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Automated blood culture systems for isolation of bacterial pathogens of bloodstream infection: The experience of Bobo-Dioulasso Teaching Hospital, Burkina Faso

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Abstract:

Background: Identification of the causative agent is an essential requirement for better treatment of bloodstream infection. The BacT/Alert 3D (BioMérieux, Marcy l'Étoile, France), is a blood culture system equipped with CO_2 sensors to monitor the growth of microorganisms in blood culture bottles designed to optimize bacterial growth. The aim of this study was to determine the performance of this equipment in detecting bacterial pathogens from patients with bloodstream infection in the context of low-and-middle-income countries (LMICs), with Bobo-Dioulasso Teaching Hospital as a case study.
Methodology: A cross-sectional study was conducted over a period of 5 months at the Sourô Sanou University Hospital, Bobo-Dioulasso, Burkina Faso, a low-income country. Blood samples from a total of 231 patients with clinical suspicion of bloodstream infections were collected and processed according to the manufacturer's instructions.
Results: Sixty-nine of the 231 blood culture samples of patients were positive, giving a bacteriological yield of 29.9%. *Escherichia coli, Salmonella* spp, and *Staphylococcus aureus* were the top three bacterial species isolated.
Conclusion: The implementation of the BacT/Alert 3D system has significantly enhanced the diagnosis of bacteraemia in the Bobo-Dioulasso Teaching Hospital. This enhancement is marked by time savings in patient care, reduced staff workload, and an increased bacteriological yield over time.

Keywords: Bloodstream infection, Automated blood culture, Low-and-middle-income-countries

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Systèmes automatisés d'hémoculture pour l'isolement des bactéries pathogènes des infections sanguines: l'expérience de Hôpital Universitaire de Bobo-Dioulasso, Burkina Faso

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Résumé:

Contexte: L'identification de l'agent causal est une démarche essentielle pour améliorer le traitement des sepsis.

Le système d'hémoculture BacT/Alert 3D (BioMérieux, Marcy l'Étoile, France) est équipé de capteurs de CO_2 pour surveiller la croissance des micro-organismes dans des flacons d'hémoculture conçus pour optimiser la croissance bactérienne. L'objectif de cette étude était de déterminer la performance de cet équipement dans la détection des pathogènes bactériens chez les patients présentant des signes d'un sepsis dans le contexte des pays à revenu faible et intermédiaire (PRFI), avec le Centre Hospitalier Universitaire (CHU) de Bobo-Dioulasso comme cas d'étude.

Méthodologie: Une étude transversale a été menée sur une période de 5 mois au CHU Sourô Sanou de Bobo-Dioulasso, Burkina Faso, un pays à faible revenu. Des échantillons de sang de 231 patients présentant une suspicion clinique de sepsis ont été prélevés et traités conformément aux instructions du fabricant.

Résultats: Soixante-neuf des 231 échantillons d'hémoculture étaient positifs, ce qui représente un rendement bactériologique de 29,9%. *Escherichia coli*, *Salmonella* spp et *Staphylococcus aureus* étaient les trois espèces bactériennes les plus fréquemment isolées.

Conclusion : L'acquisition du système BacT/Alert 3D a considérablement amélioré le diagnostic des bactériémies au CHU de Bobo-Dioulasso. Cette amélioration se reflète par un gain de temps dans les soins aux patients, une réduction de la charge de travail du personnel et une augmentation du rendement bactériologique au fil du temps.

Mots-clés: Sepsis, Hémoculture automatisée, Pays à revenu faible et intermédiaire.

Introduction:

In the realm of clinical infections, bacteraemia represent a grave concern (1). Their unchecked progression can escalate to sepsis, often marked by a graver prognosis. Mortality rates associated with sepsis span from 10 to 50%, contingent on the severity of the concomitant pathology (2). However, the clinical significance of bacteraemia is frequently underestimated in low-and-middle-income-countries (LMICs), especially in the sub-Saharan Africa. Within these regions, fevers are commonly misattributed to malaria or addressed through empirical antibiotic treatment due to limited access to microbiological diagnosis. This practice inadvertently fuels the surge of antimicrobial resistance (3–5).

