Randomized Trial of Rapid Multiplex Polymerase Chain Reaction–Based Blood Culture Identification and Susceptibility Testing

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(See the Editorial Commentary by Caliendo on pages 1081–3.)

Background. The value of rapid, panel-based molecular diagnostics for positive blood culture bottles (BCBs) has not been rigorously assessed. We performed a prospective randomized controlled trial evaluating outcomes associated with rapid multiplex PCR (rmPCR) detection of bacteria, fungi, and resistance genes directly from positive BCBs.

Methods. A total of 617 patients with positive BCBs underwent stratified randomization into 3 arms: standard BCB processing (control, n = 207), rmPCR reported with templated comments (rmPCR, n = 198), or rmPCR reported with templated comments and real-time audit and feedback of antimicrobial orders by an antimicrobial stewardship team (rmPCR/AS, n = 212). The primary outcome was antimicrobial therapy duration. Secondary outcomes were time to antimicrobial de-escalation or escalation, length of stay (LOS), mortality, and cost.

Results. Time from BCB Gram stain to microorganism identification was shorter in the intervention group (1.3 hours) vs control (22.3 hours) ($P < .001$). Compared to the control group, both intervention groups had decreased broad-spectrum piperacillin-tazobactam (control 56 hours, rmPCR 44 hours, rmPCR/AS 45 hours; $P = .01$) and increased narrow-spectrum β-lactam (control 42 hours, rmPCR 71 hours, rmPCR/AS 85 hours; $P = .04$) use, and less treatment of contaminants (control 25%, rmPCR 11%, rmPCR/AS 8%; $P = .015$). Time from Gram stain to appropriate antimicrobial de-escalation or escalation was shortest in the rmPCR/AS group (de-escalation: rmPCR/AS 21 hours, control 34 hours, rmPCR 38 hours, $P < .001$; escalation: rmPCR/AS 5 hours, control 24 hours, rmPCR 6 hours, $P = .04$). Groups did not differ in mortality, LOS, or cost.

Conclusions. rmPCR reported with templated comments reduced treatment of contaminants and use of broad-spectrum antimicrobials. Addition of antimicrobial stewardship enhanced antimicrobial de-escalation.

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Keywords. antimicrobial stewardship; blood culture; diagnostic; PCR.

Conventional methods for identification and susceptibility testing of microorganisms from blood cultures takes ≥2 days, during which time patients may be receiving ineffective or unnecessarily broad-spectrum antibiotics [1, 2]. Panel-based molecular diagnostic assays are now available for direct testing of positive blood culture bottles (BCBs), providing timelier results than conventional subculture and phenotypic susceptibility testing. Faster identification and resistance characterization of pathogens may lead to earlier administration of directed antimicrobial therapy, promote earlier de-escalation of broad-spectrum agents, and potentially result in better outcomes, fewer antibiotic-associated adverse effects (eg, *Clostridium difficile* infection), and less emergence of antimicrobial-resistant

organisms [3]. However, rapid blood culture diagnostics are "add-on" tests performed in addition to conventional testing, and therefore increase complexity of laboratory testing and cost of patient care. The Infectious Diseases Society of America has recently called for research to evaluate how novel diagnostics impact patients and healthcare systems and to identify methods to integrate novel diagnostic testing into clinical practice [4].

Studies evaluating the clinical impact of rapid blood culture diagnostics have been limited by observational study designs and use of historical controls [5–11]. Whereas studies suggest that antimicrobial use, length of stay (LOS), mortality, and/or cost may be reduced through use of rapid blood culture diagnostic tests [5, 6, 9, 11–14], practice changes other than implementation of the novel tests may have affected outcomes. Additionally, prior studies have evaluated bundled interventions in which rapid tests were implemented with antimicrobial stewardship initiatives, making it impossible to determine whether the rapid test or the stewardship intervention alone might have been effective [5, 6, 8, 11, 15, 16]. Furthermore, real-time antimicrobial stewardship is not feasible in hospitals without stewardship programs. Ideally, a randomized controlled trial (RCT) is needed to evaluate outcomes associated with use of rapid diagnostics for bacterial identification and susceptibility testing directly from positive BCBs, but to date, none has been performed.

We conducted a prospective RCT comparing antimicrobial utilization and outcomes among patients with positive blood cultures who received standard culture and antimicrobial susceptibility testing alone, or with a rapid multiplex polymerase chain reaction (PCR) panel that identifies bacteria and *Candida* species and select antimicrobial resistance genes in approximately 1 hour [17, 18]. To determine the optimal method of communicating results, PCR test results were delivered in 2 ways, with templated comments to guide antimicrobial prescribing, or with templated comments in conjunction with real-time antimicrobial stewardship team recommendations to prescribers.

METHODS

Study Design, Randomization, and Masking

The study was a prospective RCT conducted at the Mayo Clinic, Rochester, Minnesota. Eligible patients were adults and children who had positive blood cultures processed in the clinical microbiology laboratory using the Becton Dickinson BACTEC FX system between August 2013 and March 2014. Patients were randomly assigned to either standard BCB processing (control), rapid multiplex PCR with templated comments (rmPCR), or rmPCR with templated comments and real-time antimicrobial stewardship (rmPCR/AS). Patients were excluded if they had a positive blood culture in the prior week, had not provided the Minnesota state research authorization (Minnesota Statute 144.335), were previously enrolled in the study, died or were transitioned to comfort care within 24 hours of enrollment, or had a negative

BCB Gram stain. This study was approved by the Mayo Clinic Institutional Review Board with a waiver of informed consent.

