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(RESEARCH ARTICLE)

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Diagnostic advances in sepsis: A comparative analysis of syndromic approaches and molecular biology techniques in clinical bacteremia

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Abstract

Sepsis remains a leading cause of morbidity and mortality. This retrospective study, conducted at Avicenne Military Hospital in Marrakech over ten years, analyzed 3721 blood cultures, identifying 420 cases of bacteremia (11.3% positivity rate). The predominant isolates included *Staphylococcus aureus*, coagulase-negative *staphylococci*, *Escherichia coli*, *Klebsiella spp*., and *Acinetobacter baumannii*, with significant resistance patterns observed. Notably, 50% of *S. aureus* were methicillin-resistant (MRSA), while extended-spectrum beta-lactamase (ESBL) production was found in 30% of *E. coli* and *Klebsiella pneumoniae* isolates. The FilmArray multiplex PCR system was utilized to enhance rapid pathogen identification, reducing turnaround time from 72 hours to under 2 hours, and demonstrated high concordance with conventional cultures. The molecular diagnostics identified various resistant strains, including carbapenemase-producing organisms in *K. pneumoniae* and multidrug-resistant *A. baumannii*, underscoring the critical need for effective antimicrobial stewardship. Overall, this study emphasizes the evolving epidemiology of bacteremia, the rising antimicrobial resistance, and the utility of rapid molecular diagnostics in guiding timely and appropriate treatment, ultimately aiming to improve patient outcomes in sepsis management.

Keywords: Sepsis; Antibiotic Resistance; Epidemiology; Film Array; Molecular biology

1. Introduction

Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection, remains a major cause of morbidity and mortality globally. Bacteremia, defined as the presence of viable bacteria in the bloodstream, plays a critical role in the pathogenesis of sepsis and septic shock. Despite advances in medical care, the incidence of bacteremia continues to rise, largely due to increased use of invasive devices and the growing prevalence of multidrug-resistant organisms [1]. Timely diagnosis and appropriate treatment are crucial, as delayed intervention significantly worsens patient outcomes. Traditional blood cultures remain the gold standard for diagnosing bacteremia, but these tests are slow, with results taking 48 to 72 hours. Hence, the integration of molecular diagnostics, such as multiplex PCR systems, has become essential for the rapid identification of pathogens and antibiotic resistance markers [2].

This study aims to evaluate the bacteriological profile of bacteremia cases and assess the resistance patterns of isolated bacteria to various antibiotics, with a particular focus on the advantages of molecular diagnostic methods, specifically the BioFire FilmArray system.

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2. Material and methods Patients

This retrospective, descriptive study was conducted over a 10 years period (2014 to 2024) at the Microbiology Laboratory of Avicenne Military Hospital in Marrakech. The study included patients with one or more positive blood cultures and excluded those with contaminated blood cultures or redundant results (i.e., identification of the same bacteria in multiple samples from the same patient during a single episode).

Blood samples for culture were collected at the bedside of hospitalized patients. For each patient, two bottles were used—one for aerobic and one for anaerobic cultures. The bottles were transported to the laboratory and incubated using the BacT/ALERT system (VersaTrek). Positive cultures were identified using the Phoenix 100 BD automated system, and antibiotic susceptibility was determined on solid and liquid media. Since 2020, multiplex PCR (FilmArray, BioMérieux) has been employed for rapid identification of bacteria and resistance genes directly from positive blood cultures.

Data were collected and analyzed, focusing on the bacteriological profile, the resistance patterns of isolated organisms, and the clinical departments from which patients were admitted. The performance of conventional culture methods was compared with molecular diagnostics in terms of speed and accuracy.

2.1. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM SPSS Statistics, Armonk, NY, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Bacteriological Profile

Out of 3721 blood cultures performed during the study period, 11.3% were positive for bacteremia, 86.05% were negative, and 2.65% were contaminated. A total of 420 cases of bacteremia were analyzed, with a male predominance (74% males vs. 26% females). The most frequently isolated bacteria were *Staphylococcus aureus*, coagulase-negative *staphylococci*, *Escherichia coli*, *Klebsiella spp*, and *Acinetobacter baumannii*.

