Validation of an Isothermal Amplification Platform for Microbial Identification and Antimicrobial Resistance Detection in Blood: A Prospective Study

Harish M Maheshwarappa, Prasadini Guru, Reddy Sailaja Mundre, Nima Lawrence, Snehali Majumder, Alben Sigamani, CN Anupama, Sudeshna Adak

Abstract

Background: Recent advances in nucleic acid amplification technique (NAAT)-based identification of pathogens in blood stream infections (BSI) have revolutionized molecular diagnostics in comparison to traditional clinical microbiology practice of blood culture. Rapid pathogen detection with point-of-care diagnostic-applicable platform is prerequisite for efficient patient management. The aim of the study is to evaluate an in-house developed, lyophilized OmiX-AMP pathogen test for the detection of top six BSI-causing bacteria along with two major antimicrobial resistance (AMR) markers of carbapenem and compare it to the traditional blood culture-based detection.

Materials and Methods: One hundred forty-three patients admitted to the Medical Intensive Care Unit, Narayana Hrudayalaya, Bangalore, with either suspected or proven sepsis, of either gender, of age ≥18 years were enrolled for the study. Pathogen DNA extracted from blood culture sample using OmiX pReP method was amplified at isothermal conditions and analyzed in real time using OmiX Analysis software.

Results: Among the processed 143 samples, 54 were true negative, 83 were true positive, 3 were false negative, and 2 were false positive as analyzed by OmiX READ software. Gram-negative bacteria (91.3%) and gram-positive bacteria (75%) were detected with 100% specificity and 95.6% sensitivity along with the AMR marker pattern with a turnaround time of 4 hours from sample collection to results.

Conclusion: OmiX-AMP pathogen test detected pathogens with 96.5% concordance in comparison to traditional blood culture. Henceforth, OmiX-AMP pathogen test could be used as a readily deployable diagnostic kit even in low-resource settings.

Keywords: Blood stream infection, Diagnosis, Isothermal amplification, Pathogen detection, Sepsis.

Indian Journal of Critical Care Medicine (2021): 10.5005/jp-journals-10071-23761

Introduction

Blood stream infections (BSI) ranging from mild bacteremia to potentially life-threatening septic shock are posing a major healthcare burden worldwide. A delay in appropriate treatment could lead to multiorgan failure and eventual death. Overall mortality due to sepsis in developing countries like India is about 63%, of which 34% of deaths were from the intensive care unit (ICU) of hospitals.

Traditional blood culture (BC) takes 48–72 hours for pathogen detection with culture positivity rates of 10–25%. Meanwhile treating patients with high-end antibiotics like carbapenems and colistin has changed the epidemiology and susceptibility patterns of microorganisms, with a huge impact on antimicrobial stewardship. The higher turnaround time (TAT) along with the lack of sensitivity and contamination issues associated with BC testing highlight the need for a more rapid and accurate method for pathogen detection and antibiotic susceptibility patterns.

Nucleic acid amplification technique (NAAT)-based molecular diagnostic methods enable rapid pathogen identification (ID) in 2–7 hours to complement or to confirm the BC results. Recently, loop-mediated isothermal amplification (LAMP) has emerged as a point-of-care (POC) deployable technique with characteristics like better amplification efficiency, 2–3 pairs of sequence-specific primers, and requirement of simple water bath/dry bath to maintain isothermal conditions. World Health Organization has authorized LAMP-based tuberculosis test—Xpert MTB/RIF (Xpert)

source of support: This study was supported by funding provided from OmiX Research and Diagnostics Laboratories to Narayana Hrudalaya. The funding sources were grants from BIRAC, Bill and Melinda Gates Foundation, Government of Karnataka Idea2PoC to OmiX Research and Diagnostics Laboratories.

Conflict of interest: None

(Cepheid, Sunnyvale, CA, USA) in 2013, but high consumable costs, need of sophisticated instrumentation, and maintenance limits its usage as a POC test. Reaction components of LAMP in lyophilized
format have been reported for human African Trypanosomiasis and Coxiella burnetii, which require minimal technical expertise and easy workflow. However, there is no dried or lyophilized isothermal assay available commercially for the diagnosis of BSI in low resource settings.