A cornerstone for optimal management of bloodstream infections (BSIs) is the identification of the causative agent. Despite marked advancements in clinical microbiology diagnostics, blood culture remains the 'gold standard' method for diagnosis of BSIs (6). In response to the inherent limitations of manual and semi-automated blood culture techniques, automated diagnostic systems that are reliant on continuous bacterial growth monitoring have emerged. Within this array of blood culture systems, the BacT/Alert 3D system (BTA3D, BioMérieux, Marcy l'Étoile, France) stands out, employing CO_2 sensors for growth surveillance. This system employs culture media designed to address inherent blood culture challenges (7).

Ordinarily, blood is sterile and contains antimicrobial elements that suppress bacterial proliferation during infections. Augmenting BC sensitivity thus mandates substantial blood volumes drawn from 2-4 pairs of bottles (6). Regrettably, in Burkina Faso and similar LMICs, adhering to these recommendations is problematic due to patients' limited financial means and the absence of universal health insurance, leading to cost burdens. Consequently, a single blood sample for two blood culture bottles is a common practice (8).

Considering these constraints, we seek to uncover the potential contribution of this automated blood culture system in practical usage. Here, we present our experience with the BTA3D, acquired amid resource constraints in the preceding one year. This study aims to highlight the performance of this equipment in the identification of bacterial pathogens from clinical cases of BSIs within the context of the Bobo-Dioulasso Teaching Hospital, Burkina Faso.

Materials and method:

Study setting:

Burkina Faso, situated in West Africa, represents a low-income country with an estimated population of 21,840,865 in the year 2022 (9). A substantial segment of its population (45.3%) resides below the poverty threshold of annual $ 115 per adult. The country's health infrastructure is underdeveloped, particularly in terms of diagnostic capabilities within laboratories. These diagnostic deficiencies hinder the effective management of prevalent infectious diseases. Compounded by the challenges of poverty and malnutrition, the prognosis of such diseases is further complicated in this resource-constrained setting (10,11).

Study design:

This was a hospital-based cross-sectional study conducted from October 1, 2015, to February 29, 2016, on patients with clinical features of bloodstream infection from whom blood culture samples were aseptically collected and submitted for routine analysis at the Department of Bacteriology laboratory of the Bobo-Dioulasso Teaching Hospital, Burkina Faso.

Blood sampling, inoculation of culture bottle, and incubation in BacT/Alert 3D:

Blood samples were collected from hospitalized patients with extreme body temper-

atures (>38°C or ≤ 36°C) as indicative of potential BSIs. A tourniquet was applied to the arm, followed by vein palpation and antiseptic application at the puncture site.

A sterile approach was maintained after antisepsis. Blood was drawn using a needle and syringe (or butterfly needle) and dispensed into specific blood culture bottles. Approximately 20 ml of blood was collected from adults, and dispensed into one aerobic (FAN adult, BioMérieux) and one anaerobic (FAN adult anaerobic, BioMérieux) culture bottles. About 5 ml was collected from paediatric patients into a paediatric blood culture bottle (FAN paediatric, BioMérieux). Established protocols were adhered to (6,7,12). Seeded blood culture bottles were placed in the BacT/Alert 3D system (BTA3D) for incubation at 37°C for up to 7 days, optimizing microbial growth detection.

Subculture of positive blood culture bottles:

Upon detection of positive blood cultures by the BacT/Alert 3D system, Gram stain microscopic examination was performed which guided subculture on suitable agar media. Subculture media were incubated at 37°C aerobically for 18-24 hours.

