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ORIGINAL RESEARCH

Diagnostic Accuracy of the Biofire FilmArray Blood Culture Identification 2 (BCID2) for Bloodstream Infections in Limpopo, South Africa

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Background: Bloodstream infections are a leading global cause of mortality and morbidity. Timely and accurate diagnosis improves outcomes, especially in resource-limited settings. Rapid and accurate diagnostic tools are essential for effective treatment, particularly in resource-limited settings. Our study aimed to evaluate the diagnostic performance of the BCID2 assay, comparing its effectiveness to the VITEK2 at a routine lab in Limpopo, South Africa, where alternative assays are necessary to overcome resource unavailability in areas located further away from major laboratories.

Methods: This cross-sectional study was conducted from August to December 2023. A total of 247 positive blood culture bottles were tested using both BCID2 and VITEK2 systems. Statistical analysis, including specificity, sensitivity, and accuracy, was calculated using Medcalc and SPSS.

Results: The BCID2 panel demonstrated a sensitivity, specificity, and accuracy of 95.7%, 95.5%, and 96%, respectively. Of the samples, 72% (179/247) were monomicrobial and 28% (68/247) were polymicrobial, with a total of 16 off-panel microorganisms identified across both groups. blaCTX-M was the most frequent Gram-negative resistance gene detected (55.2%, 80/145), while blaVIM was the least common (0.7%, 1/145).

Conclusion: The study concluded that the BCID2 assay demonstrates excellent diagnostic accuracy, with sensitivity, specificity, and overall accuracy rates above 95%. Its ability to rapidly detect bloodstream infections and antimicrobial resistance markers makes it a highly effective tool, particularly in resource-limited settings like Limpopo, South Africa, where timely and accurate diagnosis is crucial to improving patient outcomes.

Keywords: bloodstream infections, blood culture identification 2 panel, antimicrobial resistance genes, polymicrobial infections, monomicrobial infections

Introduction

Bloodstream infections (BSIs) constitute one of the significant causes of mortality and morbidity globally.[1–3] In 2017, there were an estimated 49 million cases of sepsis and 11 million sepsis-related deaths globally.[4,5] Thus, an early and accurate diagnosis of BSI is crucial for better clinical outcomes and an increased chance of survival.[6] Blood culture remains the gold standard for BSI screening and is followed by gram staining and other culture-based methods such as VITEK® 2 (bioMérieux, Marcy-l'Étoile, France), the automated phenotypic identification system that automates biochemical testing, usually performed in individual test tubes.[7–9] The VITEK2 uses various fluorescent biochemical panels for species identification and antimicrobial susceptibility testing.[10] The method, though not considered a gold standard, is validated and widely accepted, especially in resource-limited settings. Its phenotypic profiling mechanism helps to recognize resistance mechanisms beyond those that can be genetically identified.[11] The benefit of using conventional methods is that they provide precise species identification and a profile of antimicrobial susceptibility.[7] However, the traditional blood culture technique has a long turn-around time, requiring up to 72 hours after signal-

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positive blood cultures, rendering the results potentially clinically irrelevant to treating the affected patients promptly.[2,7,11] An ideal diagnostic system would quickly identify the pathogen-causing organisms and the factors contributing to their antibiotic resistance, enabling the start of the proper pathogen-driven therapy, and consequently improving patient outcomes.[12] The VITEK2 system is a widely validated, automated microbiology tool recognized for its accuracy in pathogen identification and antimicrobial susceptibility testing.[13–15] Its comprehensive database and standardized processes make it the reference standard for evaluating assays like the BioFire® Blood Culture Identification 2 (BCID2) Panel (BioMérieux, Salt Lake City, UT, USA), which are compared for sensitivity and specificity against VITEK2's proven performance.

Several rapid diagnostic tools have emerged in recent years, including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and fluorescence in situ hybridization (FISH).[7] While these methods significantly improve detection speed, they often require multiple preparation steps and may provide limited data in polymicrobial infections.[7] Furthermore, most of these assays offer limited information on polymicrobial blood cultures and do not provide direct identification from positive blood cultures, unlike the BCID2 panel.[7]. However, molecular technology and contemporary automation have resulted in the development of molecular-based multiplex platforms, which include the Verigene (Luminex, USA), FilmArray (bioMérieux, France), and the LightCycler SeptiFast (Roche, Germany), for rapid pathogen detection directly from blood culture bottles that flagged positive, to accelerate the detection of pathogens and resistance.[7] These assays operate exceptionally well even with polymicrobial samples and give a noticeably faster time to detection than traditional methods.[7] Moreover, molecular assays differ in turn-around time to results and pathogen and antimicrobial resistance markers identification. One of the most promising molecular assays is the BioFire FilmArray Blood Culture Identification 2 (BCID2) panel which offers a significantly quicker pathogen detection time and demonstrates acceptable accuracy even with polymicrobial (PMB) samples.[7] The BCID2 panel (bioMérieux) is an in vitro fully automated microbiological diagnostic assay that uses nested multiplex PCR analysis to identify 33 pathogens, and 10 genes for antimicrobial resistance.[7]