In order to provide a cost-effective, easy-to-use diagnostic platform that can be POC deployable, OmiX Labs has developed an isothermal test for BSI called OmiX-AMP pathogen test to detect the gene signatures of top 6 bacterial pathogens and related antibiotic resistance based on Indian epidemiology. The bacterial pathogens in the panel include Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus spp., and Staphylococcus aureus along with carbapenem antibiotic-resistant markers: NDM and OXA-48. In this study, we have clinically validated the OmiX-AMP pathogen test, and concordance was established in comparison to traditional BC test results of critically ill patients. As the first study using such a platform for isothermal tests for BSI, it was preferred to first validate in BCs and compare it to the existing tests in the market.

Materials and Methods

Study Design and Ethics Approval

The study was designed to recruit 150 patients with suspected BSI, from whom blood samples were collected for standard culture by BD BACTEC FX™ system (USA). Patients admitted to medical intensive care unit with suspected/proven sepsis and ≥18 years of age of either gender were approved by the institutional review board of Narayana Hrudayalaya, Bangalore, India (protocol number-NHH/MEC-S01/A-1-2019). The study has been registered with The Clinical Trials Registry-India, with the registration number: CTRI/2019/04/018459.

BC for ID and Antibiotic Susceptibility Test (AST)

The enrolled 150 samples were 100 BC-positive and 50 BC-negative cases and the study design allowed for 90% power to detect the top 2 pathogens with 5% type I error. Blood samples were collected in BD BACTEC™ plus anaerobic and aerobic bottles separately and incubated for a period of 7 days. BC bottles that beeped positive were processed for Gram staining and subculturing in blood agar and MacConkey’s agar medium for organism ID. Meanwhile, 2 ml of blood from BC-positive bottles and BC-negative bottles (no growth after 7 days of incubation) was sent to the OmiX-AMP pathogen test platform. The information of the pathogen identified by BC and AST were kept confidential and only made available at the end of the validation study with the OmiX-AMP pathogen test results.

DNA Extraction by OmiX pReP Method

For each BC sample received, a unique ID and barcode were generated and immediately processed for DNA extraction. To the 200 µL of BC sample, 100 µL of 2% red blood cell lysis buffer was added, incubated at 95°C for 2 minutes, and centrifuged at 8000 rpm for 5 minutes. Pellet was resuspended in 75 µL of ARCIS solution-I, from which 60 µL was transferred to a fresh tube containing 60 µL of ARCIS solution-II. The suspension was incubated at 95°C for 3 minutes and centrifuged at 8000 rpm for 3 minutes. The supernatant-containing DNA was collected into a fresh tube and utilized either immediately or stored at −20°C until the amplification was performed.

Lyophilization of Master Mix for OmiX-AMP Pathogen Test

OmiX-AMP pathogen test was prepared using LAMP master mix and dispensed into plasma-treated 0.2 ml clear tubes. The dispensed formulation tubes were freeze-dried using OmiX proprietary lyophilization program in Genesis SP Scientific pilot freeze dryer. After freeze-drying, lyophilized tubes were visually checked for “white cake” appearances, assembled in OmiX-AMP ID sepsis test kit format of eight unitized panels (in duplicates) along with a positive control and a negative control in the silver pouches with a desiccant and stored at room temperature. Tris-Cl (pH 8.8)–based reaction buffer was provided with each pouch for reagent reconstitution before testing reaction. The quality of each manufactured batch of the OmiX-AMP test kit was tested with the OmiX laboratory standard control DNA samples. Quality-approved batches were used for testing clinical samples.

OmiX Assay Using OmiX-AMP Pathogen Test

For each clinical sample, a unitized panel pouch was utilized. Reconstitution buffer (20 µL) was added to each tube to reconstitute the lyophilized reagents. Then, 5 µL of DNA was added to each of the 18 reaction tubes. The reaction tubes for 1 or 2 samples (18 or 36 tubes) were then placed in the Rotor–Gene Q-device (Qiagen) and heated to 65°C for 1 hour, and a final denaturation of 2 minutes at 95°C to inactivate the enzyme. No template control run was performed at regular intervals to ensure no amplicon contamination prevailed in the lab setup. At the end of the run, the real-time fluorescence data collected on the Rotor–Gene Q-device were exported to analyze the results using OmiX Analysis software.

