Rapid and Simple Approaches for Diagnosis of *Staphylococcus aureus* in Bloodstream Infections

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A b s t r a c t

Staphylococcus aureus is an important causative pathogen of bloodstream infections. An amplification assay such as real-time PCR is a sensitive, specific technique to detect *S. aureus*. However, it needs well-trained personnel, and costs are high. A literature review focusing on rapid and simple methods for diagnosing *S. aureus* was performed. The following methods were included: (a) Hybrisep *in situ* hybridization test, (b) T2Dx system, (c) BinaxNow *Staphylococcus aureus* and PBP2a, (d) Gram staining, (e) PNA FISH and QuickFISH, (f) Accelerate Pheno™ system, (g) MALDI-TOF MS, (h) BioFire FilmArray, (i) Xpert MRSA/SA. These rapid and simple methods can rapidly identify *S. aureus* in positive blood cultures or direct blood samples. Furthermore, BioFire FilmArray and Xpert MRSA/SA identify methicillin-resistant *S. aureus* (MRSA), and the Accelerate Pheno™ system can also provide antimicrobial susceptibility testing (AST) results. The rapidity and simplicity of
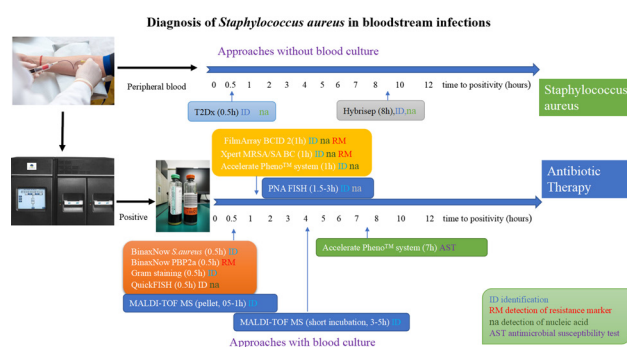
results generated by these methods have the potential to improve patient outcomes and aid in the prevention of the emergence and transmission of MRSA.

Introduction

Staphylococcus aureus (SA) is a leading cause of bacteremia. High mortality rates from 20% to 50%, frequent recurrences from 5% to 10%, and lasting impairment in more than one-third of the survivors characterize *S. aureus* bloodstream infections (BSI) (Asgeirsson et al. 2018; Kern and Rieg 2020). *S. aureus* infections are particularly problematic due to the frequent antibiotic resistance, among which methicillin resistance is the most clinically relevant (Turner et al. 2019). Patients with methicillin-resistant *S. aureus* (MRSA) bacteremia stay longer in hospitals and generate higher costs than those with bacteremia caused by methicillin-susceptible *S. aureus* (MSSA) (Tsuzuki et al. 2021).

MRSA often requires vancomycin therapy. Vancomycin use is associated with increased antimicrobial and monitoring costs and may bring about drug-induced acute kidney injury in up to 15% of patients (Pritchard et al. 2010). Furthermore, empirical anti-MRSA treatment for MSSA bacteremia can lead to poor outcomes compared with standard therapy (Jones et al. 2020). Therefore, rapid and accurate discrimination of MRSA from MSSA is essential for clinical diagnosis to facilitate a specific antimicrobial therapy (Srisrattakarn et al. 2022).

When the positive blood culture starts, conventional *S. aureus* identification takes about 48 to 72 h to complete. Recent advances in molecular and nonmolecular testing methods significantly reduced the turnaround

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time for MRSA reporting (Gonzalez et al. 2020; Sze et al. 2021; Parkes-Smith et al. 2022).

Rapid and simple techniques are required to address the challenges raised by *S. aureus* BSI, employing rapid pathogen identification and susceptibility testing to enable specific targeted antibiotic therapy on time.

This review updates recent advances in rapid and simple assays to identify *S. aureus* in bloodstream infections and discusses the advantages and limitations of these methods.