The BCID2 assay (bioMérieux, France) can identify 15 Gram-negative bacteria (GNB) (*Enterobacter cloacae* complex, *Escherichia coli, Acinetobacter calcoaceticus-baumannii* complex, *Bacteroides fragilis, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae* group, *Proteus spp., Serratia marcescens, Salmonella spp., Haemophilus influenzae, Neisseria meningitidis, Stenotrophomonas maltophilia, Pseudomonas aeruginosa*, and *Enterobacterales*), 11 Gram-positive bacteria (GPB) (*Enterococcus faecium, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus spp., Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus ludgunensis, Streptococcus spp., Streptococcus agalactiae, Streptococcus pneumoniae, and Streptococcus pyogenes)*, seven fungal pathogens (*Candida albicans, Candida auris, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei, and Cryptococcus neoformans)*, and 10 antimicrobial resistance genes (CTX-M, OXA-48-like, IMP, *mec*A/C, KPC, *mec*A/C and MREJC (MRSA), *mcr*-1, NDM, *vanA/B*, and VIM), in about an hour.[16] "Enterobacterales" in this case referred to organisms that fall under the family of *Enterobacterales*, that the assay can detect but cannot further speciate. The BCID2 has been evaluated in developed and developing countries and showed a high sensitivity (87 to 100%) and specificity (97–100%).[17–19]

Therefore, this study evaluated the diagnostic performance of the BCID2 panel in a routine laboratory setting in Limpopo Province, South Africa.

Methodology

Study Design

This was a cross-sectional study conducted from August to December 2023 in the Limpopo Province of South Africa at Pietersburg Hospital, Capricorn District. A cross-sectional design was chosen because it is well suited for diagnostic accuracy studies, allowing the performance of the BCID2 assay to be evaluated against a reference method at a single point in time using routine clinical samples.

Study Setting

The study took place at Polokwane National Health Laboratory Services (NHLS) in Pietersburg Hospital, Limpopo province. Limpopo Province is the fifth-largest province in northern South Africa and accounts for 10.6% of South Africa's population with an estimated population of 6.6 million.[20]

Study Population and Sampling Strategy

The study targeted patients suspected of having BSI in 19 regional health centres in Limpopo Province whose samples were sent to the Polokwane National Health Laboratory Services (NHLS), located in Pietersburg Hospital. The blood culture bottles were selected using the convenience sampling method whereby only positive blood culture bottles available in the laboratory were used.

Inclusion and Exclusion Criteria

All blood culture bottles that were sent to NHLS, Pietersburg Hospital, and flagged positive during the study period were included in the study. The minimum volumes required for inclusion were 10 mL of blood for aerobic bottles, 10 mL for anaerobic bottles, and 3 mL for paediatric bottles. Duplicate blood culture bottles, and blood cultures that only contained microorganisms commonly regarded as skin contaminants (*coagulase-negative Staphylococcus* (CNS), *Micrococcus spp., Corynebacterium spp., Bacillus spp*, other than *Bacillus anthracis*, and *Cutibacterium acnes)*, were excluded from the study.

Data Collection

Patient demographic information was obtained from the request forms that were submitted by clinicians. Positive blood culture bottles were retrieved from the Microbiology repository and processed according to standard procedures. Blood culture bottles were selected using convenience sampling where only those that were positive and available in the laboratory were selected. Each bottle represented a single patient, with no follow-up samples collected. The final sample size of 247 was determined by the number of positive cultures received during the study period and was not based on formal power calculations, reflecting the cross-sectional design and resource limitations. Bloodstream infections were classified according to the number of organisms present. Monomicrobial bloodstream infections in our study involved only one pathogen, while polymicrobial bloodstream infections often involved two or more distinct microorganisms, complicating treatment. Cultures were then classified accordingly in this study, with off-panel organisms being recorded separately to set aside those that are unrecognized species. On-panel organisms were those detected directly by BCID2, while off-panel organisms referred to pathogens that were identified by VITEK2 but were not covered by BCID2.

Data Analysis

BCID2 and VITEK2 assays were performed following the manufacturer's instructions. Patient demographics were obtained from patient request forms that were sent to the laboratory by the clinicians. This data was then loaded into Microsoft Excel sheet version 2402 for categorization and cleaning. The results from VITEK2 and BCID2 were also loaded into Microsoft Excel sheet version 2402 for further analysis. Statistical analysis, which included specificity, sensitivity, negative predictive values, positive predictive values, and accuracy, were calculated using Medcalc version 22.0 and SPSS version 29.0.

