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Systematic Review

Diagnostic accuracy of rapid molecular tests for detecting antimicrobial resistance in bloodstream infections: a systematic review

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ABSTRACT

Background: Rapid molecular tests (RMTs) accelerate antimicrobial resistance detection in bloodstream infections, and accuracy differs by target. This systematic review evaluated diagnostic performance. **Methods:** We searched PubMed, Scopus, Web of Science, and Embase for original studies assessing RMTs for resistance detection in bloodstream infections (BSIs) using positive blood cultures or whole blood. Eligible studies compared assays with conventional microbiology, phenotypic susceptibility testing, molecular methods, or combined standards. We extract data on assay characteristics, resistance targets, diagnostic outcomes, turnaround time, and clinical impact. **Results:** We include nine studies involving 2,439 samples. Platforms included FilmArray/BCID, Verigene BC-GP/BC-GN, BIOFIRE BCID2, T2Resistance, and the Molecular Mouse System. Performance was high for covered organisms and resistance markers. BCID2 showed 98.9% sensitivity and 99.6% specificity for organism targets, with 97.9% positive percent agreement and 99.9% negative percent agreement for resistance determinants. T2Resistance showed 100% sensitivity for several genes and shorter time to detection than conventional methods. Lower performance was reported in polymicrobial cultures and when resistance mechanisms were outside assay panels. **Conclusion:** RMTs are adjuncts for early resistance detection in BSIs and shorten time to results. They should complement conventional culture and susceptibility testing because limited target coverage, off-panel organisms, and imperfect genotype-phenotype correlation is important constraints.

Keywords: bloodstream infections; antimicrobial resistance; Rapid molecular tests; diagnostic accuracy; blood culture; sepsis

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Introduction

Bloodstream infection (BSI) is a major cause of morbidity and mortality, and their clinical course is impacted by the effectiveness of antimicrobial therapy, mainly in patients with sepsis and in those infected by resistant organisms [1]. Rapid treatment is critical because empiric therapy is ineffective in a proportion of patients, while unnecessarily broad treatment increases adverse effects and selective pressure for antimicrobial resistance (AMR) [2]. Conventional blood culture is the microbiological reference standard for BSI diagnosis, but it is limited by imperfect sensitivity, mainly after prior antibiotic exposure, which delay organism identification and final susceptibility reporting and postpone optimal treatment decisions [1,2].

RMTs have been developed to identify pathogens and selected AMR determinants from positive blood culture bottles or directly from whole blood, with the aim of providing actionable results within hours rather than days [3,4]. These technologies are attractive in BSI because early recognition of resistance genes mecA/mecC, vanA/vanB, blaCTX-M, and major carbapenemase genes support earlier escalation or de-escalation of therapy before conventional antimicrobial susceptibility testing (AST) is available [1,5]. The clinical interpretation of molecular resistance detection is not always direct, because genotype–phenotype correlation is strongest for a limited number of targets, whereas resistance in Gram-negative organisms is multifactorial and only explained by a restricted resistance-gene panel [1,6].

Current RMTs are inherently target-limited, so organisms or resistance mechanisms outside the panel will be missed, and this is relevant in polymicrobial cultures where assigning a detected resistance gene to a specific organism is difficult [1,3]. This is relevant to the findings of our review, where most included studies showed high performance for covered organisms and resistance markers, but lower performance in polymicrobial samples and incomplete detection of resistance when mechanisms extended beyond the genes interrogated by the assay panel [1]. Newer platforms have expanded organism and resistance-gene coverage, as illustrated by BCID2, which was designed with broader target content and has shown high pooled sensitivity and specificity for major bloodstream pathogens and key resistance determinants in diagnostic accuracy studies [7]. T2Resistance panel and newer positive-blood-culture assays have demonstrated the possibility of identifying major resistance genes earlier [4,7].

The available evidence is heterogeneous with respect to assay platform, specimen type, resistance targets, reference standards, and reported diagnostic accuracy measures, making it difficult to judge the performance of RMTs for AMR detection in BSI settings [3,5]. We aimed to analyze diagnostic accuracy studies to clarify how reliably these assays detect resistance markers in clinical practice, how their performance differs in platforms, and how their rapid turnaround complements culture-based methods in BSI management [8,9].