Statistical Analysis

Sensitivity, specificity, positive and negative predictive values, and their 95% confidence intervals were computed using the epir package in R-software. Cohen’s kappa was calculated as a measure of agreement between the organism identified by the OmiX-AMP pathogen test and the standard BC results. For this purpose, “negative” cases were of three types: (a) negative in culture; (b) positive in culture but negative for the six panel organisms, with a different organism identified; and (c) positive in culture but no organism identified and culture considered to have a contaminant or coagulase-negative Staphylococci (CoNS).

Results

Pathogen Identification

In the present study, a total of 100 positive and 50 negative BC samples were evaluated using the OmiX-AMP pathogen test from February 2019 to June 2019. Among the 150 BCs, 6 samples were used to standardize the process, 1 sample was not processed due to insufficient quantity, and 143 samples were considered for the study (Flowchart 1).

Of 143 subjects, 86 were men (age: 18–93 with average of 55.9 years) and 57 were women (age: 23–88.9 with average of 57.1 years). The majority of the samples were from old age of ≥60 (n = 65) followed by age of 31–59 years (n = 57). Young age people were less affected according to the study (n = 21).

Of the 143 BC samples, 89 were culture positive and 54 were culture negative as per standard BC results. Of these 89 culture-positive cases, 50 were positive for the pathogens which are part of the OmiX-AMP pathogen test, 15 were CoNS which are considered in BSI as contaminants, and 24 samples were positive for off-panel organisms (Table S1). The rate of OmiX panel organisms was 73% ([50+15]/89) and 27% of cases were not part of the OmiX-AMP panel.
The OmiX-AMP pathogen test detected 45 of 50 OmiX panel-related organisms that included *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterococcus* spp., and *S. aureus* (Table 1). Fifty-four no-growth cases in culture and fifteen cases of CoNS were reported as negative in the OmiX-AMP pathogen test. Figure 1 demonstrates the correlation between no growth to date (NH) BC results and OmiX-AMP pathogen test.

Among the 121 cases which were either of OmiX panel or negative in BC or CoNS in BC, the Cohen’s kappa measure was 0.94 with a 95% confidence interval of 0.89–0.97. This is well above the 0.80 threshold that is considered to be a statistically relevant measure of a high level of agreement. In the 143 cases, the Cohen’s kappa measure of agreement is 0.74 with a 95% confidence interval of 0.66–0.82. Table 2 illustrated the true prevalence, sensitivity, specificity, and positive and negative predictive values as computed from the 121 cases which include the OmiX panel organisms, negative in culture, and CoNS identified in culture cases. There was nearly 100% specificity, positive predictive value, and negative predictive value for all organisms. However, due to the low prevalence of some organisms, sensitivity was easily affected.

**Carbapenem Resistance Pattern Using NDM and OXA-48 as Genetic Markers**

AST results from BC for ertapenem and meropenem antibiotics identified 13 *E. coli* cases as sensitive, 1 as resistant to ertapenem, and the other 2 as resistant to both ertapenem and meropenem antibiotics, while the OmiX-AMP test detected 12 of 13 AST-sensitive samples with a sensitivity of 92.3% and the other as sensitive. The other two *E. coli* positive and resistant to ertapenem and meropenem, one was detected for NDM and the other for OXA-48 marker in OmiX-AMP test.

The OmiX-AMP test detected 11 of 12 resistant cases for OXA-48 and NDM with a sensitivity of 91.6% (11/12). Among the four sensitive cases, three were detected negative for NDM and OXA-48 markers with a specificity of 75% and 1 sensitive *K. pneumoniae* for ertapenem and meropenem was detected as positive for OXA-48 in OmiX-AMP test.

*A. baumannii* and *P. aeruginosa* were the two main non-fermenting gram-negative bacteria (GNB) covered in the OmiX-AMP pathogen panel. Among the 12 *A. baumannii* positive cases, 8 were resistant to both meropenem and ertapenem antibiotics, while the OmiX-AMP test showed that only 4 were positive for NDM marker and the other 4 samples were detected sensitive to the NDM and OXA-48 markers, resulting in false negatives. The two *P. aeruginosa* positive cases detected in both BC and OmiX-AMP pathogen test were sensitive to antibiotics with 100% specificity.

