PRE-ANALYTICAL FACTORS IMPACTING BLOOD CULTURE POSITIVITY OUTCOMES: A CROSS-SECTIONAL STUDY AT SULTAN AHMAD SHAH MEDICAL CENTRE, IIUM

BY

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ABSTRACT

Blood culture (BC) is a crucial test for diagnosing bloodstream infections (BSIs) and can be significantly influenced by pre-analytical factors. Despite its importance, concerns persist regarding the reliability of BC results, often due to contamination and variability in positivity outcomes. This study addresses the research gap concerning the impact of specific pre-analytical factors such as location, transportation time, site of collection, and sample volume on BC positivity rates at Sultan Ahmad Shah Medical Centre @ IIUM. A cross-sectional analysis of 276 BC vials from 138 patients revealed that 58% were male, with a mean age of 61.8 years. Samples for the BC analysis were collected from emergency wards (35.5%) and peripheral veins (84.8%). The overall positivity rate was 29.0%, with 34 (24.6%) positive aerobic vials and 24 (17.4%) positive anaerobic vials. Optimal blood volume (8-10 ml) was achieved in only 17.4% of cases. The mean blood volume was 6.3 ± 3.7 ml for aerobic vials and 5.9 ± 3.0 ml for anaerobic vials, both of which are considered suboptimal based on the literature review (<8 ml). The mean time to positivity (TTP) was significantly different in anaerobic cultures when comparing varying blood volumes ($p = 0.009$). Although demographics and other pre-analytical factors did not significantly predict true or false positives ($p > 0.05$), the observed trends underscore the need for improved diagnostic practices. This study provides valuable insights into the pre-analytical factors affecting BC outcomes, emphasizing the importance of refining protocols to enhance diagnostic accuracy and patient management for suspected BSIs.

ملخص البحث

مزرعة الدم (BC) هو أختبار أساسي لتشخيص عدوى مجرى الدم (BSIs)، وتتأثر بشكل كبير بالعوامل ما قبل التحليل. على الرغم من أهميتها، لا تزال هناك مخاوف بشأن موثوقية نتائج زراعة الدم، وغالبًا ما يكون ذلك بسبب التلوث وتفاوت النتائج الإيجابية. تتناول هذه الدراسة الفجوة البحثية المتعلقة بتأثير عوامل ما قبل التحليل المحددة—مثل الموقع، ووقت النقل، ومكان الجمع، وحجم العينة—على معدلات إيجابية زراعة الدم في مركز سلطان أحمد شاه الطبي @ IIUM. أظهر تحليل مقطعي لـ 276 قارورة زراعة دم من 138 مريضًا أن 58% منهم كانوا من الذكور، بمتوسط عمر 61.8 عامًا. تم جمع زراعات الدم بشكل رئيسي من أقسام الطوارئ (35.5%) والأوردة المحيطية (84.8%). كانت نسبة الإيجابية الإجمالية 29.0%، مع 34 قارورة هوائية إيجابية (24.6%) و24 قارورة لاهوائية إيجابية (17.4%). تم تحقيق حجم الدم الأمثل (8–10 مل) في 17.4% فقط من الحالات، بينما كان متوسط حجم الدم 6.3 ± 3.7 مل للقوارير الهوائية و5.9 ± 3.0 مل للقوارير اللاهوائية، والذي يعتبر دون المستوى الأمثل استنادًا إلى مراجعة الأدبيات (<8 مل). كان متوسط الوقت للوصول إلى الإيجابية (TTP) مختلفًا بشكل ملحوظ في الزراعة اللاهوائية عند مقارنة أحجام الدم المختلفة ($p = 0.009$). وعلى الرغم من أن العوامل الديموغرافية وعوامل ما قبل التحليل الأخرى لم تتنبأ بشكل كبير بالإيجابيات الحقيقية أو الزائفة ($p > 0.05$)، فإن الاتجاهات الملحوظة تؤكد على الحاجة إلى تحسين الممارسات التشخيصية. توفر هذه الدراسة رؤى قيمة حول العوامل ما قبل التحليل التي تؤثر على نتائج زراعة الدم، مع التأكيد على أهمية تحسين البروتوكولات لتعزيز دقة التشخيص وإدارة المرضى الذين يشتبه في إصابتهم بـ BSIs.

APPROVAL PAGE (MASTER'S DEGREE)

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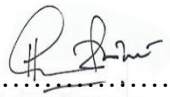
Supervisor



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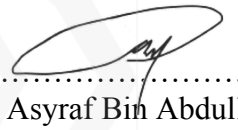

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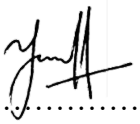

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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This thesis is dedicated to my parents, my brother Faisal, my husband Ali, my daughters, and my siblings, who have all laid the foundation for who I have become. Their unwavering love, encouragement, and support have been my constant source of strength throughout this journey. Each of them has played a pivotal role in shaping the person I am today, and I am deeply grateful for the values they have instilled in me, the sacrifices they have made, and the belief they have always shown in my abilities. This achievement would not have been possible without their guidance and presence in my life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASB	Aerobic Spore-Bearing Bacilli
BD BACTEC™ FX	Becton Dickinson BACTEC™ FX Blood Culture System
BCs	Blood Cultures
BSIs	Bloodstream Infections
CDC	The Centers for Disease Control and Prevention
CRBSI	Catheter-Associated Bloodstream Infections
CLABSIs	Central Line-Associated Bloodstream Infections
CONS	Coagulase-Negative Staphylococcus
CFU	Colony Forming Units
ED	Emergency Departments
ESRD	End-Stage Renal Disease
ISDD	Initial Specimen Diversion Device
IM	Internal Medicine
ICU	Intensive Care Unit
IL	Integrity Laboratory
IIUM	International Islamic University Malaysia
LIS	Laboratory Information System
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
PHL	Public Health Laboratory
post-hoc Tukey HSD Test	post-hoc Tukey Honest Significant Difference Test
SASMEC	Sultan Ahmad Shah Medical Centre
TTP	Time to Positivity
TRF	Test Request Form

CHAPTER ONE

INTRODUCTION

Blood cultures (BCs) are among the most frequently performed microbiological tests in hospitals worldwide. They are considered the gold standard for detecting bacteremia by identifying microorganisms such as bacteria and fungi. BCs are particularly important when there is a suspicion of sepsis or endovascular infections. The high rates of sepsis, septic shock, and related bloodstream infections (BSIs) contribute significantly to morbidity and mortality, especially in Europe and North America (Nannan Panday et al., 2019).

The effectiveness of BCs can be compromised when they are ordered based on nonspecific symptoms, such as fever or elevated white blood cell counts, which are not reliable indicators of bacteremia. National guidelines suggest a more targeted approach to ordering BCs, especially in intensive care units (ICUs), to avoid unnecessary testing (De Plato et al., 2019; Aiesh et al., 2023). Excessive ordering of BCs can lead to significant issues, as many cultures either fail to grow the organisms or become contaminated. This can lead to unnecessary antibiotic treatments, extended hospital stays, and higher healthcare costs. Many clinicians often order BCs reflexively due to concerns about potentially missing an infection (Fabre et al., 2020).

In the United States, BSIs account for an estimated 536,000 to 628,000 cases each year, resulting in 79,000 to 94,000 deaths. Physicians frequently order BCs in Emergency Departments (EDs), even in situations where bacteremia is less likely, such as pneumonia or cellulitis. Despite this practice, the rate of true-positive BCs in the ED is low, ranging from 1.4% to 12.2%, and influences treatment decisions in only 0.18% to 2.8% of cases. While the primary goal of BCs is to identify true BSIs, excessive testing often leads to contamination rates between 40% and 55% (Schinkel et al., 2022).