Stratified randomization (based on age <65 or ≥65 years, intensive care unit admission, and admission to solid organ or bone marrow transplant services) was done once a BCB signaled positive. Laboratory technologists and investigators were not blinded to study arm assignment. For all groups, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for pathogen identification of colonies isolated from positive BCBs, and rapid testing for methicillin resistance on *Staphylococcus aureus* colonies with the Alere PBP2a test (Alere, Waltham, Massachusetts) were used. Baseline institutional antimicrobial stewardship interventions were in place for all study groups including requiring infectious diseases approval for restricted antimicrobials, and Monday–Friday daytime prospective audit and feedback of select inpatient antimicrobial orders. A computer-based monitoring system that integrates pharmacy, laboratory, and microbiology databases was used to identify opportunities for audit and feedback [19] (see Supplementary Methods for details).

Intervention

The rmPCR panel used in both intervention arms was the FilmArray Blood Culture ID Panel (BioFire Diagnostics/ bioMérieux, Salt Lake City, Utah), which was performed as soon as a BCB signaled positive, 24 hours a day, 7 days a week. This assay detects *Staphylococcus* species, *S. aureus*, *Streptococcus* species, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus* species, *Listeria monocytogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Serratia* species, *Proteus* species, *Acinetobacter baumannii*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, Enterobacteriaceae, *Escherichia coli*, *Enterobacter cloacae* complex, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*, and 3 antimicrobial resistance genes, $mecA$, $vanA/B$, and bla_{KPC}. Results of the rmPCR test and templated comments regarding optimal antimicrobial therapy (Supplementary Table 1) were communicated to the service by telephone by a laboratory technologist and entered into the electronic medical record in real time. Gram stain–positive blood cultures that had negative rmPCR results were reported with Gram stain result only.

In the rmPCR/AS group, the rmPCR test was performed and reported as above, and an infectious diseases clinician or pharmacist (R. B. or C. B. T.) was paged with the result, 24 hours a day, 7 days a week. The subject's rmPCR result and medical record were reviewed and the primary service (or consulting infectious diseases physician, if applicable) contacted immediately over the 3 days following enrollment if a modification to antimicrobial therapy was deemed appropriate. Discordant results between rmPCR and conventional identification and
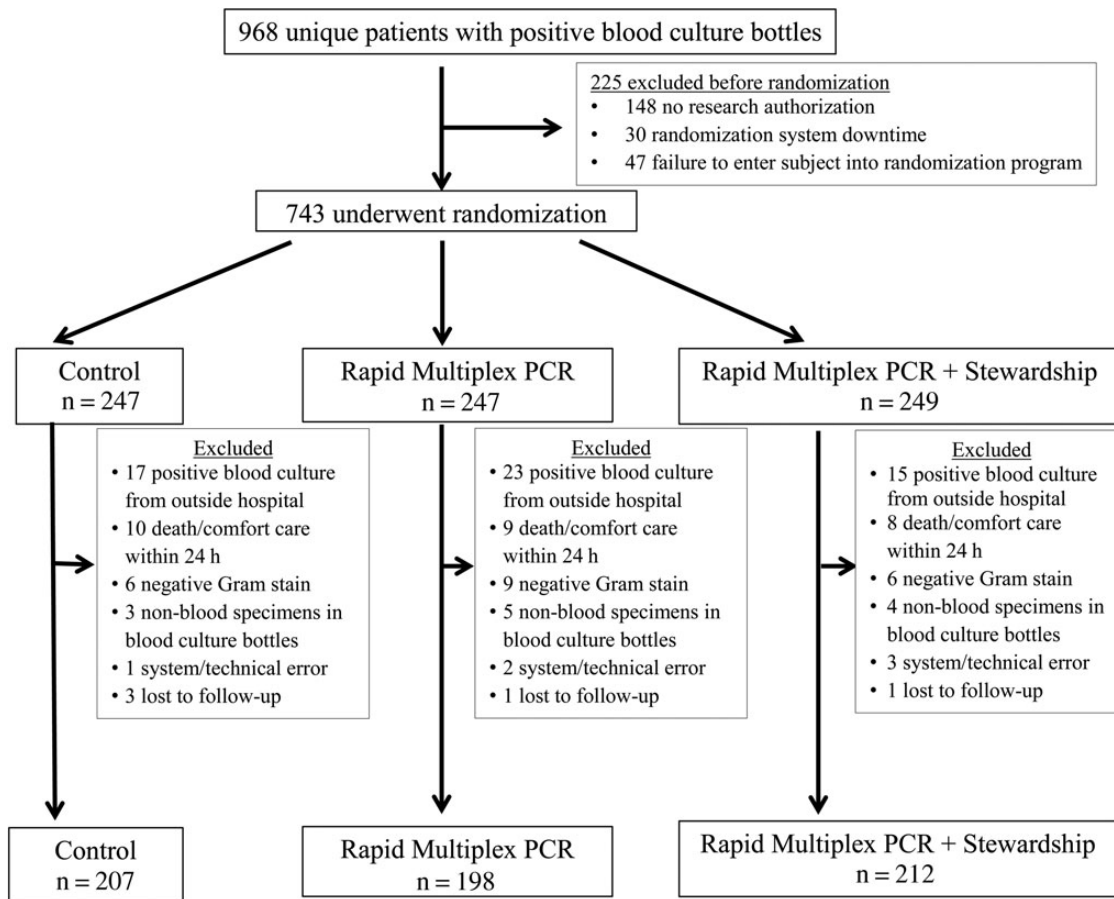
Figure 1. Participant enrollment. System/technical errors included randomization software downtime, typographical errors causing an ineligible subject to be erroneously randomized, and failure to enter an eligible subject into the randomization program. Abbreviation: PCR, polymerase chain reaction.