**Table 1: The number of samples detected for in-panel and off-panel organisms by the NH blood culture identification vs. OmiX-AMP pathogen detection**

| OmiX-AMP pathogen detection | Negative | E. coli | K. pneumoniae | A. baumannii | P. aeruginosa | Enterococcus spp. | S. aureus | Negative | Off-panel | Total |
|-----------------------------|---------|--------|---------------|--------------|--------------|------------------|----------|----------|-----------|-------|
| No growth                   | 54      | 0      | 0             | 0            | 0            | 0                | 0        | 0        | 0         | 54    |
| *E. coli*                   | 0       | 16     | 0             | 0            | 0            | 0                | 0        | 0        | 0         | 16    |
| *K. pneumoniae*             | 2       | 0      | 14            | 0            | 0            | 0                | 0        | 0        | 0         | 16    |
| *A. baumannii*              | 1       | 1      | 0             | 10           | 0            | 0                | 0        | 0        | 0         | 12    |
| *P. aeruginosa*             | 0       | 0      | 0             | 2            | 0            | 0                | 0        | 0        | 0         | 2     |
| *Enterococcus* spp.*        | 1       | 0      | 0             | 0            | 0            | 2                | 0        | 0        | 0         | 3     |
| *S. aureus*                 | 0       | 0      | 0             | 0            | 0            | 1                | 0        | 0        | 1         | 1     |
| CoNS                        | 0       | 0      | 0             | 0            | 0            | 0                | 0        | 0        | 15        | 15    |
| Other                       | 0       | 0      | 0             | 0            | 0            | 0                | 0        | 0        | 24        | 24    |
| Total                       | 58      | 17     | 14            | 10           | 2            | 2                | 1        | 39       | 0         | 143   |

**Flowchart 1: Flowchart of blood culture samples considered for the study**

150 blood culture samples
(100 blood culture positive + 50 blood culture negative)

1 unprocessed sample—sample inadequacy

6 samples utilized for OmiX-AMP test standardization

143 blood culture samples considered for the final study
(89 blood culture positive + 54 blood culture negative)
This study reports validation of an in-house developed, room temperature stable OmiX-AMP pathogen detection kit that detects bacterial pathogens in sepsis-related BSI with 96.69% sensitivity and 100% specificity with 96.5% concordance and was able to generate results in an easy-to-use format with a TAT of 4 hours using OmiX Analysis software.

Commercially available molecular-based detection systems include: SeptiFast (Roche), Magicplex Sepsis (Seegene), SeptiTest (Molzym), broad-range polymerase chain reaction (PCR) and electrospray ionization mass spectrometry (PCR/ESI-MS) (IRIDICA), and film array-based BIOFIRE to mention a few. SeptiFast claims to detect 25 BSI pathogens with a sensitivity of 68–69% and specificity of 83–93% in 4.5–6 hours. Magicplex technology could detect 27 organisms and antimicrobial resistance (AMR) markers mecA, vanA, and vanB with a sensitivity of 11–65% and specificity of 77–92%.[19,20] SeptiTTest based on broad-range PCR and sequencing detects more than 300 BSI-related pathogens with a sensitivity of 37–87% and specificity of 85.5–100%.[21,22] IRIDICA detects more than 700 pathogens along with mecA, vanA, vanB, and Klebsiella pneumoniae carbapenemase AMR markers with a sensitivity 45–83% and sensitivity range of 69–94%.[23,24] The Food and Drug Administration-approved BIOFIRE based on nested multiplex PCR has a sensitivity of 94.6% and specificity of 100% when only on-panel organisms (24 GNB, gram-positive bacteria, and yeast pathogens, as well as 3 AMR genes) were considered.[25] However, variability in results and the high cost of automation limit their utility as POC diagnostics, especially in emerging markets.

BC results for OmiX on-panel organisms revealed that GNB members—E. coli and K. pneumoniae were the most widespread pathogens (64% 32 of 50). Seventy-five percent of detected K. pneumoniae were found resistant to carbapenem panel, OXA-48 in particular (88%) that correlates with the previous studies emphasizing rapidly disseminating carbapenem-resistant E. coli and K. pneumoniae in the population.[26] BC results projected that 70% of the detected A. baumannii were resistant to both ertapenem and meropenem antibiotics, whereas OmiX panel detected resistance in only 57% (NDM) of the AMR culture-positive results. This could be due to the stabilized expression of NDM-2 gene in A. baumannii rather than NDM-1 and OXA-48–mediated resistance.[27] Prevalence of resistance among the pathogens might differ based on the magnitude of the pathogens and the kind of antibiotics being consumed in a particular geographical area.[28] Of the 24 off-panel organisms mentioned in study one, A. nosocomialis was detected as A. baumannii. It could be because the gene signatures of both the organisms display sequence similarity at the genus level as they both belong to Acinetobacter calcoaceticus–baumannii complex, which causes nosocomial infections.[29]

Apart from age and gender, this study did not include other clinical parameters and severity of illness characteristics because this is the initial clinical validation of the in-house developed OmiX-AMP pathogen test. It would be worth to include a less-prevalent ICU antibiogram—Burkholderia, Streptococcus, Enterobacter, etc., and fungal species like Candida, etc., to the OmiX-AMP panel.

