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# Improved detection of blood stream pathogens by real-time PCR in severe sepsis

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**Abstract** *Objective:* Evaluation of the technical and diagnostic feasibility of commercial multiplex real-time polymerase chain reaction (PCR) for detection of blood stream infections in a cohort of intensive care unit (ICU) patients with severe sepsis, performed in addition to conventional blood cultures. *Design:* Dual-center cohort study. *Setting:* Surgical ICU of two university hospitals. *Patients and participants:* One hundred eight critically ill patients fulfilling the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) severe sepsis criteria were included. *Interventions:* None. *Measurements and results:* PCR results obtained in 453 blood samples from 108 patients were compared with corresponding blood culture results. PCR resulted in a twofold higher positivity rate when compared with conventional blood culture (BC) testing (114 versus 58 positive samples). In 40 out of 58 PCR positive assays the results of the corresponding blood cultures were identical to microorganisms detected by PCR. In 18 samples PCR and BC yielded discrepant results. Compared with conventional blood culture the sensitivity and specificity of PCR was 0.69 and 0.81, respectively. Further evaluation of PCR results against a constructed gold standard including conventional microbiological test results from other significant patient specimen (such as bronchio-alveolar lavage fluid, urine, swabs) and additionally generated clinical and laboratory information yielded sensitivity of 0.83 and specificity of 0.93. *Conclusions:* Our cohort study demonstrates improved pathogen detection using PCR findings in addition to conventional blood culture testing. PCR testing provides increased sensitivity of blood stream infection. Studies addressing utility including therapeutic decision-making, outcome, and cost-benefit following diagnostic application of PCR tests are needed to further assess its value in the clinical setting.

**Keywords** Detection of blood stream pathogens · Real-time PCR · Blood culture · Severe sepsis

## Introduction

Sepsis is the second leading cause of death in the non-coronary ICU. Sepsis is associated with mortality rates ranging from 20% to 50% [1, 2]. Early diagnosis followed by prompt appropriate treatment [3] improves the prognosis of septic patients [4, 5]. One important therapeutical aspect is early initiation of calculated antibiotic therapy. Each hour of delay in administration of antibiotics is associated with an average 8% decrease in survival rate of septic shock [6]. Blood cultures (BC) must be obtained before antibiotic therapy [3] which should be reassessed on the basis of culture results and clinical data [7]. Timely and continuous reassessment is important, since inappropriate antibiotic therapy deteriorates outcome [8, 9], whereas adequate therapy is associated with favorable outcome [10, 11].

Typically, BC specimens become positive within 24–36 h after sampling and therapy can be optimized based on presumptive bacterial identification. A complete microbial identification and susceptibility profile, however, is usually not available before 24–72 h later. Despite advances in BC techniques [12, 13] BC positivity rates remain low and may vary significantly, depending on severity of sepsis and ongoing antibiotic treatment [14–16]. It has been suggested that nucleic-acid-based technology such as PCR is more sensitive and can also shorten the time to result when compared with conventional BC technique [17]. The development and initial evaluation of such an assay was recently reported [18, 19]. Our study objective was to evaluate the technical and diagnostic feasibility of a commercial multiplex real-time PCR-based method for diagnosis of blood stream infections in a cohort of ICU patients with severe sepsis. Under the conditions of this investigation PCR was used in addition to conventional BC diagnostics as performed according to the local standard procedures of the participating centers. Primary endpoint of this study was the positivity of findings for microbial DNA in relation to results from conventional microbiological testing of patients with severe sepsis. Secondary endpoints were differences of Sequential Organ Failure Assessment (SOFA) score, procalcitonin (PCT) and interleukin (IL)-6 plasma levels, length of stay (ICU and hospital), and survival.

## Materials and methods

### Patient cohort

This study was performed in patients from two surgical ICUs, including a total of 108 patients, covering a total of 453 paired blood samples, consisting of simultaneously obtained BC and PCR blood samples. The relevant institutional or regional review boards or ethics committees approved the research protocol and participants or legal guardians gave written informed consent. All patients included were clinically suspected of suffering from severe sepsis of bacterial or fungal origin. Severe sepsis was classified according to the ACCP/SCCM consensus conference criteria of 1998 [14]. Inclusion followed after independent decision of the physician in charge to call for a blood culture. Repeated samples per patient were allowed. The Systemic Inflammatory Response Syndrome (SIRS) criteria [temperature, heart rate, respiratory rate, and white blood cell (WBC) count] were obtained for all patients. Further data were compiled regarding antibiotic regimen, site of infection, SOFA score (degree of organ dysfunction), procalcitonin (PCT) and interleukin-6 (IL-6) plasma level, length of stay (ICU and hospital), and hospital survival. Following the prospective and observational study design, physicians in charge did not use PCR test results to guide clinical treatment.

