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FilmArray (BCID2) provides essential and timely results in bloodstream infections in small acute care hospitals without conventional microbiology services

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We have evaluated the performance of FilmArray BCID2 in reactive blood cultures in a small acute care hospital compared to conventional diagnostics at a regional microbiological laboratory. This is a retrospective observational study of BactAlert reactive blood cultures (n = 160) from Helgeland Hospital, July–December 2021, analysed by BCID2 locally and conventional culture at a regional laboratory. The overall clinical and analytic sensitivity with BCID2 were 87.2% and 97.8%, respectively. The false-negative BCID2 rate was low (n = 4; 2.9%). No false-positive BCID2 results were observed. The BCID2 data were available on average 1.88 days earlier than culture-based results, due to long transport time to the regional laboratory. The BCID2 provided results to support a significantly earlier optimized targeted antibiotic treatment in 27% of the cases according to national guidelines for empirical treatment of BSI. The high clinical and analytical sensitivity, and specificity support the use of BCID2 as a robust supplement to traditional cultivation of positive blood cultures. The significant time gain to microbial identification and detection of resistance determinants suggests a great clinical importance of BCID2 in small acute care hospitals with long transport time to conventional clinical microbiology services.

Key words: FilmArray; BCID2; blood culture; blood stream infection; antimicrobial stewardship.

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The burden of blood stream infections (BSIs) is considerable in terms of mortality, morbidity and health care costs and is predicted to increase significantly in industrialized countries due to the demographic development [1]. Fast and accurate identification of pathogens and antimicrobial susceptibility testing (AST) are crucial for therapeutic decisions, antibiotic stewardship and clinical outcome for the patients [2].

Rapid molecular tests are a promising tool complementing traditional culture-based diagnostics and may enable reduced time to targeted antimicrobial therapy [3]. This is of particular importance in patient care at small acute care hospitals in rural areas, where positive blood culture (BC) samples typically are forwarded to a regional clinical microbiology laboratory for traditional culture-based

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diagnostics. When adding transport time, pathogen identification and corresponding AST are usually not available until 2–4 days after identification of reactive BCs.

Helgeland Hospital trust (HT), a small hospital in Northern Norway, implemented the FilmArray Blood Culture Identification 2 panel (BCID2; BioMérieux, Marcy l'Etoile, France) for rapid molecular diagnostics of positive BC samples in 2021. BCID2 is a multiplex PCR-based system for detection of the most important bacterial and fungal pathogens and bacterial resistance genes in positive BCs, delivering results in about 1 h [4].

The aims of this study were to examine the performance of BCID2 at Helgeland HT compared with conventional diagnostics at the regional hospital related to species identification and AST. We also examined the average gain in time to relevant results obtained by early local BCID2 compared to standard diagnostics. Finally, we discuss the potential implications for early targeted antimicrobial therapy based on rapid BCID2 results.

MATERIALS AND METHODS

Population and study setting

Helgeland HT consists of three small acute care hospitals with a catchment area of 78 000 inhabitants (18 834 km^2) in the Nordland County of Northern Norway. The three hospitals have a total of 158 beds in somatic wards, accepting patients with acute infectious diseases including BSIs. The laboratory facilities are limited and do not support standard culture-based microbial analyses, except for a BC system BactAlert 3D (BioMérieux). Briefly, double sets of BCs (aerobic and anaerobic), primarily from two different injection sites, were incubated for 5 days, or until reported as reactive. Positive BCs were transported on weekdays to the regional microbiological laboratory at Nordland HT for standard diagnostics, approximately 300 km. Transport time vary, depending on flight times and weather conditions. Primo 2021, the BCID2 panel was implemented as a standard supplementary analysis of positive BCs in all three hospitals at Helgeland HT before transport to Nordland HT. The data used in this study are a retrospective collection of all BactAlert reactive BCs as part of patient care from July 1, 2021 through December 2021.

BCID2 testing

Reactive BCs were analysed with BCID2 at first opportunity (24/7) at Helgeland HT. In case of several (≥2) simultaneously reactive BC bottles per patient, an anaerobic bottle was preferred for BCID2. BCID2 tests were prepared and analysed on the FilmArray V2.0 system according to the instructions from the manufacturer, except from Gram-stain analyses which was not available. The run takes about 1 h. The results from the BCID2 panel were classified as 'detected' or 'not detected', transferred directly from the FilmArray platform to the electronic patient record (DIPS, Norway) and delivered to the clinician by phone once the run was completed. Without further local primary diagnostics, all reactive BC-bottles were forwarded to Nordland HT for conventional diagnostics. According to local procedures, consecutive BCID2 analysis are not to be carried out during the same patient admission. All additional reactive BCs were forwarded directly to the microbiological laboratory at Nordland HT for cultivation.

