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ORIGINAL ARTICLE

Multiplex qPCR for the early detection of sepsis pathogens and its impact on antimicrobial therapy in critically ill patients

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ABSTRACT

Background and Objectives: Sepsis is a life threatening condition caused by a dysregulated host response to infection and is associated with high morbidity and mortality worldwide. Early bacterial detection and therapy with antibiotics improve outcomes. We compared multiplex quantitative PCR (qPCR) to traditional blood culture for early pathogen detection in critically ill patients with suspected sepsis.

Materials and Methods: This prospective observational study included 200 critically ill ICU patients with suspected sepsis. Multiplex qPCR using the TRUPCR® Sepsis Panel was compared with conventional blood culture for pathogen detection. To assess sensitivity, specificity, PPV, and NPV, blood culture was used as the reference standard. Mortality, ICU stay, and antibiotic therapy time were studied. Multivariable logistic regression was adjusted for baseline severity (SOFA, APACHE II), septic shock, and antibiotic exposure.

Results: Multiplex qPCR significantly reduced the time to initiation of appropriate antibiotic therapy (5.2 vs 8.3 hours, $p<0.001$). The assay demonstrated higher sensitivity compared with blood culture for pathogen detection. qPCR positivity was associated with shorter ICU stay and lower mortality; however, these associations were interpreted after adjustment for baseline illness severity. *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* were the most frequently detected pathogens, and several antimicrobial resistance genes including bla_{CTX-M}, bla_{NDM}, and *mecA* were identified.

Conclusion: Multiplex qPCR can detect infections early and optimize antimicrobials for sepsis. These findings should be cautiously evaluated and corroborated in larger multicentre trials due to reduced specificity and observational nature.

Keywords: Sequential organ failure assessment; Multiplex quantitative polymerase chain reaction; Sepsis; Procalcitonin

INTRODUCTION

Sepsis, a condition marked by an uncontrolled reaction of the body to infection, is a major global health concern, particularly for those with severe illnesses. Despite improvements in critical care, sepsis remains a leading cause of death, especially in intensive care units (1). Early diagnosis and the use

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of antibiotics are crucial for increasing survival rates (2). Delays in targeted treatment are associated with poor outcomes.

Blood culture is the best way to diagnose BSIs. Its downsides include a 24-72-hour turnaround time, reduced susceptibility in individuals who have received antibiotics, and lower detection rates in low-microbial-burden or fastidious species (3). The process of identifying pathogens and testing their susceptibility to antimicrobials can delay the start of targeted treatment. As a result, this often leads to the use of broad-spectrum antibiotics, which can worsen the problem of antimicrobial resistance. Molecular diagnostics, such as qPCR, offer a rapid and sensitive means of pathogen detection. These assays can identify a wide array of bacterial and fungal infections, as well as antibiotic resistance genes, in blood samples within a matter of hours (4). Therefore, this faster processing time could help improve treatments that target pathogens early and support better antibiotic use.

Many studies have examined the clinical efficacy of multiplex PCR-based sepsis treatments. In patients with past antibiotic exposure, LightCycler SeptiFast detected pathogens better than blood cultures (4). The BioFire FilmArray Blood Culture Identification panel helps doctors detect organisms faster to choose the right treatments (5). Magnetic resonance technology helps the T2Dx device detect bloodstream infections in whole blood samples. This is especially useful for identifying infections that might be missed by standard culture methods (6).

Meta-analyses show that molecular diagnostics paired with blood culture improve diagnostic yield, antibiotic therapy, and clinical outcomes (7). Multiplex qPCR was compared to blood culture for detecting bloodstream infections in critically ill sepsis patients. We examined its effects on early microbial identification, antibiotic selection, and clinical outcomes. The study investigates whether multiplex qPCR can be used to diagnose sepsis in contexts where culture methods are limited.

MATERIALS AND METHODS

Design and participants of the study. We conducted a prospective observational diagnostic accuracy study to compare multiplex qPCR with conventional blood culture and evaluate its influence on antimicrobial decision-making. Patients at least eighteen years of age admitted to the intensive care unit with a clinical suspicion of infection or sepsis who fulfilled the inclusion criteria were enrolled in the study. The exclusion criteria included patients not willing to participate, patients on antibiotics (more than 48 hours before admission), and insufficient blood samples for the qPCR and culture assays. A target sample size of 200 patients was selected based on previous studies evaluating multiplex PCR in sepsis diagnostics, which reported sensitivities between 85% and 95%. This sample size was considered adequate to estimate diagnostic performance with acceptable precision in an ICU population.