### BC procedures and blood collection for the PCR test

Twenty microliters whole blood, freshly drawn according to common standards of the German Society of Medical Microbiology and Hygiene (DGHM) [20], were used for a pair of aerobic/anaerobic BC bottles (BACTEC, Becton Dickinson GmbH, Germany). Immediately after drawing blood for culture, 9 ml whole blood was then collected in sterile MONOVETTE® ethylenediamine tetraacetic acid (EDTA) K2E tubes (Sarstedt, Germany) for further analysis by PCR test. All BC were processed using semi-automated BC systems (BACTEC 9240, Becton Dickinson GmbH, Germany) according to the manufacturer's instructions and the published standards of the DGHM [20]. The BC system and the local laboratory software automatically registered time to positive BC.

### Sample preparation and PCR procedure

Preparation of DNA and PCR testing was performed from 1 ml whole blood samples using the Septi*Fast*® Lys Kit, the Septi*Fast*® Prep Kit and the LightCycler® Septi*Fast* Kit as described recently in more detail [18]. In brief, samples were mechanically lyzed and internal controls (IC) were included in each sample and in negative controls (NC). Manual extraction was performed to obtain a final extraction volume of 200 µl DNA. Eluate (50 µl) was used for subsequent real-time PCR amplification using the LightCycler 2.0 Instrument. Potential contaminations were eliminated using uracil-*N*-glycosylase. DNA amplification targets were conserved and variable parts of the internal transcribed sequence (ITS) regions of bacteria and fungi [18]. Amplified variable parts of any amplification products were then hybridized to genus- or species-

specific oligonucleotide probes and subjected to software-assisted temperature melting-peak ($T_m$) analysis using the Septi*Fast*® Software set V2.0 (Roche Diagnostics GmbH, Penzberg, Germany) to reliably identify microorganisms covered by the Septi*Fast* test panel [18]. A result was regarded as a true negative only if included ICs were measured positive. Moreover, the assay was regarded as valid only if the NC tested negative and the corresponding controls (reagent control and the IC of the NC) were detected within their assigned $T_m$ ranges. According to recent study data, time to report for the method's workflow is less then 6 h and the analytical sensitivity of the assay ranges between 3 and 100 CFU/ml, depending on the individual microorganism [18].

### Comparison of LightCycler® Septi*Fast* test results with BC findings

A blood stream infection was defined as a positive BC result, obtained and analyzed as set forth by the current DGHM procedures [20]. Interpretation of BC findings was performed according to established clinical and microbiological standards (DGHM) [20]. Whether microorganisms identified by PCR represented true infection or contamination was evaluated retrospectively by taking into account the identity of the microorganism detected and by comparing PCR results with corresponding BC findings. For common facultative and obligate bacterial pathogens except potential skin contaminants such as coagulase-negative *Staphylococcus* (CoNS) and some streptococcal species, a positive BC result was interpreted as evidence for ongoing blood stream infection (BSI). For CoNS and streptococci, positive BC results were interpreted as evidence for BSI only if BC demonstrated growth of identical organisms in two or more different samples of a given patient within 72 h. If a single positive result was reported for these microorganisms within a 72-h period the results were interpreted as probable contamination. Purpose of this study was primarily to evaluate the technical and diagnostic feasibility of PCR as an add-on to conventional BC diagnostics as routinely performed in the participating centers, no 24-h 7-days-a-week PCR service was provided throughout the investigation. Therefore, direct comparison of "time-to-result" performance between PCR and conventional blood culture methods is not part of the analysis as presented in this study.

### Analysis of clinical data in conjunction with PCR and BC findings

Sepsis patients were grouped according to BC and PCR results obtained during the patient's individual course. Negative findings throughout the clinical course by both BC and PCR led to inclusion in the PCR negative/BC negative group, positive findings by BC and PCR led to inclusion in the PCR positive/BC positive group, respectively. Single positive results for PCR without corresponding BC culture findings throughout the entire course led to inclusion in the PCR positive/BC negative group. Conversely, negative PCR and positive BC were grouped as PCR negative/BC positive. Maximum PCT level and SOFA score, and length of ICU and hospital stay for each patient's course, as well as patient survival were also recorded for this analysis.