OmiX pReP method with simplified DNA extraction protocol and OmiX assay with minimal pipetting steps using lyophilized tubes further shortened the time to 2 hours from the receipt of sample to result generation. Assay run raw data were analyzed using OmiX Analyze software that generated data in the form of amplification plots and self-populated Excel sheet with cycle threshold values and

### Table 2: Prevalence, sensitivity, specificity, and positive and negative predictive values of OmiX panel organisms in the study

| Organism     | True prevalence Rate (95% confidence interval) | Sensitivity Rate (95% confidence interval) | Specificity Rate (95% confidence interval) | Positive predictive value Rate (95% confidence interval) | Negative predictive value Rate (95% confidence interval) |
|--------------|-----------------------------------------------|--------------------------------------------|-------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| E. coli      | 0.13 (0.08, 0.21)                              | 1.00 (0.79, 1.00)                           | 0.99 (0.95, 1.00)                           | 0.94 (0.71, 1.00)                                         | 1.00 (0.97, 1.00)                                         |
| K. pneumoniae| 0.13 (0.08, 0.21)                              | 0.88 (0.62, 0.98)                           | 1.00 (0.97, 1.00)                           | 1.00 (0.77, 1.00)                                         | 0.98 (0.93, 1.00)                                         |
| A. baumannii | 0.10 (0.05, 0.17)                              | 0.83 (0.52, 0.98)                           | 1.00 (0.97, 1.00)                           | 1.00 (0.69, 1.00)                                         | 0.98 (0.94, 1.00)                                         |
| P. aeruginosa| 0.02 (0.00, 0.06)                              | 0.10 (0.16, 1.00)                           | 1.00 (0.97, 1.00)                           | 1.00 (0.16, 1.00)                                         | 1.00 (0.97, 1.00)                                         |
| Enterococcus spp. | 0.02 (0.01, 0.07)                      | 0.67 (0.09, 0.99)                           | 1.00 (0.97, 1.00)                           | 1.00 (0.16, 1.00)                                         | 0.99 (0.95, 1.00)                                         |
| S. aureus   | 0.01 (0.00, 0.05)                              | 1.00 (0.02, 1.00)                           | 1.00 (0.97, 1.00)                           | 1.00 (0.02, 1.00)                                         | 1.00 (0.97, 1.00)                                         |
pathogen detection status. Assay run and report generation took around 90 minutes in comparison to the traditional analysis time of 2–3 hours by normal PCR-based reaction setup. Overall, the cost-effective OmiX-AMP detection platform has the potential for rapid detection of bacterial pathogens in clinical samples with minimal laboratory setup at conventional laboratories in healthcare centers.

**Conclusion**

In conclusion, the developed OmiX-AMP pathogen detection test is a rapid and cost-effective detection method for identifying top six BSI-causing bacterial pathogens. With a TAT of 4 hours along with high specificity and sensitivity in detection, the OmiX-AMP pathogen detection test would accelerate the pathogen detection process with minimal laboratory setup. Further challenge was to detect BSI-related pathogens from whole blood excluding the need for BC.

**Acknowledgments**

We acknowledge the support from BIRAC (Government of India), Bill and Melinda Gates Foundation, Government of Karnataka, Menterra Social Impact Fund and Artha Lesing.