Methods

Study design

This study was designed as a systematic review of diagnostic test accuracy studies evaluating RMTs for the detection of AMR in BSIs. The review was conducted according to standard systematic review principles for diagnostic accuracy research.

Data sources and search strategy

Electronic literature search was performed in PubMed, Scopus, Web of Science, and Embase from database inception to 2025. The search strategy combined controlled vocabulary terms and free-text keywords related to BSI, sepsis, blood culture, RMTs, AMR, and diagnostic accuracy. Search terms included combinations of words such as "BSI," "bacteremia," "sepsis," "rapid molecular tests," "PCR," "multiplex PCR," "blood culture identification," "resistance gene," "antimicrobial resistance," "diagnostic accuracy," "sensitivity," and "specificity." Reference lists of relevant studies were screened manually to identify additional eligible articles.

Eligibility criteria

Studies were included if they were original research articles; evaluated a rapid molecular diagnostic assay for the detection of AMR markers in patients with suspected or confirmed BSI; used blood culture bottles or direct blood specimens as the tested sample; reported sufficient data to assess diagnostic performance (sensitivity, specificity, positive predictive value, negative predictive value, concordance, or data allowing construction of 2 × 2 tables); used conventional microbiological methods, AST, molecular reference assays, or a combination of these as the reference standard.

We exclude review articles, editorials, conference abstracts without full data, case reports, animal studies, non-English articles, or studies without extractable diagnostic accuracy data.

Study selection

All records identified through the database search were imported into a reference management program, and duplicate records were removed. Titles and abstracts were screened for relevance, followed by full-text review of eligible studies. Studies meeting the predefined inclusion criteria were included in the final review. Disagreements during the screening process were resolved through discussion and full text reassessment.

Data extraction

Data were extracted using a standardized form. The extracted variables included: first author, year of publication, country, study design, study setting, patient population, sample type, index test, target pathogens or resistance genes, reference standard, sample size, and main diagnostic outcomes. For each included study, data on true positives, false positives, false negatives, and true negatives were extracted whenever available. Additional information on clinical impact was also collected when reported.

Outcomes

The primary outcome was the diagnostic accuracy of RMTs for detecting AMR markers in BSIs. The main measures of interest were sensitivity and specificity. Secondary outcomes included turnaround time to result, agreement with conventional microbiological methods, and reported impact on antimicrobial management.

Data synthesis and statistical analysis

A descriptive summary of study characteristics and findings was first prepared. When sufficient homogeneous data were available, pooled diagnostic accuracy estimates were calculated. Sensitivity and specificity were synthesized in studies, and corresponding 95% CIs were reported. 2 × 2 contingency tables were constructed from the

original study data. Statistical heterogeneity was assessed by examining variations in study design, assay platform, specimen type, resistance targets, and reference standards. The study selection process was summarized in a PRISMA flow diagram, and the characteristics of the included studies were presented in Table 1 and 2.

Result

Nine original diagnostic accuracy studies were included in this review. These studies evaluated rapid molecular platforms for identification of bloodstream pathogens and AMR markers in 2,439 samples or enrolled cases. Most studies assessed assays performed on positive blood culture bottles, while one prospective pilot study evaluated direct whole-blood testing using the T2Resistance panel. The included platforms were heterogeneous and comprised FilmArray/FilmArray BCID, Verigene BC-GP and BC-GN, BIOFIRE BCID2, T2Resistance, and the Molecular Mouse System (MMS). The studies were conducted in single-center, multicenter, pediatric, adult, and mixed hospital settings in the United States, Sweden, Hong Kong, Croatia, Italy, and Greece.

RMTs showed high diagnostic performance for covered organisms and resistance targets. In the earliest FilmArray study, 95% of pathogens in archived blood cultures and 91% of covered pathogens in prospectively collected blood cultures were identified, with all culture-proven MRSA and VRE isolates correctly detected. In the Swedish FilmArray BCID evaluation, organism identification was achieved in 91.6% of monomicrobial samples and in 71% of polymicrobial samples. For Verigene BC-GP, organism identification was concordant with routine methods in 94.6% of cultures in one study and 95.8% in a pediatric study, with the latter reporting an overall accurate identification rate of 97.0% after repeat testing. The Verigene BC-GN assay identified all 51 Gram-negative bacilli from monomicrobial cultures in one study. Monomicrobial agreement was 89.6% for Gram-positive bacteria and 90.5% for Gram-negative bacteria, whereas performance was lower in polymicrobial cultures. The large multicenter BCID2 evaluation reported sensitivity of 98.9% and specificity of 99.6% for on-panel organism targets. The MMS study showed 98.7% sensitivity and 100% specificity for covered Gram-negative pathogens.