The BCID2 panel includes targets for one bacterial order (Enterobacterales), four bacterial genera (*Proteus* spp., *Salmonella* spp., *Staphylococcus* spp. and *Streptococcus* spp.), 21 bacterial species (*Acinetobacter calcoaceticus-baumannii* complex, *Bacteroides fragilis*, *Enterobacter cloacae* complex, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella oxytoca*, *Klebsiella pneumoniae group*, *Listeria monocytogenes*, *Serratia marcescens*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Stenotrophomonas maltophilia*, *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*), 1 fungal genus [*Cryptococcus* (*C. neoformans*/*C. gattii*)], six fungal species (*Candida albicans*, *Candida auris*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis*) and a total of 10 resistance genes encoding extended spectrum betalactamases (ESBL; CTX-M only) and carbapenemases (IMP, KPC, OXA-48-like, NDM and VIM), methicillin-resistant *Staphylococcus aureus* (MRSA; *mecA*/*C*), vancomycin resistant enterococci (VRE; *vanA*/*B*) and colistin-resistance (*mcr-1*).

Standard culture-based detection

Briefly, at Nordland HT, reactive BCs were prepared for direct mass spectrometry (MALDI-TOF, Bruker Daltonics, Bremen, Germany) and Gram-stained. One drop (50 µL) from every reactive BC-bottle was used for inoculation on each of three different agar media (chocolate, horse blood with an aztreonam 30 µg disc and lactose) and incubated at 35 °C under an atmosphere containing 5% CO_2. A fastidious anaerobic agar plate with gentamicin (10 µg) and metronidazole (5 µg) discs was added for each reactive anaerobic bottle. One drop was also inoculated for preliminary AST on agar plates suitable for the bacterial presence confirmed by Gram-stain and/or direct MALDI-TOF. If Gram-stain revealed presence of fungi, both Sabouraud and Candida Chrom-agar plates were added and incubated at 36 °C in ambient air. Final species identification and AST were performed with MALDI-TOF and the EUCAST disc-diffusion method, respectively [5]. The results were reported through the laboratory information system (CGM Analytix®, Sweden) to the electronic patient record (DIPS).

Data collection, handling and calculations

All BCs analysed with BCID2 at Helgeland HT in the period July 1, 2021 to December 31, 2021 were included in the study. BCID reports were extracted from the electronic patient record by the help of laboratory administrative services and imported into Excel® (Microsoft, USA) for further analysis. Using unique sample ID, associated

microbiologic laboratory data were retrieved by manual review of the microbiology journal for each positive BC. Time of report (hour and date) at the earliest preliminary species identification and AST for both standard culture and BCID2 was registered. The two data sets were not linked by patient identification. Hence, compliance with procedures stating that additional BCs during the same admission are to be shipped directly to microbiological laboratory were not investigated. Consecutive analysis conducted during the same admission may thus occur in the material.

Time gained using BCID2 vs conventional culture-based methods

BCID2 reports included sample ID, time of sampling, time of report and results. The time of each report release was set as parameter for 'ID from BCID2'. Time of report for preliminary identification by either Gram stain or direct MALDI-TOF at the microbiological department was used as a marker for 'first ID by conventional diagnostics'.

Test characteristics

We have separated the test characteristics into two groups, analytical and clinical sensitivity. *Analytical sensitivity* was defined as number of targets detected by BCID2 divided by in-BCID2 panel target cultivation-positive microbes. *Clinical sensitivity* was defined as number of targets detected by BCID2 divided by all cultivation-positive bacterial isolates, both in- and off-panels. *False-negative* results were defined as number of targets not detected by BCID2, divided by in-BCID2 panel cultivation-positive bacterial isolates. *False-positive* results were defined as targets detected by BCID2 but not by cultivation.