Potential confounding variables that could influence diagnostic performance were considered during data analysis. These included patient age group, hospital ward, blood culture bottle type (aerobic, anaerobic, and paediatric), prior exposure to antimicrobial therapy as documented on laboratory request forms, and the presence of polymicrobial versus monomicrobial infections. Where applicable, stratified analyses were performed to assess whether these factors influenced the sensitivity, specificity, and agreement between BCID2 and VITEK2. These variables were also considered when interpreting discordant results between the two diagnostic methods. No multivariable regression modelling was performed, as the primary objective of the study was diagnostic concordance; however, stratified assessments were used to explore the influence of relevant clinical and laboratory factors.

Ethical Considerations

Ethical approval was granted from the Turfloop Research Ethics Committee of the University of Limpopo (TREC/138/2023:PG). Consent was waived as only stored samples were used, with data anonymized. Permission to use patient samples that were stored in the Microbiology laboratory repository was obtained from the National Health Laboratory Services (NHLS) Academic Affairs and Research (AARMS) (PR2345776). There was no direct contact with the patients. Confidentiality was ensured by making the researchers sign a confidentiality form, as well as ensuring that the clinical, laboratory, and health status of the participants were not linked to their real names. The study complies with the Declaration of Helsinki.

Laboratory Methods

This cross-sectional study was carried out in Limpopo Province, South Africa, from August to December 2023. Blood samples that were flagged positive were collected from nineteen major regional health centres and processed at the Polokwane National Health Laboratory Service (NHLS) Lab in Pietersburg Hospital, Limpopo. Different types of blood culture bottles which included aerobic, anaerobic, and paediatric bottles were placed in BACTEC 9240 automatic incubator system (Becton Dickinson, USA) (Figure 1). Laboratory testing procedures BCID2 and VITEK2 testing were conducted by trained laboratory personnel following standard protocols. Blood cultures were processed in the microbiology laboratory, with BCID2 performed immediately upon sample positivity, while VITEK2 subculturing was initiated within two hours. To minimize bias, laboratory personnel conducting BCID2 and VITEK2 assays were blinded to each other's results. Gram staining was done on all samples, and sub-culturing was done on Blood, Chocolate, and MacConkey agar plates (Figure 1). Blood agar plates were incubated aerobically and anaerobically. For samples suspected of yeast, Sabouraud Dextrose agar plates were included. The plates were incubated at 37°C for a maximum of 72 hours.

For BCID2 testing, 200 µL of blood culture broth was loaded into a BioFire FilmArray BCID2 panel as per the manufacturer's instructions (Figure 1). An automated nucleic acid extraction, two-stage multiplex PCR, and DNA melting curve analysis were applied by the system.[18] BCID2 quality control test vials were used for each test batch's quality control. The VITEK2 system was also used for AST and identification of colonies (Figure 1). Colonies from sub-cultured plates were standardized to McFarland 0.5 standards using a VITEK DensiCHEK colorimeter. For Gram-positive, Gram-negative, and yeasts, specific VITEK2 cards were used, following the manufacturer's protocols. Weekly quality control was carried out with bacterial and fungal ATCC strains.

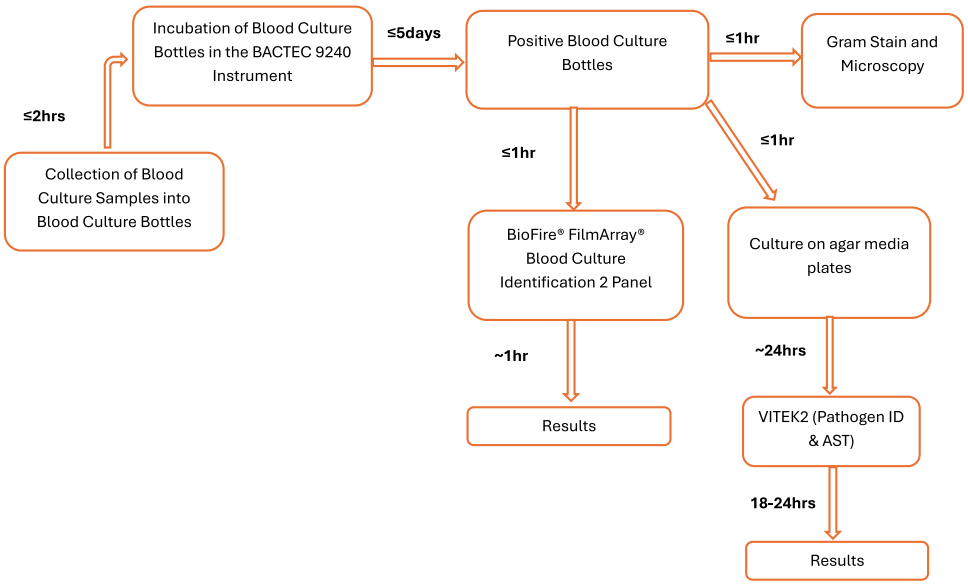

Figure 1 Laboratory methodology procedures for blood pathogen detection.