The distribution of bacteremia cases across hospital services is as follows: Internal Medicine accounts for 34.5% of cases, while Intensive Care represents the largest proportion with 47.3%. Cardiology accounts for 4.5%, and Neurology, Traumatology, Urology, and Visceral Surgery each contribute 0.9%. Finally, Neurosurgery accounts for 2.7%.

The study conducted antibiotic susceptibility testing on various bacterial isolates, employing both conventional phenotypic methods and rapid molecular identification through the BCID Panel. Findings revealed significant resistance among key pathogens, which impacts clinical treatment options. In *Staphylococcus aureus,* 50% of isolates were methicillin-resistant (MRSA), confirmed by mecA gene presence. All isolates remained susceptible to vancomycin, making it a primary treatment, while 30% displayed resistance to fluoroquinolones, limiting their use in MRSA cases. Among *Enterobacteriaceae*, extended-spectrum beta-lactamase (ESBL) production was high, with 30% of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing ESBL, reducing beta-lactam efficacy. Furthermore, carbapenemase-producing organisms were present in 15% of *Klebsiella pneumoniae* isolates, with certain strains carrying the KPC gene, necessitating alternative treatments like colistin or tigecycline. In *Acinetobacter baumannii*, 70% of isolates showed multidrug resistance, particularly to carbapenems, amikacin, and ceftazidime, with a high prevalence of OXA-type carbapenemases identified by the BCID Panel, leaving colistin and sometimes tigecycline as viable options. Lastly, in *Pseudomonas aeruginosa*, 45% of isolates were resistant to carbapenems, yet 80% remained susceptible to amikacin.

