A retrospective evaluation of a multiplex polymerase chain reaction test directly applied to blood for the management of sepsis in the critically ill

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Background. Blood culture (BC) is the established gold standard for microbiological diagnosis of bloodstream infection (BSI); however, its sensitivity is poor.
Objectives. The primary objective was to determine the sensitivity and specificity of the Magicplex Sepsis Real-time Test, a multiplex polymerase chain reaction test (mPCR), and BC to detect BSIs. Secondary outcomes included determining the prevalence of BSIs.
Methods. A retrospective review of a technical evaluation of the mPCR. Patients requiring BC had a blood sample collected for mPCR.
Results. The respective sensitivity and specificity of mPCR for the detection of BSI were 50% (*n*=7/14) and 58% (*n*=18/31), while the sensitivity and specificity using BC were 36% (*n*=5/14) and 68% (*n*=21/31), respectively. The addition of mPCR to BC increased BSI detection during sepsis from 36% to 64%.
Conclusion. The use of mPCR directly applied to blood may increase the detection of micro-organisms associated with BSIs in critically ill patients requiring BC investigation.
Keywords. polymerase chain reaction; PCR; sepsis; bloodstream infections; bood culture; critically illness; ICU.

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Contribution of the study. Our data add to a growing body of evidence indicating that mPCR applied directly to blood prior to incubation increases the detection of pathogenic bacteria among hospitalised patients for whom blood cultures are performed for suspected infection. Our study was performed in a low-to-middle income country with a higher sepsis prevalence, a greater burden of multidrug-resistant organisms and clinically defined sepsis. This strengthens the robustness and generalisability of this body of evidence.

Sepsis is defined as life-threatening organ dysfunction due to a dysregulated host response to infection.[1] The clinical appreciation of organ dysfunction is straightforward; however, pathogen identification is challenging. Early, appropriate antibiotic use is assessed as Grade 1b evidence.[2] Blood culture (BC) may have relatively poor sensitivity, as low as 32%.[3] The time to pathogen identification using BC is 48 - 72 hours, while the time to a negative BC is longer, 3 - 5 days.[4] BC contamination adds to clinical interpretation complexity.[5]

Amplification during polymerase chain reaction (PCR) allows detection of minuscule amounts of pathogen DNA within 4 - 6 hours.[6] The Magicplex Sepsis Real-time Test (Seegene, South Korea), a multiplex polymerase chain reaction test (mPCR), requires 1 mL of whole blood and has a 3 - 6-hour turnaround time. It identifies 90 pathogens, 27 at a species level and 3 resistance genes (*mecA, vanA, vanB*).[7] A technical evaluation of mPCR was performed in a multidisciplinary tertiary level intensive care unit (ICU).

Methods

Study design

A retrospective review of the technical evaluation and ICU database was performed. The mPCR's technical evaluation took place from 1 June to 31 July 2019. Approval was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (ref. no. M200251). Owing to the retrospective nature of the study, the requirement for informed consent was waived.

Study procedure

During the evaluation period, patients in whom infection was suspected, and from whom BCs were collected, were included. An additional blood sample was collected for the mPCR during the aseptic procedure. The ICU patient database was then cross-searched for clinical information, laboratory information, and antimicrobial prescriptions.

Blood cultures

BCs in the ICU are collected under strict aseptic technique as standard procedure (20 mL blood).

mPCR blood assay

All patients having BCs collected had an additional 4 mL ethylenediaminetetraacetic acid (EDTA) tube filled with blood

during the procedure. Blood samples were directly processed according to the manufacturer's instructions to yield microbial nucleic acid material.

Microorganism identification followed three steps. The first PCR step identified Gram-positive bacteria and three resistant genes in one tube, and Gram-negative bacteria and six fungi in a second tube. The second PCR step identified organisms at a genus level: *Streptococcus*, *Staphylococcus*, and *Enterococcus* spp. in the first tube; Gram-negative bacteria group A (GNB-A) and GNB-B in the second tube; and fungi, *vanA, vanB,* and *mecA* resistance genes in the third tube. A third and final PCR was not performed.

GNB-A organisms included: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Serratia marcescens*, *Bacteroides fragilis*, and *Salmonella typhi*. GNB-B organisms included: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae*, and *Enterobacter aerogenes*. Fungi included: *Candida albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *Aspergillus fumigatus*.