Sample collection. Approximately 8–10 mL of blood was collected for blood culture, and 2 mL of blood was collected for multiplex qPCR, both obtained from the same venipuncture site during the initial clinical evaluation to ensure comparability. Anaerobic and aerobic blood culture bottles were inoculated with blood and then incubated for a maximum of five days.

Nucleic acid extraction. EDTA-coated tubes were used to collect whole blood samples to avoid coagulation. For each extraction, 200–500 µL of whole blood were aliquoted into sterile 1.5 mL microcentrifuge tubes. Three volumes of RBC lysis buffer were combined with one volume of whole blood. After vortexing the sample for 10-15 seconds, it was allowed to stand for 10-15 minutes till complete erythrocyte lysis was assured.

The samples were centrifuged at 10,000 × g for five minutes. The cell pellet was kept while the disintegrated erythrocyte-containing supernatant was discarded. The pellet was resuspended in 200 µL of lysis solution, and then 20 µL of proteinase K was added. The mixture was incubated at 56°C for 30 minutes to break down proteins and release nucleic acids. An equivalent 96-100% ethanol volume was added to the lysate to precipitate DNA (8-10).

Entire constituents were transferred to the spin column; the samples were centrifuged at 8,000–10,000 × g for 1 minute. Twice, the column was centrifuged at 8,000 × g for one minute in 70% ethanol. To elute the DNA, 50-100 µL of nuclease-free water was directly put onto the column membrane. The membrane was then allowed to sit at room temperature for two minutes, followed by centrifugation for one minute at

8,000 × g; the DNA was eluted. A spectrophotometer was used to measure absorbance ratios at 260/280 nm and 260/230 nm to evaluate DNA yield and purity. DNA integrity was assessed using gel electrophoresis to ensure there was no degradation. Details are illustrated in Fig. 1.

Real-time PCR assay. The in vitro nucleic acid amplification assay TRUPCR® Sepsis Panel Kit qualitatively detects pathogens and AMR genes in blood samples. Bacteria like *Salmonella* spp., *Streptococcus pyogenes, Acinetobacter baumannii, Streptococcus agalactiae, Pseudomonas aeruginosa, Haemophilus influenzae* (A-F), *Escherichia coli, Neisseria meningitidis, Klebsiella oxytoca, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter* spp., *Listeria monocytogenes, Candida albicans, Candida lusitaniae. Sul2, dfrA1, dfrA5,* are sulfamethoxazole genes. bla_{CTX-M} gr1, bla_{TEM}, and bla_{SHV} are extended-spectrum beta-lactam resistance genes. KPC, bla_{NDM}, bla_{VIM}, bla_{OXA}, and bla_{IMP} detected carbapenem resistance, while *mecA* detected methicillin resistance and *vanA* and *vanB* vancomycin resistance.

Blood culture. Blood culture samples were reported as positive by the BacT/ALERT® system. After that, these samples underwent subculturing. The isolated colonies were suspended in 0.45-0.5% saline and matched to McFarland 0.5 turbidity for identification and inoculum production. The VITEK® 2 system was used to process and inoculate VITEK® 2 ID and AST cards for automated microbial identification and susceptibility testing. Results were available within five to eight hours after inoculation, including organism identification and MIC-based susceptibility interpretations. Quality control was carried out daily using reference strains, and interpretation was done per CLSI/EUCAST recommendations. This quick strategy facilitated prompt and focused antibiotic treatment (11, 12).

Biomarker measurement. Procalcitonin (PCT) was measured following the manufacturer's instructions using the chemiluminescence method (VITROS® XT 7600 Integrated System). A fully automated Randox Daytona Plus biochemistry analyzer was used to determine the lactate.

Data collection. Clinical data from medical records included demographics (age, sex), the source of the infection, comorbid conditions (including immunosuppression status), severity scores (SOFA and APACHE II), the presence of shock and organ dysfunction, interventions, the specifics of the antibiotic therapy (time to initiation, changes, duration), mortality, and clinical outcomes.

