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ORIGINAL ARTICLE

# Evaluation of loop-mediated isothermal amplification for the rapid identification of bacteria and resistance determinants in positive blood cultures

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**Abstract** The use of molecular assays to rapidly identify pathogens and resistance genes directly from positive blood cultures (BCs) contribute to shortening the time required for the diagnosis of bloodstream infections. In this work, loop-mediated isothermal amplification (LAMP) assays have been examined for their potential use in BC diagnosis. Three different assays were applied. The commercially available eazyplex® MRSA test detects *Staphylococcus aureus*, *S. epidermidis*, *mecA*, and *mecC*. Two in-house assays [Gram-positive (GP) and Gram-negative (GN)] have been developed for the detection of streptococci, enterococci, *vanA*, *vanB*, *Pseudomonas* spp., Enterobacteriaceae, and the $bla_{\text{CTX-M}}$ family. A total of 370 positive BCs were analyzed. LAMP test results were obtained within 30 min, including sample preparation. Amplification was measured by real-time fluorescence detection. The threshold time for fluorescence intensity values ranged from 6.25 to 13.75 min. The specificity and sensitivity of the assays varied depending on the target. Overall, from 87.7% of BCs, true-positive results were obtained, compared to routine standard diagnosis. Twenty-one tests were true-negative because of the lack of an appropriate target (5.7%). The concordance of positive test results for resistance genes with subsequent antibiotic susceptibility testing was 100%. From 15 BC bottles with mixed cultures, eazyplex® assays produced correct results in 73% of the cases. This study shows that LAMP assays are fast and cost-saving tools for rapid BC testing in order to expedite the diagnostic report and improve the antibiotic stewardship for sepsis patients.

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## Introduction

Time-saving microbiological diagnosis of sepsis remains a key challenge for the clinical laboratory. The initial use of inappropriate antibiotics increases the mortality of patients with sepsis, underlining that an early targeting of the antibiotic therapy is highly important for an improved patient care [1]. Bloodstream infections can be diagnosed by classical blood culture (BC) and culture-independent polymerase chain reaction (PCR)-based approaches. PCR conducted directly on patient blood samples can basically produce faster results and avoids the problem of high contamination rates of BCs with coagulase-negative staphylococci (CoNS) [2]. However, labor-intensive PCR assays are difficult to integrate into a continuous routine lab flow. A recently introduced commercial assay based on PCR/electrospray ionization-mass spectrometry (PCR/ESI-MS) offers reduced hands-on time but requires high investment costs and has only a limited capacity of samples that can be analyzed during a normal working day [3]. One major problem of BC diagnosis, the delay in reporting results due to conventional subculture for identification and antimicrobial susceptibility testing (AST), can be avoided by the application of rapid molecular tests or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) to identify pathogens and their key resistance markers directly from positive BC bottles [4–6]. Automated random access tests have been shown to be easily applicable as reliable tools for BC diagnosis, providing results within one or two hours [7, 8]. Such assays with a minimum of hands-on

time can contribute to implement a targeted antibiotic therapy as soon as possible [9]. The choice of an appropriate test for the routine lab depends on costs for machines and consumables, test performance, and an easy handling, allowing integration into the daily routine workflow. An alternative technology for rapid molecular assays is provided by loop-mediated isothermal amplification (LAMP), an amplification technique that uses *Bst* DNA polymerase with strand-displacing activity [10]. LAMP offers high-speed amplification within several minutes under isothermal conditions and does not require DNA purification from most clinical sample types [11, 12]. Its reliability to detect carbapenemases and extended-spectrum beta-lactamase (ESBL) genes in cultured bacteria and directly from urine samples of patients with urinary tract infection has been recently demonstrated [13–15].

This study was designed to evaluate the potential of LAMP to rapidly identify the most common pathogens and their major resistance genes from positive BC bottles within a time frame of 30 min. Three different eazyplex® LAMP assays with real-time fluorescence detection of amplificates were applied. The eazyplex® MRSA (Amplex BioSystems, Giessen, Germany) is a CE-labeled commercial test for the identification of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) from nasal and pharyngeal swabs and was used with a preliminary BC protocol. The eazyplex® Gram-positive (GP) and Gram-negative (GN) assays were developed for study purposes to detect streptococci, enterococci, and Enterobacteriaceae and *Pseudomonas*, respectively. All LAMP assays were evaluated using routine BCs as clinical samples.