susceptibility testing were reviewed in real time by the bacteriology laboratory director (R. P.).

Outcome Measures

Subjects were followed for 30 days after enrollment. The primary outcome was duration of antimicrobial therapy (in hours) in the 4 days after enrollment. Duration of antimicrobial therapy was calculated as the difference between the date and time of the antibiotic start order (or Gram stain–positive blood culture, if antibiotics were started prior to the positive culture result) and the date and time of the antibiotic stop order. Secondary outcomes included time from positive Gram stain result to first active antibiotic; time to first appropriate antibiotic escalation (initiation of 1 or more antibiotics or switch from a narrow- to a broad-spectrum antibiotic) or de-escalation (discontinuation of 1 or more antibiotics and/or switch from a broad- to a narrow-spectrum antibiotic); proportion of contaminants not treated; time to pathogen identification and blood culture clearance; LOS; mortality; antibiotic-associated toxicities; infectious disease consultation; and costs per patient [20, 21] (see Supplementary Methods for details).

Statistical Analysis

We anticipated that two-thirds of patients (130/200 per arm) would receive vancomycin or an antipseudomonal antibiotic and that the standard deviation (SD) for antibiotic duration would be about 24 hours. Thus, we would be able to detect a difference in either vancomycin or antipseudomonal antibiotic duration of at least 0.403 SD (approximately 10 hours) with 80% power at an α level of .017 (to account for 3 comparisons) using a 2-sample t test. Comparisons among 3 groups were performed using the Kruskal–Wallis test for continuous variables and Fisher exact test or χ^2 test for categorical variables. When the overall test was significant, pairwise comparisons were made using Wilcoxon rank-sum, Fisher exact, or χ^2 test, as appropriate. Analysis was performed using SAS software, version 9.3 (SAS Inc, Cary, North Carolina).

RESULTS

Patients

A total of 2646 positive blood cultures from 968 unique patients were identified, 743 patients underwent randomization, and 126

Table 1. Baseline Demographic and Clinical Characteristics According to Study Group

Characteristic	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)
Demographics			
Male sex	142 (69)	116 (59)	127 (60)
Age, y, mean ± SD	61.5 ± 19.32	61.4 ± 21.22	61.2 ± 20.08
Race, white	176 (85)	186 (93.9)	179 (84.4)
Location before admission			
Nursing home	22 (10.6)	13 (6.6)	15 (7.1)
Outside hospital	27 (13)	34 (17.2)	30 (14.2)
Patient home	114 (55.1)	103 (52)	104 (49.1)
Outpatient clinic	22 (10.6)	13 (6.6)	15 (7.1)
Location at enrollment			
Outpatient	14 (6.8)	20 (10.1)	28 (13.2)
General ward	125 (60.4)	120 (60.6)	125 (59)
Intensive care unit	68 (32.9)	58 (29.3)	59 (27.8)
Comorbidities			
Charlson comorbidity score, mean ± SD	5.3 ± 3.2	5.2 ± 4.7	4.9 ± 3.0
Myocardial infarction	17 (8.2)	16 (8.1)	9 (4.2)
Chronic heart failure	31 (15)	32 (16.2)	22 (10.4)
Cerebrovascular accident	24 (11.6)	21 (10.6)	18 (8.5)
Chronic obstructive pulmonary disease	25 (12.1)	22 (11.1)	27 (12.7)
Peptic ulcer disease	27 (13)	15 (7.6)	21 (9.9)
Lymphoma	21 (10.1)	21 (10.6)	25 (11.8)
Leukemia	15 (7.2)	21 (10.6)	21 (9.9)
Solid tumor	41 (19.8)	34 (17.2)	31 (14.6)
Diabetes mellitus	68 (32.9)	50 (25.3)	63 (29.7)
Immunosuppressant use[a]	79 (38.2)	77 (38.9)	82 (38.7)
Surgery in prior 30 d	30 (14.5)	27 (13.6)	45 (21.2)
Renal replacement therapy	16 (7.7)	13 (6.6)	14 (6.6)
Any malignancy	76 (36.7)	72 (36.4)	73 (34.4)
Chronic heart disease	81 (39.1)	87 (43.9)	73 (34.4)
Chronic renal disease	62 (30)	49 (24.7)	52 (24.5)
Chronic liver disease	35 (16.9)	24 (12.1)	33 (15.6)
Chronic lung disease	43 (20.8)	44 (22.2)	50 (23.6)
Central venous catheter	95 (45.9)	91 (46)	94 (44.3)
Acute kidney injury	53 (25.6)	35 (17.7)	37 (17.5)
Concurrent infectious syndromes[b]	97 (46.9)	87 (43.9)	108 (50.9)
Neutropenic fever	24 (11.6)	24 (12.1)	27 (12.7)
Respiratory infection	31 (15)	21 (10.6)	29 (13.7)
Urinary tract infection	15 (7.3)	17 (8.6)	19 (9)
Intra-abdominal infection	22 (10.6)	21 (10.6)	26 (12.3)
Source of bacteremia			
Central venous catheter	32 (15.5)	38 (19.2)	37 (17.5)
Urinary	31 (15)	34 (17.2)	33 (15.6)
Intra-abdominal	38 (18.4)	22 (11.1)	37 (17.5)
Skin/soft tissue	11 (5.3)	13 (6.6)	8 (3.8)
Respiratory	9 (4.3)	11 (5.6)	8 (3.8)
Bone/joint	2 (1)	4 (2)	12 (5.7)
Eyes, ears, nose, throat	1 (0.5)	2 (1)	1 (0.5)
Cardiovascular	2 (1)	3 (1.5)	2 (0.9)
Surgical site infection	0 (0)	0 (0)	2 (0.9)
Unidentified	18 (8.7)	16 (8.1)	9 (4.2)