Microbial identification and antimicrobial susceptibility testing:

Microbial identification from positive cultures was achieved through culture morphology and biochemical identification tests (13). For Enterobacterales and non-fermenting Gram-negative bacilli identification, API 20 E and API 20 NE strips (BioMérieux) were respectively utilized. For Gram-positive cocci, selective media and conventional biochemical tests were employed for identification. Culture results were evaluated by a senior biologist to distinguish between causative pathogens and contaminants.

Antimicrobial susceptibility testing (AST) was performed on pathogenic isolates by the modified Kirby-Bauer disc diffusion method in line with the 2015 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (14). Internal quality control measures were employed to assess all stages of the blood culture test, encompassing incubation, solid media preparation, Gram staining, biochemical identification and AST. This process utilized reference strains, adhering to the EUCAST guideline (14).

Data management and ethical considerations:

Data collection was conducted from the blood culture registry and recorded in Microsoft Excel software version 2015. Patient anonymity and confidentiality were rigorously maintained. The study was smoothly incorporated into the standard care delivery framework and did not interfere with patient treatment. Given its alignment with routine healthcare procedures and the absence of any negative impact on patient management, no evaluation by the Research Ethics Committee was required in accordance with national regulations.

Results:

Patients' characteristics

A total of 231 requests for blood culture were received during the study period from 231 patients, with a mean age of 41±12 years and a gender ratio of 1.17. Most blood culture requests were from the Department of Paediatric Medicine (61%), followed by Department of Infectious Diseases (11%) and the Intensive Care Units (7%).

Bacteriological yield:

Sixty-nine blood culture bottles were positive, giving a bacteriological yield of 29.9%. This yield remained approximately the same regardless of the gender or age group of the patients. One patient had positive growth in both anaerobic and aerobic bottles but only the aerobic bottle was evaluated by sub-culture. All subcultures were positive, yielding 69 bacterial isolates belonging to 10 different species as shown in Table 1**.**

The morphological groups of bacteria isolated were almost equally represented with 50.8% being Gram-negative bacilli and 49.2% Gram-positive cocci. *Escherichia coli, Salmonella* spp., and *Staphylococcus aureus* were the three most frequently isolated species. *Staphylococcus epidermidis* was isolated in 14.4% of positive subcultures.

Results of antibiotic susceptibility testing:

The antibiotic susceptibility of *E. coli, Salmonella* spp*.,* and *S. aureus* strains is as shown in Table 2. *Salmonella* spp*.*, showed overall good sensitivity to ciprofloxacin (90. 9%) and gentamicin (100.0%). They were resistant to penicillin and cephalosporins in 80.9% and 36.4% of cases respectively. *S. aureus* was resistant to cefoxitin in 5.8% of cases, which was associated with resistance to fluoroquinolones and aminoglycosides in 58.9% and 60.6% of cases respectively. *E. coli* isolates were resistant to at least one penicillin, one cephalosporin, and imipenem in 93.6%, 80%, and 60.0% of cases respectively.

Table 1: Distribution of isolated bacterial pathogens from blood cultures of patients with bloodstream infection at Sourô Sanou Teaching Hospital, Bobo-Dioulasso, Burkina Faso

Bacterial species		**Number of isolates**	**Proportion (%)**
Gram positive cocci (n=34)	*Staphylococcus aureus*	17	24.6
	Staphylococcus epidermidis	10	14.4
	Enterococcus spp.	07	10.1
Enterobacterales (n=31)	*Klebsiella pneumoniae*	01	1.5
	Citrobacter freundii	01	1.5
	Escherichia coli	15	21.7
	Enterobacter spp.	03	4.4
	Salmonella spp.	11	15.9
Non fermentative (n=4)	*Acinetobacter* spp.	02	2.8
	Pseudomonas spp.	02	2.8
Total		**69**	**100.0**

[1] n: number

Table 2: Antibiotic susceptibility of the three main bacteria pathogens isolated from blood cultures of patients with bloodstream infection at Sourô Sanou Teaching Hospital, Bobo-Dioulasso, Burkina Faso