**Orcid**

Harish M Maheshwarappa https://orcid.org/0000-0001-9377-3421
Prasadini Guru https://orcid.org/0000-0002-7876-3150
Reddy Sailaja Mundre https://orcid.org/0000-0003-0983-1647
Nima Lawrence https://orcid.org/0000-0002-7318-8791
Snehal Majumder https://orcid.org/0000-0002-2745-493X
Alben Sigamani https://orcid.org/0000-0002-6927-1947
CN Anupama https://orcid.org/0000-0002-9375-8368
Sudeshna Adak https://orcid.org/0000-0001-8241-4976

**Supplementary Material**

Table S1: List of off-panel organisms detected in blood culture and considered negative in OmiX-AMP pathogen test

| Off-panel organisms | Number |
|---------------------|--------|
| Bacillus pumilus     | 1      |
| Burkholderia cepacia | 7      |
| Morganella morganii msp. morganii | 1 |
| Aeromonas caviae    | 1      |
| Streptococcus constellatus | 1 |
| Streptococcus pyogenes | 1 |
| Acinetobacter nosocomialis | 1 |
| Acinetobacter radioresistens | 1 |
| Ralstonia mannitolitica | 1 |
| Elizabethkingia meningoseptica MDR | 1 |
| Pseudomonas monteilii MDR | 1 |
| Enterobacter cloacae XDR | 1 |
| Candida species      | 1      |
| Candida tropicalis   | 2      |
| Candida orthopsilosis| 1      |
| Candida albicans     | 1      |
| Candida glabrata     | 1      |
| Total               | 24     |

**References**

1. Fleischmann C, Scherag A, Adhikari NKJ, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated sepsis—current estimates and limitations. Am J Respir Crit Care Med 2016;193:253–272. DOI: 10.1164/rccm.201504-07810OC.
2. Abubakar II, Tillmann T, Banerjee A. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study. Lancet 2015;385:117–171. DOI: 10.1016/S0140-6736(14)61682-2.
3. Lueangarun S, Leelarasamee A. Impact of inappropriate empiric antimicrobial therapy on mortality of septic patients with bacteremia: a retrospective study. Interdiscip Perspect Infect Dis 2012;2012:765205. DOI: 10.1155/2012/765205.
4. Chatterjee S, Bhattacharya M, Subhash Kumar T. Epidemiology of adult-population sepsis in India: a single center 5 year experience. Indian J Crit Care Med 2017;21(9):573–577. DOI: 10.4103/ijccm.IJCCM_240_17.
5. Patel R, Vetter EA, Harmsen WS, Schleck CD, Fadel HJ, et al. Optimized pathogen detection with 30- compared to 20-milliliter blood culture draws. J Clin Microbiol 2011;49:4047–4051. DOI: 10.1128/JCM.01314-11.
6. Riedel S, Carroll KC. Early identification and treatment of pathogens in sepsis: molecular diagnostics and antibiotic choice. Clin Chest Med 2016;37(2):191–207. DOI: 10.1016/j.ccm.2016.01.018.
7. Gehel K, Jojera A, Soni S, Gang S, Sabnis R, Desai M. Bacteriological profile and drug resistance patterns of blood culture isolates in a tertiary care nephrology teaching institute. Biomed Res Int 2014;2014:153747. DOI: 10.1155/2014/153747.
8. Dat VQ, Vu HN, Nguyen TH, Nguyen HT, Hoang LB, Tien V, et al. Bacterial bloodstream infections in a tertiary infectious diseases hospital in Northern Vietnam: aetiology, drug resistance, and treatment outcome. BMC Infect Dis 2017;17:493. DOI: 10.1186/s12879-017-2582-7.
9. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial gram-negative pathogens from North and South Indian hospitals. J Med Microbiol 2017;66:999–1004. DOI: 10.1099/jmm.0.000513.
10. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis 2004;4:751–760. DOI: 10.1016/S1473-3099(04)01205-8.
11. Leggieri N, Rida A, Francois P, Schrenzel J. Molecular diagnosis of bloodstream infections: planning to (physically) reach the bedside. Curr Opin Infect Dis 2010;23:311–319. DOI: 10.1097/QCO.0b013e32833bf4c6.
12. Murray PR, Masur H. Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Crit Care Med 2012;40(12):3277–3282. DOI: 10.1097/CCM.0b013e318278e771.
13. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucl Acids Res 2000;28(12):E63. DOI: 10.1093/nar/28.12.e63.
14. Parida M, Samaranagahal S, Dash PK, Rao PV, Morita K. Loop-mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. Rev Med Virol. 2008;18(6):407–421. DOI: 10.1002/rmv.593.
15. World Health Organization. Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva: World Health Organization; 2011.
16. Hayashida K, Kajino K, Hachaambwa L, Namangala B, Sugimoto C. Direct blood dry LAMP: a rapid, stable, and easy diagnostic tool for Human African Trypanosomiasis. PLoS Negl Trop Dis 2015;9:e0003578. DOI: 10.1371/journal.pntd.0003578.
17. Chen HW, Ching WM. Evaluation of the stability of lyophilized loop-mediated isothermal amplification reagents for the detection of Coxiella burnetii. Heliyon 2017;3(10):e00415. DOI: 10.1016/j.heliyon.2017.e00415.