Statistical analysis. Means with standard deviations (SD) or medians with interquartile ranges (IQR) were used for continuous variables. The groups (qPCR positive vs. negative) were compared using the Mann-Whitney U test or Student's t-test for continuous variables. The chi-square test or Fisher's exact test was used for categorical variables and when expected cell counts were less than 5. Multiplex qPCR

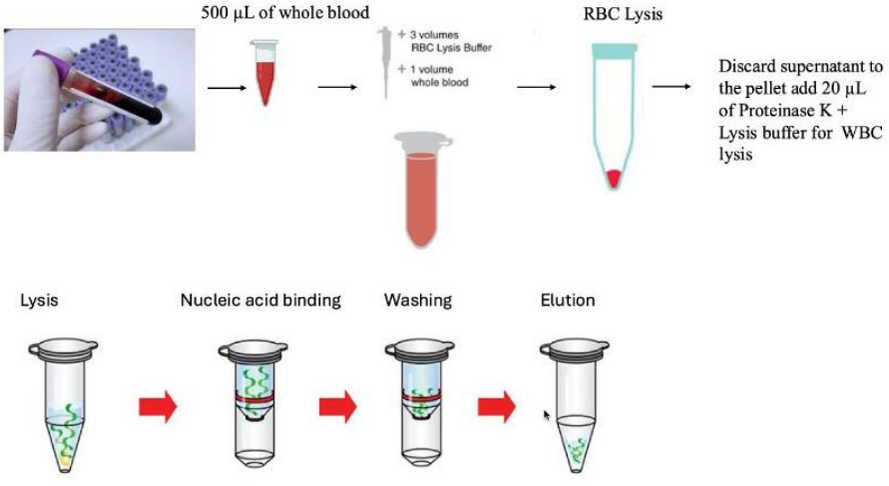

Fig. 1. Diagrammatic illustration of Whole blood extraction procedure.

diagnostic performance was measured versus blood culture. As the comparator, blood culture data were used to assess sensitivity, specificity, PPV, and NPV.

The cutoff value for statistical significance was $p<0.05$. To account for baseline differences in illness severity between groups, a multivariable logistic regression model was constructed to identify independent predictors of ICU mortality. To account for baseline differences in illness severity between groups, a multivariable logistic regression model was developed to identify independent predictors of ICU mortality. The model included qPCR positivity, SOFA score, APACHE II score, septic shock presence and prior antibiotic exposure. Adjusted odds ratios (aORs) with 95% confidence intervals were reported.

Ethical considerations. The institutional review board granted ethical approval (134X/11/13/2024), and written informed consent was collected from the patient attendant.

RESULTS

Patient characteristics. The study included 200 individuals with a mean age of 62.0 ± 16.8 years. Men accounted for 70% (n = 140). Of these, a positive qPCR result was reported in 142, and 58 had a negative one. There was no significant difference between the two groups in terms of age (61.5 ± 16.2 vs. 63.5 ± 17.4 years; p=0.46) or gender distribution (70.4% vs. 69.0%; p=0.84) (Table 1).

Source of infection. Pneumonia was the most common source of infection in 25% of patients. The prevalence was higher in the group with a positive qPCR result, although it was not statistically significant (28.2% vs. 17.2%; p=0.11). Intra-abdominal infections were found in 22% of patients, with similar percentages between groups (22.5% vs. 20.7%; p=0.77). Infections of the urinary tract and skin-soft tissues did not differ statistically substantially across the groups (Table 1).

Comorbidities and clinical factors. 45% of patients had comorbid issues, and their distribution was the same across all groups (p=0.97). Forty per cent of patients had a shock; this group was more likely to have positive qPCR results (42.3% vs. 34.5%; p= 0.30). Twenty-five per cent of the sample had immunosuppression (p=0.86), and no statistically significant differences existed between groups (Table 1).

Severity scores and organ dysfunction. Patients with a positive qPCR exhibited higher SOFA scores (median 8 (6–10)) compared to the negative group (7 (5–9); p=0.0001), indicating more organ dysfunction. Furthermore, APACHE II scores were higher in the positive group (18.1 ± 7.5 vs. 16.2 ± 7.0), but this difference was not statistically significant (p= 0.10) (Table 1).