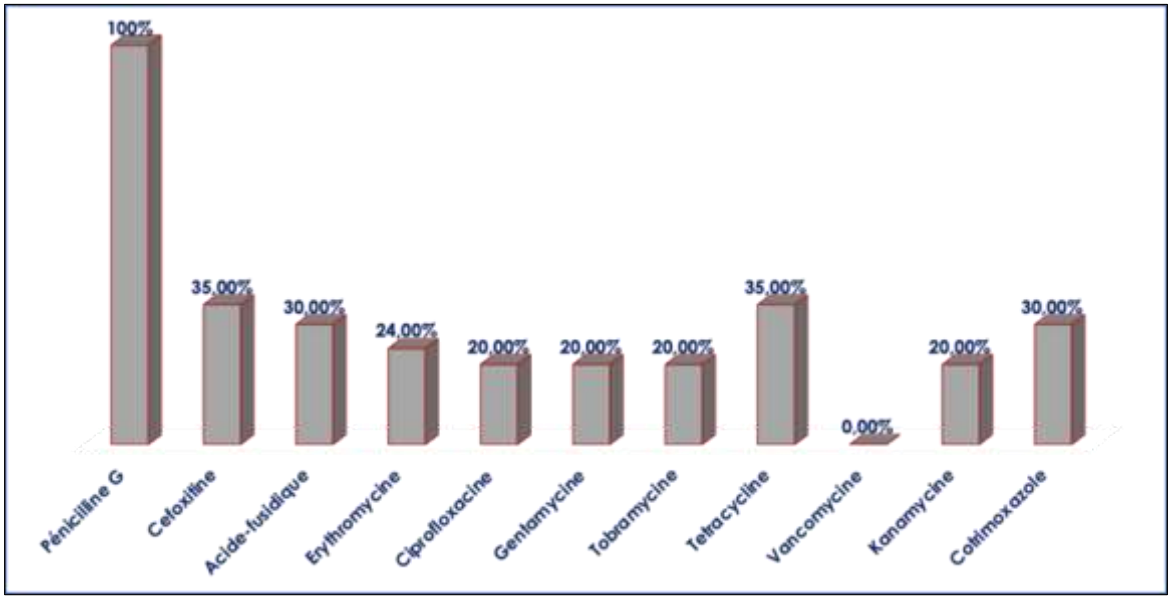


Figure 1 Antibiotic resistance profiles of *Staphylococcus aureus*

3.2. Comparative Analysis: BCID Panel vs. Conventional Techniques

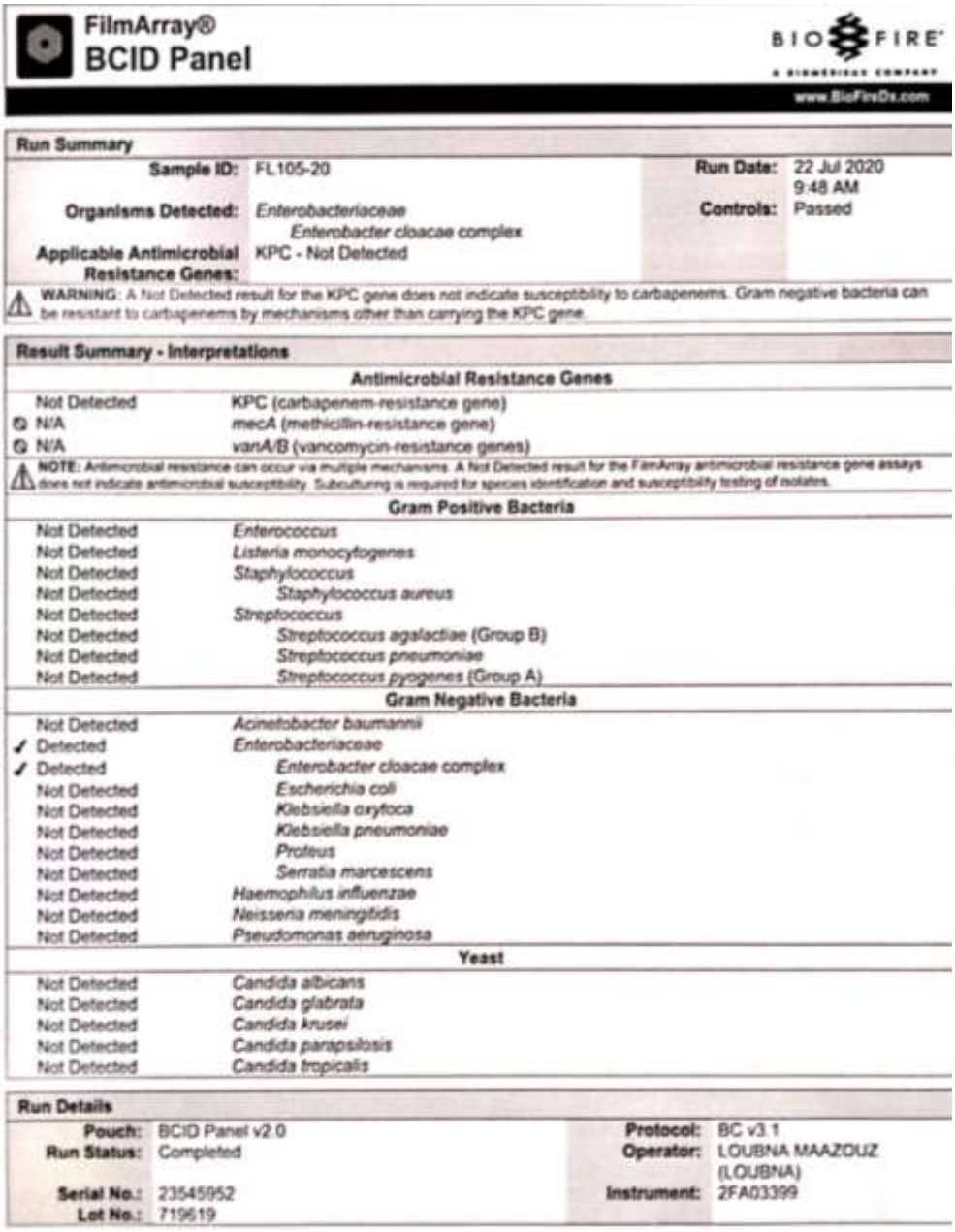
The integration of multiplex PCR (FilmArray) significantly reduced the time to result from an average of 72 hours (for conventional cultures) to less than 2 hours. This rapid turnaround enabled quicker initiation of targeted antimicrobial therapy.

The FILMARRAY system enables the identification of:

- **Gram-positive bacteria:** including Enterococcus, Listeria monocytogenes, Staphylococcus spp., S. aureus, Streptococcus spp., S. agalactiae, S. pneumoniae, and S. pyogenes.
- **Gram-negative bacteria:** such as *A. baumannii*, H. influenzae, N. meningitidis, Ps. aeruginosa, Enterobacter cloacae complex, E. coli, K. pneumoniae, K. oxytoca, Proteus spp., and S. marcescens.
- **Five species of *Candida.***

Additionally, FILMARRAY detects resistance genes, including mecA, vanA/B, and KPC.