## Materials and methods

### Bacterial strains and in-house LAMP assays

Specific primer sets were designed using LAMP Designer software v1.10 (PREMIER Biosoft International, Palo Alto, CA, USA). For the different species or genera, the following target genes were chosen for primer design: GP assay: *Enterococcus* spp. (tufA), *E. faecalis* (EF0027, coding for a phosphosugar-binding transcriptional regulator protein), *Streptococcus pneumoniae* (lytA), *Streptococcus* spp. (tufA); GN assay: *Escherichia coli* (yfiL), *Klebsiella pneumoniae* (ydhS), *Pseudomonas aeruginosa* (oprL) and 16s rDNA for Enterobacteriaceae (Table 1). A total of 19 Gram-positive and 64 Gram-negative isolates and reference strains purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) or obtained from the National Reference Laboratory for Multidrug Resistant Gram-negative Bacteria (Bochum, Germany) were used to evaluate the specificity of the primer sets of the in-house GP and GN LAMP assays

**Table 1** Analytes detected by the eazyplex® assays used in this study[a]

| MRSA | GP | GN |
|---|---|---|
| *S. aureus* | *E. faecalis* | *E. coli* |
| *S. epidermidis* | *Enterococcus* spp. | *K. pneumoniae* |
| *mecA* | *S. pneumoniae* | Enterobacteriaceae |
| *mecC* | *Streptococcus* spp. | *Pseudomonas* spp. |
| | *vanA* | $bla_{\text{CTX-M-1}}$ group |
| | *vanB* | $bla_{\text{CTX-M-9}}$ group |

[a] An inhibition control was included in each assay

(Table 1). A small single colony was suspended in 500 μL of resuspension and lysis fluid (RALF, Amplex BioSystems) and boiled for 2 min. 25 μL of the suspension were added to each of the tubes in the eazyplex® test strip. The strip was gently knocked to remove air bubbles and loaded into the Genie II machine (OptiGene Ltd., Horsham, West Sussex, UK; purchased from Amplex BioSystems). Tests were run at 65 °C for 20 min.

### Collection and processing of BCs

Clinical samples were BCs submitted as part of routine patient care to the laboratory from the University Hospital Jena between April and August 2015. Blood samples collected in BD BACTEC Plus Aerobic/F and Lytic/10 Anaerobic/F bottles (BD Diagnostics, Heidelberg, Germany) were incubated on a BACTEC FX instrument (BD Diagnostics). Positive BCs were sampled aseptically, Gram-stained, and streaked onto Columbia sheep blood agar, chocolate agar, Drigalski lactose agar, and Schaedler agar (Oxoid, Thermo Fisher Scientific) for overnight incubation at 37 °C. In parallel, an aliquot was prospectively tested using the LAMP eazyplex® assays MRSA, GN, and GP according to the results of Gram staining. Only one positive BC bottle per patient was tested.

### LAMP testing of BCs

25 μL of BC broth were mixed with 500 μL of RALF and boiled for 2 min. After centrifugation at 4000 rpm for 1 min, 25 μL of the supernatant were added to each tube of the eazyplex® test strip containing the lyophilized master mix. The strip was gently knocked to remove air bubbles and loaded into the Genie II machine. Tests were run at 65 °C for 20 min. Amplification was measured by real-time fluorescence detection using a DNA intercalating dye. Depending on the result of Gram staining, the MRSA, GP, and GN tests were used for Gram-positive cocci in clusters, Gram-positive cocci arranged in chains or as diplococci, and Gram-negative rods, respectively.