Methods

The authors searched PubMed and Google Scholar with the following terms: bloodstream infections, *S. aureus*, rapid diagnostics, bacteremia, and blood cultures. English and Japanese literature for randomized clinical trials (RCTs), meta-analyses, systematic reviews, and observational studies were evaluated (1999–2022). The author agreed with the quality of included studies.

Direct detection of *S. aureus* from blood samples

Hybrisep – an *in situ* hybridization method. Hybrisep is an *in situ* hybridization-based assay which detects specific bacterial DNA in smears enriched in polymorphonuclear neutrophils (PMN) from the blood of suspected BSI individuals. At an early stage of sepsis, neutrophils ingest bacteria, and bacterial DNA can be detected in the blood smears by an *in situ* hybridization (ISH) method. In 1999, an ISH assay targeted phagocyted bacteria from blood was developed by collecting the blood and preparing neutrophil-enriched smears. The neutrophils were then permeabilized and incubated with specific probes (Shimada et al. 1999). Digoxygenin-labelled probes and anti-digoxygenin-alkaline phosphatase conjugates were utilized. BCIP (5-bromo-4-chloro-3-indolyl-phosphate) with NBT (nitro blue tetrazolium) was used as a substrate for ALP and allowed the signal visualization. These signals were observed in the cell cytoplasm under a microscope. Blue-colored dots represented the phagocytized bacteria in neutrophils, and it was assumed a positive result. The first available kit Hybrisep (Fuso Pharmaceuticals, Japan), provided five probes, including those specific to *S. aureus*. In 2014, Enomoto et al. (2014) introduced a new ISH assay that contained a universal probe targeting 59 species of 35 genera. The hybridization and washing procedures were performed automatically by Hybristat. The whole process takes about eight hours.

The ISH assay was used to investigate blood smears from 60 patients with suspected sepsis (Kudo et al. 2009). Nine *S. aureus* strains were detected with ISH assay, while only one blood was found to be positive for *S. aureus* by conventional culture. Discrepancies were found to be due to the effect of antibiotic treatment. Three of the nine patients with positive *S. aureus* blood cultures underwent antibiotic therapy before blood sampling. Therefore, the ISH method appeared less affected by antibiotic treatment than blood culture.

Over 40% of patients can take antibiotics before blood collection (Roh et al. 2012). Although new blood culture bottles contain neutralization of antimicrobial substances, the elimination effect for antibiotics is limited by the concentration of antibiotics in the blood (Mitteregger et al. 2013). ISH depends on the hybridization performed in neutrophils; thus, a reduced number of neutrophils resulting from immunosuppressive therapy may decrease the sensitivity of the ISH assay. As an independent-culture method, ISH is beneficial for managing patients with BSI, despite the absence of *S. aureus* in blood culture. So far, the Hybrisep kit and Hybristat are available only in Japan. Only limited studies on ISH for *S. aureus* detection in blood have been reported (Kudo et al. 2009). Further studies of ISH for *S. aureus* detection in blood should be conducted through multicenter cooperation.

T2Dx magnetic resonance assay. The T2Dx system (T2 Biosystems, USA), an automated instrument, uses a method based on magnetic resonance changes in the water proton T2 relaxation signal in the presence of a magnetic field (Neely et al. 2013). The protocol includes a pathogen-specific amplification step, and the amplicons are hybridized into specific probe-enriched nanoparticles (Paolucci et al. 2010). The T2Bacteria Panel enables multiplex detection of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). De Angelis et al. (2018), upon evaluation of 140 suspected BSI samples, detected two of two *S. aureus* strains with the T2Bacteria Panel and confirmed this finding by blood culture. The remaining 138 samples were negative when using the T2Bacteria Panel and a paired blood culture. The sensitivity and specificity of T2Dx for *S. aureus* identification was 100% compared to the blood culture. Another study by Drevinek et al. (2021) found a good sensitivity of the T2Dx system. In 55 samples, two of two *S. aureus* strains were detected by the T2Bacteria Panel, but the corresponding blood culture detected only one strain. Nguyen et al. (2019) conducted a prospective multicenter study involving 1,427 samples. The T2Dx system and blood culture identified 41 and 16 *S. aureus* strains, respectively. The turnaround time of the T2Dx system was shorter than that of the blood culture (3.6–7.7 h vs. 38.5–71.7 h, respectively). It demonstrates that the T2Dx system has value over blood culture in patients receiving antimicrobial therapy.