FilmArray identified all culture-proven MRSA and VRE cases in the developmental study. Verigene BC-GP showed concordant mecA and vanA detection in most relevant isolates, and the pediatric evaluation reported correct detection of mecA in all MRSA and 98.0% of MRSE isolates and correct identification of vancomycin-resistant E. faecium. In the Hong Kong study, the Verigene assay showed 100% sensitivity for MRSA, VRE, and carbapenem-resistant Acinetobacter, and 84.4% sensitivity for ESBL-producing Enterobacteriaceae based on blaCTX-M detection. BCID2 show a 97.9% positive percent agreement and 99.9% negative percent agreement for AMR determinants. The T2Resistance study detected 25 resistance genes in 59 enrolled patients and showed 100% sensitivity for blaCTX-M-14/15, blaNDM/blaIMP/blaVIM, blaAmpC, and mecA/mecC, with slightly lower sensitivity for blaKPC at 87.5%. The MMS resistance assay showed 100% sensitivity and 100% specificity for covered Gram-negative resistance markers.

Verigene BC-GP results were available 31 to 42 hours earlier than phenotypic identification and 41 to 50 hours earlier than susceptibility results in one study, while the pediatric study showed mean

reductions of 30.1 hours for identification and 37.3 hours for resistance detection. Verigene BC-GN provided organism identification 24 hours earlier, and medical management changes occurred in 31.8% of patients an average of 33 hours sooner. The T2Resistance panel reduced median time to resistance detection to 4.4 hours versus 58.34 hours for blood culture with final AST and was associated with antibiotic discontinuation and escalation events. The MMS study reported shorter time to results and indicate that empirical therapy would change in half of cases.

Table 1. Characteristics of the included studies

Study	Country and setting	Study design	Index test	Specimen and sample size	Comparator
Blaschke 2012 [10]	USA; University of Utah Primary Children's Medical Center	Clinical evaluation Prospectively collected samples	Developmental FilmArray BC panel (>25 pathogens, 4 resistance genes; 1 h)	Positive blood cultures; 102 archived blood cultures and 111 prospectively collected blood cultures from adults and children with bacteremia	culture and susceptibility testing
Altun 2013 [11]	Sweden; Karolinska University Laboratory serving 3 tertiary-care hospitals	Prospective clinical evaluation	FilmArray BCID panel (19 bacteria, 5 yeasts, 3 resistance genes)	Positive blood culture bottles; 206 bottles	Conventional microbiology methods, MALDI-TOF/Vitek2, disc diffusion; PCR for discordant mecA
Wojewoda 2013 [12]	USA	Clinical evaluation	Verigene BC-GP nucleic acid test	BacT/Alert FA positive blood cultures with Gram-positive cocci; 186 cultures	Routine laboratory identification and susceptibility profiles; discrepant analysis with biochemical testing, cefoxitin disk, repeat BC-GP
Mestas 2014 [13]	USA; Children's Hospital Los Angeles pediatric tertiary-care center	Clinical performance evaluation in pediatric patients	Verigene BC-GP assay	Positive blood cultures from pediatric patients; 203 cultures from 203 patients	Conventional ID and susceptibility workup; discordant results confirmed by MALDI-TOF or 16S rRNA sequencing; mecA confirmation by cefoxitin/PCR
Hill 2014 [14]	USA; Kaiser Permanente, Portland, Oregon	Consecutive clinical sample evaluation	Verigene BC-GN assay	Inpatient positive blood cultures with Gram-negative bacilli; 54 patient cultures, with 51 monomicrobial cultures analyzed for on-panel organism accuracy;	Standard culture ID and AST