To minimise potential sources of bias, laboratory personnel performing the BCID2 and VITEK2 assays were blinded to the results of the alternative method. BCID2 testing was performed immediately after blood culture positivity, while VITEK2 testing was conducted independently following routine subculture procedures. Sample processing followed standardized, predefined laboratory protocols, and all positive blood cultures were tested consecutively during the study period to reduce selection bias. Internal quality control materials were run according to manufacturer recommendations, and external quality assurance was maintained using standard ATCC reference strains. Data entry and statistical analyses were performed independently to reduce reporting and analytical bias.

Results

A total of 247 positive blood culture bottles were collected. From the 247 blood culture bottles, 289 isolates were identified. The majority of the participants were males, constituting 53.4% (132/247) with a median age of 18.44 (IQR 0 to 36) years. Most samples belonged to the age group of infants that had less than 1 year of age, and the 21 to 40 age group with 49% (121/247) and 19.4% (48/247), respectively, while the 60 years and above age had the least number of people with 8.9% (22/247) (Table 1).

The majority of the samples 78.1% (193/247) were gram-negative bacteria (GNB) while 17.4% (43/247) were gram-positive bacteria (GPB), and 4.5% (11/247) were yeasts organisms (Table 1). A total of 72% (179/247) of the blood cultures were monomicrobial. Among these, nine isolates were identified as microorganisms that are not included in the BCID2 panel (ie., off-panel organisms). Additionally, 28% (68/247) of the blood cultures were polymicrobial, and seven off-panel organisms were identified among these. In total, 16 off-panel microorganisms were detected by VITEK2 across all samples. The highest number of samples was collected from ICU and paediatrics unit patients, 33.6% (83/247) and 29.1% (72/247), respectively (Table 1).

From the 144 samples in which AMR genes were detected, a total of 200 AMR genes were identified (Table 1). Of the 247 blood culture samples tested, AMR genes included in the BCID2 panel were not detected in 103 samples (41.7%), which may reflect susceptible organisms or resistance mechanisms not targeted by the panel. It is important to note that AMR detection was assessed per isolate, and not all isolates harbored resistance genes. Among Gram-negative isolates with detected AMR genes (n = 145), *bla*CTX-M was the most common, identified in 55.2% (80/145), and this proportion refers specifically to isolates with detected AMR genes, not all Gram-negative organisms. Overall, Gram-negative AMR genes accounted for 72.5% (146/200) of all AMR genes identified, with *bla*VIM being the least common at 0.7% (1/145). The *bla*VIM gene is a carbapenemase gene typically found in Gram-negative bacteria (GNB) and not in Gram-positive organisms.

Among the 27.5% (54/200) Gram-positive AMR genes, mecA/C was most prevalent (83.6%, 46/55), followed by mecA/C and MREJ (MRSA) at 12.7% (7/55), and vanA/B in 1.8% (1/55) of the isolates (Table 1).

The organism identification performance of the BCID2 as compared to the VITEK2 for Gram-negative Bacteria showed a minimum sensitivity, specificity, accuracy, negative predictive values, and positive predictive values of 95.7%, 95.5%, 96%, 99.5%, and 33%, respectively (Table 2), while for Gram-positive Bacteria it was 100%, 96%, 96.3%, 100%, and 50% (Table 2), respectively, and for Yeasts it was 100%. 98%, 98.4%. 100%, and 44%, respectively (Table 2). A summary of the performance indices is shown in Table 2, while a detailed organism-level analysis has been provided in the Appendix 1.

There was one sample whereby VITEK2 identified the pathogen as *Acinetobacter baumannii*, whilst the BCID2 identified it as *Serratia marcescens*. There was also one sample whereby the VITEK2 identified *Escherichia coli*, which the BCID2 could not detect. The BCID2 could not identify the *Salmonella spp., Proteus spp., and* some *Streptococcus spp*., to their species level.

Some pathogens were identified by the VITEK2 that were not on the BCID2 panel. A total of 16 BCID2 off-panel microorganisms were identified in this study, namely *Cronobacter sakazakii group (1), Pluralibacter gergoviae (1), Pseudomonas fluorescens (1), Sphingomonas paucimobilis (2), Pseudomonas putida (1), Aeromonas hydrophila (2), Acinetobacter spp. (4), Kluyvera ascorbata (1), Aerococcoccus viridans (1), Kocuria kristinae (1*), and *Leuconostoc mesenteroides ssp. dextranicum (1)*.