### Comparison of LightCycler® Septi*Fast* test results with a constructed gold standard

In the course of microbiological screening for potential infectious foci in the individuals with severe sepsis additional microbiological samples were taken from various body sites and subjected to conventional culture and additional microbiological testing. For assessment of the diagnostic plausibility of available PCR results, the status of infection in the sepsis cohort was further analyzed in the light of a constructed gold standard generated from such supplementary data from additional microbiological samples. For this in-depth analysis a positive PCR result from the patient's blood was regarded as a true positive only if the detected microorganisms were present also in the corresponding BC and/or in other supplementary materials of the patient expected to be sterile under healthy conditions (i.e., bronchio-alveolar lavage fluid, intra-abdominal swabs, urine, etc.) obtained $\pm 2$ days from the onset of the septic episode.

### Statistical methods

Comparisons were made by $2 \times 2$ contingency tables. Differences between groups were analyzed by one-way analysis of variance (ANOVA) using the GraphPad Prizm 4.0 statistical software package.

## Results

### Study and control population characteristics

A total of 108 patients with severe sepsis from the two study sites were included in the study. All patients were under antibiotic coverage at the beginning and throughout the observational study. The corresponding demographics are depicted in Table 1. The most common underling causes of severe sepsis were peritonitis, pneumonia, and severe sepsis following cardiovascular surgery (Table 1). Most frequent comorbidities were chronic renal failure

**Table 1** Characteristics and causes of sepsis of the study patients

| | All | Female | Male |
|---|---|---|---|
| Patients (*n*) | 108 | 36 | 72 |
| Age, mean (range), years | 58.37 (18–84) | 60.10 (19–84) | 57.42 (18–84) |
| Survival (*n*) | 65 (60%) | 25 (69%) | 40 (56%) |
| Nonsurvival (*n*) | 43 (40%) | 11 (31%) | 32 (44%) |
| Underlying cause of sepsis | Number | | |
| Abdominal sepsis (peritonitis, necrotizing pancreatitis, cholangitis or cholecystitis) | 35 | | |
| Sepsis following cardiovascular surgery | 28 | | |
| Pneumonia/ARDS | 23 | | |
| Tissue infection following trauma | 15 | | |
| Osteomyelitis | 2 | | |
| Genitourinary infection | 2 | | |
| Mediastinitis | 2 | | |
| Catheter related | 1 | | |
| Total | 108 | | |

**Table 2** Chronic comorbidities of study patients ($n = 108$)

| | No.[a] | Total (%)[a] |
|---|---|---|
| Neoplasms (acute or chronic lymphoma, acute or chronic leukemia, metastatic solid cancer) | 20 | 18.5 |
| Liver failure (biopsy-proven cirrhosis, documented variceal hemorrhage or portal hypertension, hepatic ascites or hepatic encephalopathy) | 18 | 16.7 |
| New York Heart Association class III or IV heart failure | 55 | 50.9 |
| Chronic renal failure (serum creatinine >1.5 of upper normal limit) or renal replacement therapy | 49 | 45.4 |
| Diabetes mellitus (non-insulin-dependent and insulin-dependent) | 21 | 19.4 |

[a] Patients ($n = 108$) presented multiple comorbidities. Sums of individual comorbidities ($n = 163$) and relative comorbidity fractions of the whole study population are given

and New York Heart Association class III or IV heart failure (Table 2).

## Analysis of PCR compared with BC

PCR results of 453 blood samples were compared with the results of conventional BC that had been obtained in parallel at each individual time point of sampling. In septic patients, BC were positive in 58 samples (12.8%). In the concomitantly drawn EDTA blood samples PCR yielded 114 positive results (25.2%; Table 3). PCR thus resulted in a twofold higher recovery rate compared with conventional BC (Fig. 1a). Of 58 positive BC findings, 40 corresponded directly to the PCR test results. Based on this, the negative predictive value of PCR versus blood culture as a gold standard was 0.95, sensitivity was 0.69, and specificity was 0.81. However, 18 samples of positive BC showed different PCR results.