Table 1 continued.

Characteristic	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)
Complicated bloodstream infection[c]	14 (6.8)	21 (10.6)	13 (6.1)
Source control within 5 d[d]	44 (21.3)	46 (23.2)	57 (26.9)
Contact isolation for MRSA, VRE, or *Clostridium difficile*	51 (24.6)	40 (20.2)	43 (20.3)
Possible contaminant[e]	63 (30.4)	55 (27.8)	62 (29.3)
Severity of illness			
APACHE II score, mean ± SD[f]	18.3 ± 8.2	17.4 ± 7.8	16.4 ± 7.3
Requiring mechanical ventilation	21 (10.1)	23 (11.6)	16 (7.5)
Hypotension[g]	80 (38.6)	76 (38.4)	63 (29.7)
Pitt bacteremia score, mean ± SD[f]	2.0 ± 2.5	2.0 ± 2.3	1.6 ± 2.0
Infectious diseases consultation within 72 h of enrollment	103 (49.8)	97 (49)	96 (45.3)
On active antibiotic at the time of enrollment[h]	99 (69)	102 (71)	113 (75)

Data are presented as No. (%) unless otherwise specified. There were no significant differences in baseline characteristics between the study groups with the exception of race ($P = .005$).

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction; SD, standard deviation; VRE, vancomycin-resistant *Enterococcus* species.

[a] Received cytotoxic agents within prior 6 weeks, >15 mg prednisone for >1 week in prior 4 weeks, or any other immunosuppressant within 2 weeks of blood culture.

[b] A patient was classified with concurrent infection other than neutropenic fever when a site of infection was documented in the medical record (eg, pneumonia, intraabdominal infection), and culture from the concurrent infection site grew at least 1 organism that was not isolated from blood.

[c] Positive blood culture after 3 days of effective antimicrobial therapy, metastatic infection, or infective endocarditis.

[d] Catheter removal or surgical drainage procedure.

[e] Growth of common contaminant (eg, coagulase-negative *Staphylococcus* species) from a single blood culture set when ≥2 blood culture sets were collected, except among subjects suspected to have true bacteremia associated with central venous catheters or devices.

[f] Excludes outpatients.

[g] Requiring vasopressors or systolic blood pressure decrease by >20 mm Hg.

[h] Active antibiotic defined as an agent to which the blood culture organism was susceptible by conventional antimicrobial susceptibility testing.

(17%) were excluded, leaving 617 patients included in the study (Figure 1). Compared to nonrandomized subjects, randomized patients were slightly older (61.3 vs 56.7 years, $P = .01$), but not different by sex. Clinical and demographic characteristics were similar between the groups (Table 1).

Microbiology

Blood cultures grew 54.8% gram-positive bacteria, 32.6% gram-negative bacteria, 2% *Candida* species, and 10.5% multiple organisms. One-third of organisms isolated (29.2%) were considered contaminants. Among subjects with rmPCR testing, 81% of organisms isolated were detectable by the rmPCR panel. Study groups did not differ in terms of the distribution of microorganisms or the proportion that were contaminants or detectable by rmPCR (Supplementary Table 2).

**Rapid Multiplex PCR Performance and Stewardship Interventions**

Among subjects with pathogens represented on the rmPCR panel, median time from Gram stain result to organism identification was shorter in both intervention groups (both 1.3 hours) vs the control group (22 hours) ($P < .0001$; Figure 2). Most discrepancies between rmPCR and standard culture and

susceptibility results occurred because microorganisms were not represented on the rmPCR panel (78/410 [19%]). In 13 of 410 (3.2%) cases, there were discrepancies in organism identification or susceptibility result (Table 2).