Potential implications of BCID2 results on early targeted antimicrobial therapy

The Norwegian guidelines for antibiotic treatment of septicaemia recommend intravenous benzylpenicillin and gentamicin as empirical treatment of sepsis with unknown focus, with a few exceptions [6]. According to the current prevalence of AMR in bacterial pathogens found in Norwegian BCs, several BCID2 targets are likely to predict suboptimal outcome without adjustment of the standard empirical treatment, due to either intrinsic or acquired resistance [6, 7]. This includes the following in-panel species and resistance-gene targets: *B. fragilis*, *E. faecalis*, *E. faecium*, *H. influenzae*, *P. aeruginosa*, *S. aureus*, *S. maltophilia*, ESBL- or carbapenemase producing Enterobacterales (CTX-M, IMP, KPC, OXA-48-like, NDM and VIM), vancomycin resistance (*vanA*/*B*), methicillin resistance [*mecA*/*C* and SCC*mec* right extremity junction (MREJ)] and seven different fungal targets. The potential changes to targeted antimicrobial therapy based on the BCIDs results were calculated on these assumptions. Concerning the potential for de-escalation based upon BCID2-results alone, we only considered the monobacterial presence of *S. pneumoniae*, taking into consideration the current recommended empirical treatment. We did not investigate the actual antibiotic therapy registered in patient records.

Ethics statement

The hospital data protection officer accepted the study as a quality assurance project using de-identified data sets from the laboratory data systems DIPS (Helgeland HT) and Analytix (Nordland HT). The data material originated from BCs samples collected during routine patient management without requiring additional sampling. As such, the study did not require ethics committee approval or informed consent from the patients.

RESULTS

The primary data extraction included 160 BCID2 analyses. The distribution of microbes and resistance genes covered three out of four bacterial genera, 14 of 21 bacterial species, 0 of 7 fungal genera and two of 10 resistance genes BCID2 in-panel targets.

Conventional culture-based results

The overall findings are presented in Table 1 and Fig. 1. Briefly, standard cultivation showed bacterial growth in 148 out of 160 (92.5%) reactive BC-sets. The 12 culture-negative BC-sets were also negative by Gram stain microscopy and considered false BactAlert reactive (12 out of 160; 8%). Polymicrobial growth was seen in seven out of 148 (5%) positive BC-sets. A total of 156 bacterial strains were detected. Fungi were not observed. The dominant species and bacterial groups were *E. coli* (n = 33), *S. aureus* (n = 22), *Streptococcus* spp. (n = 17), *Enterococcus* spp. (n = 14), *K. pneumoniae* (n = 11) and anaerobic bacteria (n = 8).

BCIDs panel results and concordance with conventional culture

The complete results are presented in Fig. 1 and Table 1. Shortly, the 12 false reactive BactAlert BCs were all negative by BCID2. In total 131 out of 148 (88.5%) of culture-positive BCs had a positive BCID2 for at least one of the family-, genus- or species-related BCID2 targets. Polymicrobial findings were precisely identified at species level by BCID2 in four BCs, all confirmed by culture. Monomicrobial BCID2 results were correctly obtained at the species or genus level in 125 out of 141 (88.6%) monoculture confirmed BCs. In summary, concordance in microbiological identification at the genus or species level was defined as clinical sensitivity and calculated as 87.2% (129 out of 148). A total of 136 out of 156 (87.2%) bacterial strains were accurately detected by BCID2. 140 out of 156 bacterial strains proven by culture was in-panel microbes, leading to an analytic sensitivity of 97.1% (136 out of 140).

Table 1. Concordance and discrepancies in BCID2-based order, genus, species and resistance-gene detection in culture confirmed BC bottles (n = 148)