Rapid detection
of *S. aureus* from positive blood cultures

BinaxNOW *Staphylococcus aureus* test with Binax-Now PBP2a assay. The principle of BinaxNow *Staphylococcus aureus* (Alere, USA) and BinaxNowPBP2a (Alere SAS, France) assays is an immunochromatographic test for the identification of *S. aureus* and the determination of methicillin resistance, respectively. BinaxNow *Staphylococcus aureus* test was FDA approved for direct identification of *S. aureus*. It uses polyclonal antibodies to qualitatively detect an *S. aureus*-specific protein in a positive blood culture (Dhiman et al. 2013). Qian et al. (2014) collected 104 blood cultures from 2012 to 2013 that were tested with this method. The blood cultures included 41 *S. aureus* (14 MRSA, 27 MSSA), 60 CoNS (coagulase-negative *Staphylococci*), three other Gram-positive cocci in pairs and clusters (one of *Micrococcus* species, one anerobic Gram-positive coccus, and one of *Gemella* species). The BinaxNow *Staphylococcus aureus* test showed 97.6% sensitivity, 100% specificity, and positive and negative predictive values of 100% and 98.4%, respectively, upon *S. aureus* identification in positive blood culture. The performance was equally efficient in both aerobic and anaerobic bottles. Only one false-negative result was found due to antibiotic treatment of the patient and the resulting reduction in the antigen's level. Dhiman et al. (2013) evaluated 319 positive blood cultures containing clusters of Gram-positive cocci using the BinaxNow *Staphylococcus aureus* test. The study achieved 95.8% of sensitivity and 99.6% of specificity. One false-positive result occurred in the blood culture containing a presumptive *Staphylococcus lugdunensis* isolate not later confirmed. One false-negative result was confirmed by repeated testing. It might have resulted from a decrease in the bacterial concentration below the detection limit (5.42×10^8 cells/ml). One study combined BinaxNow *Staphylococcus aureus* for species identification and BinaxNow PBP2a assay to detect methicillin resistance (Heraud et al. 2015). Seventy-nine positive blood cultures with Gram-positive cocci in clusters were tested. A sensitivity of 94% and specificity of 98% in identifying *S. aureus* and 100% sensitivity and specificity of methicillin resistance detection were reported. These tests are valuable alternatives for the diagnosis of MRSA bacteremia.

Gram staining. Gram staining is an essential technique in microbiology. It was invented by Danish bacteriologist Hans Christian Gram in 1882 (Wu and Yang 2020). Gram staining distinguishes Gram-positive from Gram-negative organisms based on differences in their cell walls. The thick cell walls of Gram-positive bacteria retain the purple crystal violet-iodine complex after treatment with ethanol (O'Toole 2016). Once a blood culture is detected as positive by an automated, con-tinuously monitoring blood culture system, a smear is Gram stained, providing a quick presumptive identification of pathogens. It might be helpful in directing the bread antibiotic therapy (Boyanova 2018). Gram staining is a crucial but easy, rapid, and inexpensive method for pathogen detection in a positive blood culture. A series of evaluating criteria were developed based on the type of blood culture bottle, bacterial species, and cluster characteristics (Murdoch and Greenlees 2004). These criteria may help an experienced microbiological technician to distinguish *S. aureus* from CoNS in positive blood cultures. It allowed for achieving the overall sensitivity and specificity of 89% and 98%, respectively. A prospective observational study with 118 blood samples showed that a pink-colored "oozing sign" could also be used to distinguish *S. aureus* from CoNS (Hadano et al. 2018). The study demonstrated a sensitivity and specificity of 78.7% and 95.0%, respectively. Compared to the previous study by Murdoch and Greenlees (2004), this method focused on the "oozing sign" regardless of blood culture bottle type, organism species, and cluster characteristics. Therefore, it is simpler and more suitable than the previous method for application in routine work. A significant advantage of Gram staining is to be a good alternative for a community hospital without a diagnostic microbiology laboratory. The quality of Gram staining depends on the skills and can vary over time and between technicians. With the development of automated Gram staining systems, the comparability and consistency of results can be improved in laboratories (Baron et al. 2010). It also holds for new automated image acquisition systems as a convolutional neural network-based classification of Gram staining results (Smith et al. 2018). Integrating these techniques with previous evaluation criteria in *S. aureus* identification may make the Gram staining method more uncomplicated and accurate. Early identification of *S. aureus* in blood cultures is vital because the clinical signs of *S. aureus* bacteremia may be unspecific and therefore, the patients may be no symptomatic early in the course of the infection (Mitchell and Howden 2005).