Study	Country and setting	Study design	Index test	Specimen and sample size	Comparator
				plus 14 known carbapenemase test organisms	
Siu 2015 [15]	Hong Kong; 4 public hospitals	Prospective multicenter diagnostic performance study	Verigene BC-GP + BC-GN assays	Positive blood cultures; 364 total (114 Gram-positive, 250 Gram-negative); TAT subset 125 cultures	Conventional culture-based methods; discrepant testing with 16S/yggE sequencing and PCR
Rhoads 2023 [16]	Multicenter; USA, Greece, Italy	Retrospective and prospective multicenter clinical study	BIOFIRE BCID2 panel	Positive blood culture samples; 1,093 enrolled, 1,074 analyzed	Standard-of-care results, sequencing, PCR, and reference-laboratory AST
Walsh 2024 [4]	Italy and Greece; Perugia General Hospital and Evangelismos General Hospital	Prospective observational pilot study	T2Resistance (T2R) panel on T2Dx	Whole blood from patients with suspected sepsis/BSI; 59 enrolled patients	AST, phenotypic identification, and standard molecular resistance detection assays
Tićac 2025 [7]	Croatia; Western Croatian clinical hospital center	Prospective hospital-setting evaluation	Molecular Mouse System (MMS) gram-negative ID and resistance cartridges	Positive blood culture samples with microscopically detected GNB; 80 BSI episodes	Culture-based methods with Vitek 2 ID/AST

Table 2. Main findings and outcomes

Study	Main findings on organism detection	Main findings on AMR detection	Clinical outcome
Blaschke 2012 [10]	In archived cultures, 95% of 109 pathogens identified by FilmArray; in prospective cultures, 84/92 (91%) panel-covered pathogens identified	Among 25 S. aureus and 21 Enterococcus spp., the assay identified all culture-proven MRSA and VRE	Rapid panel-based detection from positive blood cultures; no formal stewardship impact analysis reported in the abstract
Altun 2013 [11]	FilmArray identified microorganisms in 153/167 (91.6%) monomicrobial samples; all target organisms detected in 17/24 (71%) polymicrobial samples; 3/206 (1.5%)	Resistance genes were included in the panel, but separate AMR estimates were not clearly reported in the abstract-level results	Reproducible on repeat testing and after storage; no formal clinical-impact analysis reported

Study	Main findings on organism detection	Main findings on AMR detection	Clinical outcome
Wojewoda 2013 [12]	Initial BC-GP organism identification concordant with routine methods for 94.6% of blood cultures; S. pneumoniae identification was weak (40% correct)	mecA detection for 69 blood cultures with S. aureus or S. epidermidis was concordant with susceptibility testing; vanA agreed with susceptibility for 45/46 E. faecalis/E. faecium cultures	BC-GP results were available 31–42 h earlier than phenotypic identification and 41–50 h earlier than susceptibility results
Mestas 2014 [13]	Overall concordance for organism identification was 95.8% (206/215); after repeat testing, accurate identification reached 97.0%	Correct mecA detection in 100% of MRSA and 98.0% of MRSE isolates; 100% correct identification of vancomycin-resistant E. faecium isolates	Mean TAT decreased by 30.1 h for organism identification and 37.3 h for resistance-marker detection
Hill 2014 [14]	BC-GN identified all 51 Gram-negative bacilli from monomicrobial patient blood cultures	Identified all 14 carbapenemase resistance markers in known test organisms No resistance markers detected in the 54 patient isolates, in agreement with susceptibility testing	Median ID TAT decreased by 24 h 26 min
Siu 2015 [15]	Overall identification agreement in monomicrobial cultures: 89.6% for Gram-positive and 90.5% for Gram-negative bacteria; in polymicrobial cultures: 62.5% and 53.6%, respectively; specificities ranged 98.9%–100%	100% sensitivity for MRSA, VRE, and carbapenem-resistant Acinetobacter; 84.4% sensitivity for ESBL-producing Enterobacteriaceae based on blaCTX-M detection	Verigene results were 40.5–99.2 h faster than conventional methods MRSA and VRE were identified 91–99 h earlier
Rhoads 2023 [16]	Overall sensitivity 98.9% (1,712/1,731) and overall specificity 99.6% (33,592/33,711) for organism targets; 118 off-panel organisms identified by SoC in 10.6% of samples	PPA 97.9% (325/332) and NPA 99.9% (2,465/2,467) for AMR genes; AMR markers in Enterobacterales correlated with phenotypic susceptibility	Sample-to-answer time 1 h
Walsh 2024 [4]	T2R detected 25 resistance genes among 59 enrolled patients; per-patient sensitivity/specificity were 92.3%/84.1%, improving to 94.7%/97.4% after adjudication	Sensitivity versus AST was 100% for blaCTX-M-14/15, blaNDM/blaIMP/blaVIM, blaAmpC, and mecA/mecC, and 87.5% for blaKPC	Median time-to-positive T2R was 4.4 h versus 58.34 h to final AST There were 32 discontinuations of unnecessary antibiotics and 17 escalations, including 6 starts of ceftazidime/avibactam for blaKPC-positive cases
Tićac 2025 [7]	MMS GNB identification assay sensitivity 98.7% and specificity 100% for covered pathogens; one K. aerogenes was identified only at family level	Resistance-marker detection showed 100% sensitivity and 100% specificity; correctly identified CTX-M-1/9 in 17/20,	Time to result was shorter than routine culture methods