Organism	No. of microorganisms detected by culture/ BCID2				Preferred antimicrobial[1]	Proportions (%)	National proportions(%) in BCs[2]
	+/+	−/+	+/−	−/−			
Gram-positive	73	0	10	73		53.2	50.9
Staphylococcus spp.	43	0	2	111		28.8	31.0
S. aureus	20	0	2	134	Kloxacillin	14.1	10.6
S. epidermidis	8	0	0	148		5.1	
S. lugdunensis	0	0	0	156		0	
Streptococcus spp.	17	0	0	139		10.9	11.8
S. agalactiae	0	0	0	156		0	1.7
S. pneumoniae	5	0	0	151	Penicillin, monotherapy	3.2	1.6
S. pyogenes	0	0	0	156		0	0.8
E. faecalis	11	0	1	144	Ampicillin	7.7	3.5
E. faecium	2	0	0	154	Vancomycin	1.3	1.1
L. monocytogenes	0	0	0	156		0	
Other Gram-positive bacterias[3], not included in the BCID2	0	0	7	149		4.5	3.5
Gram-negative	61	0	4	91		41.7	41.6
Enterobacterales	56	0	1	99		36.5	37.9
E. cloacaea complex	1	0	0	155		0.6	1.7
E. coli	33	0	0	123		21.2	30.7
K. aerogenes	0	0	0	156		0	
K. oxytoca	4	0	1	151		3.2	1.3
K. pneumoniae group	11	0	0	145		7.1	3.4
Proteus spp.	2	0	0	154		1.3	1.6
Salmonella spp.	0	0	0	156		0	
S. marcescens	3	0	0	153		1.9	
A. calcoaceticus-baumannii complex	0	0	0	156		0	
H. influenzae	1	0	0	155	Cefotaxime	0.6	0.2
N. meningitidis	0	0	0	156		0	0.02
P. aeruginosa	3	0	0	153	Piperacillin/ Tazobactam	1.9	1.9
S. maltophilia	1	0	0	155	Trimethoprim/ Sulfamethoxazole	0.6	
Other Gram-negative bacterias[4], not included in the BCID2	0	0	3	153		1.9	1.6
Anaerobic	2	0	6	148		5.1	6.4
B. fragilis	2	0	0	154	Metronidazole	1.3	1.8
Other anaerobic bacterias[5], not included in the BCID2	0	0	6	150		3.8	4.2
Fungi						0	1.2
C. albicans	0	0	0	156			
C. auris	0	0	0	156			
C. glabrata	0	0	0	156			
C. krusei	0	0	0	156			
C. parapsilosis	0	0	0	156			
C. tropicalis	0	0	0	156			
C. neoformans	0	0	0	156			
Resistance genes							
ESBL (CTX-M)	1	0	0	155	Meropenem		
MRSA (mecA/C, MREJ)	0	0	0	156			
MRSE[6] (mecA/C)	3	0	0	153			
Carbapenemases (IMP, KPC, OXA-48-like, NDM, VIM)	0	0	0	156			

Discrepancies between BCID2 and conventional cultivation was observed in 19 out of 148 (12.8%) BCs, three in-panel and 16 off-panel bacterial targets. Discordance was observed in three out of seven polymicrobial BCs, 16 out of 141 (11.3%) monoculture BCs and 20 out of 156 (12.8%)

Table 1 *(continued)*

Organism	No. of microorganisms detected by culture/ BCID2				Preferred antimicrobial[1]	Proportions (%)	National proportions(%) in BCs[2]
	+/+	−/+	+/−	−/−			
Colistin resistance (mcr-1)	0	0	0	156			
VRE (vanA/B)	0	0	0	156			

The table shows distribution of all microorganisms detected by cultivation (n = 156), concordance (+/+) and discrepancy (−/+, +/−) between culture and BCID2, compared with the total number of other occurring microbes in the material (−/−).

[1]Preferred antimicrobial; Either in accordance to the Norwegian guidelines for antibiotic treatment in hospitals [5], and/or expected treatment failure due to either intrinsic resistance or a high prevalence of acquired resistance to the standard empirical treatment of septicaemia in Norway [5].

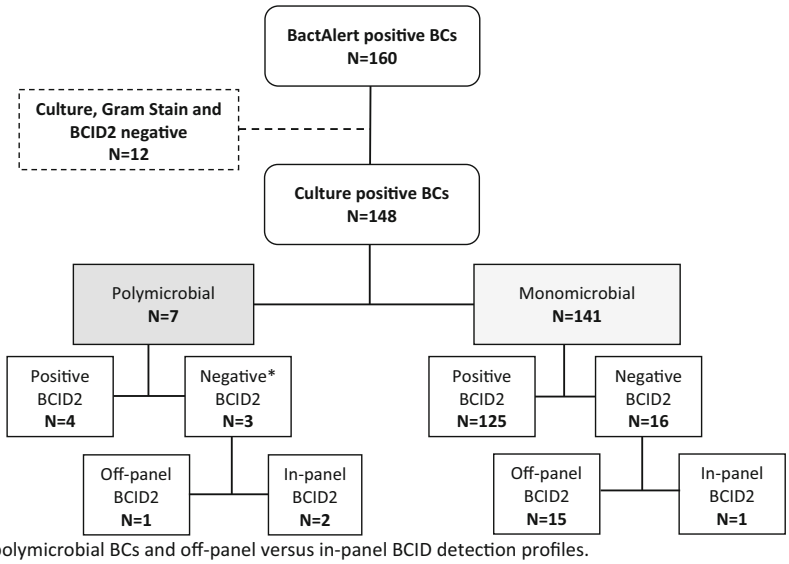
[2]General distribution in Norwegian BCs in 2020, NORM-VET 2020 [8].