PNA FISH and QuickFISH assays. Fluorescent *in situ* hybridization (FISH) is a diagnostic technique using a probe (e.g., peptide nucleic (PNA) probe) to target the 16S rRNA gene of *S. aureus* directly in the blood culture smears. Fluorescence *in situ* hybridization using the PNA probes is a useful diagnostic tool for pathogen-directed therapy (Weaver et al. 2019). PNA probes have unique performance characteristics that render PNA FISH to be applied widely to positive blood cultures. The first commercially available kit was PNA FISH (OpGen®, USA, previously AdvanDx). González et al. (2004) evaluated 285 blood cultures containing Gram-positive cocci similar to staphylococci using PNA FISH. The results showed 100% sensitivity,

and 99.4% specificity, and their positive and negative predictive values were 99.2% and 100%, respectively. The PNA probe recognizes both MRSA and MSSA. In ten positive blood cultures mixed with other bacteria, *S. aureus* was correctly identified. Only one weak positive hybridization was obtained with a positive blood culture of *Staphylococcus schleiferi*. Oliveira et al. (2003) performed a blinded comparison of *S. aureus* PNA FISH in eight centres with three types of blood culture bottles produced by different manufacturers (ESP, BACTEC, and BacT/Alert). The sensitivity ranged from 98.5% to 100%, the specificity was between 98.5% and 99.1%.

Even a potentially interfering substance as charcoal did not affect the performance of *S. aureus* PNA FISH. The total analysis time is up to 2.5 h, and hands-on time is below 30 min. QuickFISH was launched in 2013 (Carretto et al. 2013), a modified PNA FISH version with several timesaving innovations. It takes less than 30 min. because procedure does not include washing or mounting of slides, and the hands-on time is about 5 min. Deck et al. (2012) tested 722 positive blood cultures containing Gram-positive cocci in cluster using the *Staphylococcus* QuickFISH method. The sensitivity was 99.5%, and the combined specificity was 89.5%. Two false positive and seven false negative results were found. The discrepancies between the *Staphylococcus* QuickFISH method and the standard method were due to human errors or to the limited sequence alignment of the probes designed. The *Staphylococcus* QuickFISH method can speed up the *S. aureus* identification in blood cultures. It would help in the patient's management when catheter-related BSI is suspected. Early catheter removal is recommended when *S. aureus* is detected to decrease the risks of persistent bacteremia and hematogenous complications.

Accelerate Pheno™ system. The fully automated Accelerate Pheno™ system provides rapid identification and susceptibility of microorganisms in positive blood samples (Cenci et al. 2020). The system combines FISH for bacterial identification and an automated microscope for assessing bacterial growth rates and calculating the MIC value (Marschal et al. 2017). Molecular methods and MALDI-TOF MS can also identify microorganisms in positive blood cultures and their susceptibility phenotype in a few hours. However, these methods must provide the susceptibility phenotype that clinicians request to make a therapeutic decision. This need drove Accelerate Diagnostic™ (USA) to develop an Accelerate Pheno™ system (APS). The multiplex fluorescence *in situ* hybridization using target-specific probes is a diagnostic strategy APS uses to identify on-panel microbes. The minimum inhibitory concentration (MIC) is evaluated after analyzing the bacterial morphokinetic growth pattern in the presence of a given antimicrobial agent (Marschal et al. 2017).