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Table 1 Patient Demographics, Gram Stain, and Antimicrobial Resistance in Limpopo (August to December 2023) (N=247)

Category	Percentage
Gender (n=247)	
Females	(107/247) 43.3%
Males	(132/247) 53.4%
Unknown	(8/247) 3.2%
Age (n=247)	
Infants (<1)	(121/247) 49%
1 to 20	(28/247) 11.3%
21 to 40	(48/247) 19.4
41 to 59	(24/247) 9.7%
60 and above	(22/247) 8.9%
Unknown	(4/247) 1.6%
Wards (n=247)	
Casualty/OPD	(29/247) 11.7%
ICU	(83/247) 33.6%
Pediatrics	(72/247) 29.1%
Other wards	(46/247) 18.6%
Unknown wards	(17/247) 6.9%
Gram Stain	
Blood culture bottles with Gram-positive bacteria	(43/247) 17.4%
Blood culture bottles with Gram-negative bacteria	(193/247) 78.1%
Blood culture bottles with Yeasts	(11/247) 4.5%
Antimicrobial Resistance (AMR) Genes (N=200)	
Gram-negative Bacteria AMR genes (N=145)	
*bla*CTX-M	(80/145) 55.2%
*bla*NDM	(39/145) 26.9%
*bla*OXA-48-like	(26/145) 17.9%
*bla*VIM	(1/145) 0.7%
Gram-positive Bacteria AMR genes (N=55)	
mecA/C	(46/55) 83.6%
vanA/B	(1/55) 1.8%
mecA/C and MREJ (MRSA)	(7/55) 12.7%

Table 2 Summary of BCID2 Diagnostic Performance by Organism Group Based on Gram Stain

Organism Group Based on Gram Stain	Sensitivity	Specificity	Accuracy	Negative Predictive Value	Positive Predictive Value
Gram-negative Bacteria	95.7%	95.5%	96%	99.5%	33%
Gram-positive Bacteria	100%	96%	96.3%	100%	50%
Yeasts	100%	98%	98.4%	100%	44%

Cohen's Kappa Analysis of BCID2 versus VITEK2

Cohen's kappa coefficients were calculated for each organism to assess the level of agreement between BCID2 and VITEK2. Kappa values ranged from 0.0 (slight agreement; eg., *Bacteroides fragilis, Candida krusei, Candida tropicalis*) to 1.0 (perfect agreement; eg., *Salmonella group, Haemophilus influenzae, Listeria monocytogenes, Candida glabrata*). Some organisms, such as *Enterococcus faecium* (κ = 0.717) and *Candida albicans* (κ = 0.742), demonstrated substantial agreement. Most organisms demonstrated substantial to almost perfect agreement (κ = 0.61–1.00). For example, *Klebsiella pneumoniae* group (κ = 0.954), *Pseudomonas aeruginosa* (κ = 0.933), and *Staphylococcus aureus* (κ = 0.924) showed almost perfect agreement (Table 3).

For certain organisms, such as *Candida parapsilosis*, Cohen's kappa could not be meaningfully interpreted. This was primarily due to the very low number of positive cases and skewed distributions, which limit the stability of kappa estimates. Although percent agreement and predictive values could still be reported for these organisms, the corresponding kappa values are considered unreliable and should be interpreted with caution.

When pooling all organisms, the overall Cohen's kappa was 0.875, indicating almost perfect agreement between BCID2 and VITEK2 across the dataset (Table 3).

Table 4 compares the turnaround times for organism identification using the BioFire FilmArray BCID2 panel and the conventional subculturing with VITEK2 method. The BCID2 panel provided results within approximately 1.5 hours after blood culture positivity, whereas the traditional subculturing and VITEK2 workflow required 48–96 hours.

Table 3 Cohen's Kappa Coefficients for Agreement Between BCID2 and VITEK2

Organism	TP	FP	TN	FN	Kappa	Interpretation
Acinetobacter calcoaceticus-baumannii complex	45	9	192	1	0.875	Almost perfect
Bacteroides fragilis	0	4	243	0	0.0	Slight
Enterobacter cloacae complex	24	4	219	0	0.914	Almost perfect
Escherichia coli	22	4	220	1	0.887	Almost perfect
Klebsiella aerogenes	1	2	244	0	0.497	Moderate
Klebsiella pneumoniae group	55	4	188	0	0.954	Almost perfect
Proteus spp.	5	1	241	0	0.907	Almost perfect
Salmonella group	4	0	243	0	1.0	Almost perfect
Serratia marcescens	6	2	239	0	0.853	Almost perfect
Haemophilus influenzae	1	0	246	0	1.0	Almost perfect
Neisseria meningitidis	0	0	247	0		Not calculable
Pseudomonas aeruginosa	33	4	210	0	0.933	Almost perfect
Enterococcus faecalis	24	9	214	0	0.822	Almost perfect
Enterococcus faecium	14	10	223	0	0.717	Substantial
Listeria monocytogenes	1	0	246	0	1.0	Almost perfect
Staphylococcus aureus	20	3	224	0	0.924	Almost perfect
Streptococcus agalactiae	4	2	241	0	0.796	Substantial
Streptococcus pneumoniae	1	1	245	0	0.665	Substantial
Streptococcus pyogenes	0	0	247	0	-	-
Candida albicans	6	4	237	0	0.742	Substantial
Candida glabrata	1	0	246	0	1.0	Almost perfect
Candida krusei	0	2	245	0	0.0	Slight
Candida parapsilosis	4	5	240	0	0.607	Invalid*
Candida tropicalis	0	1	246	0	0.0	Slight
Cryptococcus neoformans	0	0	247	0	-	-
Overall	**271**	**71**	**5833**	**2**	**0.875**	**Almost perfect**