[3]Other Gram-postitive bacteria: *Actinomyces oris*, *Actinotignum schaali* (n = 2), *Bacillus cereus* (n = 2), *Cutibacterium acnes* and *Micrococcus luteus*.

[4]Other Gram-negative bacteria: *Cardiobacterium hominis* (n = 2) and *Moraxella catarrhalis*.

[5]Other anaerobic bacteria: *Bacteroides vulgatus*, *Bifidobacterium longum*, *Clostridium perfringens*, *Fusobacterium* spp., *Fusobacterium nucleatum* and *Parvimonas micra*.

[6]MRSE is not included in the panel, but is added by the authors if mecA og mecC was detected together with *Staphylococcus epidermidis*.



polymicrobial BCs and off-panel versus in-panel BCID detection profiles.

Fig. 1. Flow chart describing the overall number of positive BCs, monomicrobial vs polymicrobial BCs and off-panel vs in-panel BCID detection profiles. *One or more microbes not detected.

culture-verified strains. We observed no false-positive BCID2 findings. Discordant in-panel BCID2 target findings are summarized in Table 2, including two *S. aureus* of which one was observed in a polymicrobial BC, as well as one polymicrobial BC containing *K. oxytoca* and *E. faecalis*. BCID2 off-panel positive BC results (n = 16) are outlined in Table 3, and included one polymicrobial and 15 monomicrobial BCs. In the polymicrobial BCs only one off-panel species (*B. vulgatus*) was not detected by BCID2. Many of the off-panel targets were anaerobic species (n = 6). Five of the monomicrobial off-panel target positive BCs were considered 'possible contamination' by the clinical microbiology laboratory (*Bacillus cereus* n = 2, *Actinomyces oris* n = 1, *Cutibacterium acnes* n = 1, *Micrococcus luteus* n = 1).

BCID2 performance for the most prevalent bacterial order, genera and species: Staphylococci and Enterobacterales

*Staphylococc*us spp. and Enterobacterales cover 102 out of 156 (65.3%) of all bacteria detected by culture in this study. The overall concordance between BCID2 and conventional culture in detection of

Table 2. Discordant BCID2 in-panel target findings (n = 3) compared to culture

Sample no.	Bottle type tested with BCID2	BCID2	Culture	Gram stain	MALDI-TOF ID
5	Aerobic	Negative	Monomicrobial	GPCC	*S. aureus*
16	Aerobic	Negative	Polymicrobial	GNR, GPCP	*K. oxytoca*, *E. faecalis*
163	Missing information	Enterobacterales, *K. pneumoniae group*	Polymicrobial	GNR	*K. pneumoniae*, *S. aureus*

GPCC, gram-positive cocci in clusters; GNR, gram-negative rod; GPCP, Gram-positive cocci in pairs or chains.

Table 3. BCID2 off-panel target positive BC results (n = 16)

Sample no.	Bottle type for BCID2	BCID2	Cultivation	Gram stain	MALDI-TOF ID	Preferred antimicrobial
1	Aerobic	Negative	Monomicrobial	GPR	*A. oris*	
2	Aerobic	Negative	Monomicrobial	GPR	*B. cereus*	Vancomycin
29	Anaerobic	Negative	Monomicrobial	GPR	*Actinotignum schaalii*	
33	Anaerobic	*Enterococcus faecalis*, Enterobacterales, *Escherichia coli*	Polymicrobial	GPCP, GNR	*E. faecalis*[1] *E. coli*[1] *Bacteroides vulgatus*	
38	Aerobic	Negative	Monomicrobial	GPCC	*M. luteus*	
64	Anaerobic	Negative	Monomicrobial	GPR	*A. schaalii*	
68	Anaerobic	Negative	Monomicrobial	GPR	*Clostridium perfringens*	
94	Anaerobic	Negative	Monomicrobial	GNC	*Parvimonas micra*	
107	Anaerobic	Negative	Monomicrobial	Possible fungal elements	*Bifidobacterium longum*	
108	Aerobic	Negative	Monomicrobial	GNC	*Moraxella catarrhalis*	Cefotaxime
109	Anaerobic	Negative	Monomicrobial	GNR	*Fusobacterium* spp.	Metronidazole
126	Aerobic	Negative	Monomicrobial	Neg	*C. acnes*	
128	Anaerobic	Negative	Monomicrobial	GVR	*Fusobacterium nucleatum*	Metronidazole
138	Aerobic	Negative	Monomicrobial	GNR	*Cardiobacterium* spp.	
149	Aerobic	Negative	Monomicrobial	GNR	*Cardiobacterium* spp.	
154	Aerobic	Negative	Monomicrobial	GPR	*B. cereus*	