## Analysis of discrepancies

Thirteen out of 18 discrepant cases (72%) could be resolved either by not being in the panel covered by PCR such as *Pantoea agglomerans* ($n = 4$) and *Proprionibacterium* spp. ($n = 2$), or because they were judged as contaminating skin flora ($n = 7$; Table 3). However, five positive BC yielding growth of *Serratia marcescens* ($n = 1$), *Enterococcus faecalis* ($n = 1$), *Enterococcus faecium* ($n = 2$), and *Candida glabrata* ($n = 1$) did not match the corresponding PCR result (Table 3). Despite evidence of real infection as judged by independent clinical review the negative PCR finding remained unresolved.

## Analysis of PCR results versus a constructed gold standard

PCR results were also analyzed against a constructed microbiological gold standard. This constructed gold standard included BC findings and/or additional microbiological test results from other patient materials expected to be sterile under healthy conditions and obtained $\pm 2$ days from onset of the corresponding septic episode. The rationale for introducing such a constructed gold standard was to further elucidate the plausibility of the unopposed positive PCR. PCR detected 74 pathogens which were not confirmed by the corresponding BC

analysis. However 51 of these 74 PCR results matched microbiological test results from additional specimens. These additional results included detection of *Enterobacter cloacae/aerogenes* ($n = 6$), *Escherichia coli* ($n = 5$), *Klebsiella oxytoca/pneumoniae* ($n = 7$), *Proteus mirabilis* ($n = 1$), *Pseudomonas aeruginosa* ($n = 5$), *Serratia marcescens* ($n = 1$), *Stenotrophomonas maltophilia* ($n = 1$), *Enterococcus faecalis* ($n = 6$), *Enterococcus faecium* ($n = 8$), *Staphylococcus aureus* ($n = 3$), *Streptococcus* spp. ($n = 1$), *Aspergillus fumigatus* ($n = 4$), and *Candida albicans* ($n = 2$) by both PCR and conventional culture from primarily sterile body materials other than blood (Table 3). When compared with the constructed gold standard the sensitivity of PCR increased to 0.83, the specificity increased to 0.93, and the negative predictive value to 0.95.

### *Analysis of the clinical course of the study population*

The 108 sepsis patients were grouped according to matching results of BC and PCR results during their clinical course. Positive BC findings with concomitant positive PCR results ($n = 40$ samples) were present in 16 out of 108 patients during the septic episode. PCT plasma level, SOFA score, length of ICU, and hospital stay in these patients were significantly increased, while survival was decreased, when compared with the 66 patients of the cohort with negative findings in 321 BC and parallel PCR samples ($p < 0.05$; Table 4).

## Discussion

In this observational study we investigated the implementation and potential add-on utility of a new PCR test compared with conventional BC diagnostics. To date, BC still play a pivotal role in the diagnosis of sepsis and are regarded as the microbiological gold standard for the detection of bloodstream infections in patients with clinical sepsis and fever of unknown origin [17]. The practical value of BC in the diagnosis of sepsis, however, is clearly impaired due to lengthy detection time. Commonly the positive yield does not exceed 5–30% in septic patients, mainly depending on the severity of the disease and localization of the infection [21]. Likewise, the sensitivity of BC is known to be poor for many slow-growing and fastidious organisms [22]. As shown in this study PCR-based assay technology holds promise to circumvent some methodological drawbacks of conventional culture and to enhance detection of bacteria and fungi in patients with suspected sepsis. Compared with conventional BC diagnostics, PCR testing resulted in a twofold higher positivity

**Table 3** Microbiological findings by blood culture and PCR

| Gram-negative organisms | BC only | BC + PCR | PCR only | PCR + suppl. cultures |
|---|---|---|---|---|
| *Enterobacter cloacae* or *aerogenes* | 0 | 7 | 13[a] | 6 |
| *Escherichia coli* | 0 | 3 | 16 | 5 |
| *Klebsiella oxytoca* or *pneumoniae* | 0 | 1 | 14[a] | 7 |
| *Pantoea agglomerans* | 4 | Not in panel | Not in panel | 0 |
| *Proprionibacterium* spp. | 2 | Not in panel | Not in panel | 0 |
| *Proteus mirabilis* | 0 | 0 | 1 | 1 |
| *Pseudomonas aeruginosa* | 0 | 1 | 6 | 5 |
| *Serratia marcescens* | 1 | 1 | 5 | 1 |
| *Stenotrophomonas maltophilia* | 0 | 2 | 3 | 1 |
| Gram-positive organisms | BC only | BC + PCR | PCR only | PCR + MiBi |
| Coagulase-negative *Staphylococci* | 7[b] | 7 | 12[c] | 0 |
| *Enterococcus faecalis* | 1 | 8 | 15 | 6 |
| *Enterococcus faecium* | 2 | 2 | 10 | 8 |
| *Staphylococcus aureus* | 0 | 5 | 8 | 3 |
| *Streptococcus* spp. | 0 | 0 | 1 | 1 |
| Yeast/fungi | BC | BC + PCR | PCR | PCR + MiBi |
| *Aspergillus fumigatus* | 0 | 0 | 4 | 4 |
| *Candida albicans* | 0 | 3 | 5 | 2 |
| *Candida crusei* | 0 | 0 | 1 | 0 |
| *Candida glabrata* | 1 | 0 | 0 | 0 |
| $\sum$ of all | 18 | 40 | 114 | 51 |