Notes: Cohen's Kappa Interpretation Scale: κ < 0.00 → Poor agreement (worse than chance), κ = 0.00–0.20 → Slight agreement, κ = 0.21–0.40 → Fair agreement, κ = 0.41–0.60 → Moderate agreement, κ = 0.61–0.80 → Substantial agreement, κ = 0.81–1.00 → Almost perfect agreement.* For Candida parapsilosis, the kappa value is marked as "Invalid" because the very low number of positive cases and highly skewed distribution made the statistic unstable and not meaningful for interpretation.

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Table 4 Comparison of Time to Result Between BCID2 Panel and Subculturing+VITEK2

Method	Average Time to Results After Blood Culture Positivity
BioFire FilmArray BCID2 Panel	1.5hrs
Subculturing + VITEK2	48-96 hours

Discussion

This study underscores the importance of rapid and accurate diagnostics for bloodstream infections (BSIs). The BCID2 Panel demonstrated excellent diagnostic accuracy with minimal detection failures, aligning closely with conventional methods. These findings highlight the study's aim to advance early and accurate detection of BSIs, emphasizing its potential to enhance patient outcomes and reduce healthcare system burdens. This aligns with broader efforts to improve clinical management and address the high mortality and morbidity associated with severe infections.

The current study showed that the BCID2 offers a high identification accuracy, as compared to the conventional method, the VITEK2. Additionally, the BCID2 shortened the identification time from 48–72 hours to about an hour, which is similar to what was also reported in studies conducted in France and United Arab Emirates by Caméléna et al and Senok et al, respectively.[16,21]

The overall concordance between culture methods and BCID2 for pathogen identification was very good at 96% and it was similar to what was reported in a study conducted in Germany by Berison et al, who reported 94% concordance.[17] This high concordance rate is encouraging; however, it should be noted that not all BCID2 targets were found in our samples, making it impossible to assess how well they performed. All off-panel organisms yielded a negative result with the BCID2. Beyond concordance, agreement between BCID2 and VITEK2 was further evaluated using Cohen's kappa coefficients. The overall kappa value was 0.875, indicating almost perfect agreement between the two methods. Most organisms demonstrated substantial to almost perfect agreement ($\kappa = 0.61–1.00$), supporting the reliability of BCID2 in accurately identifying bloodstream pathogens. However, a few organisms showed only slight agreement, underscoring the importance of continued evaluation of BCID2 performance across diverse microbial targets.

The microbial profile of our study revealed that the predominant organism was found to be 78% (193/247) of GNB, while only 17% (43/247) were found to be GPB and 4% (11/247) were yeasts. This was similar to the results obtained in a study conducted in China by Li et al, whereby they obtained 76.7% GNBs and 23.3% GPBs.[22] Additionally, a study in France by et al highlighted the BCID2 panel's high diagnostic reliability.[16]

The monomicrobial and polymicrobial BSIs found in our study were 72% (179/247) and 28% (68/247), respectively. Similarly, in studies conducted in Hong Kong and France by Sze et al and Caméléna et al, respectively, they also obtained a high number of monomicrobials, 87.1% and 85.5%, respectively, as compared to polymicrobial, 12.9% and 14.5%, respectively.[16,23] A total of 21% (52/247) with polymicrobial infections were detected by the BCID2 assay but were missed by the VITEK2 assay. The isolates were not picked by the VITEK2 which reported the result as monomicrobial. The inability to pick some poly-microbials by the VITEK 2 assay may prevent proper care of patients. In cases of polymicrobial infections, the presence of dominant organisms might overshadow others on agar plates which may lead to misdiagnosis and affect the choice of organisms picked for VITEK2 identification testing. A study conducted by Manyahi and colleagues, in 2020 highlighted that PMB BSIs are associated with high mortality rates; roughly twice that of monomicrobial infections, and patients with polymicrobial infections stay longer in hospitals than those with monomicrobial BSI.[24] Thus, an assay must be able to detect polymicrobial BSIs, as VITEK2 detects polymicrobials from culture plates, but overgrowth can mask others. BCID2 detects multiple organisms directly from broth pre-culture.