Interventions. The qPCR -positive group had significantly higher median PCT levels (40.0 ng/ml (15.0-100.0)) than the negative group (20.0 ng/ml (7.0-50.0); p=0.002). Additionally, the lactate levels in the positive group were higher (4.2 mmol/l (3.0-8.0) vs. 3.0 mmol/l (2.0-4.5); p=0.003), which would suggest a higher burden of metabolic abnormalities associated with sepsis. Leukocyte numbers did not significantly differ across the groups (p=0.36) (Table 1).

Blood culture-based real-time PCR assay sensitivity and specificity. Blood culture and multiplex qPCR were compared for diagnostic performance. Table 2 shows qPCR and blood culture contingency tables. In 200 patients, qPCR discovered 131 true-positive, 49 true-negative, 11 false-positive, and 9 false-negative cases. Multiplex qPCR detected pathogens with 93.6% sensitivity and 81.7% specificity. The 92.3% PPV indicated that most qPCR-positive results were bloodstream infections. Negative qPCR results reduced infection risk by 84.5%. Statistically significant correlation (χ^2 test, $p < 0.001$) between qPCR and blood culture data shows strong agreement. Given the 11 false-positive and 9 false-negative results, molecular testing should supplement culture-based approaches. We examined pathogen type and clinical outcomes. Patients with *Acinetobacter baumannii*, *Candida auris* infections were linked to more fatalities and longer ICU stays. These results should be interpreted with caution due to the low number of cases for each infection.

Clinical decision-making was significantly impacted by q PCR data. The time taken to initiate the appropriate antibiotic treatment was lesser for patients with positive qPCR results (5.2 hours) than for those with negative results (8.3 hours, $p < 0.001$). Additionally, compared to 51.7% of patients with negative qPCR results, 84.5% of patients with positive qPCR results saw a change in antibiotic therapy ($p < 0.001$). Pa-

Table 1. Characteristics of Patients in the Study (n = 200)

Characteristic	All Patients (n = 200)	Positive qPCR (n = 142)	Negative qPCR (n = 58)	p-value
Age (years, Mean ± SD)	62.0 ± 16.8	61.5 ± 16.2	63.5 ± 17.4	0.46
Male, n (%)	140 (70.0)	100 (70.4)	40 (69.0)	0.84
Source of Infection, n (%):				
CNS	14 (7.0)	4 (2.8)	10 (17.2)	0.001
Pneumonia	50 (25.0)	40 (28.2)	10 (17.2)	0.11
Intra-abdominal	44 (22.0)	32 (22.5)	12 (20.7)	0.77
Urinary Tract	40 (20.0)	30(21.1)	10(17.2)	0.53
Skin-soft tissue	20 (10.0)	12 (8.5)	8 (13.8)	0.26
Unknown	22 (11.0)	14 (9.9)	8 (13.8)	0.41
Other Comorbidities, n (%)	90 (45.0)	64 (45.1)	26 (44.8)	0.97
Shock, n (%)	80 (40.0)	60 (42.3)	20 (34.5)	0.30
Immunosuppression, n (%)	50 (25.0)	36 (25.4)	14 (24.1)	0.86
Antibiotic Before Blood Culture, n (%)	120 (60.0)	88 (62.0)	32 (55.2)	0.38
Number of Dysfunctional Organs (mean ± SD)	3.0 ± 1.1	3.2 ± 1.2	2.9 ± 1.0	0.14
APACHE II Score (mean ± SD)	17.5 ± 7.3	18.1 ± 7.5	16.2 ± 7.0	0.10
SOFA Score (median (IQR))	8 (6–12)	8 (6–10)	7 (5–9)	0.0001
Mechanical Ventilation, n (%)	90 (45.0)	70 (49.3)	20 (34.5)	0.04
CRRT, n (%)	50 (25.0)	36 (25.4)	14 (24.1)	0.86
PCT (median (IQR), ng/ml)	25.5 (8.0–80.0)	40.0 (15.0–100.0)	20.0 (7.0–50.0)	0.002
Leukocyte Count (mean ± SD, G/l)	16.7 ± 10.2	17.2 ± 10.3	15.5 ± 10.0	0.36
Lactate (median (IQR), mmol/l)	3.6 (2.2–6.0)	4.2 (3.0–8.0)	3.0 (2.0–4.5)	0.003

Table 2. Diagnostic performance of multiplex qPCR compared with blood culture

	Blood Culture Positive	Blood Culture Negative	Total
qPCR Positive	131	11	142
qPCR Negative	9	49	58
Total	140	60	200

tients with positive qPCR results spent less time in the critical care unit (9.8 days) than those with negative qPCR results (11.8 days, p = 0.02).