[a] Indistinguishable for PCR method due to ITS-sequence homologies between *E. cloacae* and *E. aerogenes*, and between *K. oxytoca* and *K. pneumoniae*, respectively

[b] samples of coagulase-negative *Staphylococci* were regarded as contamination

[c] Seven further positive PCR results were regarded as contamination and not included

**A**

Sepsis cohort (n=108)

| PCR | | Blood culture: Positive | Blood culture: Negative | Blood culture: Σ |
|---|---|---|---|---|
| | Positive | 40 | 74 | 114 |
| | Negative | 18 | 321 | 339 |
| | Σ | 58 | 395 | 453 |

**B**

Sepsis cohort (n=108)

| PCR | | Constructed Gold Standard: Positive | Constructed Gold Standard: Negative | Constructed Gold Standard: Σ |
|---|---|---|---|---|
| | Positive | 91 | 23 | 114 |
| | Negative | 18 | 321 | 339 |
| | Σ | 109 | 334 | 453 |

**Fig. 1** **a** Analysis of PCR results compared with BC findings in sepsis patients (study group). **b** Analysis of PCR results compared with a constructed gold standard, comprising positive BC findings or detected organisms in additional patient specimen (e.g., bronchio-alveolo lavage, urine, swabs)

rate in our cohort of patients with clinical sepsis (Fig. 1a). To further investigate the clinical plausibility of PCR results, we also included additional laboratory data and culture results obtained from additional body sites that were clinically incriminated to be focus of ongoing infection in these patients (constructed gold standard, Fig. 1b). Interestingly, in 51 of 74 conventional samples (69%) the additional microbiological findings paralleled the PCR results obtained directly from the blood of the corresponding patients (Fig. 1b) and, thus, may be interpreted as an important additional clue for the involvement of the recovered microorganisms in the ongoing septic infection in these individuals. Finally, the remaining 23 PCR-positive but discrepant constructed gold standard-negative samples remain unresolved at this point. Such inconclusive results may be interpreted as being either false-positive PCR assays or false-negative constructed gold standard findings. False-positive PCR results, however, may also originate from the amplification of free DNA released from nonviable or killed bacteria and fungi, thereby mimicking ongoing infection. Future interventional trials utilizing PCR-guided therapy may enlighten the relevance of PCR-positive but BC (or constructed gold standard) negative findings in the clinical context of the ICU.

Timely and adequate antimicrobial treatment is key for improved outcome in patients with pneumonia, meningitis, and sepsis [8, 23]. Recently Kumar et al. showed that from the onset of hypotension in patients presenting with septic shock, each hour of delay in adequate antimicrobial treatment on average was associated with 8% decrease in survival rate [6]. Similarly, improved survival in sepsis patients with early adequate therapy has been demonstrated in several other studies [4, 9–11]. The diagnosis of bacteraemia by BC, however, can be delayed for 12–48 h even in common rapid growers depending on individual growth kinetics and initial inoculum, thereby complicating a more timely diagnosis in patients with sepsis. Moreover, the recovery rate of conventional cultures is clearly impaired after initiation of antimicrobial treatment despite current BC systems having been modified in an attempt to reduce the effect of antimicrobials in the BC bottle [24]. Although a comparative time-to-result analysis of PCR and BC was not part of this study, the given turnaround time of 6 h for the PCR assay [18] may turn out to be an important add-on of PCR technology compared with the 12–48 h time interval necessary to generate initial BC findings [25, 26]. Another possible advantage of a DNA-based detection system is that the microorganisms causing sepsis do not have to be viable at the time of sampling, thereby also including phagocytized or partly digested bacteria within white blood cells. Thus, PCR may be advantageous particularly in patients receiving antibiotics.