Study definitions and research assumptions

Sepsis was defined using Sepsis-3 definition.[1] Infection was defined by a combination of clinical presence plus an associated biomarker increase above the upper reference limit (two of three markers: white cell count (WCC), C-reactive protein (CRP) and procalcitonin (PCT). BC and mPCR were considered concordant if the organism cultured from blood was an organism present in the corresponding mPCR group in the second PCR step. Appropriate clinical response was defined as a 40% reduction in either PCT or CRP over the following 48 hours. This was based on a daily decrease of 20% for 4 days reaching a 20%-of-peak threshold, allowing termination of antibiotics.[8] Appropriate antibiotic choices defined for the study included vancomycin for *Enterococcus* (provided the *vanA* and *vanB* genes were absent) and meropenem/imipenem for a GNB in group B with additional trimethoprim-sulfamethoxazole for group A. *S. aureus*-appropriate therapy included vancomycin or linezolid if the *mecA* gene was identified. Owing to limited fungal identification, only bacteria were analysed. Because of the retrospective nature of the study, mPCR results were not available to treating clinicians and antibiotic therapy was not actually changed; hence we refer to the comparative model as hypothetical.

Study outcomes

Primary outcome

To determine the sensitivity and specificity of mPCR and BC in detecting BSIs among patients with sepsis.

Secondary outcomes

To determine the prevalence of BSI, the concordance rates between BC and mPCR, the frequency of different implicated pathogens, and the frequency of inappropriate antibiotic therapy based on hypothetical knowledge from mPCR results availability at 12 hours compared with BC results at 72 hours.

Data analysis

Non-normal data were described using median and interquartile range (IQR). Independent medians and percentages were compared using the Mann-Whitney *U*-test and χ^2 test, respectively. Frequencies were described using numbers and percentages. Analysis was performed on Statistica version 13. 5.0.17 (TIBCO Software, USA).

Results

There were 45 episodes (35 patients) of clinically suspected infection or sepsis, each with simultaneous BC and mPCR testing performed. Fourteen patients met the definition of sepsis, 14 had a study-defined infection and 17 had clinically suspected infection. The patient demographics are provided in the Table 1. The number and percentage (*n* (%)) of suspected sites of infection were the lungs (*n*=19; 42.2%), abdomen (*n*=15; 33.4%), pelvis (*n*=5; 11.1%), skin/soft tissue (*n*=2; 4.4 %) and other sites (*n*=4; 8.9% %).

Primary outcome

The sensitivity and specificity of mPCR for the detection of BSI among patients with sepsis was 50% (*n*=7/14) and 58% (*n*=18/31) respectively, while the sensitivity and specificity of BC was 36% (*n*=5/14) and 68% (*n*=21/31) respectively. When mPCR and BC positivity rates were compared, mPCR was more likely to detect a positive BSI, relative risk 1.82 (confidence interval 1.01 - 3.27). Three positive BCs among patients without sepsis were due to contamination and were included in the BC-negative group.

Secondary outcomes

Prevalence of bloodstream infections

The prevalence of BSI using BC in this study was 27%. A combination of BC and mPCR yielded the highest prevalence (Table 2). The concordance rates between BC and mPCR are provided in Table 2.

There were 14 microorganisms (2 fungal) cultured on blood and 25 identified on mPCR. Gram-negative bacteria were the most frequently identified bacterial pathogens (Fig. 1). Three contaminants were cultured on blood; two coagulase-negative *Staphylococcus*, and one *Micrococcus* spp.

Hypothetical comparative model

Availability of mPCR results was assumed at 12 hours post collection (mPCR 12h) compared with actual BC results at 72 hours (BC 72h). Two patients (8%) in the mPCR 12h group would have been on inappropriate antimicrobial therapy compared with 6 (23%) in the BC 72h group (*p*=0.25).

Discussion

In this technical evaluation, the sensitivity for detecting BSI in critically ill adult patients with sepsis in the ICU was 50% for mPCR

Table 1. Patient demographics

Characteristic	Median (IQR)*	IQR, *n*
Age (years)	36 (28 - 59)	35
Weight (kg)	70 (60 - 84)	35
Height (cm)	170 (165 - 180)	34
Apache II score	16 (8 - 22)	35
Comorbidities, *n* (%)		
None	15 (43)	
HIV	7 (20)	
Hypertension	4 (11)	
Diabetes	3 (9)	
Chronic kidney disease	2 (6)	
Other	4 (11)	
Diagnostic category, *n* (%)		
Medical	9 (26)	
Surgical	6 (17)	
Obstetrics and gynaecology	3 (9)	
Trauma	11 (31)	
Other	6 (17)	

IQR = interquartile range.
*Unless otherwise specified.