False negative discrepancies between the BCID2 and the VITEK2 were found on 18 (6%) isolates. Of the total of 18 (6%) cases that the BCID2 failed to identify correctly, 16 (5.5%) were off-panel microorganisms, while two were on-panel microorganisms. Of the 16 off-panel microorganisms identified, *Cronobacter sakazakii, Pluralibacter gergoviae, Pseudomonas fluorescens, Sphingomonas poucimobilis, Pseudomonas putida, Kocuria kristinae*, and *Leuconostoc mesenteroides spp dextranicum*, have been previously reported as rare causes of clinical human infections.[25–30] As

a result, the BCID2 Panel identified clinically significant infections with a reasonable degree of agreement with conventional approaches and minimal detection failures overall, which agrees with the study that was conducted in Norway by Harboe-Sjåvik et al.[19] Our study also classified the organisms as rare causes of BSIs.

In addition to overall diagnostic accuracy, BCID2 demonstrated a clear advantage in the detection of polymicrobial bloodstream infections. This superior performance is largely due to its multiplex PCR-based technology, which enables the simultaneous identification of multiple bacterial and fungal targets directly from positive blood culture bottles. In contrast, VITEK2 depends on culture-based isolation, where dominant or fast-growing organisms may suppress the detection of slower-growing or less abundant pathogens, leading to potential under-recognition of mixed infections. This capability is particularly important in resource-limited healthcare settings, where delayed or missed identification of polymicrobial infections can significantly impact patient outcomes. By reducing the need for repeated subcultures and shortening the time to comprehensive pathogen identification, BCID2 supports earlier targeted therapy and strengthens antimicrobial stewardship efforts in low-resource environments.

The discrepancies of the BCID2 on-panel isolates included a case where BCID2 identified the organism as *Serratia marcescens,* while VITEK2 identified it as *Acinetobacter baumannii*. While the BCID2 panel has shown to perform high diagnostic accuracy as compared to the VITEK2, it is also necessary to highlight some cases where specific organisms displayed significantly higher false positive rates. For example, *Acinetobacter baumannii, Enterococcus faecium, Enterococcus faecalis* and *Candida parapsilosis* showed greater than twenty percent false positives, which is likely a result of cross-reactivity, contamination or detection of non-viable DNA.[2,31] Other studies evaluating the BCID2 panel have described similar aspects where detection of non-viable organisms or residual DNA may result in false positive results.[2,31] This underscores the importance of interpreting molecular findings together with culture results and clinical context to prevent misdiagnosis.[31] Another discrepancy was seen when the VITEK2 identified the organism as *Escherichia coli* but BCID2 did not detect it. This could happen due to low quantities of bacteria present in the sample, of which culture can amplify the quantity of bacterial populations to detectable levels. VITEK2 uses phenotypic analysis through biochemical tests, while BCID2 uses molecular methods for specific genetic detection. As a result, low or degraded DNA would cause BCID2 to miss targets. These differing techniques may account for the differences in detection of the results.

Additional discrepancies were due to the further identification of organisms by the BCID2. It should be noted that colonies of distinct coagulase-negative staphylococcal species can frequently be mistaken for one another.[17] As a result, it is not impossible to assume that choosing the incorrect colony for a VITEK2 species analysis could account for at least some discrepant results.[17] Furthermore, it is likely that other gram-positive organisms that were only identified by molecular BCID2 analysis were prevented or concealed from growing due to the increased proliferation of Gram-negative bacteria.[17] Discrepancies between BCID2 and VITEK2 systems reveal key differences in pathogen detection and resistance profiling. BCID2 excels in detecting pathogens like *Candida auris* and *Candida parapsilosis*, which VITEK2 occasionally misindentifies, likely due to its reliance on viable cultures. However, BCID2's sensitivity can lead to false positives from contamination or DNA remnants. The BCID2 assay showed some false positives, including 9 for *Acinetobacter baumannii* and *Enterococcus faecalis*, 10 for *Enterococcus faecium*, and 5 for *Candida parapsilosis*. These may result from the detection of non-viable DNA, contamination, or subclinical organisms, emphasizing the need for cautious interpretation alongside conventional methods and clinical correlation. This was seen in this study when the BCID2 picked additional organisms, which were not picked by the VITEK2.

The observed discrepancies between BCID2 and VITEK2 have important clinical implications. Failure to detect certain pathogens by either method may result in inappropriate empirical therapy, delayed initiation of targeted antimicrobial treatment, or unnecessary broad-spectrum antibiotic use. In critically ill patients, particularly those with sepsis, delayed or incorrect antimicrobial therapy has been associated with increased morbidity and mortality. Conversely, false-positive results may lead to overtreatment and contribute to antimicrobial resistance. The rapid detection capability of BCID2 may allow earlier modification of therapy in cases where VITEK2 fails to promptly identify pathogens. Understanding and addressing these discrepancies is therefore essential to optimise clinical decision-making, improve patient outcomes, and support antimicrobial stewardship, particularly in resource-limited healthcare settings.