Patients with positive qPCR results showed a considerably lower mortality rate (16.9%) than those with negative qPCR results (27.6%, p = 0.03). Additionally, patients with positive qPCR results had a shorter duration of mechanical ventilation (4.6 days) than those with negative results (6.0 days, p = 0.04).

Continuous Renal Replacement Therapy (CRRT) utilization did not differ significantly between groups (p = 0.86). Targeted antibiotic therapy was much more common in the positive qPCR group (80.9%) than in the negative group (25.9%, p < 0.001) (Table 3).

Because baseline severity differed between groups, multivariable logistic regression was used to examine independent ICU mortality factors. Even after adjusting for SOFA, APACHE II, septic shock, and antibiotic exposure, qPCR positive was independently related with decreased mortality (Table 4). Higher SOFA score and septic shock were both associated with a higher risk of mortality.

Distribution of microorganisms and antimicrobial resistance genes. In 142 qPCR-positive patients, 130 organisms were identified, suggesting polymicrobial diseases. The most common pathogen was *Escherichia coli* (n = 33), followed by *Klebsiella pneumoniae* (n = 26). *Acinetobacter baumannii* was found in 16, *Pseudomonas aeruginosa*, *Enterococcus* spp., and *Staphylococcus aureus* in 13 (Fig. 2). CoNS was the least common pathogen (n = 7), while *Candida auris* was found in 10. *E. coli* and *K. pneumoniae* were common in bloodstream and urinary tract infections, while *A. baumannii* was common in ventilator-associated pneumonia. *Enterococcus* spp. and *Staphylococcus*

Table 3. Impact of q PCR on Clinical Decision-Making

Clinical Decision-Making Parameter	All Patients (n = 200)	Positive qPCR (n = 142)	Negative qPCR (n = 58)	p-value
Time to Initiate Appropriate Antibiotic Therapy (hours, mean ± SD)	6.5 ± 2.1	5.2 ± 1.9	8.3 ± 2.4	< 0.001
Change in Antibiotic Therapy (n, %):	150 (75.0)	120 (84.5)	30 (51.7)	< 0.001
Duration of Empiric Antibiotic Therapy (days, mean ± SD)	3.0 ± 1.5	2.3 ± 1.1	4.0 ± 1.8	< 0.001
ICU Stay Duration (days, mean ± SD)	10.5 ± 5.2	9.8 ± 4.7	11.8 ± 5.8	0.02
Mortality Rate (n, %):	40 (20.0)	24 (16.9)	16 (27.6)	0.03
Duration of Mechanical Ventilation (days, mean ± SD)	5.2 ± 3.0	4.6 ± 2.7	6.0 ± 3.4	0.04
Use of CRRT (n, %)	50 (25.0)	36 (25.4)	14 (24.1)	0.86
Rate of Targeted Antibiotic Therapy (n, %)	130 (65.0)	115 (80.9)	15 (25.9)	< 0.001

Table 4. Multivariable Logistic Regression Analysis for ICU Mortality

Variable	Adjusted Odds Ratio (aOR)	95% CI	p-value
qPCR positive	0.52	0.28–0.96	0.038
SOFA score	1.18	1.07–1.31	0.001
APACHE II score	1.05	1.01–1.10	0.021
Septic shock	2.21	1.16–4.19	0.016
Prior antibiotic use	1.12	0.62–2.03	0.70

aureus caused severe nosocomial infections. *Candida auris* multidrug- resistant fungal infections in critically unwell individuals were noted. In *Escherichia coli*, trimethoprim resistance genes *dfrA1* and *dfrA5* were detected in 21.2% and 24.2% of cases, respectively. bla_{CTX-M} gr 1 was present in 36.4%, bla_{TEM} in 6.1%, and bla_{SHV} in 9.0% of the samples. Carbapenemase *genes reported in the isolates were* bla_{NDM} *(9.0%),* bla_{OXA-48} (6.1%), bla_{KPC} (6.1%), bla_{VIM} (3.0%), and bla_{IMP} (6.1%) respectively.