The molecular method showed high concordance with conventional cultures, though discrepancies were noted in cases where culture results were negative due to prior antibiotic use, while PCR detected bacterial DNA. For instance, *Enterobacter cloacae* and *Enterococcus spp.* were identified by PCR despite negative culture results, likely due to slow growth or antibiotic suppression.



FilmArray®
BCID Panel

BIOFIRE
www.BioFireDx.com

Run Summary

Sample ID:	FL105-20	**Run Date:**	22 Jul 2020 9:48 AM
Organisms Detected:	*Enterobacteriaceae* *Enterobacter cloacae complex*	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:	KPC - Not Detected		

⚠ **WARNING:** A Not Detected result for the KPC gene does not indicate susceptibility to carbapenems. Gram negative bacteria can be resistant to carbapenems by mechanisms other than carrying the KPC gene.

Result Summary - Interpretations

	Antimicrobial Resistance Genes
Not Detected	KPC (carbapenem-resistance gene)
⊖ N/A	*mecA* (methicillin-resistance gene)
⊖ N/A	*vanA/B* (vancomycin-resistance genes)

⚠ **NOTE:** Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

	Gram Positive Bacteria
Not Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)

	Gram Negative Bacteria
Not Detected	*Acinetobacter baumannii*
✓ Detected	*Enterobacteriaceae*
✓ Detected	*Enterobacter cloacae complex*
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*

	Yeast
Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details

Pouch:	BCID Panel v2.0	**Protocol:**	BC v3.1
Run Status:	Completed	**Operator:**	LOUBNA MAAZOUZ (LOUBNA)
Serial No.:	23545952	**Instrument:**	2FA03399
Lot No.:	719619		

Figure 2 Example of detection of *Enterobacter cloacae* using the BCID Panel v2.0

4. Discussion

Blood culture is still the most reliable laboratory diagnostic test for bloodstream infections, despite not being the most sensitive method.

Our study reports a positivity rate of 9.3%, which aligns closely with that of Aiesh et al. (2023) at 9.4%, but is lower than the rates seen in Tajima et al. (2021) and Tenderenda et al. (2022), which report 12.1% and 11.5%, respectively. Interestingly, Lalezari et al. (2019) shows the lowest positivity rate at 6.3%, potentially indicating differing study parameters or sample characteristics.

In terms of contaminants, our series shows one of the lowest rates at 2.65%, suggesting a strong sample quality or effective contamination controls. This is notably lower than Tenderenda et al. (2022), which reports the highest contaminant rate at 9.5%. Tajima et al. (2021) presents the lowest contaminant rate at 1.3%, suggesting either stringent contamination controls or sampling protocols that minimize external interference [3-7].

Our study aligns closely with findings reported in recent research concerning antibiotic resistance patterns in *Staphylococcus aureus, Enterobacteriaceae, Acinetobacter baumannii, and Pseudomonas aeruginosa* in ICU settings.

Concerning *Staphylococcus aureus,* we observed a 50% methicillin resistance rate (MRSA) among *S. aureus* isolates, with all remaining susceptible to vancomycin. Similarly, Andrei et al.[8] found universal vancomycin susceptibility in *S. aureus*, including MRSA, but reported resistance to penicillin and oxacillin across nearly all isolates. Other studies show congruent patterns, such as 66% resistance to oxacillin and additional resistance to erythromycin [9]. These findings collectively highlight the persistent efficacy of vancomycin and underscore MRSA as a significant clinical challenge.

In *Enterobacteriaceae* (including *E. coli* and *K. pneumoniae*), We reported high extended-spectrum beta-lactamase (ESBL) production in *E. coli* (30%) and a 15% rate of carbapenemase-producing organisms in *K. pneumoniae*. Ahmed M.K. et al. reported similar findings, with *K. pneumoniae* as a predominant ICU pathogen, documenting ESBL rates of up to 65% and significant carbapenem resistance [9]. Likewise, Wang et al. identified carbapenem-resistant *K. pneumoniae* harboring KPC and NDM genes [10].