**Table 2** Performance of eazyplex® assays for the identification of bacterial reference strains

| Bacterial strains | No. | Identification level | Correctly identified (*n*) | Incorrectly identified (*n*) | Negative test result (*n*) |
|---|---|---|---|---|---|
| *E. casseliflavus* | 1 | *Enterococcus* spp. | 1 | 0 | 0 |
| *E. gallinarum* | 1 | *Enterococcus* spp. | 1 | 0 | 0 |
| *E. faecalis* VRE, *vanB* | 3 | *E. faecalis*, *Enterococcus* spp., *vanB* | 3 | 0 | 0 |
| *E. faecium* VRE, *vanA* | 1 | *Enterococcus* spp., *vanA* | 1 | 0 | 0 |
| *E. faecium* VRE, *vanB* | 2 | *Enterococcus* spp., *vanB* | 2 | 0 | 0 |
| *S. anginosus* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. agalactiae* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. constellatus* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. dysgalactiae* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. intermedius* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. mitis* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. parasanguinis* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. pneumoniae* | 1 | *S. pneumoniae*, *Streptococcus* spp. | 1 | 0 | 0 |
| *S. pyogenes* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. salivarius* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *S. sanguinis* | 1 | *Streptococcus* spp. | 1 | 0 | 0 |
| *A. baumannii* | 1 | – | – | 0 | 1 |
| *Citrobacter* spp. | 3 | Enterobacteriaceae | 2 | 1 | 0 |
| *Enterobacter* spp. | 6 | Enterobacteriaceae | 6 | 0 | 0 |
| *E. coli* | 12 | *E. coli*, Enterobacteriaceae | 12 | 0 | 0 |
| *E. coli*, CTX-M-positive | 11 | *E. coli*, Enterobacteriaceae, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$ | 11 | 0 | 0 |
| *K. oxytoca* | 3 | Enterobacteriaceae | 3 | 0 | 0 |
| *K. pneumoniae* | 10 | *K. pneumoniae*, Enterobacteriaceae | 10 | 0 | 0 |
| *K. pneumoniae,* CTX-M-positive | 2 | *K. pneumoniae*, Enterobacteriaceae, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$ | 2 | 0 | 0 |
| *Morganella morganii* | 1 | Enterobacteriaceae | 1 | 0 | 0 |
| *Proteus mirabilis* | 1 | Enterobacteriaceae | 1 | 0 | 0 |
| *Salmonella enterica* | 2 | Enterobacteriaceae | 2 | 0 | 0 |
| *S. marcescens* | 2 | Enterobacteriaceae | 2 | 0 | 0 |
| *P. aeruginosa* | 7 | *Pseudomonas* spp. | 6 | 1[a] | 0 |
| *P. fluorescens* | 2 | *Pseudomonas* spp. | 1 | 1[a] | 0 |
| *P. alcaligenes* | 1 | *Pseudomonas* spp. | 0 | 1[a] | 0 |

[a] Tested positive for both *Pseudomonas* spp. and Enterobacteriaceae

### Conventional species identification and AST

Isolates sampled from positive BCs were identified by Vitek MS (bioMérieux, Nürtingen, Germany). Preliminary resistance patterns by disk diffusion assay were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. AST was performed using Vitek 2 and minimal inhibitory concentration (MIC) interpretation according to EUCAST criteria. The production of ESBLs was verified by the combination disk method using cefotaxime (CTX) vs. CTX+clavulanic acid (CV) and cefpodoxime (CPD) vs. CPD+CV. The LAMP results on CTX-M were verified for phenotypic ESBL strains using the Verigene BC-GN array (Nanosphere, Northbrook, IL, USA; purchased from Thermo Fisher Scientific, Wesel, Germany). For staphylococci, cefoxitin disk diffusion assay was performed to confirm beta-lactam resistance.

## Results and discussion

### Overview

To investigate the potential of LAMP for BC diagnosis, three different assays were applied (Table 1). The eazyplex® MRSA assay is a commercial CE-labeled test. For the GP