In the rmPCR/AS group, investigators made 159 recommendations for the following: antibiotic de-escalation (58%), antibiotic escalation (18%), optimization of antibiotic dose or duration (15%), and infectious diseases consultation (9%); 78% of recommendations were accepted within 24 hours. In contrast, the baseline stewardship program in place for all groups identified fewer audit and feedback opportunities (50 in control, 26 in rmPCR, and 34 in rmPCR/AS) and made fewer recommendations to modify therapy (7 in control, 0 in rmPCR, and 6 in rmPCR/AS).

Antimicrobial Utilization

Within the first 4 days after enrollment, duration of vancomycin was not different between groups. However, among subjects with bloodstream infections caused by organisms not requiring vancomycin therapy (eg, monomicrobial cultures of methicillin-susceptible *S. aureus*, *S. pyogenes*, *S. agalactiae*, or gram-negative or fungal organisms), the median duration
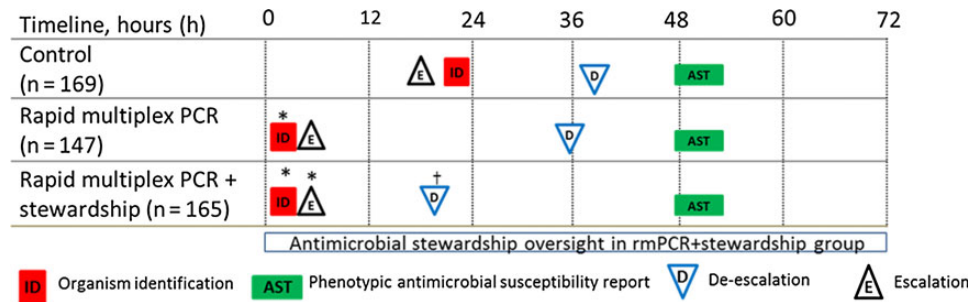
Figure 2. Comparison of time to organism identification, availability of phenotypic antimicrobial susceptibility results, and first appropriate modification of antimicrobial therapy for the subset of study subjects with organisms represented on the rapid multiplex polymerase chain reaction (rmPCR) panel (n = 481). Time 0 is when the positive Gram stain result was reported. Median time in hours (interquartile range [IQR]) to organism identification: control 22.3 (17–28), both rmPCR and rmPCR + stewardship 1.3 (0.9–1.6); de-escalation: control 39 (19–56), rmPCR 36 (22–61), rmPCR + stewardship 20 (6–36); escalation: control 18 (2–63), rmPCR 4 (1.5–24), rmPCR + stewardship 4 (1.8–9). *P < .05 vs control; †P < .05 vs control and rmPCR groups.

of vancomycin use was shorter in the rmPCR (0 hours) and rmPCR/AS (0 hours) groups compared with the control group (8.2 hours, P = .03) (Table 3). Conversely, for subjects with bloodstream infections caused by vancomycin-susceptible enterococci, vancomycin use was greater in the rmPCR (70 hours) and rmPCR/AS (82 hours) groups than the control group (20 hours, P = .037). Duration of narrow-spectrum β-lactam use (cefazolin, nafcillin, oxacillin) was greater in both the rmPCR (71 hours) and rmPCR/AS (85 hours) groups than in the control group (42 hours, P = .04). Among all

Table 2. Discrepancies Between Rapid Multiplex Polymerase Chain Reaction Assay and Standard Culture/Phenotypic Antimicrobial Susceptibility Testing

Rapid Multiplex PCR Panel Result	Standard Culture and Phenotypic Susceptibility Testing	No.
Single/few organisms detected	Multiple organisms detected	6
1. *Klebsiella pneumoniae*	1. *K. pneumoniae, Staphylococcus epidermidis*	
2. *Enterococcus* species	2. *Enterococcus faecium, Staphylococcus hemolyticus, Micrococcus luteus*	
3. *Enterobacter cloacae, Enterococcus* species (*vanA/B* positive)	3. *Enterococcus faecalis* (vancomycin susceptible), *E. faecium* (vancomycin resistant), *E. cloacae, Streptococcus mitis, S. epidermidis, Acinetobacter* species	
4. (bottle a) *Streptococcus agalactiae* (bottle b) *Escherichia coli*[a]	4. (bottle a) *S. agalactiae, E. coli* (bottle b) *E. coli, Proteus* species	
5. *Streptococcus* species, *Haemophilus influenzae*	5. *Haemophilus sputorum, Neisseria subflava, Streptococcus salivarius, Streptococcus viridans* group, *Streptococcus mitis*	
6. *Enterococcus* species (*vanA/B* negative)	6. *E. faecium* (vancomycin susceptible), *S. anginosus*	
Discrepancy in susceptibility result		4
1. *Enterococcus* species (*vanA/B* negative)	1. *Enterococcus casseliflavus* (vancomycin MIC, 8 µg/mL)	
2. *Enterococcus* species (*vanA/B* positive)	2. *E. faecium*, vancomycin MIC 2 µg/mL, isolate *vanA* positive by PCR	
3. *Staphylococcus* species (*mecA* positive)	3. *Staphylococcus capitis*, oxacillin MIC 0.25 µg/mL, isolate *mecA* negative by PCR	
4. *Staphylococcus* species (*mecA* negative)	4. Coagulase-negative *Staphylococcus* species, oxacillin 1 µg/mL, isolate *mecA* negative by PCR	
Discrepancy in organism identification		3
1. *Enterococcus* species and *Staphylococcus* species (*mecA* positive)[b]	1. *S. epidermidis*	
2. Negative	2. *Enterobacter* species	
3. *K. pneumoniae*	3. *Enterobacter aerogenes*	