GPR, gram-positive rod; GPCC, gram-positive cocci in clusters; GPCP, gram-positive cocci in pairs or chains; GNR, gram-negative rod; GNC, Gram-negative cocci, Neg, negative; GVR, gram-variable rod.
[1]Detected by BCID2.

Staphylococcus spp. was 43 out of 45 (95.6%), including *S. aureus* (20 out of 22) and *S. epidermidis* (eight out of eight). The remaining 15 coagulase-negative staphylococci [CoNS; *S. hominis* (n = 10), *S. capitis* (n = 1), *S. haemolyticus* (n = 1), *S. pettenkoferi* (n = 1), *S. simulans* (n = 1), *S. warnerii* (n = 1)] detected by cultivation do not have species-specific targets in the panel. *S. lugdunensis* was not detected. With the exception of two BCs containing *S. epidermidis* and one BC containing *S. simulans*, all other CoNS where considered as contamination by the regional laboratory.

For Enterobacterales we observed concordance between BCID2 and culture-based detection in 56 out of 57 (98.2%) of culture verified BCs, including *E. coli* (33 out of 33), *K. pneumoniae* group (11 out of 11) and *K. oxytoca* (4 out of 5). BCID2 failed to detect one *K. oxytoca* in a polymicrobial BC.

Test characteristics

The overall clinical sensitivity was 87.2% (129 out of 148) per positive BC and 87.2% (136 out of 156) for bacterial strains. The overall analytic sensitivity was and 97.7% (129 out of 132) per in-panel cultivation-positive BC and 97.1% (136 out of 140) for bacterial strains. No false-positive BCID2 were observed, resulting in 100% specificity.

Concordance between BCID2s panel results and conventional culture in antimicrobial resistance detection

The results are summarized in Table 1. One ESBL (CTX-M)-producing *E. coli* was detected by both BCID2 and cultivation. Three methicillin-resistant isolates of *S. epidermidis* (*mec*A/C-positive) were

detected by both methods. MRSA, VRE or carbapenemase producing strains were not detected by either method.

The potential implications of BCID2 results for early targeted antimicrobial therapy

A total of 54 out of 156 (34.6%) bacterial species or resistance phenotypes [*S. aureus* ($n = 22$), *S. pneumoniae* ($n = 5$), *E. faecalis* ($n = 12$), *E. faecium* ($n = 2$), *H. influenzae* ($n = 1$), *P. aeruginosa* ($n = 3$), *S. maltophilia* ($n = 1$), *B. fragilis* ($n = 2$), *B. cereus* ($n = 1$), *M. catarrhalis* ($n = 1$), *Fusobacterium* spp. ($n = 1$) *F. nucleatum* ($n = 1$), *B. vulgatus* ($n = 1$), ESBL-producing *E.coli* ($n = 1$)] (CTX-M) confirmed by cultivation supported a change (optimization or de-escalation) from standard empirical to targeted antimicrobial treatment [6]. 49 out of 54 had in-panel targets, whereof 46 (93.9%) were correctly identified by BCID2. Preferred changes in antimicrobial therapy are listed in Tables 1 and 3.

Average gain in time by BCID2 results compared to conventional culture

The average time of transportation from Helgeland HT to Nordland HT for all BactAlert reactive 160 BCs was 1.35 days (median 1.03 days) with a range [0.15–3.73]. The BCID2 results were available on average 1.88 days (median 1.59 days, range [0.23–7.08]) earlier than the first preliminary culture-based results.