**Table 3** Results of the eazyplex® MRSA, GP, and GN tests for BCs

| | True-positive (*n*) | True-negative (*n*) | False-positive (*n*) | False-negative (*n*) | No. positive/no. tested (%) (95% CI[a]) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Sensitivity | Specificity | PPV[b] | NPV[c] |
| MRSA | | | | | | | | |
| *S. aureus* | 31 | 106 | 2 | 0 | 100 (88.8–100) | 98.2 (93.5–99.8) | 93.9 (79.8–99.3) | 100 (96.6–100) |
| MRSA[d] | 6 | 25 | 0 | 0 | 100 (54.1–100) | 100 (86.3–100) | 100 (54.1–100) | 100 (86.3–100) |
| *S. epidermidis* | 72 | 60 | 1 | 6 | 92.3 (84–97.1) | 98.4 (91.2–100) | 98.6 (92.6–100) | 90.9 (81.3–96.6) |
| *mecA* (CoNS) | 73 | 30 | 0 | 3 | 96 (88.9–99.2) | 100 (88.4–100) | 100 (95.1–100) | 90.9 (75.1–98.1) |
| GP | | | | | | | | |
| *E. faecalis* | 12 | 58 | 1 | 0 | 100 (73.5–100) | 98.3 (90.9–100) | 92.3 (64–99.8) | 100 (93.8–100) |
| *Enterococcus* spp. | 41 | 27 | 2 | 1 | 97.6 (87.4–99.9) | 93.1 (77.2–99.1) | 95.4 (84.2–99.4) | 96.4 (81.6–99.9) |
| VRE[e] | 3 | 39 | 0 | 0 | 100 (29.2–100) | 100 (91–100) | 100 (29.2–100) | 100 (91–100) |
| *S. pneumoniae* | 2 | 69 | 0 | 0 | 100 (15.8–100) | 100 (94.8–100) | 100 (15.8–100) | 100 (94.8–100) |
| *Streptococcus* spp. | 20 | 50 | 0 | 1 | 95.2 (76.2–99.9) | 100 (92.9–100) | 100 (83.2–100) | 98 (89.6–100) |
| GN | | | | | | | | |
| *E. coli* | 97 | 58 | 2 | 0 | 100 (96.3–100) | 96.7 (88.5–99.6) | 98 (92.9–99.8) | 100 (93.8–100) |
| *E. coli* CTX-M[f] | 17 | 80 | 0 | 0 | 100 (89.5–100) | 100 (95.5–100) | 100 (81.6–100) | 100 (95.4–100) |
| *E. coli* ESBL[g] | 17 | 78 | 0 | 2 | 89.5 (66.9–98.7) | 100 (95.3–100) | 100 (80.5–100) | 97.5 (91.2–99.7) |
| *K. pneumoniae* | 11 | 143 | 2 | 1 | 91.7 (61.5–99.8) | 98.6 (95.1–99.8) | 84.6 (54.6–98.1) | 99.3 (96.2–100) |
| *K. pneumoniae* ESBL[h] | 4 | 7 | 0 | 0 | 100 (39.8–100) | 100 (59–100) | 100 (39.8–100) | 100 (59–100) |
| Enterobacteriaceae | 134 | 12 | 11 | 2 | 98.5 (94.8–99.8) | 52.2 (30.6–73.2) | 92.4 (86.8–96.2) | 100 (73.5–100) |
| *Pseudomonas* spp. | 9 | 148 | 0 | 0 | 100 (66.4–100) | 100 (97.5–100) | 100 (66.4–100) | 100 (97.5–100) |

[a] CI, confidence interval

[b] PPV, positive predictive value

[c] NPV, negative predictive value

[d] No. of *mecA*-positive/no. of *S. aureus*

[e] No. of *vanA*-/*B*-positive/no. of *Enterococcus* spp

[f] No. of CTX-M-positive/no. of confirmed *E. coli* strains carrying CTX-M

[g] No. of CTX-M-positive/no. of ESBL strains of *E. coli*

[h] No. of CTX-M-positive/no. of ESBL strains of *K. pneumoniae*

and GN in-house assays, verification was performed using reference strains selected from our collection of bacterial strains (Table 2). All enterococci and streptococci strains were correctly identified. For Gram-negatives, there were a small number of incorrectly identified *Pseudomonas* strains that showed signals for both *Pseudomonas* spp. and Enterobacteriaceae.

For the evaluation of clinical samples, a total of 370 positive BCs were investigated. The initial Gram staining was used to apply only the appropriate eazyplex® assay strip, thereby saving costs. The results of LAMP were compared with routine diagnosis species identification and AST. The sensitivity, specificity, and positive and negative predictive values of the eazyplex® assays for clinical BCs are summarized in Table 3. The eazyplex® LAMP tests demonstrated high sensitivity and specificity for the identification of species and resistance genes in BCs. One MRSA and two GN tests reported an invalid result (0.8%). Overall, 532/553 (96.2%) fluorescence signals of targets of the eazyplex® assays represented true-positive results, compared to routine species identification as the gold standard.