The faster turnaround time of BCID2 has important implications for clinical decision-making. Rapid identification of pathogens directly from positive blood cultures enables clinicians to initiate earlier targeted therapy, rather than relying solely on broad-spectrum empirical treatment. This is particularly critical in polymicrobial bloodstream infections, where delayed or incomplete organism identification may result in suboptimal antimicrobial coverage. In addition, the ability of BCID2 to rapidly detect key antimicrobial resistance markers supports timely optimisation of therapy, contributes to antimicrobial stewardship, and may reduce the emergence and spread of resistant organisms. In resource-limited healthcare settings, these time savings are especially valuable, as they can directly influence patient outcomes by reducing treatment delays and improving the precision of antimicrobial prescribing.

While the study highlights the technical advantages of BCID2, its implementation in Limpopo also depends on economic feasibility, including instrument costs, consumable prices, and personnel training requirements. These financial considerations may limit widespread adoption in resource-constrained facilities despite clear clinical benefits. Future cost-effectiveness analyses are therefore needed to determine whether BCID2 offers sufficient value to justify routine implementation in the province.

However, the BCID2 similar to all other molecular assays relies on genetics-based mechanisms of resistance, which fail to recognize non-genetic causes of resistance and generally account for a relatively few genes, the hot-spot mutations or frequent mutations, leaving other crucial unknown and compensatory genes that may contribute to drug susceptibility results.[2]

Strengths of the Study

The study was a multi-centre study, with samples drawn from 19 different health centres, providing diversity in population and pathogen ID.

Limitations of the Study

The study included 247 positive blood culture bottles and did not use a formal power calculation, as the sample size was determined by the number of positive cultures available during the study period. While this approach is common in diagnostic accuracy studies conducted in routine laboratory settings, the relatively modest sample size may limit statistical power, particularly for rare pathogens and antimicrobial resistance genes, and may reduce the precision of some performance estimates. Consequently, the findings should be interpreted with caution, and larger studies are warranted to confirm these results.

Data collection was limited to the Limpopo region, which may limit the generalizability of results to other areas; results may potentially reflect a unique distribution of pathogens and resistance genes. Since this was a cross-sectional study, it was not possible to assess the resurgence of resistant pathogens or the long-term performance of the BCID2 system. Longitudinal studies are needed to provide a clearer understanding of the dynamics of bloodstream infection pathogens and their associated antimicrobial resistance genes, thereby informing the development of more effective diagnostic and treatment strategies. The study's primary analysis was based on blood culture bottles rather than individual isolates, which complicates performance interpretation in polymicrobial infections. Future analyses will re-express diagnostic accuracy based on isolate-level data to improve comparability with other studies. A genotypic method (BCID2) was compared with a phenotypic method (VITEK2), which could have possibly led to the misidentification and missing of some resistance markers and misinterpretation of the AST results by the VITEK2. Because no third reference method (eg., 16S rRNA sequencing) was used, discrepant results between BCID2 and VITEK2 could not be resolved. Some BCID2 "false positives" may have been true positives that VITEK2 missed, which likely underestimated the BCID2's specificity and accuracy. Future studies should include an independent gold-standard method to more accurately assess performance. The type of sampling method used in the study could have possibly introduced selection bias.

Although blinding and standardized workflows were used to minimise bias, the use of convenience sampling may still have introduced some selection bias.

The limited sample size and restriction to a single province may reduce the generalizability of our findings, particularly for rare pathogens and resistance genes that were underrepresented. These constraints may also affect the precision of diagnostic performance estimates, meaning that some accuracy measures should be interpreted with caution.

Future studies involving larger, multi-provincial cohorts and longitudinal surveillance are needed to validate these results and better characterize pathogen diversity and resistance patterns across South Africa.

Recommendations

In the future, the BCID2 should be used in laboratories to rapidly and accurately identify pathogens that cause BSIs and antimicrobial resistance genes so that treatment will be administered early to patients, thereby reducing the mortality and morbidity rates of BSIs within the province. However, an additional detection method has to be added to identify pathogens and AMR genes not covered by the BCID2 panel. Furthermore, future research should aim to determine the cost-effectiveness and technical competence required for the adoption of the BCID2 panel for routine use in resource-limited settings.

Conclusion

The BCID2 assay demonstrates excellent diagnostic accuracy, with sensitivity, specificity, and overall accuracy rates above 95%. Its ability to rapidly detect bloodstream infections pathogens and antimicrobial resistance markers makes it a highly effective tool, particularly in settings where timely and accurate diagnosis is crucial to improving patient outcomes, however there is a need for formal cost effectiveness analysis for implementation in resource limited setting.

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Data Sharing Statement

The authors confirm that data is available from the corresponding author I.R upon request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

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