18. Warhurst G, Maddi S, Dunn G, Ghrew M, Chadwick P. Diagnostic accuracy of SeptiFast multi-pathogen real-time PCR in the setting of suspected healthcare-associated bloodstream infection. Intensive Care Med 2015;41(1):86–93. DOI: 10.1007/s00134-014-3551-x.

19. Carrara L, Navarro F, Turbau M, Seres M, Morán I, Quintana I, et al. Molecular diagnosis of bloodstream infections with a new dual-priming oligonucleotide-based multiplex PCR assay. J Med Microbiol 2013;62:1673–1679. DOI: 10.1099/jmm.0.064758-0.

20. Ziegler I, Fagerstrom A, Stralin K, Molling P. Evaluation of a commercial multiplex PCR assay for detection of pathogen DNA in blood from patients with suspected sepsis. PLoS One 2016;20;11(12):e0167883. DOI: 10.1371/journal.pone.0167883.

21. Wellinghausen N, Kochem AJ, Disque C, Mühl H, Gebert S, Winter J, et al. Diagnosis of bacteremia in whole-blood samples by use of a commercial universal 16S rRNA gene-based PCR and sequence analysis. J Clin Microbiol 2009;47(9):2759–2765. DOI: 10.1128/JCM.00567-09.

22. Rogina P, Skvarc MM, Stubljar DD. Diagnostic utility of broad range bacterial 16S rRNA gene PCR with degradation of human and free bacterial DNA in bloodstream infection is more sensitive than an in-house developed PCR without degradation of human and free bacterial DNA. Mediators Inflamm 2014;2014:108592. DOI: 10.1155/2014/108592.

23. Jordana-Lluch E, Carolan HE, Giménez M, Sampath R, Ecker DJ, Quesada MD, et al. Rapid diagnosis of bloodstream infections with PCR followed by mass spectrometry. PLoS One 2013;8:e62108. DOI: 10.1371/journal.pone.0062108.

24. Jordana-Lluch E, Giménez M, Quesada MD, Rivaya B, Marcó C, Domínguez MJ. Evaluation of the broad-range PCR/ESI-MS technology in blood specimens for the molecular diagnosis of bloodstream infections. PLoS One 2015;10:e0140865. DOI: 10.1371/journal.pone.0140865.

25. Southern TR, Van Schooneveld TC, Bannister DL, Brown TL, Crisman AS, Buss SN. Implementation and performance of the BioFire FilmArray® blood culture identification panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. Diagn Microbiol Infect Dis 2015;81(2):96–101. DOI: 10.1016/j.diagmicrobio.2014.11.004.

26. Nagaraj S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. Indian J Med Microbiol 2012;30:93–95. DOI: 10.4103/0255-0857.93054.

27. Rahman M, Prasad KN, Gupta S, Singh S, Singh A, Pathak A, et al. Prevalence and molecular characterization of New Delhi metallo-beta-lactamases in multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii from India. Microb Drug Resist 2018;24(6):792–798. DOI: 10.1089/mdr.2017.0078.

28. Singh SK, Sengupta S, Antony R, Bhattacharya S, Mukhopadhyay C, Ramasubramanian V, et al. Variations in antibiotic use across India – multicentre study through global point prevalence survey. J Hosp Infect 2019;103(3):280–283. DOI: 10.1016/j.jhin.2019.05.014.

29. Pourabbas B, Firouzi R, Pouladfar G. Characterization of carbapenem-resistant Acinetobacter calcoaceticus-baumannii complex isolates from nosocomial bloodstream infections in southern Iran. J Med Microbiol 2016;65(3):235–239. DOI: 10.1099/jmm.0.000219.

30. Tian Y, Zhao Y, Chen B, Chen S, Zeng R, Hu B, et al. Real-time PCR assay for detection of Dickeya fangzhongdai causing bleeding canker of pear disease in China. J Integr Agric 2020;19:898–905. DOI: 10.1016/S2095-3119(19)62881-9.