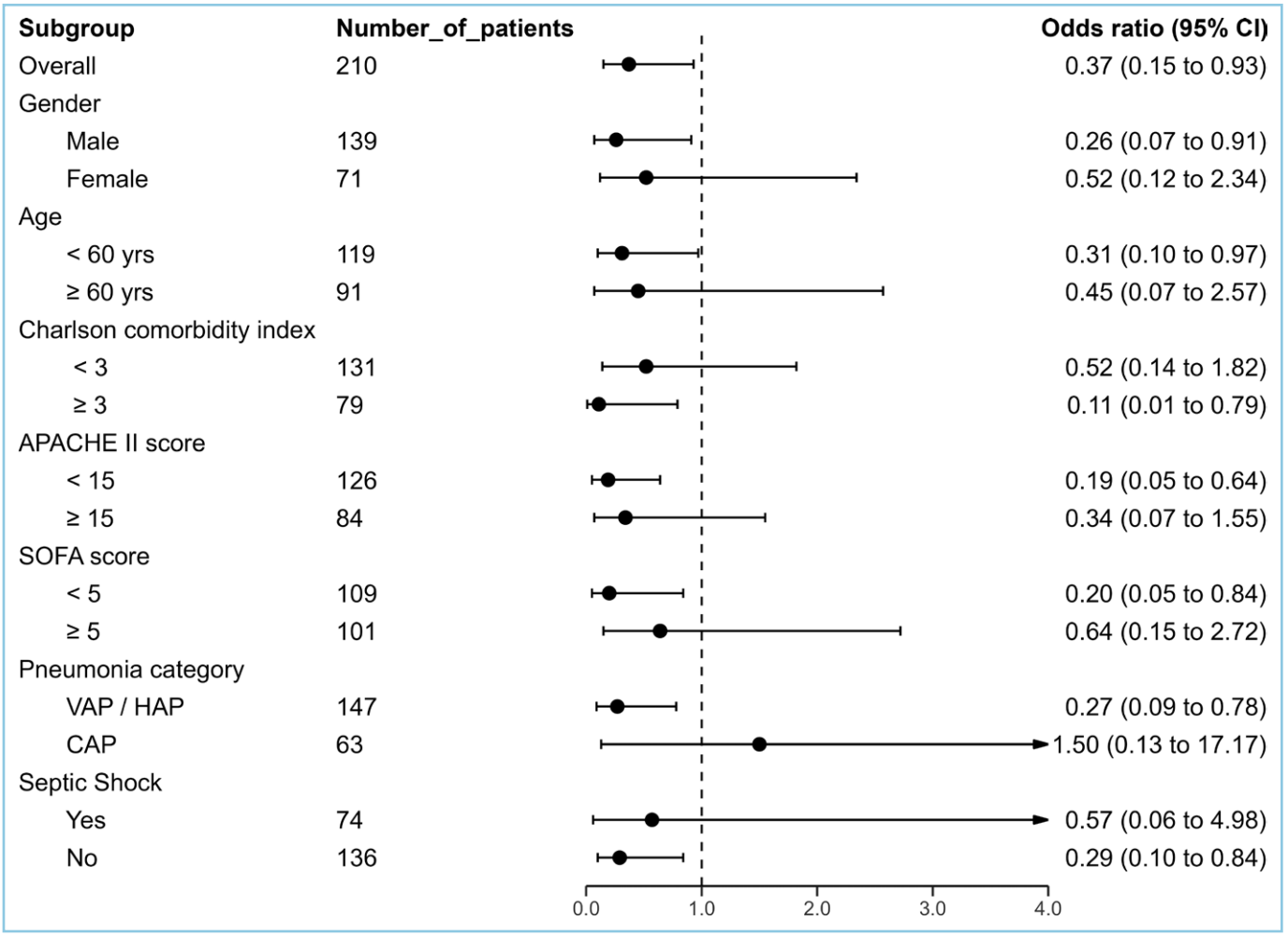

Figure 3. Impact of appropriate antibiotic therapy following multiplex polymerase chain reaction guidance on ICU mortality. Subgroup analysis by gender, age, severity scores, and pneumonia categories. APACHE II = Acute Physiology and Chronic Health Evaluation II, CAP = community-acquired pneumonia, HAP = hospital-acquired pneumonia, SOFA = Sequential Organ Failure Assessment, VAP = ventilator-associated pneumonia.

of 94% (95% CI, 91–95%) and specificity of 98% (95% CI, 97–98%) (28). Despite differences in microbial ecology—where *S. aureus* and *Pseudomonas aeruginosa* were the predominant microorganisms in this meta-analysis—these results align with our study's findings.

We observed a high proportion of FPs, resulting in a low PPV, which aligns with previous studies reporting PPV values ranging from 40% to 60% (12, 18, 25, 28–30). This observation can be attributed to several factors. First, the high sensitivity of mPCR allows for the detection of trace amounts of bacterial DNA, including that from colonizing flora or nonviable bacteria no longer causing active infection and therefore not detected by culture. In addition, fastidious organisms, such as *H. influenzae* or *S. pneumoniae*, likely contributed to the low PPV observed (18). Indeed, these microorganisms displayed the lowest PPV values. Second, variability in sample collection, preservation (fresh vs. frozen), handling, and processing may also contribute to FP. Notably, the sampling process was not standardized in our study. Regarding sample type, studies suggest that BAL is associated with the best mPCR diagnostic performance (11, 24, 28, 31). In our study, the most commonly performed respiratory sample was PDS. The mPCR diagnostic performance for PDS has not been previously reported. Third, the administration of antibiotics before sampling can inhibit microbial growth in culture. Previous studies have shown that 50–76% of patients with FP mPCR results had received antibiotics before sample collection (11, 32). In our cohort, more than 60% of patients had received antibiotics prior to respiratory sampling, which was associated with a two-fold increase in the likelihood of negative cultures (18, 30, 31). Furthermore, the delay between respiratory sampling and antibiotic initiation was notably long, with a median of 48 hours. This delay may be explained by the initiation of antibiotics before intubation in patients with CAP or HAP. Last, although culture is still considered the gold standard for bacterial microbiological diagnosis, its reliability remains questionable and may be challenged by the greater accuracy of mPCR (28).