### Staphylococci

A total of 140 BCs that showed growth of Gram-positive cocci in clusters were analyzed with the eazyplex® MRSA assay. *Staphylococcus aureus* was diagnosed with high accuracy (Table 3). Of the two specimens with a false-positive signal for *S. aureus*, *S. epidermidis* (1) and *S. hominis* (1) were grown. All cases of MRSA infections were correctly identified by the detection of *mecA*. No isolates with *mecC* could be found. Of the 78 *S. epidermidis* isolates that were identified phenotypically, six were not detected by eazyplex® (92.3% sensitivity). Twenty-nine BCs with Gram-positive cocci in clusters gave no species result in the eazyplex® assay but 17 of them were tested positive for *mecA*. Subcultures revealed CoNS other than *S. epidermidis* in 28 cases and *Stomatococcus* spp. in one case. All *mecA* signals for BCs with CoNS including *S. epidermidis* were concordant with the subsequent detection of oxacillin resistance. Two oxacillin-resistant strains that were found to be *mecA*- and *mecC*-negative were defined as false-negative results to calculate the sensitivity of *mecA*/*mecC* detection for the identification of beta-lactam resistance in CoNS in general (Table 3). One MRSA eazyplex® test reported an invalid result. Because *S. hominis* which was subcultured from the BC bottle is not covered by the targets of the assay, this result was excluded from the performance analysis.

### Streptococci and enterococci

A total of 71 BCs were tested with the GP assay (Table 3). In 43 cases, *Enterococcus* spp. was detected. One false-positive result for *E. faecalis* came from a BC with growth of *S. dysgalactiae* ssp. *equisimilis* that also showed a *Streptococcus* spp. signal in the same test strip. eazyplex® assays that were positive for *Enterococcus* spp. but negative for *E. faecalis* revealed *E. faecium* (27), *E. avium* (2), and two false-positive test results for BCs that contained *S. mitis* and *S. parasanguinis*. The detection of *vanA* by the GP assay was concordant with phenotypic identification of vancomycin resistance for 13/13 *E. faecium* isolates (Table 3). No isolates were positive for *vanB*. Streptococci were identified in 20 BCs. There was only one false-negative test result for a BC that contained *S. anginosus*. True-positive eazyplex® test results revealed *S. agalactiae* (2), *S. dysgalactiae* spp. *equisimilis* (5), *S. mitis* (4), *S. oralis* (1), *S. parasanguinis* (3), *S. sanguinis* (3), and *S. pneumoniae* (2). Both cases of *S. pneumoniae* were also correctly identified by eazyplex® at the species level in the same test strip. One BC with a negative GP test result contained *Peptostreptococcus asaccharolyticus*. For seven further negative tests, the initial Gram stain was misinterpreted, resulting in the application of the GP test for BCs that contained CoNS.

**Table 4** Time to result of the eazyplex® assays

| | Threshold time [min; mean values (SD)] | |
|---|---|---|
| | True-positive | False-positive |
| MRSA | | |
| *S. aureus* | 7 (1.5) | 17 |
| *mecA* (*S. aureus*) | 11.5 (4) | – |
| *S. epidermidis* | 11.75 (2.75) | 18.75 |
| *mecA* (CoNS) | 9.75 (2.5) | – |
| IC | 7.5 (1) | – |
| GP | | |
| *E. faecalis* | 6.75 (1) | 17.5 |
| *Enterococcus* spp. | 10 (4) | 17.5 |
| *vanA* | 7 (1) | – |
| *vanB* | – | – |
| *S. pneumoniae* | 7 | – |
| *Streptococcus* spp. | 13.5 (3.25) | – |
| IC | 9.5 (0.75) | – |
| GN | | |
| *E. coli* | 7.25 (1.75) | 15 |
| *K. pneumoniae* | 13.75 (2.5) | 10.75 |
| $bla_{\text{CTX-M-1}}$ group[a] | 8.5 (4) | – |
| $bla_{\text{CTX-M-9}}$ group[b] | 6.25 (1) | – |
| Enterobacteriaceae | 6.25 (1.75) | 15.75 (2.5) |
| *Pseudomonas* spp. | 8.25 (1.5) | – |
| IC | 9 (1.75) | – |