Bacterial isolates	**Percentage of isolates sensitive to antibiotics**										
	AMC	AX	CAZ	GM	CP	CN	IM	L	ER	FOX	PG
E. coli (n=15)	20	6.6	20	26.6	66.6	46.6	40	-	-	-	-
Salmonella spp (n=11)	36.3	9.1	72.7	63.6	90.9	100	45.4	-	-	-	-
S. aureus (n=17)	-	-	-	-	41.1	29.4	-	52.9	17.6	5.8	17.6

AX: amoxicillin; AMC: amoxicillin + clavulanic acid; PG: penicillin G; CZ: ceftriaxone; CAZ: Ceftazidime; GM: gentamicin; CP: ciprofloxacin; IM: imipenem; FOX: Cefoxitin; ER: erythromycin; L: Lincomycin; -: Not performed

Discussion:

This study aimed to comprehensively assess the utility of the automated BacT/Alert 3D system (BTA3D) in comparison to manual detection systems for BSIs within the specific setting of the Bobo-Dioulasso Teaching Hospital, one year following equipment acquisition. The investigation predominantly centered on elucidating potential improvements in bacteriological yield, elucidating the advantages of the BTA3D, addressing contamination reduction, streamlining patient care, reducing staff workload, and mitigating healthcare costs in the context of limited resources.

Enhanced bacteriological performance:

Within the period of study, a total of 231 blood cultures were processed, with 69 of them testing positive, giving a bacteriological yield of 29.9%. This reported yield aligns closely with findings from comparable studies utilizing automated systems (15,16). For instance, Okomo et al., (17) conducted research in Gambia in 2011, reporting a bacteriological yield of 27.1%. This yield outperformed analogous studies by Ki-Zerbo et al., (18), Ouédraogo et al., (19) and Bahwere et al., (20) which reported yields of 19.8%, 18.3% and 15.9% respectively. Noteworthy is that these studies were conducted within similar contextual constraints and employed manual detection methods.

The substantial deviation of approximately 10 percentage points underscores the pivotal contribution of automated systems in elevating bacteriological performance. The pronounced efficiency witnessed with the BTA3D system can be attributed, in part, to the composition of the blood culture media. In contrast to the studies cited above, the BTA3D blood bottles encompass a unique blend of saponin, resin, absorbent polymeric bile, sodium polyanetholsulfonate, and complex amino acid substrates (7,12). Saponin functions as a lytic agent, facilitating the release of intracellular microorganisms without impeding their growth. The presence of resin neutralizes antimi-

crobial activity in blood and augments surface area, thereby promoting microbial growth in biofilms. These attributes collectively augment the positivity rate of the automated method (2,12). Conversely, the hemoline media employed in studies by Ouedraogo et al., (18) in Burkina Faso in 2012 and Bahwere et al., (20) in Congo in 2001 lack some of the key components present in the BTA3D blood culture bottles.

Superiority of the BTA3D:

Our series exhibited a marginal preponderance of Gram-negative bacilli (GNB) at 50.8% (comprising Enterobacterales and non-fermenting organisms) juxtaposed with 49.2% Gram-positive cocci (GPC). These findings parallel those reported by Elouennass et al., (21) in Morocco in 2008 with 49.3% GNB and 46.8% GPC. However, Karlowsky et al., (22) in the US in 2004 reported an opposing trend with GPC dominance (78.1%). The divergence in results can be attributed to the distinct bacterial ecology and population characteristics.

The prevalence of children and infants in our study population likely contributes to a higher propensity for GNB-associated bacteraemia such as salmonellosis, in line with findings from other developing countries (21,23). Comparing the BTA3D to other automated blood culture systems highlights its discernible efficacy in detecting Enterobacterales. This proficiency is particularly advantageous in regions where limited hygiene and water access culminate in gastroenteritis and subsequent bacteraemia (5,17).

