

Original Article

Assessment of Adequate Blood Culture Incubation Time to Accentuate Clinically Relevant Results from Blood Culture Instrument and Media System in a Tertiary Care Hospital Lahore

Mehwish Saba Aslam^{1*}, Muhammad Abid Farooque¹, Irfan Malik², Aneela Khawaja¹, Namra Yunus¹, Ammarah Mehmood¹

¹Combined Military Hospital Lahore, Pakistan.

²Armed Forces Institute of Pathology Rawalpindi, Pakistan.

*Corresponding Author: *Mehwish Saba Aslam*; Email: *dr.mehwish2005@gmail.com*

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ABSTRACT

Background: Bloodstream infections necessitate rapid identification and management. Automated blood culture systems have revolutionized diagnostic microbiology by expediting pathogen detection and enabling effective clinical interventions. This study assesses the optimal incubation period for blood cultures in a tertiary healthcare setting, aiming to enhance diagnostic accuracy and support antibiotic stewardship.

Objective: To evaluate the adequacy of incubation times in blood cultures for producing clinically significant results using an automated system in a tertiary care setting.

Methods: This cross-sectional descriptive study was conducted at the Microbiology Section of Combined Military Hospital, Lahore, from October 2022 to March 2023. All patient blood samples processed through the Bactec/Alert system were studied. Positive samples were cultured on Blood and MacConkey agar and incubated at $35 \pm 2^\circ\text{C}$ for 24 hours. Positivity rates, contamination, and patient demographics were analyzed using the Laboratory Information Management System.

Results: Of the 8,216 blood culture bottles processed, 490 (5.96%) were flagged as positive. Early detection within the first 24 hours was noted in 41.63% (n=204) of cases, with subsequent detections on the second day at 58.65% (n=258), the third day at 5.30% (n=26), and the fourth day at 0.20% (n=1). The isolated pathogens included gram-positive organisms (16.32%, n=80), gram-negative organisms (80.20%, n=393), and *Candida* spp. (3.46%, n=17).

Conclusion: Timely and effective blood culturing is crucial for the diagnostic confirmation of bacteremia, enabling appropriate pathogen identification and antimicrobial therapy. Shortening detection times can significantly impact clinical outcomes, reduce healthcare costs, and promote antimicrobial stewardship.

Keywords: Antimicrobial Stewardship, Bactec Alert, Blood Culture, Bloodstream Infection, Coagulase Negative Staphylococcus (CONS).

INTRODUCTION

Bloodstream infections (BSIs) represent a severe clinical challenge, as they involve the isolation of pathogenic microorganisms from the blood. Defined by hypotension, signs of organ dysfunction, and systemic manifestations, severe sepsis can escalate to septic shock when hypotension persists despite fluid resuscitation or requires management with vasopressors. These conditions may result in widespread organ failure, tissue damage, and ultimately, death (1). Timely and accurate identification of pathogens through blood cultures is crucial as it enables clinicians to initiate appropriate treatments promptly.

In clinical microbiology, the evolution of continuous monitoring blood culture systems has been pivotal in diagnosing life-threatening conditions such as endocarditis, fungemia, and bacteremia. Traditionally, blood culture bottles were incubated for up to seven days before being reported as "no growth." However, with advancements in technology, a reduced incubation period of five days is now

recommended for contemporary systems, though recent studies suggest that even shorter periods may suffice for most clinically relevant organisms (3, 4).

The diagnostic capabilities in tertiary care settings have significantly benefitted from the advent of automated systems like the BacT/Alert (bioMérieux, France), which continuously monitors cultures for signs of microbial growth. This system, along with others that utilize CO₂-based detection via colorimetric or fluorescence methods, has improved the accuracy and timeliness of BSI detection. The integration of resin-containing media in culture bottles, such as the BacT/Alert FAN Plus, enhances the recovery of organisms, allowing for the effective pairing of aerobic and anaerobic bottles to capture a broad spectrum of pathogens (5).

This study aims to assess the optimal incubation period for blood culture bottles in a high-tech diagnostic setup of a tertiary care hospital in Lahore. The goal is to determine the duration that maximizes the recovery of clinically relevant microorganisms while minimizing contamination, thereby supporting better clinical outcomes and patient care.

METHODS

This study received approval from the Institutional Review Board of the Microbiology Department at Combined Military Hospital, Lahore, with the approval number 432/2023 dated February 6, 2023. Blood culture specimens were collected from patients across various specialties from both inpatient and outpatient departments, ensuring a comprehensive representation of clinical scenarios. Upon receipt in the laboratory, each specimen was meticulously identified, labeled, and cross-verified with corresponding request forms to maintain the integrity of the data.