Despite progress in understanding factors that affect BC yield, such as blood volume and, site of collection, pre-analytical time, current practices remain inefficient, with a pathogen recovery rate of only 7% (Nannan Panday et al., 2019; Fabre et al., 2020). This ongoing inefficiency exists even with advancements in BC methodologies, which have transformed the diagnostics of BSIs. Innovations such as continuous monitoring systems, resin-based media to counteract antibiotic interference, and novel detection technologies like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have improved pathogen identification. Additionally, techniques like nucleic acid amplification and PCR have made it possible to identify specific pathogens quickly, allowing for more targeted treatment (Cheng et al., 2019; Doern et al., 2019; Evangelista & Ferreira, 2022).

In conclusion, the persistent low efficiency and yield of BCs, even with the advancements in technology, emphasizes the need to investigate pre-analytical factors that may contribute to these challenges. This study aims to explore these factors to improve BCs outcomes and ultimately enhance patient care.

1.1 PROBLEM STATEMENT

In healthcare settings, accurate diagnosis of BSIs is essential for proper patient management. However, challenges persist regarding the reliability of BC results, particularly due to contamination and varying positivity outcomes. While BC positivity rates at Sultan Ahmad Shah Medical Centre (SASMEC) in Malaysia were reported to be in the range of 15% and 20% in 2021 and 2022, respectively, with contamination rates ranging from 5% to 20%, there is a gap in research exploring the pre-analytical factors contributing to these outcomes. No comprehensive study has examined how specific factors, such as sample volume, collection site, ward location, and transportation time, affect the contamination and positivity rates at SASMEC. This study aims to investigate the pre-analytical factors influencing BC positivity outcomes at SASMEC to enhance diagnostic accuracy and improve patient management for those with suspected BSIs.

1.2 SIGNIFICANCE OF STUDY

BSIs represent a formidable global health threat, significantly impacting morbidity and mortality. The precision of BC results is intricately tied to expertise and adherence to best practices. Proficient BC practices are pivotal for minimizing false results and accurately identifying true BSIs. Aseptic techniques during blood collection, proper skin disinfection, sufficient blood volume, and prompt transportation are crucial components of robust BC practices. Facilities with well-established protocols, trained staff, and stringent quality controls are more likely to yield accurate results. Conversely, suboptimal practices may compromise sensitivity and specificity, increasing the risk of false negatives and delayed treatment. The reliability of these diagnostic tests is inherently linked to diligence and proficiency in following best practices, which directly affect the clinical utility. Rapid identification of pathogens and timely initiation of appropriate antimicrobial therapy is crucial in minimizing mortality risk and avoiding unnecessary antibiotic usage. (De Plato et al., 2019; Lin et al., 2022).

This study at SASMEC aimed to bridge critical gaps in BC understanding and implementation. A meticulous examination of current practices sought to identify areas for improvement, focusing on factors such as location, transportation time, site of collection, and sample volume within SASMEC. The research aimed to propose evidence-based recommendations for incorporation into clinical guidelines and practice standards. Additionally, addressing pitfalls in current practices sets the stage for tailored training initiatives, elevating BC standards. The study seeks to enhance BC positivity outcomes and contribute to advancements in patient care by enabling swifter and more accurate diagnoses.

1.3 RESEARCH QUESTIONS

1. What is the distribution of key pre-analytical factors (locations, transportation time, site of collection, and volume of sample) for BC collection?

2. Do different collection locations and collection sites affect the positivity rates of BCs?

3. Is there a significant association between transportation time and BC positivity rates?

4. What is the impact of varying sample volumes on the likelihood of BC positivity rates?

5. Which pre-analytical factors are significant predictors of BC positivity rates?

1.4 OBJECTIVES

1.4.1 General Objectives

To evaluate the impact of pre-analytical factors on the BC positivity outcome at SASMEC.

1.4.2 Specific Objectives

1. To determine the distribution of key pre-analytical factors (locations, transportation time, site of collection, and sample of volume) for BC collection.

2. To investigate the association between pre-analytical factors and BC positivity rates.

3. To identify and analyze specific pre-analytical factors that serve as predictors of BC positivity rates.

1.5 HYPOTHESIS

The BC positivity in SASMEC is significantly affected by pre-analytical factors such as locations, transportation time, collection site, and sample volume. The hypothesis suggests that optimizing these factors can enhance the positivity of BC outcomes at SASMEC.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 BLOOD CULTURE COLLECTION TECHNIQUE & PROCEDURE: ENHANCING QUALITY AND REDUCING CONTAMINATION

Obtaining BCs is a critical process that significantly influences the diagnostic yield. As such, careful practices are essential to minimize contamination. Proper cleaning of the venipuncture site, avoiding peripheral intravenous lines, and adhering to The Centers for Disease Control and Prevention (CDC) disinfection guidelines are crucial (Chela et al., 2019; Pandit et al., 2021). The CDC recommends using alcohol followed by chlorhexidine for skin disinfection before allowing sufficient time for the skin to dry. Adequate blood volume is essential for detecting bacterial or fungal bloodstream infections, with recommendations of 20-30 ml per set for adults and adjusted volumes for children based on age and weight. It is advisable to collect a minimum of two sets of BCs and sometimes up to four sets in suspected endocarditis cases. Each set should include one aerobic and one anaerobic vial (De Plato et al., 2019; Lin et al., 2022).

In cases of catheter-associated bloodstream infections (CRBSI), submitting the catheter tip to the microbiology lab alongside a peripheral venipuncture BC is required. Although the timing of BC collection is less critical than volume, it remains important. Ideally, it should be collected at intervals during febrile episodes, if possible, due to the intermittent nature of bacteremia. Technologically, BCs typically show growth within 48 hours and rarely require monitoring beyond five days (Chela et al., 2019; Pandit et al., 2021).

Furthermore, the adoption of novel blood collection devices, such as the Initial Specimen Diversion Device (ISDD), has demonstrated reduced BC contamination rates with enhanced culture quality. ISDD diverts initial blood portions during venipuncture to lower contamination rates in Emergency Departments (ED), decreasing false positives, unnecessary antibiotic use, and healthcare costs. Cost-benefit analyses indicate that ISDD implementation in EDs saves costs per patient by reducing unnecessary tests, treatments, and interventions associated with contaminated cultures. Overall, ISDD adoption represents a cost-effective strategy to improve patient care and hospital outcomes (Skoglund et al., 2019). Moreover, implementing quality control measures like regular audits and feedback to collectors effectively maintains low contamination rates and optimizes BCs utilization (Halstead et al., 2020).

2.1.1 Blood Culture Collection Guidelines at SASMEC

BCs are crucial diagnostic tools that should only be collected when there is a clinical suspicion of septicemia. It is essential that BCs are not used for routine assessments or to investigate localized infections. The focus should be on ensuring that specimens are collected in adequate volumes, performing the appropriate number of BCs, and adhering to strict aseptic techniques. Blood should be drawn before administering antibiotics, ideally as the patient's temperature begins to rise. Peripheral veins should be the primary sites for blood collection unless a catheter-related BSIs is suspected. In such cases, blood should be drawn concurrently from both the peripheral vein and the catheter to ensure that the same volume is collected from each site.

At SASMEC, specific guidelines for BC collection are followed to ensure accuracy and reliability. The collection process begins with preparing a blood collection set while maintaining aseptic non-touch techniques. Venipuncture sites must be disinfected with an antiseptic agent in a central-to-peripheral motion to allow sufficient contact time for maximum efficacy. Prior to venipuncture, the cap of the BC vial should be removed, and the rubber stopper should be cleaned thoroughly. Additionally, the alcohol needs to be allowed to evaporate for about 30 seconds before inoculating the vial. Blood is then injected into each vial through the rubber cap with a needle, and the inoculated vials should be gently inverted two to three times to mix the contents.