DISCUSSION

In this study we have examined the performance of BCID2 compared to standard culture-based diagnostics and the potential therapeutic implications of rapid BCID2-findings in the treatment of bloodstream infections in a rural hospital setting. The material consisted of consecutive reactive BCs ($n = 160$) collected during 6 months in 2021. We detected a predominance of *Staphylococcus* and Enterobacterales species. Only one ESBL-producing Enterobacterales. MRSA or VRE were not detected. The overall findings correspond to the relative distribution of species and low occurrence of antimicrobial resistance in BSIs in Norway in 2020 [7]. Thus, the material and results seem representative for a Norwegian context.

Concordance BCID2 and conventional culture

We observed a high concordance in BCID2 in-panel target results compared to routine diagnostics. BCID2 detected 136 out of 140 (97.1%) in-panel bacterial targets and all in-panel resistance gene targets including one $bla_{\text{CTX-M}}$. There was an excellent concordance for *Staphylococcus* (95.5%, 43 out of 45) and Enterobacterales (98.1%, 54 out of 55), the most prevalent microbes in the material. We observed no false-positive BCID2 results. The high analytic sensitivity and specificity are consistent with the high concordance between BCID2 and culture-based diagnostics in previous studies [8, 9]. A systematic review on the performance of the BCID2 panel found only few false-positives in the 10 studies included, mainly S. epidermidis, without further specification [10].

Analytic sensitivity and in-panel target discrepancies

Our findings are in line with the analytic sensitivity listed in the manufacturer's instruction manual [4]. The overall analytic sensitivity in our material is calculated at 97.8% (137 out of 140). BCID2 failed to detect two out of 22 *S. aureus* isolates, one from a monomicrobial BC and another from a polymicrobial BC which also contained *K. pneumoniae*, where the latter was correctly identified. The BCID2 also failed to detect both in-panel targets in a polymicrobial BC containing *K. oxytoca* and *E. faecalis*. False-negative BCID2 in-target results have been published specifically for *E.coli* but not for the three bacterial targets we encountered [11]. Possible explanations regarding false-negative PCR-results include user and/or technical errors as well as challenges related to target sensitivity [12]. Two of the three false-negative BCID2 results occurred in polymicrobial BCs. Unevenly distributed microbes within a polymicrobial BC may reduce test sensitivity. Challenges regarding correct and complete species identification in polymicrobial BCs are reported in previous studies using BCID2 [13]. We re-examined the false-negative results by spiking separate BC-bottles with colonies from the actual isolates (*S. aureus* ($n = 2$), *K. oxytoca*, and *E. faecalis*). The corresponding reactive BCs were then reanalysed with BCID2, and all strains were detected. Thus, the false-negative BCID2-results could not be explained by rare genotypes or mutations within the PCR-target region. We suggest that the most probable cause of missed detection of one *S. aureus* in a polymicrobial BC also containing *K. pneumoniae* could be a significant higher abundance of *K. pneumoniae* and a correspondingly low abundance of *S.aureus* at the time of BCID2 analysis. This suggestion was also supported by Gram stain at the regional microbiology laboratory, where only gram-negative rods were visualized on BC-admission.

Although limited by small numbers, our observations indicate a higher false-negative rate in polymicrobial (two out of six) BCs compared to

monomicrobial (one out of 126) BCs. In a meta-analysis of ten BCID2 performance studies the concordance with standard culture in polymicrobial BCs ranged between 50 and 100% in five of the included studies, where the number of polymicrobial BCs in each study vary between 2 and 35 [10].

Clinical sensitivity and off-panel targets

We observed an overall high clinical sensitivity of BCID2 in our study (87.2%). This observation is in line with the theoretical calculation that the BCIDs panel would cover 89% of all bacterial genera and species found in Norwegian BSIs in 2020 distributions [7]. Thus, in a Norwegian context with predictable and low levels of AMR, rapid genus and species-specific level identification is of high significance to support relevant changes in empirical antimicrobial treatment in a majority of patients. Several studies have discussed the need for an additional expansion of the panel, including pan-*Bacteroides* and pan-*Clostridium* [8]. We do not consider limitations in the current BCID2 panel to be a significant weakness, when used as a supplement to traditional cultivation-based methodology.