[a] $n = 16$

[b] $n = 5$

**Table 5** Comparison of subcultures with eazyplex® test results for the identification of polymicrobial BCs

| Case | eazyplex® | | Subculture |
|---|---|---|---|
| | Assay performed[a] | Results | |
| 1 | MRSA | *S. aureus*, *S. epidermidis*, *mecA* | *S. aureus*, MSSA; *S. epidermidis*, FOX[b]-resistant |
| 2 | MRSA | *S. aureus* | *S. aureus*, MSSA; *S. epidermidis*, FOX-resistant |
| 3 | MRSA | *S. aureus*, *S. epidermidis* | *S. hominis*, FOX-sensitive; *S. epidermidis*, FOX-sensitive |
| 4 | MRSA | *S. epidermidis* | *S. epidermidis*, FOX-sensitive; *S. hominis*, FOX-sensitive |
| 5 | MRSA | Negative | *S. epidermidis*, FOX-resistant; *S. salivarius* |
| 6 | MRSA; GP | *mecA*; *Streptococcus* spp. | *S. haemolyticus*, FOX-resistant; *S. mitis* |
| 7 | GP | *E. faecalis*, *Enterococcus* spp. | *E. faecalis*, VAN[c]-sensitive; *S. epidermidis*, FOX-resistant |
| 8 | GP | *Enterococcus* spp. | *E. faecium*, VAN-sensitive; *S. epidermidis*, FOX-resistant |
| 9 | GP | *Streptococcus* spp. | *S. parasanguinis*; *S. epidermidis* |
| 10 | GP; GN | *Enterococcus* spp.; *E. coli*, Enterobacteriaceae | *E. avium*, VAN-sensitive; *E. coli*, CTX[d]-sensitive; *C. koseri* |
| 11 | GP; GN | Negative; *E. coli*, Enterobacteriaceae | *S. anginosus*; *E. coli*, CTX-sensitive |
| 12 | GN | *E. coli*, Enterobacteriaceae, CTX-M-9 | *E. coli*, ESBL; *Raoultella ornithinolytica* |
| 13 | GN | *E. coli*, *K. pneumoniae*, Enterobacteriaceae | *E. coli*, CTX-sensitive; *K. pneumoniae*, CTX-sensitive |
| 14 | GN | *E. coli*, *K. pneumoniae*, Enterobacteriaceae, CTX-M-1/15 | *E. coli*, ESBL; *K. pneumoniae* |
| 15 | GN | *E. coli*, Enterobacteriaceae, *P. aeruginosa* | *E. coli*, CTX-sensitive; *P. aeruginosa* |

[a] The eazyplex® assays were performed according to the result of Gram stain: GP cocci in clusters, MRSA test; GP in chains, BloodScreen GP; GN rods, BloodScreen GN