It is imperative to avoid using expired BC vials and to accurately label each one to match the request form. Specimens should be transported to the laboratory immediately at room temperature, avoiding refrigeration, and should not be sent via pneumatic tubes. Additionally, care must be taken not to cover the vials' barcodes with patient labels. These practices align with SASMEC's guidelines and are designed to enhance the reliability of BC results.

2.2 IMPORTANCE OF PROPER BLOOD CULTURE TECHNIQUES

Proper BC collection techniques are essential to mitigate the risk of contamination that can lead to extended hospital stays, increased costs, unnecessary antibiotic treatments, and the rise of antibiotic resistance (Liaquat et al., 2022). The use of sterile techniques is critical in minimizing contamination from normal bacterial commensals on the skin during sample collection (De Plato et al., 2019). This becomes particularly challenging in patient groups such as those with morbid obesity, difficult venous access, end-stage renal disease (ESRD), and critically ill patients with extended hospital stays, where technical difficulties in obtaining reliable cultures are common (Chela et al., 2019).

Contamination in BC results in false-positive outcomes, where non-pathogenic microorganisms are mistakenly identified as pathogens. This may lead to inappropriate treatments and potential patient harm (De Plato et al., 2019; Doern et al., 2019). Common contaminants include *Staphylococci* (CoNS), aerobic spore-bearing bacilli (ASB), and *Viridians* group *streptococci*, introduced during specimen collection or processing (Krisanapan & Chaiwarith, 2019). The clinical significance of these contaminants requires confirmation through multiple positive BC sets with concordant results within a 24-hour period (Doern et al., 2019).

Moreover, prior antimicrobial therapy significantly impacts BC outcomes by reducing the sensitivity to detect pathogens, potentially leading to false-negative results (Cheng et al., 2019; Hirosawa et al., 2023). Studies have shown a notable decrease in positive cultures after antibiotic administration, highlighting the challenge of interpreting BC results in patients with prior antimicrobial exposure (Cheng et al., 2019). Clinicians must carefully consider these factors to accurately diagnose BSIs and guide appropriate treatment decisions in clinical practice.

2.3 DETERMINATION AND IMPACT OF TRUE POSITIVE AND FALSE NEGATIVE BLOOD CULTURES

A BC test is considered true positive when a microbe that may be the etiological agent of BSI is cultivated, and the results are consistent with the patient's clinical condition (Tenderenda et al., 2022). According to Salimiyan et al. (2021), true bacteremia is defined as a positive BC (>15 CFU/mL) with signs and symptoms of infection (e.g., fever and chills). A true positive BC is distinguished by the growth of bacteria in multiple blood sample sets, as well as the presence of corresponding clinical signs. A positive test result indicates the presence of germs in the patient's blood, the most commonly harmful bacteria associated with a potentially serious infection (Tenderenda et al., 2022).

In contrast, a true negative BC test is one where no microorganisms are detected in the BC samples, and the results are consistent with the patient's clinical condition (Tenderenda et al., 2022). False-negative BC tests occur when no microorganisms grow in the BC vials, even though the patient has BSIs. This result is inconsistent with the patient's clinical signs and symptoms, indicating an infection despite the negative test result (Nguyen et al., 2019; Ter et al., 2021). Consequently, false-negative BC results can lead to misdiagnosis, delayed therapy, and increased risk of morbidity and mortality due to bacteremia. Potential reasons for false-negative results include collecting an inadequate blood volume, insufficient number of BC sets, sampling after antibiotic therapy has started, and infections caused by microorganisms that are difficult to detect with routine BC methods (Doern et al., 2019; Fabre et al., 2022).

2.4 FALSE POSITIVE BLOOD CULTURES: CHALLENGES, COSTS, AND CLINICAL IMPLICATIONS

Blood culture contaminations (BCCs) are the clinical term for false-positive BCs, which are frequently caused by human microbiota contamination. In these cases, isolated microorganisms are classified as skin contaminants rather than pathogens. Distinguishing true BSIs from contamination remains a persistent challenge for physicians and microbiologists. Despite advancements in culture techniques and automated identification processes, some laboratories report BCC rates approaching 50% of total positive BCs (Hemeg et al., 2020; Tenderenda et al., 2022).

According to CLSI recommendations from the United States, contamination rates in adult healthcare settings should not exceed 3% of total BCs (Doern et al., 2019; Sacchetti et al., 2022). False-positive BC events contribute significantly to increased laboratory costs, with additional testing expenses rising by up to 20%. They also lead to unnecessary antibiotic prescriptions and are associated with a 40% increase in antimicrobial spending.

Furthermore, BCCs result in extended hospital stays, sometimes up to 5 days (Tenderenda et al., 2022). Another study by Klucher et al. (2022) found that patients with BCC incurred significantly higher hospital charges. On average, these patients faced $7,132 more in hospital costs than those with true-negative BCs ($36,007 vs. $28,874; $p < 0.0001$). These increased costs align with previous findings in the literature, highlighting the financial impact of BCC on healthcare systems.

2.4.1 Criteria for Identifying Contamination

There is no definitive method to distinguish between 'true pathogens' and 'contaminants' in BCs. False-positive BCs typically involve the isolation of commensal skin flora bacteria such as *Micrococcus* species, *Bacillus* species (excluding *B. anthracis*), coagulase-negative *Staphylococci* (CoNS), *Corynebacterium* species, and *Propionibacterium acnes*. These microorganisms can transfer from the patient's skin, immediate surroundings, healthcare workers' hands, or equipment used during sample collection (Doern et al., 2019; Hemeg et al., 2020; Tenderenda et al., 2022).

While often considered contaminants in a single BC without clinical significance, they may indicate infection if two or more positive BC sets yield consistent results within 24 hours (De Plato et al., 2019; Lin et al., 2022). Additionally, the time-to-detection metric can aid in distinguishing between contaminants and pathogens, as contaminants generally exhibit slower growth rates than true pathogens (Ombelet et al., 2019)

Clinicians and laboratory protocols differentiate true positive, true negative, and false positive BC results by considering several critical factors essential for patient management and treatment. These factors include identifying the isolated microorganism, reviewing medical records, counting the number of positive cultures, noting the time to positivity, checking for the presence of a permanent catheter during the hospital stay, and evaluating the patient's clinical status, history, and laboratory findings such as leukocytosis, fever, leukopenia or fever (Ombelet et al., 2019; Hemeget al., 2020; Lin et al., 2022; Aiesh et al., 2023; Palavecino et al., 2024).

The type of isolated organism plays a pivotal role; certain pathogens such as *Listeria*, *S. aureus*, *Brucella*, *Haemophilus*, and *Enterobacteriaceae* are typically associated with severe infections (Azad & Patel, 2024), whereas CoNS, *Propionibacterium*, *Corynebacterium*, and *Bacillus* are more likely contaminants (Ombelet et al., 2019). Multiple positive BC samples increase the likelihood of true BSIs, while contaminants often exhibit delayed time-to-detection due to lower microbial load (Osaki et al., 2020; Tenderenda et al., 2022).

Furthermore, multiple BC sets are recommended, each typically comprising aerobic and anaerobic vials (Henning et al., 2019). Many studies have demonstrated enhanced sensitivity with multiple sets, as contaminants typically affect single vials, whereas true infections were more likely to manifest across multiple samples (Tenderenda et al., 2022). For instance, the isolation of skin commensal pathogens like CoNS from at least two sets of blood samples indicates clinical significance. Meanwhile, growth in one of two or one of four vials without other positive microbiological samples within five days is deemed as contamination (De Plato et al., 2019; Henning et al., 2019; Lin et al., 2022). In conclusion, each positive BC result is assessed for contamination using established criteria, with false-positive cultures coded as negative (Nannan et al., 2019).