Another aspect, not addressed in our study, is the diagnostic cutoff for mPCR. Some authors have suggested that increasing the mPCR threshold above 10^5 copies/mL could improve the test's PPV and specificity (30, 31). However, studies have demonstrated only a weak correlation between mPCR DNA copy numbers and CFU in culture (33, 34). Therefore, further research is required to establish a threshold that reliably distinguishes colonization from infection (32).

Our study suggested a significant improvement in the appropriateness of empirical antibiotic therapy with mPCR, consistent with previous studies. However, while most prior studies focused on the potential or simulated impact of mPCR, our research assessed its real-world effects. For instance, a British study of 323 CAP episodes suggested that molecular testing could lead to de-escalation in 77% of cases and escalation in 5.9% (9). Similarly, two French multicenter studies involving both CAP and HAP/VAP found that mPCR could enable early de-escalation in 40% and escalation in 20% of cases (13, 14). Randomized controlled trials (RCTs) on mPCRs impact on antibiotic therapy management remain scarce. The INHALE trial (the impact of using FilmArray Pneumonia Panel molecular diagnostics for hospital-acquired and ventilator-associated pneumonia on antimicrobial stewardship and patient outcomes in UK Critical Care), which included 545 HAP/VAP patients from 13 British ICUs, showed that antibiotic therapy appropriateness was significantly higher in the mPCR group at 24 and 72 hours (76% and 73% vs. 56% and 59%; $p < 0.001$). Surprisingly, despite this improvement, the pneumonia cure rate was lower in the mPCR group (56.7% vs. 64.7%) (35, 36). Similarly, RCTs by Poole et al (15) and Darie et al (16) found that mPCR improved antimicrobial treatment but had no impact on clinical outcomes. Additionally, the MultiCoV RCT (use of a respiratory MULTIplex PCR andprocalcitonin to reduce antibiotic exposure in patients with severeconfirmed COVID-19 pneumonia) during the COVID-19 pandemic found no benefit of an mPCR/procalcitonin strategy on antibiotic exposure or clinical outcomes (17).

In contrast to these studies, which were mostly conducted in high-income countries, our research revealed a significant association between mPCR-guided therapy and improved outcomes. One likely factor is the low rate of adequate empirical antibiotic therapy in our setting (38.7%), compared with the 56–77% reported in high-income settings (13, 16, 37). Furthermore, this study was conducted in the African context, which is distinct due to its limited medical resources, higher rates of nosocomial infections, and greater antimicrobial resistance (38, 39). Even after

mPCR-guided adjustments, there was room for further optimization in 35% of cases, particularly with regard to de-escalation. Figure 2 clearly shows the gap between physician-prescribed and expert-recommended antibiotic therapy. Physicians may be more hesitant to de-escalate due to patient severity, limited expertise in interpreting mPCR results, and varying confidence in the test (25). This highlights the importance of antimicrobial stewardship (AMS) measures to support the use of mPCR. However, the protective effect of appropriate post-mPCR antibiotic therapy was not observed in CAP patients, likely due to the already higher rate of empiric antibiotic therapy appropriateness (62%) in this subgroup. The small sample size (n = 63) limits any definitive conclusions.

This study has several limitations. First, a formal sample size calculation was not performed due the exploratory nature of the study and logistical constraints across participating centers in a resource-constrained setting. Second, microbiological diagnostics, particularly culture methods, were not standardized across the participating laboratories due to logistical constraints, which may have resulted in lower culture performance. Third, the study was neither controlled nor randomized, which could weaken the findings related to the impact of mPCR on outcomes. However, unlike RCTs, this study reflects "real-life" conditions in Africa, making the results more applicable to clinical practice. Forth, we did not collect additional outcome data, such as clinical cure rates, antibiotic exposure, or length of stay, which could limit the scope of our analysis. Last, including both CAP and HAP/VAP cases may have introduced heterogeneity into the study population. However, the mPCR panel is designed to address both types of pneumonia, and our primary objective was to evaluate its impact on antibiotic therapy appropriateness. Furthermore, we included only patients under invasive mechanical ventilation to minimize heterogeneity. Future studies may explore the potential benefits of designing mPCR panels tailored specifically to each context (CAP vs. HAP/VAP) (35).

CONCLUSIONS

In conclusion, in this multicenter study conducted in a lower-middle-income country, mPCR was associated with improved antimicrobial use in critically ill patients with pneumonia. The findings highlight the value of rapid molecular diagnostics in guiding appropriate antibiotic therapy. Additionally, the study underscores the need for enhanced education on AMS and the optimal use of mPCR to maximize its clinical benefits.

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Drs. Aissaoui and Madani were involved in conception and design of the study. Drs. Aissaoui, Derkaoui, Hachimi, Bouchama, Dendane, Doumiri, ElAidaoui, Ziadi, Essafti, Khaddouri, Oualili, Khallouki, Berdai, Boukatta, Kohen, El Adib, Mroune, and Oudrhiri Safiani were involved in acquisition of data. Drs. Aissaoui, Bouchama, Madani, Soraa, and Abouqal were involved in analysis and interpretation of the data, Drs. Aissaoui, Bouchama, and Abouqal were involved in drafting and critical revision of the article. All authors provided final approval of the version submitted for publication.

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