Multiple resistance gene expression such as *sul2 + dfrA1* (3.8%), bla_{CTX-M} gr 1 + bla_{TEM} (6.1%), bla_{CTX-M} gr 1 + bla_{NDM} (15.4%), and bla_{CTX-M} gr 1 + bla_{SHV} (9.0%) were reported in *Escherichia coli* isolates.

In *Klebsiella pneumoniae* (n=26) isolates, bla_{CTX-M} gr 1 was 15.4%. A high prevalence of carbapenemase genes was also seen, including bla_{NDM} (42.3%), bla_{KPC} (15.4%), and bla_{OXA-48} (11.5%). Multiple resistance gene expression bla_{CTX-M} gr 1 + bla_{NDM} (11.5%) and bla_{KPC} + bla_{NDM} (3.8%) were also observed.

In *Pseudomonas aeruginosa*, bla_{TEM} (15.4%) and bla_{SHV} (23.1%) were observed among the ESBL genes. Two carbapenemase genes conferring mutations were reported: bla_{NDM} (38.4%) and bla_{VIM} (7.7%). In 15.4% of isolates, multiple resistance gene expression of bla_{VIM} + bla_{NDM} was observed. In *Acinetobacter baumannii*, the prevalence of carbapenemase genes was high, including bla_{OXA-23} (50%) and bla_{NDM} (12.5%). Multiple resistance gene expression of bla_{OXA-23}+ bla_{NDM} (12.5%) was seen in 12.5% of samples. In *Enterococcus* spp. vancomycin resistance genes, *vanA* (30.8%) and *vanB* (23.1%) were seen. In 30.8% of instances, a dual mutation of bla_{TEM} + *vanA* was seen. In *Staphylococcus aureus*, 53.8% of the isolates had the methicillin-resistant *mecA* gene. In 7.7% of instances, a dual mutation of *mecA* + *vanA* was reported. In CoNS, 42.8% of isolates had the *mecA* gene, and 28.6% of patients had a dual mutation of bla_{TEM} + *mecA* (Fig. 3).

DISCUSSION

This study suggests multiplex qPCR may help critically ill patients detect and treat sepsis early. Rapid molecular diagnostics are improving pathogen detection, especially when delayed diagnosis may negatively impact clinical results. Compared to standard blood cultures, multiplex qPCR was more sensitive and it helps initiate antimicrobial therapy faster. In addition, the assay detected antimicrobial resistance genes simultaneously, which helped optimize antimicrobial therapy in ICUs (16-18). The study population's demographic characteristics were in accordance with prior reports that detailed the epidemiology of sepsis in critically ill patients. Most patients were male with a mean age of 62 years. Similar demographic patterns have been reported in several ICU-based studies, suggesting that older

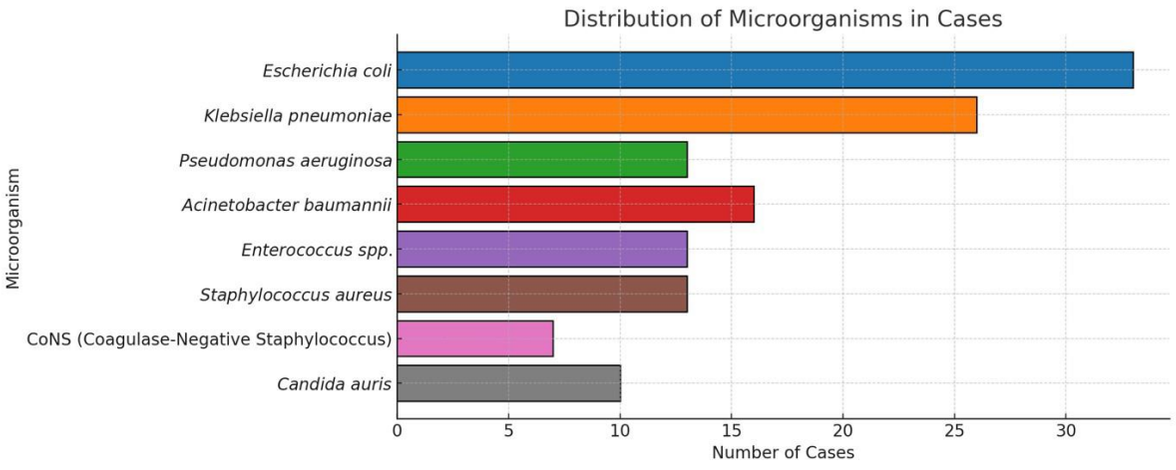

Fig. 2. Microorganism distribution in sepsis patients identified by multiplex qPCR.
The bars indicate the number of pathogens found in qPCR-positive samples. The number of identified organisms may exceed the number of ill patients due to polymicrobial illnesses.