2.5 PRE-ANALYTICAL FACTORS INFLUENCING BLOOD CULTURE OUTCOMES

The accuracy and reliability of BC results are significantly influenced by pre-analytical factors. In particular, the aseptic technique plays a critical role in minimizing contamination during sample collection since it can reduce false-positive results and ensure precise microbial identification. Moreover, proper aseptic practices can help avoid situations where clinicians are misled by erroneous infection indicators, which are vital for guiding appropriate antibiotic therapy and enhancing patient care quality (Doern et al., 2019; Sacchetti et al., 2022).

2.5.1 Aseptic Technique

Contamination often stems from skin microflora due to inadequate aseptic technique, insufficient skin disinfection, poor technique execution, and inadequate hub disinfection (Sacchettietal., 2022; Aiesh et al., 2023). To mitigate contamination risks, adherence to standard precautions during sampling that involve meticulous maintenance of aseptic conditions and strict adherence to procedural protocols are necessary (Hemeg et al., 2020; Tenderenda et al., 2022).

Achieving aseptic blood collection requires specialized knowledge and training. It has been demonstrated that when trained phlebotomists are used for BCs, the contamination rates are significantly reduced. A meta-analysis of five large United States hospital studies confirmed that trained phlebotomists have lower contamination rates compared to non-phlebotomists, with a combined odds ratio of 2.58. This highlights the importance of professional expertise for accurate diagnostic outcomes (Doern et al., 2019). This practice not only supports appropriate antibiotic therapy but also prevents unnecessary treatments and improves patient care through timely and accurate diagnosis of BSIs (Sacchetti et al., 2022).

2.5.2 Time of Collection

Another critical factor influencing BC accuracy is the timing of collection, which includes periodicity and fever timing. Clinical training for healthcare providers has emphasized the rapid collection of BCs before administering antibiotics, particularly for septic shock patients, to enhance the chances of isolating causative microorganisms and increase the sensitivity that can aid in identifying BSIs (Fabre et al., 2022; Tenderenda et al., 2022).

In contrast, delayed collection, especially post-antibiotic administration, may yield false-negative results due to the suppressive effects of medication on microbial growth. Timely and appropriately timed BC collection is crucial for optimizing diagnostic precision that can inform effective treatment strategies for patients with suspected BSIs (Cheng et al., 2019; Lin et al., 2022).

A study by Cheng et al. (2019) involving 325 adults with severe sepsis or septic shock in the Emergency Department found that 31.4% of patients had positive results for one or more microbial pathogens in BCs taken before antibiotic administration. In comparison, post-antimicrobial BCs showed positivity in only 19.4% of cases. The absolute difference in the proportion of positive BCs between pre- and post-antimicrobial testing was 12.0% ($p < 0.001$). The sensitivity of post-antimicrobial cultures was 52.9%, and when considering results from other microbiological cultures, microbial pathogens were detected in 67.6% of cases. In this research, the endocarditis presence and *S. aureus* isolation were the sole independent predictors of post-antibiotic positive BCs.

In summary, timely BC collection before antibiotic administration is essential to enhance diagnostic accuracy, reduce false-negative results, and inform effective treatment strategies. If the collection is delayed or if BCs are taken after antibiotic administration, lower positivity rates are to be expected. The situation underscores the need for prompt sampling in clinical practice.

2.5.3 Location of Collection

Another significant factor influencing BC test results is the location within hospitals. BC collection practices vary widely depending on specific hospital areas. The excessive use of BCs in ED has been linked to reduced effectiveness and increased contamination rates, which can adversely affect patient care. According to Schinkel et al. (2022), BCs are frequently obtained from EDs patients suspected of infections, even in cases where the likelihood of bacteremia is low, such as pneumonia or cellulitis.

As a result, the yield of BCs collected in ED tends to be low, with true-positive rates globally ranging from 1.4% to 12.2%, excluding contamination. Additionally, the hemodialysis unit shows significantly higher BCC rates, likely due to its crowded and mechanized environment (Aiesh et al., 2023).

BCC rates are often elevated in EDs due to the urgency of cases, patient dehydration, agitation, and inadequate hygiene practices. Another study identified an 11.7% contamination rate in their ED, significantly higher than the average of approximately 2.5% observed in other hospital departments (Sacchetti et al., 2022).

Moreover, contamination rates exceeding internationally acceptable levels have been observed in the Internal Medicine (IM) and Intensive Care Unit (ICU) departments, negatively impacting BSI detection (Tenderenda et al., 2022). In summary, BC collection practices vary significantly across different hospital locations. EDs and specialized units like hemodialysis and ICU exhibit higher contamination rates, potentially compromising diagnostic accuracy and patient care.

2.5.4 Transportation Time (Pre-Analytical Time)

The transportation time of BC vials is crucial for effective microbial detection. Pre-analytical time, defined as the interval from the collection of a BC sample to its entry into the incubator, plays a crucial role in the diagnostic process. It is recommended that vials be placed into automated systems within two to four hours of collection and promptly transported to laboratories. However, not all medical facilities can provide optimal sample transportation within one to two hours. Samples taken after hours are stored at room temperature (21-25°C) inwards and transported in a similar manner.

When recommended timelines are exceeded, BC yield is reduced, microbial growth is limited, and the start of appropriate antimicrobial therapy is delayed, highlighting the need to minimize turnaround times (Doern et al., 2019; Skusa et al., 2021).

Several challenges make it difficult to adhere to the recommended timeframe of two to four hours for collecting and transporting blood samples to the laboratory. Firstly, varying laboratory hours and limited staffing, especially on weekends and holidays, further delay sample processing (Kufa et al., 2020; Simundic et al., 2020). Additionally, transportation delays exacerbate these challenges, prolonging transit times due to logistical constraints or inadequate infrastructure (Tripathi et al., 2020; Zaman et al., 2021). On top of that, extended transport times to the laboratory may result in delayed time-to-result (Skusa et al., 2021).

Furthermore, insufficient research has explored the relationship between BC collection timing during hospital admission and BC yield. Nannan et al. (2019) investigated the impact of pre-analytical time on BC yield and found no significant difference based on various pre-analytical time cut-off values. The median pre-analytical time observed was 15 hours, suggesting no notable effect on BC yield. However, further evidence is needed to fully understand these challenges.

2.5.5 Collection Site

The selection of an appropriate site before initiating skin antisepsis for BC collection is critical for accurate BSIs. Generally, peripheral venipuncture is preferred due to its reliability (De Plato et al., 2019). However, recent years have witnessed increased contamination rates associated with BCs drawn through indwelling catheters. Hospitalized patients, particularly those with long-term intravenous access via central lines, Peripherally Inserted Central Catheters (PICCs), ports, or dialysis lines, often require cultures from both these sites and peripheral veins to comprehensively evaluate BSIs. According to the guidelines, sending cultures from both the catheter tip and peripheral blood to the microbiology lab enhances diagnostic accuracy for catheter-associated BSIs (Chela et al., 2019).

Even though peripheral venipuncture remains the preferred method, arterial blood sampling is a viable alternative when suitable peripheral veins are unavailable. Nonetheless, using existing intravenous catheters, port-a-caths, or central venous catheters for BC collection increases contamination risks. The issue highlights the importance of meticulous microbiological interpretation (De Plato et al., 2019).