Abbreviations: MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.

[a] Gram stain results differed between 2 blood culture bottles collected on the same day for this subject. Both bottles were evaluated according to the study arm assignment of bottle a, which signaled positive first.

[b] Heavy growth of coagulase-negative staphylococci may lead to cross-reactivity with *Enterococcus* species; thus, the rapid multiplex PCR result was not reported.

Table 3. Antibiotic Utilization Among All Study Subjects in the First 96 Hours Following Enrollment

Outcome	Control	Rapid Multiplex PCR	Rapid Multiplex PCR + Stewardship	P Value Comparing 3 Groups
Duration of therapy[a], h				
Vancomycin				
All patients (n = 357)	44 (22–72)	42 (21–93)	42 (19–90)	.92
Organisms not requiring vancomycin[b] (n = 169)	8.2 (0–26)	0 (0–16)	0 (0–3)[c]	.032
Vancomycin-susceptible enterococci (n = 32)	20 (1–59)	70 (48–88)[c]	82 (40–96)[c]	.037
Methicillin-susceptible *Staphylococcus aureus* (n = 42)	23 (20–53)	11 (0–26)	8 (0–44)	.2
Nafcillin, oxacillin, or cefazolin (n = 50)	42 (24–57)	71 (51–79)[c]	85 (42–92)[c]	.035
Piperacillin-tazobactam (n = 214)	56 (39–82)	44 (27–74)[c]	45 (19–78)[c]	.012
Cefepime (n = 181)	55 (28–96)	71 (43–96)	58 (32–96)	.56
Antibiotic modifications				
Time to first appropriate de-escalation[d] (n = 344)	34 (21–55)	38 (22–66)	21 (7–37)[c,e]	<.0001
Time to first appropriate escalation[f] (n = 122)	24 (3–67)	6 (2–36)	5 (2–22)[c]	.04
Time to administration of active antibiotics (n = 123)[g]	11 (2–51)	6 (2–31)	4 (2–20)	.55
Contaminated blood cultures not treated or treated for <24 h, No. (%)[h]	47 (75)	49 (89)[c]	57 (92)[c]	.015

Data are presented as median (IQR) unless otherwise specified.

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[a] Duration of therapy (hours) was calculated as the difference between the date and time of the antibiotic start order (or Gram stain–positive blood culture, if antibiotics were started prior to the positive culture result) and the date and time of the antibiotic stop order, for subjects who received the specified antibiotics, according to the organisms identified and study group. Shorter duration of broad-spectrum antibiotics, longer duration of narrow-spectrum antibiotics, faster antibiotic escalation or de-escalation, and less treatment of contaminants were considered favorable outcomes.

[b] Organisms not requiring vancomycin included monomicrobial cultures with methicillin-susceptible *Staphylococcus aureus*; groups A, B, C, or G streptococci; *Streptococcus anginosus* species group; or gram-negative or fungal organisms.

[c] Statistically significant compared to control group.

[d] From positive Gram stain to 96 hours after enrollment. De-escalation included discontinuation of 1 or more antibiotics and/or switching from a broad- to a narrow-spectrum antibiotic.

[e] Statistically significant comparison between the 2 intervention groups.

[f] From positive Gram stain to 96 hours after enrollment. Escalation included initiation of 1 or more antibiotics and/or switching from a narrow- to a broad-spectrum antibiotic.

[g] From positive Gram stain to start of active antibiotic among patients not on active therapy at enrollment; excludes patients with contaminated blood cultures.

[h] Contaminated blood cultures were defined as growth of organisms such as coagulase-negative staphylococci from a single blood culture set when ≥2 blood culture sets were collected, except among subjects suspected to have true bacteremia associated with central venous catheters or devices.

subjects, the median duration of piperacillin-tazobactam was shorter in the rmPCR (44 hours) and rmPCR/AS (45 hours) groups compared with control (56 hours, *P* = .012). Utilization of other antibiotics was not different between groups.