The specimens were then incubated in a Bactec/Alert system, which continuously monitored for signs of microbial growth. Specimens flagged as positive were immediately inoculated onto primary culture media, specifically blood agar and MacConkey agar, and incubated at an optimal temperature of 35±2°C for 24 hours to promote growth. Following incubation, isolates were identified based on colony characteristics, Gram staining, and biochemical reactions, adhering to standardized microbiological techniques.

Antimicrobial susceptibility testing was conducted in accordance with the 2022 Clinical and Laboratory Standards Institute (CLSI) guidelines. This step was crucial for determining the effective antimicrobial agents against the identified pathogens, thereby facilitating targeted therapeutic interventions. This methodological rigor ensured that the study's findings would provide actionable insights into optimizing blood culture practices within clinical settings (2).

All laboratory staff and technicians who assisted in this research contributed significantly to the successful completion of the study. Their efforts were instrumental in managing the workflows, processing samples, and ensuring the accuracy of the data collected. Their expertise and dedication were pivotal in facilitating the study's objectives and outcomes.

RESULTS

In this study, a total of 8,216 blood culture bottles were processed using the Bactec/Alert system, of which 490 (5.96%) were flagged as positive for microbial growth. Analysis of these positive cultures revealed a significant presence of Gram-positive cocci, with *Staphylococcus aureus* accounting for half of these isolates (50%), followed by Coagulase-negative Staphylococci (43.75%), and a smaller proportion of *Enterococcus* species (6.25%), as depicted in the pie chart (Figure 1).

Furthermore, the investigation into Gram-negative rods showed that they constituted a significant portion of the bacterial isolates. Out of the total positive cultures, 393 instances were identified as Gram-negative rods, representing a substantial diversity in pathogen types.

The study also examined the time to positivity of these cultures, which is critical for timely clinical intervention. The findings, presented in Table 3, illustrate the varying durations required for different pathogens to reach detectable levels. Notably, *Candida* spp. were isolated from 17 samples, accounting for 3.46% of the positive cultures, highlighting the system's capability to detect both bacterial and fungal pathogens effectively.

These results underscore the importance of an efficient and reliable blood culture system in diagnosing bloodstream infections, which is crucial for initiating appropriate and timely treatment interventions. The data also suggest potential areas for further refinement in clinical practices and microbiological techniques to enhance the diagnostic yield of blood culture systems.

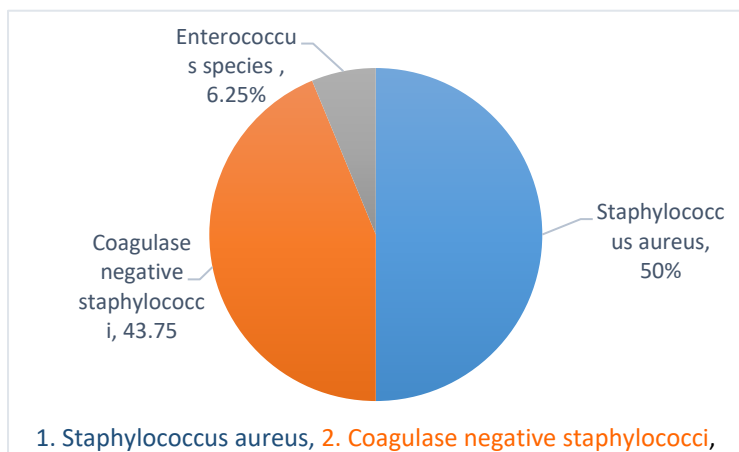


Figure 1 Frequency distribution of Gram Positive Cocci isolated from positive culture blood samples

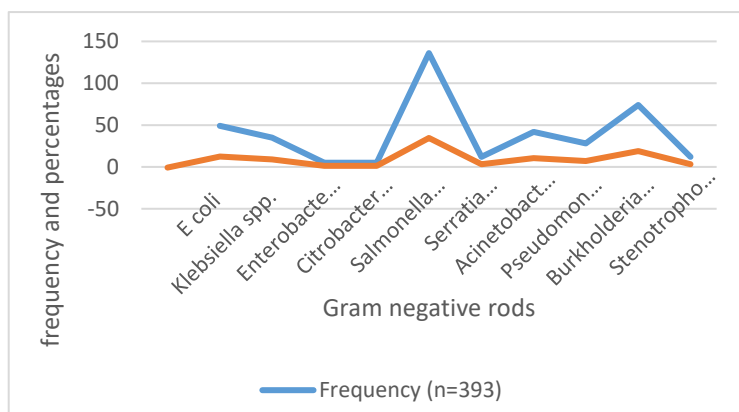


Figure 2 Frequency distribution of the Gram negative rod (n=393) from total Positive blood cultures (n=490)