Guidelines for diagnosing central line-associated bloodstream infections (CLABSIs) recommend, when feasible, simultaneous sampling from both the catheter and a contralateral peripheral vein to assess differential time to positivity or microbial load. This dual-site approach is crucial in clinical practice to accurately diagnose CLABSIs despite concerns about potential false-positive results and unnecessary antibiotic use. Studies reveal higher rates of skin commensals, such as *S. epidermidis*, in cultures from central lines compared to those from peripheral sites used in non-CLABSI evaluations (Pandit et al., 2021; Kovoor et al., 2022).

Current clinical practices for febrile ICU patients with central lines often involve obtaining multiple sets of cultures from both peripheral veins and central lines. Experts recommend three to four sets to better differentiate between infection and contamination. Clinical trials suggest that using only two sets may fail to detect up to 15% of bloodstream infections. While IDSA guidelines advocate for dual-site cultures to diagnose CLABSI, many institutions warn against relying solely on central line cultures due to higher contamination risks (Kovoor et al., 2022).

Meta-analyses by Halstead et al. (2020) indicate higher contamination rates in cultures collected via lines compared to those from venipuncture, despite procedural benefits such as reduced needle sticks and ease of collection. Another study identifies catheters as the primary source of positive BCs, with gram-positive bacteria, particularly *Staphylococcus* species, comprising a significant proportion. Gram-negative bacteria, such as *Escherichia coli*, are also frequently isolated from cultures, highlighting the complex microbial landscape associated with catheter-related infections (Aiesh et al., 2023).

It is crucial to ensure a thorough disinfection of the catheter connection using appropriate chemicals like 2% chlorhexidine in 70% isopropyl alcohol before blood collection is performed through venous catheters. This protocol, which includes at least 30 seconds of contact time and thorough drying of the disinfectant, helps minimize contamination risks and enhances the reliability of BC results (De Plato et al., 2019; Pandit et al., 2021).

2.5.6 Volume of Blood

Among all the factors influencing BC outcomes, the volume of blood collected is critical for accurately recovering microorganisms in patients suspected of BSIs. The sensitivity of BCs significantly depends on the volume obtained, as adult patients often present with a low microbial load, typically ranging from less than 1 to 10 cfu/ml (Ombelet et al., 2019). Fabre et al. (2022) report that the detection rate in blood cultures increases by 3% for each additional milliliter of blood collected. This improvement is attributed to the generally low concentrations of microorganisms present in BSIs among adults. Consequently, collecting a larger volume of blood can significantly enhance the likelihood of successfully identifying pathogens.

CLSI guidelines recommend using four 10 ml vials to detect 90 to 95% of bacteremia and up to 60 ml of blood to detect 99% of organisms, as noted by Callado et al. (2023). Insufficient filling of BC vials often leads to false-negative results due to the low microbial load in BSIs. It is recommended that at least two sets (aerobic and anaerobic) of BC samples be collected, totaling 30 to 40 mL in adults. For children, age and weight considerations determine the optimal volume. In cases such as suspected endocarditis, up to four sets may be required (De Plato et al., 2019; Lin et al., 2022).

Correct vial filling should be monitored through visual inspection or weighing at the laboratory. The weight of the culture vials can provide insight into whether the correct volume of blood has been collected, aiding in the accurate diagnosis of BSIs. Including an anaerobic vial in each set is recommended, as it yields more pathogens than two aerobic vials (Chela et al., 2019; De Plato et al., 2019).

Adequate blood volume can be obtained either through a multi-sampling strategy, with at least two separate sets collected 10 to 12 minutes apart, or a single-sampling strategy, where all required blood is drawn at once. The multi-sampling strategy is preferred for diagnosing conditions like infective endocarditis, which, per the modified Duke Criteria, requires multiple samples over 24 hours. The single-sampling strategy is advantageous for ICU and ED patients due to its efficiency and reduced contamination risk (De Plato et al., 2019). Furthermore, inadequate blood volumes, especially from patients with challenging venous access, compromise sterility and increase contamination risks, impacting culture accuracy and diagnostic reliability (Palavecino et al., 2024).

A recent study conducted in 2022 involving 5,732 BC vials showed an overall positivity rate of 9.4%. Vials filled with 8 to 10 mL of blood had the highest positivity rate at 18.1%, while those containing 3 to 8 mL had a positivity rate of 9.1%. Vials with less than 3 mL exhibited the lowest positivity rate of 5.5%, and those with more than 10 mL had a positivity rate of 12.7%. These findings are consistent with earlier studies from Taiwan and South Korea that discovered that BC vials were frequently filled with less than the suggested 8 to 10 mL (Lin et al., 2022).

Optimal blood volume, typically 8 to 10 mL, significantly increases the likelihood of positive BCs for detecting bacteremia (Henning et al., 2019). Studies have shown that BC vials filled with insufficient volume, averaging 2.7 mL, often yield low isolation rates or false-negative results, potentially excluding significant bacteremia and leading to clinical and economic implications for hospitals (Sastry et al., 2020).

On the contrary, overfilled BC vials can lead to false-positive results, as carbon dioxide produced by leukocyte respiration in large blood volumes can trigger positive signals in automated systems (De Plato et al., 2019; Sastry et al., 2020). False positives not only consume unnecessary time and resources but can also lead to unwarranted antibiotic administration (Tenderenda et al., 2022). Including the percentage of cultures with inadequate blood volume in the BC collection report can highlight a target area for educational interventions that have proven effective in reducing both BCC and the incidence of false-negative BCs (Palavecino et al., 2024).

Furthermore, the volume of blood affects the time to positivity (TTP) in culture bottles. TTP refers to the duration from when the BC vials are placed in the system to when a positive signal is detected in the automated BC system. A study conducted by Sastry et al. (2020) observed that vials with optimal blood volumes (16.72 hours) detected true pathogens the fastest, followed by overfilled vials (17 hours) and suboptimal volumes (21.5 hours). True pathogen isolation rates were highest with optimal blood volumes (88.62%), slightly lower with overfilled volumes (86.43%), and lowest with suboptimal volumes (70.01%). False positive rates were 4.1% for overfilled, 1.6% for suboptimal, and 1.4% for optimal volumes.

Another study conducted by Lambregts et al. (2019) at Leiden University Medical Center, Netherlands, found that the median TTP was 15.7 hours. In BC with prolonged TTP, anaerobic bacteremia was more common. However, there was no significant difference in TTP between Gram-negative and Gram-positive bacteremia. In more than 85% of the cases, the TTP was less than 24 hours (Lambregts et al., 2019).

Generally, anaerobic vials tend to have higher blood volumes than aerobic vials, possibly due to sampling order preferences. However, inconsistent blood volumes per vial often result from insufficient knowledge about optimal volumes and limitations of blood collection devices. Many healthcare providers mistakenly believe that 3 to 7 mL is adequate per culture vial and use 10 mL syringes for collections, leading to inadequate distribution between bottles. Additionally, physicians frequently order BCs alongside other tests, causing the blood drawn into a 10 mL syringe to be distributed across multiple tubes, reducing the volume deposited into each culture vial (Lin et al., 2022).

Further studies are required to assess the appropriate volume needed to achieve optimal pathogen recovery from BCs without compromising patient safety. Physicians should consider both volume and clinical context when requesting BCs concurrently with other blood tests (Mortensen et al., 2024). In conclusion, the volume of blood collected for culture is a critical factor in the effective recovery of microorganisms in patients with suspected BSIs. Ensuring optimal blood volumes not only improves the sensitivity and accuracy of BCs but also reduces the likelihood of false-negative and false-positive results. Adhering to recommended guidelines and educating

healthcare providers on the importance of proper blood volume collection can significantly enhance diagnostic reliability and patient outcomes.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 STUDY DESIGN

This study employed a cross-sectional study design, facilitating systematic observation and data collection at a single point in time. The design provided a snapshot of BC practices and their outcomes in terms of positivity.