Charnot-Katsikas et al. (2017) investigated 232 blood cultures using APS and compared the results with the standard method. After considering the discrepant results, the sensitivity and specificity for *S. aureus* were 94.7% and 99.0%, respectively.

The categorical agreement between APS and standard antimicrobial susceptibility tests (AST) was 98.5%. Only two minor errors were found upon conventional testing of 68 *S. aureus* strains. When using APS for blood culture, the turnaround time was shortened to about 23.47 h, 41.86 h, and 25.5 min. for identification, susceptibility, and hands-on time, respectively. Lutgring et al. (2018) demonstrated that the sensitivity and specificity of *S. aureus* detection using APS in positive blood cultures were 96.9%, and 95.2%, respectively. The categorical agreement between APS and culture-based AST was 100% upon *S. aureus* testing. Since the identification and AST were performed in 1.5 h and 7 h, respectively, as opposed to a few days with conventional testing, it would decrease morbidity and mortality of patients with BSI. It is due to the switch from broad-spectrum empiric therapy to targeted specific antibiotic therapy since it decreases adverse effects and the emergence of multi-drug resistance organisms. However, APS has low performance for correct identification, and AST results in polymicrobial blood cultures. It is also of a high cost compared to standard methods. All these APS characteristics should be considered for a diagnostic and treatment decision.

MALDI-TOF MS. One of the soft ionization techniques, a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), generates a protein-based spectral profile or "fingerprint" unique to a given species of pathogen. The microorganism identification relies on the characteristic spectrum of each species.

MALDI-TOF MS has revolutionized microorganism identification and is now widely used in routine laboratory testing. Two main commercial systems are used worldwide, the Vitek MS (bioMerieux, France) and Microflex LT Biotyper (Bruker Daltonics, USA). The detection limit is 10^8 CFU/ml of positive blood culture broth (Christner et al. 2010).

S. aureus identification from positive blood culture with MALDI-TOF MS can be conducted according to two protocols: (a) after short-term incubation on a solid medium or (b) directly from the positive blood culture pellet. Curtoni et al. (2017) reported reliable species identification in less than 5 h. The identification of *S. aureus* was successful at 85.7% and 100% after 3 h and 5 h of incubation, respectively. It means that MALDI-TOF MS can be a reliable, easy, and rapid method for the *S. aureus* identification from positive blood culture when performed after a short-term culture of bacteria on a solid medium. Direct identification from blood

culture involves the protein extraction after bacterial lysis and filtration (Foster 2013; Lin et al. 2018), use of a Sepsityper® kit (Bruker) or serum separator tube (Zengin and Bayraktar 2021), and in-house saponin-based bacterial extraction (Hu et al. 2020; Ponderand et al. 2020). Tsuchida et al. (2018) compared an in-house lysis filtration protocol with a Sepsityper® kit for *S. aureus* identification. Out of a total of 19 *S. aureus* strains, 17 were identified to species level by a lysis filtration protocol, two to genus level, whereas 11 of these strains were identified to species level and four to genus level. Four strains could not be identified by the Sepsityper® kit. The results showed that Sepsityper® kit failed to identify completely Gram-positive cocci in positive blood cultures.

Another study employed a serum separator tube followed by MALDI-TOF MS for *S. aureus* identification (Zengin and Bayraktar 2021). A total of 36 *S. aureus* strains were confirmed by a short-term incubation routine identification (SIRID) method and 32 strains from 36 monomicrobial cultures were correctly identified by direct rapid identification (RID) method using a serum separator tube followed by MALDI-TOF MS. The mean turnaround time of RID method was significantly lower compared to SIRID method (2.86 h vs. 19.49 h, $p < 0.001$). This study indicated that the serum separator tube method was suitable for the rapid identification of *S. aureus* in a blood culture.