The proportion of subjects with any de-escalation of antimicrobial therapy after Gram stain or rmPCR result was higher in the rmPCR/AS group (24.1%) compared with the rmPCR (14.7%) and control (12.1%) groups (*P* = .003). Time from Gram stain result to first appropriate antimicrobial de-escalation was shorter in the rmPCR/AS group (21 hours) compared with the control (34 hours) and the rmPCR (38 hours) groups (*P* < .001) (Table 3). Time from Gram stain result to first appropriate antimicrobial escalation was shorter in the rmPCR/AS group (5 hours) than in the control group (24 hours, *P* = .04; Table 3). Results were similar among the subset of subjects with organisms on the rmPCR panel (Figure 2). In the groups with

rmPCR testing, time to antimicrobial de-escalation or escalation did not change significantly over the course of the study. The proportion of contaminant blood cultures that was not treated or treated for <24 hours was higher in the rmPCR (89%) and rmPCR/AS (92%) groups vs control (75%, *P* = .015; Table 3). Antimicrobial utilization was not different among patients with and without infectious diseases consultation.

Other Outcomes

There were no differences in clinical or microbiologic outcomes among the groups (Table 4). Among the few patients with discrepant rmPCR and conventional culture results, no adverse consequences were observed. Both intervention groups had increased test costs but similar hospitalization costs compared with the control group. Antimicrobial costs tended to be lower for both intervention groups than for the control group,

Table 4. Comparison of Clinical, Microbiologic, and Cost Outcomes According to Study Group

Outcome	Control (n = 207)	Rapid Multiplex PCR (n = 198)	Rapid Multiplex PCR + Stewardship (n = 212)	P Value Comparing 3 Groups
Clinical outcome				
Disposition				.12
Home	68 (32.9)	62 (31.3)	78 (36.8)	
Home with outpatient antimicrobial therapy	39 (18.8)	52 (26.3)	38 (17.9)	
Nursing home/skilled nursing facility	63 (30.4)	42 (21.2)	54 (25.5)	
Hospice/comfort care	12 (5.8)	8 (4)	7 (3.3)	
Death	11 (5.3)	11 (5.6)	8 (3.8)	
Length of stay (entire hospitalization), d, median (IQR)	8 (5–15)	8 (5–15)	8 (5–16)	.60
Length of stay (after enrollment), d, median (IQR)	7 (4–12)	6 (4–12)	7 (4–12)	.61
Intensive care unit admission within 14 d after enrollment	16 (7.7)	5 (2.5)	10 (4.7)	.06
Length of stay in intensive care unit (after enrollment), d, median (IQR)	3 (2–4)	2 (1–5)	3 (2–4)	.90
30-day mortality	22 (10.6)	20 (10.1)	18 (8.5)	.74
30-day attributable mortality	7 (3.4)	7 (3.5)	2 (0.9)	.42
30-day readmission for infection with same organism	6 (2.9)	6 (3)	8 (3.8)	.88
Toxicity/adverse drug reaction[a]	3 (1.4)	3 (1.5)	2 (0.9)	.82
Microbiologic outcomes				
Blood culture clearance within 3 d after enrollment	147 (71)	131 (66.2)	146 (68.9)	.79
Acquisition of *Clostridium difficile* or multidrug-resistant organisms[b] within 30 days after enrollment	15 (7.2)	16 (8.1)	21 (9.9)	.62
Cost per hospitalized patient, mean (median)				
Overall hospitalization costs	$65 450 ($27 192)	$66 887 ($23 935)	$68 729 ($29 064)	.78
Test costs	$5377 ($2082)	$5680 ($2585)[c]	$5743 ($2774)[c]	<.001
Antimicrobial costs	$2194 ($990)	$1932 ($866)	$1741 ($890)	.65

Data are presented as No. (%) unless otherwise specified.

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[a] Toxicities include seizures, *Clostridium difficile* infection, hepatitis, myelosuppression, renal insufficiency, prolonged QTc interval, and rash that occurred within 2 weeks following enrollment and were documented in the medical record.

[b] Multidrug-resistant organisms including vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, extended-spectrum cephalosporin-resistant Enterobacteriaceae, and *Pseudomonas aeruginosa* and *Acinetobacter* species resistant to ≥3 antibiotic classes.

[c] Statistically significant compared to control group.

but this difference was not statistically significant. Total costs were not significantly different between intervention and control groups in sensitivity analysis of rmPCR test cost.

DISCUSSION

We report the first prospective RCT to demonstrate benefit of an rmPCR-based blood culture diagnostic test. We found that use of the rmPCR test led to more judicious antibiotic use. Additionally, we delivered rapid test results along with templated comments guiding interpretation and antimicrobial prescribing, and separately evaluated this approach with and without real-time antimicrobial stewardship. Both rapid test reporting strategies reduced unnecessary antibiotic use, although rmPCR testing combined with antimicrobial stewardship resulted in the most rapid antibiotic de-escalation.

In earlier observational studies, rapid pathogen identification methods have been associated with decreased mortality, LOS, and cost [5, 6, 9, 11–14]. However, these studies were limited by their retrospective designs, use of historical controls [5, 6, 10, 22], lack of randomization, and failure to match subjects by severity of illness [7]. In contrast, in this trial we did not observe differences in clinical or cost outcomes between patients in intervention and control arms. This may be due to the use of different study designs, the fact that this trial was not powered to detect differences in LOS, mortality, or cost, and/or the fact that 70% of our study subjects were receiving at least 1 active agent at time of enrollment and were generally being overtreated, rather than undertreated. In addition, our institution's baseline antimicrobial stewardship program, low resistance rates, high rate of infectious diseases consultation (48%), and baseline use of advanced technologies including MALDI-TOF MS and

rapid PBP2a testing, even in the control arm, may have reduced differences between the control and intervention arms.