3.2 STUDY SETTING AND SAMPLE COLLECTION

The study took place at SASMEC@IIUM. It involved collaboration with the Medical Microbiology Laboratory, Department of Pathology and Laboratory Medicine. The sample collection lasted two months, while the overall research duration spanned one year, concluding at the end of July 2024.

3.2.1 Inclusion Criteria

1. Adult patients aged 18 years and older.

The study focuses on adult patients aged 18 years and older to ensure a consistent sample group that can simplify the overall methodology and improve clinical relevance. Working with adults in BC studies is beneficial for several reasons: they can provide larger blood volumes, have a higher occurrence of bloodstream infections, face fewer technical challenges during sample collection, and raise fewer ethical issues compared to pediatric patients.

2. One set of BCs per patient, one aerobic and one anaerobic.

Excluding patients with more than one set of BCs helps reduce the risk of contamination, streamlines the study design, and maintains a clear emphasis on initial diagnostic accuracy. This approach also helps manage resources more efficiently, lowers costs, and minimizes the need for invasive procedures, ultimately improving patient comfort and the reliability of the study.

3.2.2 Exclusion Criteria

1. Myco/F lytic bottles.
2. Clinical specimens other than blood.

3.3 SAMPLE SIZE

The sampling design for this research utilized convenience sampling. The sample size was calculated to detect a 10% difference in positivity rates based on unpublished data from SASMEC, which indicated contamination rates ranging from 5% to 20% and BC positivity rates ranging from 15% to 20%. The study used sample size calculation to compare proportions, considering a 5% Type I error rate and statistical power of 80%, with a 95% confidence interval. A total sample size of 276 participants was required. The formula for sample size estimation, proposed by Kang (2021), was utilized.

$$n = (Z\alpha/2+Z\beta)2 * (p1(1-p1)+p2(1-p2)) / (p1-p2)2 \quad \text{[Eq. 1]}$$

Where for the sample size n, Z α/2 was the critical value of the normal distribution at α/2 (considering a 5% alpha-type error rate, the critical value is 1.96), Zβ was the critical value of the normal distribution at β (considering a statistical power of 80%, the critical value is 0.84) and p1 and p2 were the expected sample proportions of the two groups (5% & 15% respectively). In this study, 5% and 15% proportions were used based on expected positivity rates derived from prior unpublished data at SASMEC. The formula underwent review in consultation with a statistician.

3.4 ETHICAL CONSIDERATIONS

In the subsequent phases of the study, ethical considerations were actively pursued, and approvals were sought from the Kulliyyah Research Committee (KRC), IIUM Research Ethics Committee (IREC), and SASMEC Research Committee. It is noteworthy that in this study, the collection of data involved retrieving information from the laboratory information system and the BACTEC incubation system within the laboratory setting. Notably, the collected data were used exclusively for clinical research purposes.

3.5 DATA COLLECTION

A systematic approach was employed to gather information on BC and pre-analytical factors and positivity outcomes. BC request forms were reviewed to record blood volumes. Blood volumes were determined by weighing the BC bottles, a process carried out after receiving training and supervision from the laboratory staff. The weight of the filled vials was compared to the standard empty vial weight, and the difference was used to calculate the volume of blood collected. The samples were then placed in an automated continuous monitoring system (BACTEC™ FX system; BD, Sparks, MD) and incubated for up to five days or until a positive signal was detected. Positive signals prompted microorganism identification using standard procedures, including Gram staining and MALDI-TOF Mass Spectrometry (Bruker Daltonik, Bremen, Germany), along with susceptibility testing. Vials without a positive signal after five days were deemed negative. Results, including microorganism identification and antibiotic susceptibility, were integrated into the Laboratory Information System (LIS), and the incubation duration until growth was detected in the Becton Dickinson BACTEC™ FX BC System (BACTEC™ FX).

The definition of true positive BCs was included to clarify the study's findings. A true positive BC was defined as a culture that grew a microbe identified as the etiological agent of a BSI, consistent with the patient's clinical condition. This definition was supported by Tenderenda et al. (2022) and Salimiyan et al. (2021), highlighting that true bacteremia is characterized by a positive BC and clinical symptoms such as fever and chills.

This definition was chosen to provide a consistent framework for evaluating BC results throughout the study. By establishing clear criteria for true positives, the study effectively differentiated between genuine BSIs and potential contaminants, thereby enhancing the reliability of the findings. In short, including this definition ensured clarity and coherence in the methodology and aligned it with established definitions in the literature.

3.5.1 Review of Blood Culture Request Forms

To assess pre-analytical parameters and contextual information related to BC requests, a systematic review of BC request forms was conducted for each BC request (see APPENDIX I). The information collected from these forms included patient demographic details (name, age, gender, barcode of lab vials, and request number), collection site (peripheral vein vs. central line), location (ED, Internal Medicine, General Surgery, ICU, Orthopaedics, Others), and transportation time (within six hours vs. more than six hours). This comprehensive review ensured consideration of key factors that might influence BC outcomes.

3.5.2 Observation and Measurement of Blood Volume Inoculated into the Blood Culture Vials

The weight of the BC vial was a key factor in calculating the blood volume inoculated into the bottle. Accurate volume calculations were made by measuring the weight of the empty and filled vials and converting the weight difference to volume by dividing it by the density of blood (1.055 g/mL). Additionally, the type of BC bottle, whether aerobic or anaerobic, was an important factor in this calculation. After receiving the samples, each BC vial was identified by barcode and request and individually weighed to a 0.1 g accuracy using an electronic high-precision scale (Mettler Toledo ME54T/00 Standard Analytical Balances) before the inoculated BCs were incubated in the BACTEC™ FX BC System (Becton Dickinson & Company, New Jersey, USA).

In studies by Henning et al. (2019), Kim et al. (2023), and Whelan et al. (2024), the blood volume in the BC vial was determined by subtracting the weight of the empty vial from the weight of the filled vial. The weight of the removable cap (0.41 g) was added to this calculation. This measurement was converted to volume using the weight-to-volume conversion factor for blood (1.055 g/mL). Briefly, the estimated blood volume inoculated in each vial was calculated using the formula: volume = (weight of vial filled with blood - mean weight of 50 empty vials) / density of blood (1.055 g/mL). The mean (± standard deviation, SD) weight for Aerobic/F and Non-Aerobic/F vials was 60.27 (± 0.15) and 65.57 (± 0.16) g, respectively.

3.5.3 Usage/Secondary Data Obtained From the Laboratory Information System (LIS) for the Blood Culture Test Result

Secondary data were obtained from LIS for the BC test results. The results of BC samples were collected, including information such as test outcomes (whether true positive or false positive), distribution of microorganisms, and the duration of incubation until growth in the BACTEC™ FX system. These meticulous methods and processes gathered comprehensive and optimal data on BC practices, blood volume, and microbial detection outcomes. This systematic approach ensured the reliability of the collected data and contributed to the overall success of the research study.

3.6 DATA ANALYSIS

Using SPSS software, statistical analysis was conducted. Initially, descriptive statistics were used to summarize the data, and categorical data were reported as percentages. Following this, univariate analysis, utilizing chi-square or Fisher's exact test as appropriate, assessed the association between each pre-analytical factor and BC positivity rates. Finally, logistic regression determined odds ratios, confidence intervals, and p-values for each predictor variable to provide insights into their impacts on BC positivity while controlling for other variables. This comprehensive analytical approach was conducted with a predefined level of statistical significance set at 5%.