MALDI-TOF MS has been proposed to detect strains susceptible or resistant to several antibiotics. Some studies reported accurate discrimination of *S. aureus* isolates susceptible to vancomycin (Mather et al. 2016). Recently, Liu et al. (2021) presented the machine learning algorithms for rapid MALDI-TOF MS-based MRSA screening. By selecting 38 *m/z* features for the classifying model and coupling it with machine-learning algorithms, a rapid and simple method to distinguish MRSA from MSSA was established. This method makes resistance detection more convenient and effective in routine microbiology laboratories.

FilmArray system. The FilmArray is a multiplex-PCR-based system that combines samples the preparation, PCR amplification, detection, and analysis (Peker et al. 2018). The BioFire FilmArray BCID2 panel can simultaneously identify 43 pathogens and ten antimicrobial resistance genes (Cortazzo et al. 2021). It takes about 2 min. of hands-on time and 1 h turnaround time. Sze et al. (2021) compared BioFire FilmArray BCID2 with MALDI-TOF MS for the *S. aureus* identification in blood cultures and reported both sensitivity and specificity of 100%. Furthermore, two out of two MRSA were detected by the BCID2 panel. Holma et al. (2022) presented similar results. These findings showed that the BioFire FilmArray BCID2 proves to be a good tool for the early detection of MRSA and the administration of effective antimicrobial therapy.

Xpert MRSA/SA BC assay. The Xpert MRSA/SA BC assay is based on a multiplex PCR. It targets the junction between *S. aureus* conserved open reading frame (*orfx*) and the SCC containing the *mec*A gene (SCC *mec*A). It enables Xpert MRSA/SA BC to detect and identify MRSA and MSSA in 1 h (Belmekki et al. 2013). A prospective study at two clinical centers investigated the impact of rapid detection of *S. aureus* in positive blood cultures on patient management (McHugh et al. 2020). In 264 blood cultures, 39 were positive for *S. aureus,* and one strain was identified as MRSA. Compared with the culture method, Xpert MRSA/SA BC has 100% sensitivity and 100% specificity for identifying *S. aureus* and 100% specificity for MRSA detection. For the Xpert MRSA/SA BC assay, the median turnaround time from blood culture processing to the final result was 1.7 h, compared to the 25.7 h turnaround time of the culture method. Xpert MRSA/SA BC results allowed early specific therapy for *S. aureus* and de-escalation of antimicrobial therapy for MSSA. Reddy and Whitelaw (2021) explored whether Xpert MRSA/SA BC assay can be utilized as an antimicrobial stewardship tool. Of 231 samples tested, concordance was 100% between the Xpert MRSA/SA BC assay and culture method. It can discriminate MRSA from MSSA and significantly reduce the laboratory turnaround time. This study showed the potential benefit of time reduction for the appropriate therapy introduction in most patients with *S. aureus* BSI.

Conclusions

Timely and targeted specific antibiotic therapy of *S. aureus* BSI improves survival and decreases the length of hospital stay and adverse effects of antibiotics. Although new approaches are utilized for rapid identification and AST, conventional methods are still indispensable.

Several rapid and simple methods, such as PNA FISH, MALDI-TOF MS, and the Accelerate Pheno™ system, are being used to diagnose positive blood cultures. PNA FISH and QuickFISH are well-validated methods. Utilization of MALDI-TOF MS directly from blood culture pellets is still in progress. The pre-treatment of the sample is being recognized as a standard approach. The Accelerate Pheno™ system provides not only rapid identification but also susceptibility reports (as the MIC values), while the low throughput of one sample per machine hinder this method from wide utilization. Gram staining integrated with machine-learning-based image analysis may lead to the rapid identification of *S. aureus* from blood culture. The BinaxNow *Staphylococcus aureus* and BinaxNow PBP2a appear to be a golden pair for the rapid identification and determination of methicillin resistance of *S. aureus*. The Hybrisep *in situ* hybridization and T2Dx assays

have a high detection positive rate compared to blood cultures. Limited studies have been here reported and validated. Further validation and studies should be performed to determine its performance for the rapid identification of *S. aureus* from blood cultures. FilmArray BCID2 and Xpert MRSA/SA BC are very useful tools in routine microbiology laboratories for rapid discrimination of MRSA from MSSA.