The rmPCR test enabled clinicians to quickly initiate "pathogen-directed" therapy and appropriately scale up or scale down antibiotic therapy, as needed. Specifically, in both intervention groups, we observed increased narrow-spectrum antibiotic use, less unnecessary vancomycin use, decreased treatment of blood culture contaminants, and more timely antibiotic escalation compared with the control group. Because timely initiation of effective therapy is a critical step in the management of patients with sepsis [1, 23–25], reducing time to appropriate antibiotic escalation by 14 hours, as observed in both intervention arms (Figure 2), is clinically significant. Antibiotic de-escalation occurred nearly 1 day (19 hours) faster in the rmPCR/AS group compared with control (Figure 2), which is almost a 25% reduction in broad-spectrum antibiotic days of therapy, as median duration of piperacillin-tazobactam or meropenem therapy in the control group was 4 days. This is likely to be significant at a population level; models estimate that a 5% reduction in broad-spectrum antibiotic use among hospitalized patients would result in a 26% decrease in *C. difficile* infection rates [26]. Among subjects with vancomycin-susceptible enterococci, vancomycin use increased in both intervention groups because of prompt de-escalation from daptomycin to vancomycin. In contrast, cefepime use did not decrease in the intervention groups, likely because this drug is commonly used for management of suspected infection in neutropenic hosts, in whom antibiotic de-escalation is often not indicated, despite negative blood cultures.

We observed that provider response differed according to how rapid test results were delivered. Others have noted that despite the availability of a rapid test result (eg, presence or absence of *mecA*), providers may fail to modify antibiotic therapy without pharmacist intervention [8, 27]. Observational studies have found favorable outcomes when rapid testing was implemented together with antimicrobial stewardship interventions [5, 6, 11–16]. To our knowledge, no prior studies have implemented a rapid blood culture diagnostic test using electronic decision support in the form of templated comments to guide prescribing, as done in this study. In the group that received the rmPCR test results delivered with templated comments communicated verbally and in the medical record, we observed more rapid antibiotic escalation, more narrow-spectrum antibiotic use, and less treatment of contaminants compared with the control group. Templated comments are an inexpensive and effective means of communicating straightforward results to providers, such as when coagulase-negative staphylococci or *Micrococcus* species represent contaminants, or when a *Staphylococcus* species is methicillin susceptible or resistant.

When the rmPCR test result was delivered with guidance from the antimicrobial stewardship team, there was also more frequent and timelier antibiotic de-escalation. We speculate that this is in part because notification from a stewardship team member might prompt a busy clinician to act on a test result more so than a telephone call from a laboratory technologist. Additionally, in complex clinical scenarios when critically ill or immunocompromised patients or patients with polymicrobial infections are on multiple broad-spectrum antibiotics, providers may prefer discussion with infectious disease specialists prior to modifying antimicrobial management. Although in this study the stewardship team provided feedback to providers 24 hours a day, most antibiotic de-escalation occurred during the day because housestaff preferred not to contact supervising providers at night regarding nonurgent de-escalation questions. Thus, around-the-clock antimicrobial stewardship team oversight, which is costly, may not be necessary. Rather, we estimate that at our institution, an additional 1–2 hours of stewardship effort (during the daytime) would be sufficient for daily review of positive blood cultures.

Unlike other rapid PCR-based platforms that target a limited number of organisms, the rmPCR test detected multiple targets, characterizing >80% of positive blood cultures and providing accurate results nearly a day faster than standard techniques. Additional advantages of the rmPCR test studied are that it is a closed system, can be used whether blood cultures contain gram-positive or gram-negative bacteria or yeast, and does not require significant technologist training or time. Known limitations of the test include its lack of sensitivity in detecting all organisms in polymicrobial cultures (Table 2), and the limited susceptibility information provided for gram-negative bacteria. The rmPCR test studied is "add-on" testing that does not replace conventional BCB subculture workup methods.

This study has limitations, including that it was performed at a single center and may not be generalizable to other institutions with different patient populations, prescribing and stewardship practices, and antimicrobial resistance rates. The study was not powered for subgroup analyses or to detect differences in secondary outcomes including LOS and mortality. Investigators were not blinded to study arm, and infectious diseases "curbside" consultations were not captured. We did not include a control group without rmPCR testing but with stewardship of all positive blood cultures. We were unable to account for contamination between study arms, although rarely were multiple patients in the intervention arms cared for by the same clinical service at the same time. Despite these limitations, this is the first prospective RCT to evaluate the value of a rapid diagnostic test for blood culture pathogen identification and to compare strategies to communicate rapid test results to clinicians.

In conclusion, rapid pathogen and susceptibility detection directly from blood cultures implemented with templated comments or antimicrobial stewardship oversight can optimize

antibiotic prescribing for bloodstream infections. To influence clinical decision making, rapid results should be delivered with real-time decision support (using automated systems or antimicrobial stewardship programs) that assists clinicians to interpret and act on results.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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