3.7 STUDY WORKFLOW

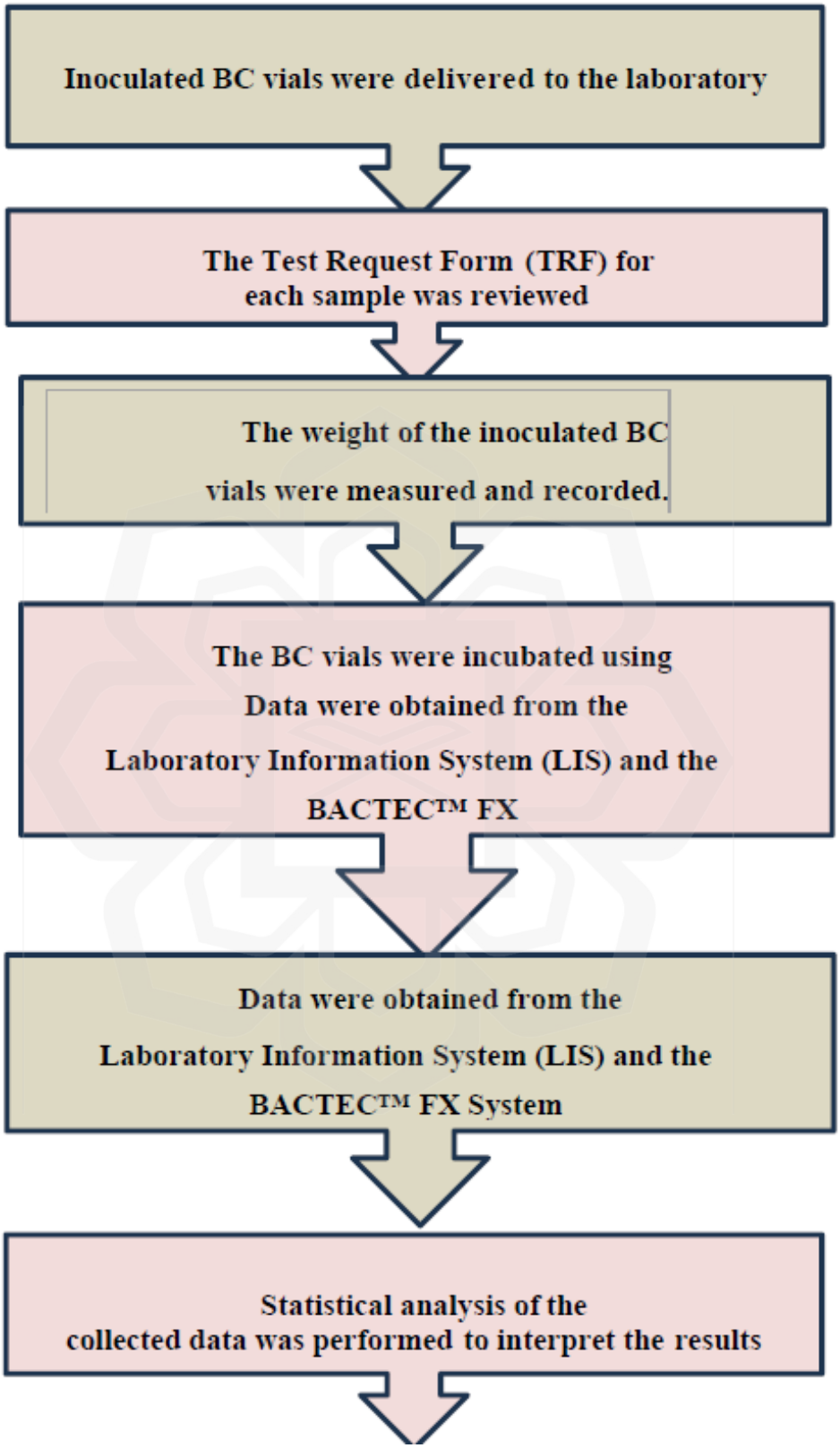

Figure 3.1 Study Workflow

CHAPTER FOUR

RESULTS

4.1 CHARACTERISTICS OF THE DISTRIBUTION OF DEMOGRAPHIC AND PRE-ANALYTICAL FACTORS IN BLOOD CULTURE SAMPLES

In this study, a total of 138 patients were included over two months. Out of these 138 cases, 80 (58%) were males and 58 (42%) were females. The mean age of all the study participants was 61.8 ± 14.4 years, ranging from 19 to 87 years. Most of the patients (88.4%) were 50 years or older. Patients admitted to various wards of the hospital were included. Among these, 49 (35.5%) presented to the ED, followed by 38 (27.5%) in the Internal Medicine Department and 18 (13.0%) in the General Surgery Department. Infection was suspected in 95 (68.8%) patients based on the information provided in TRF.

The samples were obtained from a central line or catheter in 21 (15.2%) cases, and in the remaining 117 (84.8%) cases, samples were drawn from peripheral veins. The pre-analysis time was recorded, the mean was 10.14 hours, and only 52 (37.7%) samples were analyzed within six hours. An optimal blood volume (8-10 ml) sample was obtained in 24 (17.4%) cases. Table 4.1 shows the distribution of various demographic and pre-analytical factors of BC samples of the study participants.

Table 4.1 Distribution of Demographic and Pre-analytical Factors of Blood Culture Samples (N= 138 Patients)

Factors		**N (%)**
Gender	Female	58 (42.0%)
	Male	80 (58.0%)
Age Group	18-29 years	7 (5.1%)
	30-39 years	6 (4.3%)
	40-49 years	8 (5.8%)
	50 years and above	117 (84.8%)
Ward / Location	Emergency Department	49 (35.5%)
	Internal Medicine	38 (27.5%)
	General Surgery	18 (13.0%)

Factors		N (%)
	ICU	17 (12.3%)
	Orthopaedics	11 (8.0%)
	Others	5 (3.6%)
Diagnosis	Non-Infectious	43 (31.2%)
	Infectious	95 (68.8%)
Collection Site	Central	21 (15.2%)
	Peripheral	117 (84.8%)
Pre-analytical time	< 6 hours	52 (37.7%)
	6 - 12 hours	33 (23.9%)
	> 12 hours	53 (38.4%)
Volume of blood	Inadequate (<3 ml)	14 (10.1%)
	Sub-optimum (3 - 7 ml)	82 (59.4%)
	Optimal (8 - 10 ml)	24 (17.4%)
	Over-filled (>10 ml)	18 (13.0%)

4.2 DISTRIBUTION OF MICROORGANISMS ISOLATED FROM POSITIVE BLOOD CULTURE VIALS

BCs were positive in 34 (24.6%) aerobic vials and 24 (17.4%) anaerobic vials. Overall, BC positivity (either aerobic or anaerobic vial) was observed in 40 (29.0%) cases. The mean + SD TTP in samples taken in aerobic vials was 16.9 + 13.1 hours, and in the anaerobic vials was 14.8 + 16.8 hours. Among the microorganisms isolated from aerobic and anaerobic vials, Gram-positive bacteria were the most common in both media. In the aerobic vial, Gram-positive bacteria were isolated in 47.06% of the positive cultures, and in the anaerobic vial, they were isolated in 54.17% of the positive cultures. Figure 4.1 represents the proportion of various types of microorganisms in aerobic and anaerobic media.