Study	Main findings on organism detection	Main findings on AMR detection	Clinical outcome
		detected KPC, NDM, OXA-23, six OXA-48, and one mcr-1 positive sample	Retrospective review suggested empirical therapy have changed in half of patients

Discussion

In the present review, RMTs showed high diagnostic performance for on-panel pathogens and resistance determinants, and this pattern is in line with broader evidence showing that blood culture molecular panels achieve high accuracy for the major BSI targets while shortening time to result compared with conventional workflows [3]. The strong performance you found for BCID2, including high agreement for organism targets and resistance determinants, is supported by the BCID2 diagnostic meta-analysis, which reported pooled specificity above 97% for most targets and high pooled sensitivity for Enterobacterales, Staphylococcus aureus, Streptococcus spp., blaCTX-M, carbapenemases, and mecA/C with MREJ [3,17].

Our results show that diagnostic performance is not uniform in all situations, because polymicrobial cultures remained a weak point in older FilmArray and Verigene studies, and this limitation has been highlighted in the literature for multiplex molecular panels used from positive blood cultures (She et al. 2019). This issue is important because polymicrobial specimens make it harder not only to identify every organism present, but also to determine which resistance gene belongs to which organism, so apparent molecular positivity not always translate into therapeutic interpretation in mixed infections (Banerjee et al. 2021).

Our findings on AMR detection are biologically plausible, as the best-performing markers in the included studies were those with direct genotype–phenotype relationships, mecA and van genes, whereas broader Gram-negative resistance prediction was less complete when resistance depended on mechanisms outside the tested panel [1]. This explain why our review found excellent detection for MRSA, VRE, and several carbapenemase targets, but lower sensitivity for ESBL-producing Enterobacterales when detection relied mainly on blaCTX-M, because third-generation cephalosporin resistance can be mediated by non-CTX-M ESBLs or AmpC overexpression [2].

The whole-blood T2Resistance study in our review is important because it indicate that direct resistance-gene detection move testing even earlier in the clinical pathway, with results available within a few hours and high sensitivity for several major resistance genes, although performance was still lower for blaKPC and the evidence base is much smaller than for positive-blood-culture assays [2]. Another important message from our findings is that faster results are the most consistent clinical advantage of these assays, because multiple included studies showed large reductions in time to identification or resistance detection [8].

The literature shows that speed alone is not enough, and the clinical effect of rapid diagnostics is greatest

when results are integrated into an active antimicrobial stewardship response, with network meta-analysis showing that rapid diagnostics combined with stewardship reduced mortality, length of stay, and time to optimal therapy [17]. Our review findings supports the current view that RMTs are valuable adjuncts for early targeted management of BSIs, especially when resistant pathogens are suspected, but they should still be interpreted with culture, phenotypic AST, local epidemiology, and stewardship input because limited target coverage, polymicrobial complexity, off-panel organisms, and incomplete genotype–phenotype correlation is important constraints on clinical use [1,6,9].

Conclusion

RMTs showed high diagnostic performance for covered bloodstream pathogens and resistance markers, with major reductions in time to results compared with conventional methods. BCID2, T2Resistance, and MMS show strong accuracy, while earlier FilmArray and Verigene studies supported clinical utility. Performance declined in polymicrobial cultures, and resistance detection is limited when mechanisms were outside assay panels or genotype-phenotype correlation was incomplete.

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