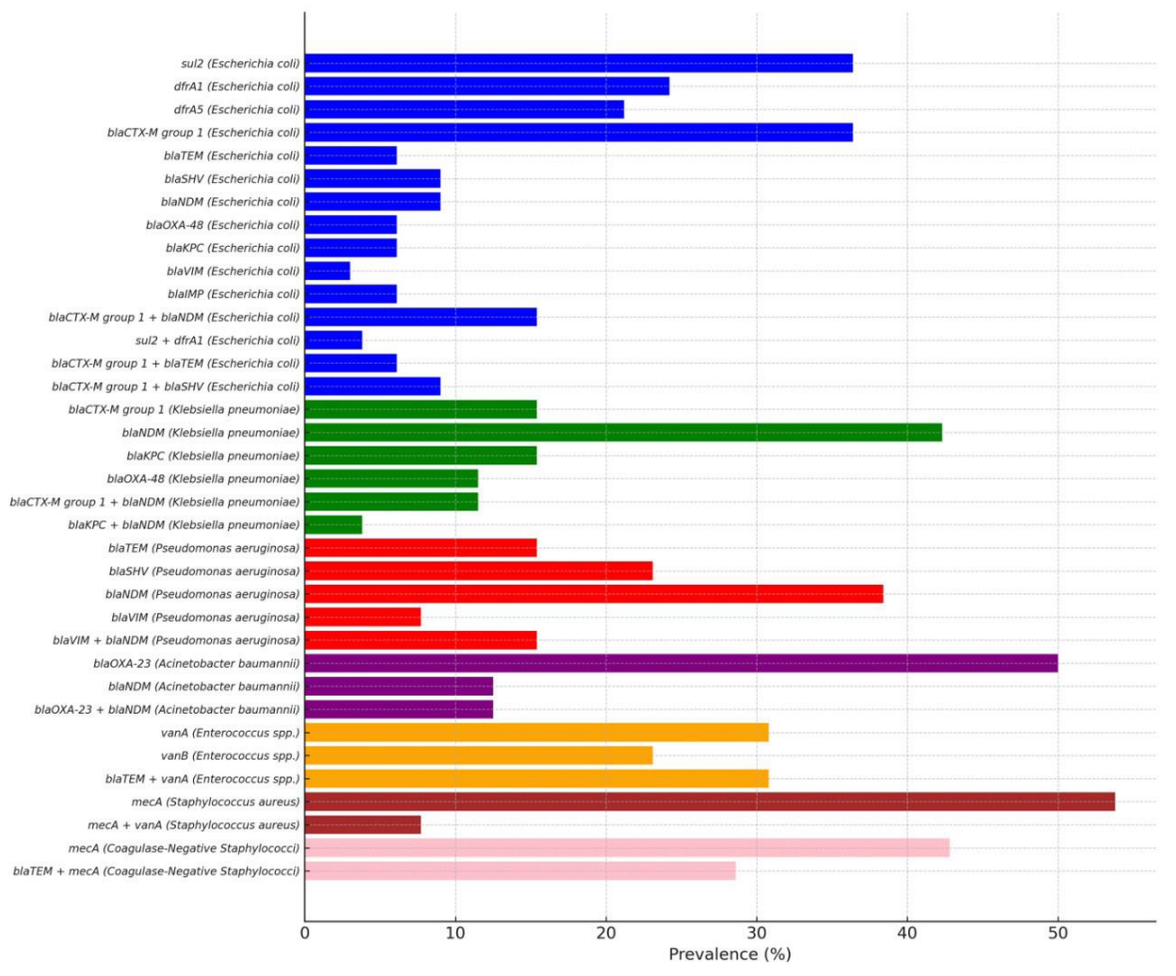

Fig. 3. Prevalence of Resistance Genes across Different Organisms

age groups and male patients are more frequently affected by severe infections and sepsis (13, 19). In this investigation, age and sex distributions did not differ substantially between qPCR-positive and qPCR-negative groups, suggesting that these characteristics did not affect test diagnostic performance. As in previous epidemiological studies of sepsis (13, 20), pneumonia was the most common source of infection, followed by intra-abdominal and urinary tract infections. These findings emphasize early detection and tailored therapy for high-risk populations. Research is needed to understand these trends and improve patient care. Positive qPCR results were associated with greater SOFA and lactate levels, suggesting the link between organ failure, hyperlactatemia, and worse sepsis outcomes (14, 15). Our multiplex qPCR analysis demonstrated similar diagnostic performance when compared to previous research. Using blood culture as the reference standard, qPCR indicated 85% to 95% pathogen detection sensitivity (18, 21). Molecular techniques can detect microbial DNA even in individuals who have received antibiotics or have low pathogen loads, making them more sensitive (17). Earlier research (18, 22) found that qPCR had lesser specificity than conventional blood cultures. Non-viable organisms, transitory bacteremia, and sample processing contamination can cause false-positive results. Molecular results should be interpreted alongside clinical findings and conventional microbiological approaches because of this restriction. Molecular detection alone may expose patients to unneeded antimicrobials, underlining the need for implementation of antimicrobial stewardship programs (36). Besides diagnostic benefits, multiplex qPCR was linked to earlier targeted antimicrobial therapy in our group. Within hours, pathogen and resistance gene identification can help clinicians change empiric antibiotic regimens. These data should be interpreted cautiously. Positive qPCR results were associated with shorter ICU stays and reduced fatality rates, however these findings were based on univariate analysis and may be affected by baseline illness severity. Multivariable regression analysis was used to adjust for severity indicators such SOFA, APACHE II, and septic shock to reduce confounders. After correction, qPCR positive was still associated with better clinical outcomes, but the study's observational methodology makes these findings associative rather than causative. Antimicrobial resistance gene patterns in this study represent global and regional trends. Enterobacterales isolates had high rates of ESBL-associated