Every approach has its characteristics (summarized in Table I and II). Each laboratory should con-

Table I
Characteristics and performance of different methods for the identification of *S. aureus* in the blood or blood cultures.

Assays	Resistance markers	Sensitivity	Turnaround time (h)	Specificity	References
Hybrisep	none	NA	NA	8	Shimada et al. 1999
T2Dx	none	100%	100%	3–5	De Angelis et al. 2018
BinaX Now *S.aureus*	none	97.6%	100%	0.5	Qian et al. 2014
BinaX Now PBP2a	yes	100%	100%	0.5	Heraud et al. 2015
Gram staining	none	78.7%	95%	0.5	Hadano et al. 2018
PNA FISH	none	100%	99.4%	1.5–3	González et al. 2004
QuickFISH	none	99.5%	89.5%	0.5	Deck et al. 2012
Accelerate Pheno™ system	susceptibility	94.7%	99%	ID1/AST 7	Charnot-Katsikas et al. 2017
MALDI-TOF MS	in development	85.7–100%	NA	0.5–1 (pellet)/3–5 (short incubation)	Foster 2013; Curtoni et al. 2017
FilmArray BCID2	yes	100%	100%	1	Holma et al. 2022
Xpert MRSA/SA BC	yes	100%	100%	1	McHugh et al. 2020

FISH – fluorescent *in situ* hybridization, PNA – peptide nucleic acid, MALDI-TOF MS – matrix-assisted laser desorption/ionisation-time of flight mass spectrometry, NA – not available

Table II
Comments on rapid and simple methods for the identification of *S. aureus* from the blood or blood cultures.

Assays	Automated	Personnel experience	Equipment requirement	Through-put	Cost	Comments
Hybrisep	yes	yes	proprietary equipment	low	moderate	+: rapid, sensitive and specific –: limited number of publications
T2Dx	yes	no	proprietary equipment	low	high	+: rapid, sensitive, and specific –: no resistance markers
BinaX Now *S. aureus*	no	no	no	low	low	+: rapid, sensitive and specific –: cross-reaction with *S. lugdunensis*
BinaX Now PBP2a	no	no	no	low	low	+: rapid, sensitive, and specific –: no identification
Gram staining	yes	yes	generic equipment	low	low	+: rapid, simple –: variable sensitivity and specificity
PNA FISH	no	yes	generic equipment	low	high	+: rapid, sensitive, and specific –: no resistance markers
QuickFISH	no	yes	generic equipment	low	high	+: rapid, sensitive, and specific –: no resistance markers
Accelerate Pheno™ system	yes	no	proprietary equipment	low	high	+: rapid, sensitive, and specific, with AST results –: low throughout
MALDI-TOF MS	no	yes	proprietary equipment	high	low	+: rapid, sensitive, and specific, cost-effective –: enrichment, not standardization
FilmArray BCID2	yes	no	proprietary equipment	low	high	+: rapid, sensitive, and specific, with resistance results –: low throughout
Xpert MRSA/SA BC	yes	no	proprietary equipment	low	high	+: rapid, sensitive, and specific, with resistance results –: expensive

FISH – fluorescent *in situ* hybridization, PNA – peptide nucleic acid, MALDI-TOF MS – matrix-assisted laser desorption/ionisation-time of flight mass spectrometry

sider some important factors such as turnaround time, panels of antibiotics offered, and hands-on time when searching for an appropriate method (Sze et al. 2021). Polymicrobial infections in BSI are not infrequent. It is essential to select an accurate method for such samples (Abat et al. 2015). Direct identification of *S. aureus* from blood rather than blood culture should be a direction of future development to maximize the benefits of rapid identification of *S. aureus* from blood cultures. Rapid identification methods combined with rapid AST determination are also necessary (Pliakos et al. 2018). Simple and rapid identification of *S. aureus* from blood culture plus phenotype AST will significantly impact the optimizing BSI management.

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Author contributions

RD and PW performed the literature search and data analysis. RD and PW drafted the work. All authors critically revised the work.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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