Specificity

The high specificity (100%) indicates high laboratory technical robustness with a low risk of environmental contamination, cross-contamination, carry-over or other known sources of error in molecular diagnostics [12]. The clinical microbiology laboratory assessed five bacterial species other than CoNS in culture-positive BCs as 'possible contamination', all off-panel targets. To define the putative clinical significance of possible contaminants, multiple BCs must be obtained in sequence and assessed in relation to patient characteristics and additional laboratory information [14]. In addition to unnecessary use of diagnostic tools and laboratory services, contamination can lead to unnecessary antimicrobial usage, unnecessary additional patient care measures and increased length of stay [14]. Thus, this suggests that rapid multiplex PCR such as BCID2 has the necessary resilience to be used outside a traditional microbiology laboratory environment, supporting antimicrobial stewardship and high-quality patient care.

Empirical vs targeted antimicrobial therapy

Taken into consideration the overall detection of bacterial genera and species in our material, the recommended empirical use of benzylpenicillin and gentamicin in national guidelines would have suboptimal or no effect in 40 of 148 (27.0%) BCs and in 40 of 140 (28.6%) BCID2 detected in-panel microbes. *S. aureus* was the most frequent pathogen in this group, accounting for 50% (20 out of 40) of those. About 70% of *S. aureus* BC-isolates in Norway produce beta-lactamase, and empirical therapy with benzylpenicillin + gentamicin is therefore not considered appropriate. Betalactamase-stable penicillins (kloxacillin) would be the antibiotic of choice based on the low levels of MRSA [7]. Regarding de-escalation, correct identification of five *S. pneumoniae* strains could justify removing gentamicin treatment and continuing with benzylpenicillin alone as the prevalence of penicillin-resistance in Norwegian invasive *S. pneumoniae* is very low (1.7%) [7].

The Norwegian empirical treatment guidelines for BSI differs from many international guidelines. Gentamicin plays a central role as resistance levels in invasive *S. aureus* (0.5%), *E.coli* (6.7%) and *Klebsiella* spp. (5.2%) are fairly low [7]. A recent single-centre study of BSI outcomes in Norway supported the notion that empirical antimicrobial therapy in accordance with national guideline recommendations was associated with better outcome compared to discordant empirical therapy [15]. The preferred use of narrow spectrum antibiotics and antibiotics that are less prone to gut microbiota dysbiosis is of importance to slow down the development of AMR [16]. To optimize the resistance gene BCID2 targets in a Norwegian context, we would suggest adding determinants covering the most prevalent aminoglycoside resistance determinants in Enterobacterales supporting earlier targeted antibiotic treatment [6, 17].

Time gain

The importance of timely and effective antimicrobial therapy in BSI is well documented [18–20]. Several studies have calculated the time gain of BCID2 compared to culture-based diagnostics, with mean gain ranging from 9.7 h to at least 1 day [9, 21]. In our study, the use of BCID2 provided genus and/or species identification on average nearly 2 days earlier than the first preliminary culture-based results. The extended time gain in our material compared to others can be explained by transportation times alone. In our setting, the climate presents weather-related logistical challenges when transporting samples over more than 300 km, passing both sea and mountains. The combination of small acute care hospitals and challenging geography enhances the importance of diagnostic tools that enables rapid microbial identification. The considerably reduced

time to species identification of the most common microbes is likely to significantly support improved antibiotic stewardship. In a setting with less challenging logistics but a higher occurrence of resistant microbes, the time gain would be of great importance.

Strengths and weaknesses

We consider the sample size, strain diversity, and overall results in our study as sufficient to support the continued use of BCID2 as a supplement to standard culture-based diagnostics in our setting. The results are consistent with those from previous studies. However, additional data are needed to further evaluate the overall analytic sensitivity for each microbe. The results might have been altered if the microbe diversity and AMR found in our material covered a wider part of the panel, due to variation of analytic sensitivity between the in-panel targets [4].

The study did not include a review of patient records. Thus, the clinical indication for sampling, antibiotic use and patient characteristics are unknown. Implementation or training of laboratory staff have not been investigated and may have influenced sources of technical compliance. The actual impact of BCID2 results on antimicrobial stewardship and patient outcome are topics for upcoming studies.

Our current strategy includes BCID2 testing of one bottle only from each BC set. Additional polymicrobial BCs would probably have been detected if more reactive BC-bottles had been tested by BCID2. However, considered the overall results the cost of extended testing would most likely exceed the benefits.

CONCLUSION

The overall clinical sensitivity, high degree of analytical sensitivity and specificity strongly support that the BCID2 method is a robust supplement to traditional cultivation of positive BCs in our context. The significant time gain to microbial identification and detection of clinically important resistance determinants suggests a great clinical importance of BCID2 in small acute care hospitals with long transport time to clinical microbiology services

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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