Clearly, little is known about the kinetics and clinical and therapeutic relevance of bacterial DNA present in the blood of patients with sepsis over time in the course of an invasive blood stream infection. The potential influence of circulating bacterial DNA on the severity and outcome of blood stream infections, however, is underlined by the findings of our study demonstrating that PCT plasma levels and SOFA scores were significantly higher, length of ICU and hospital stay were longer, and survival was

**Table 4** Clinical data of patients grouped according to PCR and blood culture findings

| | Mean PCT (ng/ml) | Mean SOFA (Score) | LOS-ICU (days) | LOS-hospital (days) | Survival (rate) | *p* |
|---|---|---|---|---|---|---|
| PCR−/BC− ($n = 66$) | 1.7 (0–56.0) | 11 (2–20) | 17 (1–89) | 23 (1–93) | 66 | <0.05 |
| PCR+/BC+ ($n = 16$) | 12.1 (0.4–139.9) | 16 (12–20) | 36 (8–87) | 38 (8–90) | 33 | |

*LOS-ICU* length of stay on ICU, *LOS hospital* length of stay in hospital

clearly decreased in a subgroup of patients revealing positive findings in both PCR testing and BC.

Although rapid and specific, an important limitation of the current PCR assay, however, arises from the fact that some pathogens that are not part of the detection panel, such as *Pantoea agglomerans* and *Proprionibacterium* spp., were missed by PCR but recovered by BC. In five other cases microorganisms that are part of the PCR detection panel were not detected by PCR but were detected in BC. These cases comprised *Serratia marcescens* ($n = 1$), *Enterococcus faecalis* ($n = 1$), *Enterococcus faecium* ($n = 2$), and *Candida glabrata* ($n = 1$). Of note, all bacterial isolates in the BC became positive after prolonged culture periods, clearly pointing to a low-level in vivo bacteraemia at the time point of sampling. The results of quantitative BC studies have shown that most episodes of clinically significant bacteraemia in adults are characterized by low numbers of circulating bacteria. Werner et al. found that 54% of BC from adult patients with staphylococcal and streptococcal endocarditis contained between 1 and 30 CFU/ml of blood [27]. Moreover, Kreger et al. described that 73% of 77 patients with Gram-negative bacteremia had BC that contained <10 CFU/ml of blood [28]. An analytical assay sensitivity of 3–30 CFU/ml [18] and a predefined detection panel of 25 different BSI pathogens, thus, limit to some extent the diagnostic capabilities of the current assay in patients with sepsis when compared with a theoretical sensitivity of one CFU per culture bottle in conventional blood culture after inoculating $\sim$10 ml whole blood. Increasing the starting volume of the PCR test may help to increase the stochastic probability of picking up a pathogen in cases of low-level bacteraemia. In addition, the current test could be improved by adding an additional assay component for the detection of consensus DNA of bacteria or fungi, thereby providing identification of pathogens which are not primarily part of the PCR panel. The feasibility of assays diagnosing bacteria and fungi by consensus DNA detection has been described previously [29].

Summing up, the novel PCR-based assay system, at present, cannot fully substitute conventional BC also because a more general susceptibility testing of the recovered organisms is not yet possible. Nevertheless, the clinical value of classical BC techniques was also clearly impaired, in part due to the low recovery rate in our sepsis cohort. Moreover, BC show a prolonged time-to-result interval, as it usually takes at least 12–48 h to provide the clinician with Gram stain results and species identification.

As shown in the present study, novel real-time PCR-based molecular biological assays for rapid detection of important pathogens causing BSI can overcome some limitations of the conventional culture-based microbiological techniques, especially when it comes to more rapid detection and species identification of blood stream pathogens. As such, molecular test systems may be seen as a valuable add-on, providing important additional information to the treating physicians very early in the course of disease, thereby potentially tailoring the antibiotic coverage in septic patients. Whether the assay will indeed significantly contribute to more rapid initiation of better-tailored antimicrobial therapy and improved patient management in conjunction with other laboratory markers, however, awaits further evaluation of the test in interventional clinical studies.

## Conclusions

Our study demonstrates good concordance of PCR results with BC. Moreover, we confirmed the increased sensitivity of PCR-based detection of bloodstream infections, as shown in other studies. These results are indicative of potential clinical utility of PCR-based pathogen identification in patients with severe sepsis. Studies addressing changes in clinical decision-making, outcome, and cost-benefit by the utilization of PCR results, however, are needed.

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