genes ($bla_{\text{CTX-M}}$) and carbapenemase genes (bla_{NDM}). These findings support earlier research from India and other high-burden locations indicating prevalent carbapenem-resistant pathogens in ICUs (23-25). Resistance determinants like *mecA*, *vanA*, and bla_{NDM} highlight the necessity of quick molecular diagnostics in antimicrobial stewardship and infection management. Multidrug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Candida auris* are worrisome. These organisms are common sources of healthcare-associated infections and often cause treatment failure and death (26-29). Rapid molecular testing can identify such species, enabling earlier infection management and antimicrobial treatment. This study highlights the TRUPCR® Sepsis Panel's clinical value in a low- and middle-income ICU. Many molecular platforms have been tested in high-resource healthcare systems, but evidence from resource-limited contexts is scarce (18, 33). Our findings support the use of fast molecular diagnostics in critically unwell patients as an adjuvant to culture. Several limitations should be noted despite these findings. This study was conducted at one tertiary-care centre, which may restrict generalizability. Second, the observational methodology restricts the conclusions about qPCR use and clinical outcomes. Third, the multiplex panel might overlook species not in the assay because it only detects predefined pathogens and resistance genes (32-34). Fourth, the study did not assess the cost-effectiveness of routine multiplex molecular testing. Despite multivariate analysis, residual confounders from unmeasured clinical factors cannot be ignored. Multiplex qPCR appears to be a useful supplemental diagnostic tool for rapidly detecting infections and antibiotic resistance genes in critically ill sepsis patients. Molecular diagnostics can improve antibiotic stewardship and tailored therapy when coupled with blood culture and phenotypic susceptibility testing (35-37).

CONCLUSION

Multiplex qPCR is a promising supplementary diagnostic method for detection of infections and antibiotic resistance genes in critically ill sepsis patients. This study found that multiplex qPCR identified pathogens faster and aids in modification of anti-

biotic therapy faster than blood culture. Due to its reduced specificity and observational nature, these findings should be considered cautiously. Molecular diagnostics ought to supplement microbiological methods rather than supplant them. The clinical efficacy of multiplex qPCR in sepsis treatment must be evaluated in larger multicentre studies, incorporating multivariable analyses and cost-effectiveness evaluations.

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