Table 2. Prevalence of bloodstream infection positivity (not number of organisms)

Category	BC positive,* *n/N* (%)	mPCR positive, *n/N* (%)	Combination, *n/N* (%)	Concordance to blood culture, *n/N* (%)
All episodes	12/45 (27)	20/45 (44)	25/45 (56)	7/12 (58)
Sepsis episodes	5/14 (36)	7/14 (50)	9/14 (64)	3/5 (60)
Study-defined infection episodes†	9/28 (32)	13/28 (46)	18/28 64)	4/9 (44)
No sepsis	-	-	-	4/7 (57)

BC = blood culture; mPCR = multiplex polymerase chain reaction test.
*BC positive excludes contaminant.
†Study-defined infection episodes includes sepsis and non-sepsis.

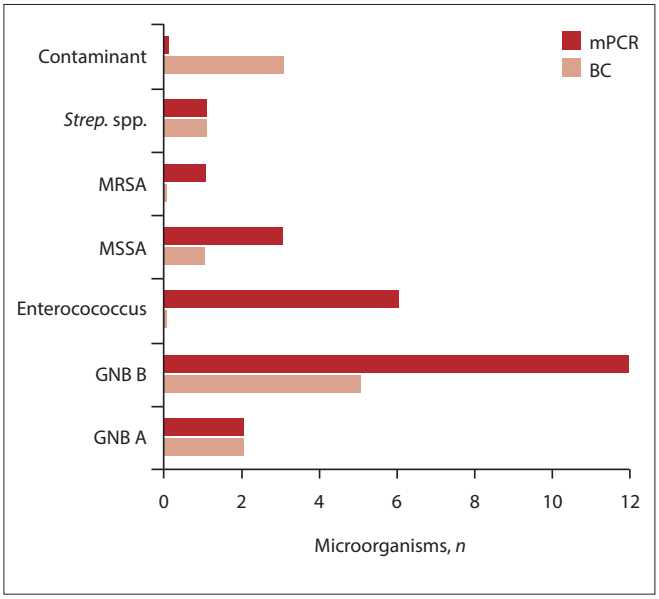

*Fig. 1. Prevalence of bloodstream infections. (*Strep. = Streptococcus*; MRSA = methicillin-resistant* Staphylococcus aureus*; MSSA = methicillin-susceptible* Staphylococcus aureus*; GNB-B = Gram-negative bacteria group B; GNB-A = Gram-negative bacteria group A, mPCR = multiplex polymerase chain reaction test; BC = blood culture.)*

and 36% for BC. A study by Carrara *et al.*[9] showed that the same mPCR kit had a sensitivity of 65%. They included hospitalised patients and sepsis was not explicitly defined. A combination of mPCR and BC demonstrated a 68% prevalence of BSI compared with 64% in our study. Zboromyrska *et al.*[10] demonstrated a sensitivity of 65% with the mPCR test. However, their clinical assessment was not defined. The combination of mPCR and BC resulted in a 64% prevalence of BSI.[10] Ljungström *et al.*[11] evaluated the mPCR in emergency department suspected sepsis. They found a sensitivity of 64% using a rigorous clinical definition. Ziegler *et al.*[12] evaluated the mPCR kit's technical aspects without defined clinical outcomes. They found a 46% prevalence of BSI using BC and mPCR.

A systematic review illustrated the lack of a uniform reference against which the mPCR test (Magicplex) has been evaluated.[13] BC sensitivity and poorly defined clinical outcomes limit the interpretation of current data.

There was a high prevalence (27%) of BSI diagnosed by BC in our study. Previously published prevalences varied between 10 and 17%.[7,10-12] Our study was performed in a low- to middle-income country and may reflect a higher sepsis burden.[14]

Another advantage of mPCR was the low (6%) incidence of BC contaminants. This is in keeping with other published data showing a higher contamination rate for BC (10%) compared with mPCR (4.8%).[8]

The real value of mPCR may be when the pathogen yield is expected to be low, e.g. antimicrobial treatment.[15] Our study supports the complementary role of mPCR by nearly doubling the number of BC-diagnosed infections with only 1 mL of unincubated blood.

We found a concordance rate between BC and mPCR of around 60%, similar to previous studies (60 - 80%).[9-11] It is this lack of complete concordance which facilitates the complementary role of mPCR to BC.

In the hypothetical comparative model, mPCR in addition to BC could potentially reduce the number of patients on inappropriate therapy. This trend is hypothesis generating.

Study limitations and strengths

The retrospective, observational nature and small study size (report) are important limitations. We performed 2 of the 3 PCR steps, limiting a more direct comparison of microorganisms identified. However, we used established clinical definitions with objective measures to support clinically defined outcomes in a specific clinical setting (ICU).

Conclusions

The use of mPCR directly applied to blood may increase the detection of microorganisms associated with BSIs in critically ill patients requiring BC investigation.

Declaration. Results from this study were shared with staff members in the ICU in an informal presentation.

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Conflicts of interest. None.

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