Contamination mitigation:

The issue of contamination in blood culture is a critical concern in the diagnosis of bloodstream infections. Coagulase-negative staphylococci, while capable of causing BSIs, are often indicative of contamination, accounting for approximately 70% of cases (27). In our study, we observed a coagulase-negative staphylococci rate of 14.4%, which could be closely aligned with the actual contamination rate within our study. However, it significantly exceeds the expected 2-5% rate reported in the literature for developed countries (28). Conversely, when compared to 19.3% contamination rate reported by El Kettani et al., (29) in 2017, this finding exhibits a slight discrepancy.

This minor variation may be attributable to variances in sampling techniques and the inherent higher risk of contamination associated with manual detection methods. Daily manual assessment of blood cultures involves frequent handling of culture bottles, increasing susceptibility to contaminants from commensal and environmental human flora, particularly enteric bacteria (28).

Clinical efficiency and staff workload:

The study yielded zero false positives when compared to subculture results. This outcome is particularly promising, as it enables prompt validation of clinical suspicions regarding the infectious origin of fever without awaiting complete identification and antimicrobial susceptibility testing (30). Such an approach expedites treatment modifications while awaiting comprehensive results. Additionally, the automated detection capability curtails laboratory personnel workload.

Manual methods necessitate daily visual scrutiny of blood culture bottles to identify those conducive to bacterial growth, engendering a higher likelihood of errors, especially among inexperienced staff. The BTA3D, with its CO_2 sensor-based detection mechanism, minimizes error risk and work burden, thus optimizing resource allocation within LMICs (24).

The reduction of costs of care for sepsis in the context of poverty:

The augmented sensitivity and swifter etiological diagnosis of fevers facilitated by the BTA3D system can potentially mitigate inpatient treatment costs. Given the absence of widespread blood culture availability in Burkina Faso, febrile syndromes are often treated empirically, leading to inappropriate use of expensive broad-spectrum antibiotics (5), contributing to both exacerbation of antimicrobial resistance and impoverishment of the family due to the lack of universal health insurance (11).

By enhancing the likelihood of identifying the causative agent in a timelier manner, the BTA3D can contribute to shorter hospital stays and more judicious resource utilization, thereby alleviating the financial burden associated with ineffective treatments.

Study limitations and perspectives:

Subcultures of bottles reported negative by BTA3D could not be performed due to lack of resources. Such cultures would help determine the false negative rate. Therefore, a study comparing the BTA3D to another device in the same line would better highlight the strengths and weaknesses of this equipment. In addition to the large volume of blood required, this experiment requires financial resources that are lacking in the Burkina Faso context.

Conclusion:

Blood culture continues to hold its significance as a crucial diagnostic tool for establishing the microbiological basis of bacteraemia. However, manual detection methods in LMICs exhibit suboptimal performance and are burdened with time-intensive processes.

The integration of the BTA3D automated detection system has emerged as a transformative step in enhancing the diagnosis of bacteraemia, as evidenced by the observed yield of 29.9% at the Bobo-Dioulasso Teaching Hospital.

This study underscores the substantial improvements brought about by the BTA3D, addressing both efficiency and accuracy concerns associated with conventional manual methods. Nonetheless, a comprehensive evaluation that directly compares the BTA3D with another device within the same domain would provide a more nuanced understanding of its capabilities and limitations. Such an assessment holds the potential to contribute to the advancement of bacteraemia diagnosis and management strategies, offering valuable insights for clinical practice in LMICs and beyond.

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Contributions of authors:

AN was involved in the data analysis, and writing of the manuscript; MK was involved in data compilation; ODK, BS, and CY were involved in the writing of the manuscript; ASO was involved in the supervision of the project and correction of the manuscript; IT, JZ, and AO were involved. All authors approved the final manuscript submitted.

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Conflicts of interest:

Authors declare no conflict of interest.

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