For *Acinetobacter baumanni, w*e found that 70% of isolates displayed multidrug resistance, particularly against carbapenems. This aligns with Andrei et al., who noted that *A. baumannii* presents increasing resistance in ICUs, often involving OXA-type carbapenemase genes. Our findings and these corroborative studies demonstrate the critical treatment challenge posed by *A. baumannii*, where only last-resort antibiotics like colistin remain effective for many strains.

Approximately 45% of *P. aeruginosa* isolates showed carbapenem resistance, while 80% remained susceptible to amikacin. Wang et al. reported similar resistance trends, with carbapenem resistance common, although amikacin retains effectiveness against many isolates [8,9].

In summary, our study aligns with current research indicating a rise in resistance, particularly with carbapenem-resistant *K. pneumoniae,* alongside high rates of MRSA in *S. aureus*. Vancomycin remains effective against MRSA, while the treatment options for multidrug-resistant *A. baumannii* and ESBL-producing *Enterobacteriaceae* are often restricted to colistin and tigecycline. These consistent findings emphasize the critical need for effective antimicrobial stewardship and development of new antibiotics to manage ICU-related infections and mitigate rising antimicrobial resistance.

The FilmArray BCID and the updated BCID2 assays offer rapid pathogen identification from positive blood cultures, reducing turnaround time to under 2 hours, a significant improvement over traditional cultures that average 72 hours. The original BCID system demonstrated high concordance with conventional culture results, though it occasionally detected bacterial DNA in culture-negative samples likely affected by prior antibiotic use. The updated BCID2 showed an 88.3% concordance rate with standard-of-care methods across 180 evaluated blood cultures, particularly excelling in monobacterial detections, with 91.9% concordance for Gram-positive and 96.0% for Gram-negative pathogens. Both versions identify a wide range of Gram-positive bacteria (e.g., *Enterococcus spp., Staphylococcus spp.,* and *Streptococcus spp*.) and Gram-negative bacteria (e.g., *E. coli, K. pneumoniae,* and *Ps. aeruginosa*), with BCID2 further enhancing accuracy and expanding to include carbapenemase and extended-spectrum beta-lactamase (ESBL) resistance markers like blaCTX-M, blaVIM-2, and blaOxa-48-like. Limitations are seen with both assays in polymicrobial blood cultures, where BCID2 had a 38.7% discordance rate, often detecting additional pathogens or failing to differentiate them accurately. Though BCID2 provides an advanced rapid detection system, the multifactorial nature of β-lactam resistance in Gram-negative organisms requires complementary phenotypic susceptibility assays to address resistance that molecular testing alone cannot predict, as it missed some cephalosporin resistance mechanisms [11].

Concerning *staphylococci, S. aureus* bacteremia is relatively common and associated with a high mortality rate, requiring the fastest possible initiation of appropriate therapy. In contrast, coagulase-negative *staphylococci* are mainly blood culture contaminants. Differentiating *S. aureus* from coagulase-negative *staphylococci* is therefore essential, especially since, in this specific case, direct blood examination revealing Gram-positive cocci does not contribute to therapeutic decision-making. Additionally, rapid agglutination tests for detecting coagulase, which identify *S. aureus*, can only be performed from colonies, requiring a 24-hour incubation. The FilmArray system enables direct identification of staphylococcal species, thereby reducing unnecessary antibiotic use, especially in cases where a coagulase-negative *staphylococcus* is identified, as with this blood culture. We must continually emphasize that any overuse of antibiotics (notably glycopeptides prescribed in cases of MRSA) has medical, economic, and microbial ecological consequences [12].

5. Conclusion

Bacteremias are frequent conditions in hospitals, and their progression is generally unfavorable in the absence of effective antibiotic treatment. They are a major source of mortality and morbidity, as well as considerable additional costs. Given the emergence and increase of bacterial resistance to antibiotics, up-to-date studies of the epidemiological profile of bacteria and the evaluation of their susceptibility profiles are necessary to rationalize the initial antibiotic therapy in bacteremias. The FilmArray BCID test (bioMérieux) enables rapid determination of the identification and certain antibiotic resistance markers of a bacterial strain present in a positive blood culture, which provides valuable support.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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