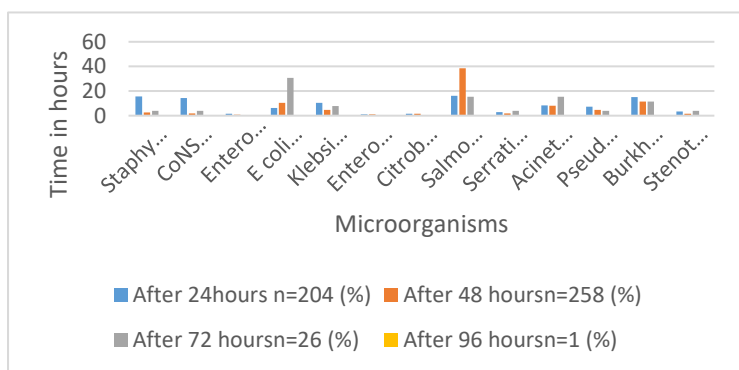


Figure 3 Positivity of Bacterial isolates from blood cultures in relation with time

such as the timing of culture initiation, transport durations, and the rapid communication of results, play a crucial role in the effectiveness of blood culture systems. These logistical factors, combined with timely infectious disease consultations and integrated antimicrobial stewardship programs, are essential for optimizing the management of bloodstream infections.

The study's strengths include its comprehensive sampling across various specialties and its utilization of advanced blood culture systems, which enhance the reliability of the findings. However, limitations such as potential variations in sample handling and the intrinsic limitations of culture-based diagnostic methods, which may not detect fastidious or non-culturable pathogens, should be considered. Additionally, the study highlights the need for continuous evaluation of diagnostic techniques to ensure their accuracy

DISCUSSION

Blood cultures serve as a critical diagnostic tool in the management, monitoring, and diagnosis of bacteremic patients. The early identification of pathogens and the subsequent antimicrobial susceptibility testing for bloodstream infections are vital for improving medical outcomes. Within hospital settings, the evaluation of bloodstream infections forms an essential component of the diagnostic process for patients with febrile illnesses. A significant proportion of positive blood cultures are associated with genuine bloodstream infections, emphasizing the importance of rapid pathogen identification to facilitate the early commencement of antimicrobial therapy. Such early intervention enhances the prospects for effective antimicrobial stewardship and optimizes patient management (6, 7).

During the study, various organisms were isolated, indicating a diverse microbiological landscape. The epidemiological prevalence of gram-positive bacteremia was identified at 16.32%, a figure slightly higher than those reported in previous studies (8). Specifically, Staphylococcus aureus was the most frequently identified gram-positive organism, although its prevalence in this study was lower compared to findings by Odutola et al., 2019, which documented a higher incidence (9). This discrepancy might reflect variations in sample demographics or methodological differences. The contamination rates found in this study were consistent with those reported by Idrees et al., 2023, particularly for Coagulase-negative Staphylococci (CoNS), underlining the persistent challenge of distinguishing between contamination and true infection (7).

Gram-negative bacilli constituted the majority of isolates in this study, a finding that diverges significantly from the rates reported in other regions (11, 12). This study's high incidence of Salmonella spp. among enterobacteriales contrasts with the lower frequencies documented in other research, suggesting possible regional variations in pathogen prevalence or differences in detection capabilities.

Furthermore, the time to culture positivity was generally between 24 to 72 hours, comparable to the durations observed in other studies (18, 19). The operational logistics involved in the pre-analytical and post-analytical phases,

and effectiveness in clinical settings, as well as the importance of tailoring antimicrobial stewardship strategies to specific pathogen profiles and resistance patterns observed in the local context.

CONCLUSION

The findings from this study underscore the critical diagnostic efficacy of properly implemented blood culture systems, which significantly enhance patient management and facilitate effective antibiotic stewardship. Careful consideration of the operational execution, potential limitations, and strategic utilization of these systems is imperative. Healthcare providers must leverage this knowledge to optimize the use of blood cultures in clinical settings, ensuring that the systems are not only effective in detecting pathogens but also instrumental in guiding targeted treatment strategies. This approach will ultimately improve patient outcomes and contribute to the judicious use of antibiotics, addressing both the immediate needs of patient care and the broader challenge of antibiotic resistance.

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