[b] FOX, cefoxitin

[c] VAN, vancomycin

[d] CTX, cefotaxime

### Gram-negatives

A total of 159 BCs that showed growth of Gram-negative rods were analyzed with the GN assay (Table 3). Two false-positive results for *E. coli* were identified as *K. oxytoca* and *A. junii* by subculture diagnosis. Two false-positive results for *K. pneumoniae* were caused by cross-reactions of *E. coli* with the *K. pneumoniae* target. In both cases, *E. coli* was correctly identified in parallel. One false-negative *K. pneumoniae* sample only showed an Enterobacteriaceae signal. All *E. coli* and *K. pneumoniae* isolates that were tested positive for CTX-M genes by eazyplex® GN were subsequently confirmed as ESBL strains by phenotypic AST. Of the 19 *E. coli* ESBL isolates, two (10.5%) were CTX-M-negative and, therefore, could not be identified as ESBL by the GN assay. In Table 3, the CTX-M results are also evaluated in relation to the phenotypic identification of an ESBL strain in order to calculate the predictive values of the CTX-M target for identifying ESBL resistance. For *K. pneumoniae*, the concordance of ESBL resistance with detection of CTX-M was 100%. Only CTX-M beta-lactamases were considered as resistance markers in this study because of a very low incidence of carbapenemase-producing Enterobacteriaceae at the Jena University Hospital. It should be noted that other eazyplex® LAMP assays have also been successfully applied to detect carbapenemase genes in BCs that were spiked with carbapenem-resistant bacterial isolates [16]. There were 25 BCs that were true-positive for the Enterobacteriaceae family-level assay but showed no species-specific test result. Subculture identification revealed *K. oxytoca* (3), *C. braakii* (1), *C. farmeri* (1), *C. freundii* (1), *C. koseri* (2), *E. aerogenes* (1), *E. cloacae* (7), *P. mirabilis* (5), and *S. marcescens* (3). However, there were also 11 false-positive eazyplex® GN results for Enterobacteriaceae that were caused by BCs containing *P. aeruginosa* (6), *M. osloensis* (1), *A. baumannii* (1), *A. junii* (1), and *H. influenzae* (2). To cover a broad range of species of Enterobacteriaceae, ubiquitous primer targets of 16S rDNA must be chosen. Potential cross-reactions to other bacteria could not be fully excluded but false-positive signals typically occurred at significantly later threshold times than specific signals and specificity can be increased by reducing the running time cut-off (Table 4). When a cut-off of 15 min was defined, the specificity of the Enterobacteriaceae signal was increased to 91.3% (true-positive: 133, true-negative: 21, false-positive: 2, false-negative: 3). For two BCs that showed growth of *P. mirabilis*, the GN assay was invalid. All cases of *P. aeruginosa* infections were correctly identified as *Pseudomonas* spp. From nine BC bottles with a negative valid GN result, non-fermenters (5) and *Bacteroides* spp. (4) that are not covered by the eazyplex® primers were subcultured.

### Time to result of LAMP for BC analysis

The threshold time for fluorescence intensity values ranged from 6.25 to 13.75 min (Table 4). The internal controls were detected between 7.5 and 9 min. With the exception of *K. pneumoniae*, false-positive signals were typically detected later than true-positive signals [mean values (SD): 15.25 (3.5) min, $n = 21$, vs. 8.5 (3.5) min, $n = 532$; $p < 0.0001$, unpaired $t$-test].

### Mixed infections

From 15 BC bottles, mixed subcultures were obtained. Depending on the results of Gram staining, one or two eazyplex® tests were performed (Table 5). In 11 cases, eazyplex® assays produced correct results including the determination of resistance genes, compared to subculture identification (73%). It should be noted that, in two cases, *S. epidermidis* was not recognized because only the GP assay was applied based on the interpretation of the Gram stain. There were three cases for which the eazyplex® MRSA assay could not correctly differentiate mixed infections with staphylococci, including one false-positive test result for *S. aureus*.

## Conclusions

The impact of rapid blood culture (BC) diagnostic tests on the health care of sepsis patients has been demonstrated in recent studies [17, 18]. The results of this study show that loop-mediated isothermal amplification (LAMP), as an alternative technique to polymerase chain reaction (PCR) assays, is a powerful tool for the rapid identification of common bacterial pathogens and their major resistance genes directly from positive BCs. LAMP is easy to handle and needs only a short running time for amplification when coupled to fluorescence real-time detection. There is no need for DNA purification because *Bst* polymerase tolerates serum and heparin [13, 14]. In principle, testing positive BCs with eazyplex® LAMP offers the possibility to generate results within half an hour, including Gram stain and sample preparation. Another advantage is that no expensive equipment is needed. Limitations of this study include the restricted pathogen panel of the assays. The eazyplex® strip format allows only a maximum of seven target genes and an inhibition control. The LAMP assays applied in this study only detect the most frequent sepsis pathogens and key resistance genes for staphylococci, enterococci, and Enterobacteriaceae. However, when the local hospital antibiogram is taken into consideration, these tests allow specific antibiotic treatment recommendations about one day earlier in comparison to classical BC diagnosis, thereby contributing to improved antibiotic stewardship for sepsis patients.

**Compliance with ethical standards**

**Ethical approval** The study protocol for the evaluation of LAMP eazyplex® assays for clinical BCs was reviewed and approved by the ethics committee of the Friedrich Schiller University of Jena (4400-04/15).

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