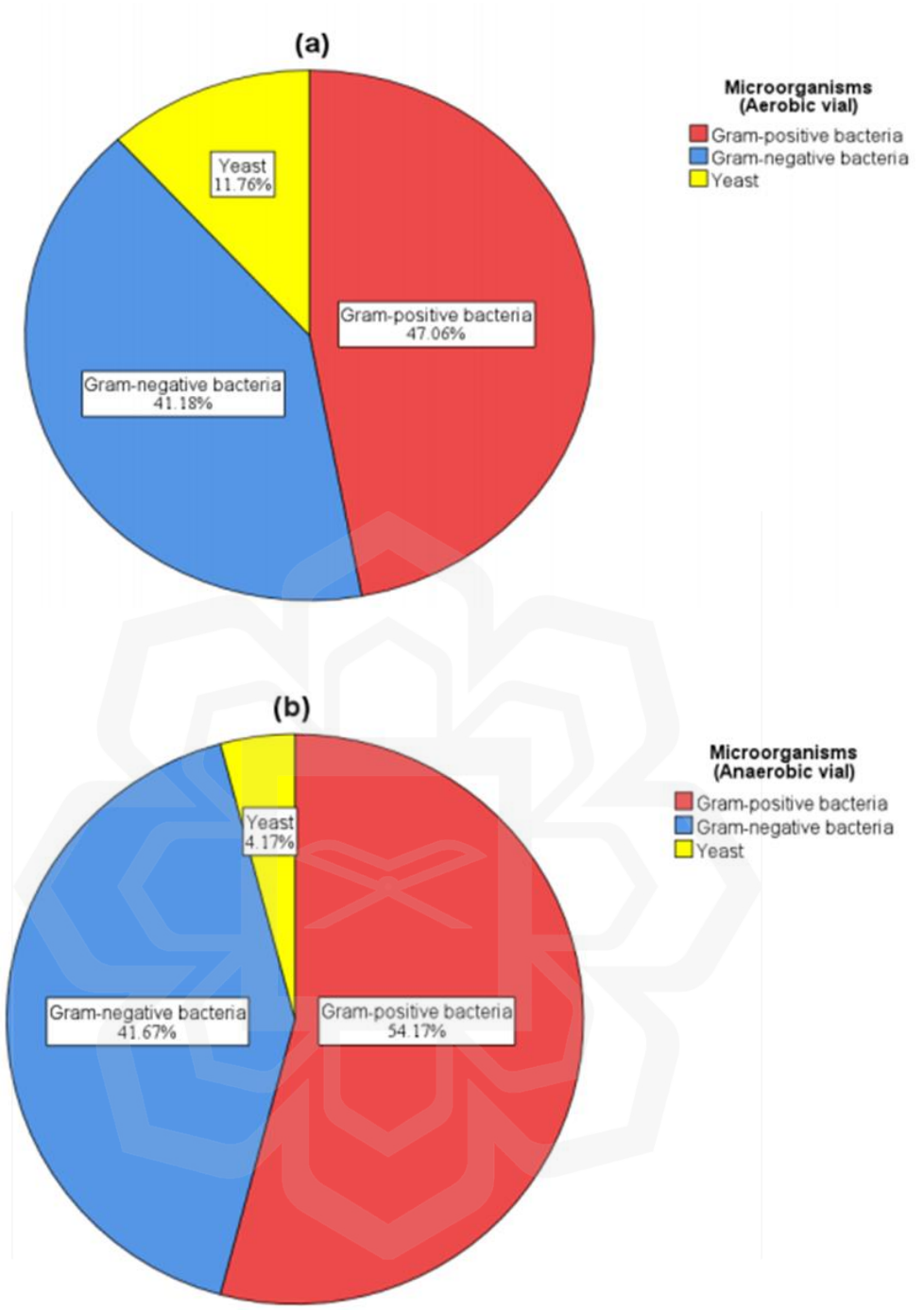

Figure 4.1 Pie Chart Showing Frequency of Various Types of Microorganisms in Blood Cultures Stored in (a) Aerobic Vials and (b) Anaerobic Vials

In the aerobic vial, *Staphylococcus aureus* was the most common Gram-positive bacteria, which was isolated in 8 (23.5%) cultures, followed by *Coagulase-negative staphylococcus* (CONS) in 7 (20.6%) cultures. Among the Gram-negative bacteria, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were most common and were isolated in 3 (8.8%) cultures each. *Candida tropicalis* was the most common yeast that was isolated in 2 (5.9%) cultures. In the anaerobic vial, the most common Gram-positive

bacteria were *Staphylococcus aureus* and CONS, which were isolated in 7 (29.2%) and 3 (12.5%) cultures, respectively. *Escherichia coli* and *Klebsiella pneumonia* were the most common Gram-negative bacteria and were isolated in 3 (20.5%) cultures each. The only yeast isolated from an anaerobic vial was *Candida glabrata,* which was isolated in a single culture. Table 4.2 and Table 4.3 show the most common microorganisms isolated from aerobic and anaerobic vials.

Table 4.2 Microorganisms Isolated from Blood Culture Stored in Aerobic Vials (N=138 Vials)

Gram-positive bacteria	**16 (47.1%)**
Staphylococcus aureus	8 (23.5%)
Coagulase-negative staphylococcus (CONS)	7 (20.6%)
Corynebacterium tuberculostearicum	1 (2.9%)
Enterococcus faecium	1 (2.4%)
Gram-negative bacteria	**14 (41.2%)**
Klebsiella pneumonia	3 (8.8%)
Pseudomonas aeruginosa	3 (8.8%)
Burkholderia cepacia	2 (5.9%)
Escherichia coli	2 (5.9%)
Haemophilus influenza	1 (2.9%)
Salmonella spp	1 (2.9%)
Citrobacterkoseri	1 (2.9%)
Stenotrophomonas maltophilia	1 (2.9%)
Yeast	**4 (11.8%)**
Candida tropicalis	2 (5.9%)
Candida albicans	1 (2.9%)
Candida glabrata	1 (2.9%)
Total	**34 (100 %)**

Table 4.3 Microorganisms Isolated from Blood Culture Stored in Anaerobic Vials (N= 138 Vials)

Gram-positive bacteria	**13 (54.2%)**
Staphylococcus aureus	7 (29.2%)
Coagulase-negative staphylococcus (CONS)	3 (12.5%)
Bacillus cereus	1 (4.2%)
Enterococcus faecalis	1 (4.2%)
Corynebacterium tuberculostearicum	1 (4.2%)
Gram-negative bacteria	**10 (41.6%)**
Escherichia coli	3 (12.5%)
Klebsiella pneumonia	3 (12.5%)
Bacteroides fragilis	1 (4.2%)
Enterobacter cloacae	1 (4.2%)
Salmonella spp	1 (4.2%)
Stenotrophomonas maltophilia	1 (4.2%)
Yeast	**1 (4.2%)**
Candida glabrata	1 (4.2%)
Total	**24 (100 %)**

4.3 ANALYSIS OF TRUE AND FALSE POSITIVE BLOOD CULTURES IN AEROBIC AND ANAEROBIC VIALS

Out of 40 positive BC samples, 26 (65.0%) had clinical signs of infection and were labeled as true positive cultures. The culture samples of 14 (35.0%) patients without clinical signs of BSI were labeled as false-positive cultures. Thus, the overall true positive rate was 18.8%, and the false positive rate was 10.1% among the 138 cases. The association of various microorganisms in the aerobic and anaerobic vial with BC positivity (true/false positive) was assessed. Gram-negative bacteria had the highest percentage of true positive cultures in both aerobic and anaerobic vials. In the aerobic vials, 78.6% of the Gram-negative cultures were true positive, and in the anaerobic vial, this percentage was 90. Additionally, there was a 100% true positive rate for yeast in both aerobic and anaerobic vials. Table 4.4 shows the association of various microorganisms with BC positivity.

Table 4.4 Association of Microorganisms with Blood Culture Positivity in Aerobic and Anaerobic Vials

Microorganisms	Positive blood cultures (N= 40)		p-value
	True Positive	False Positive	
Aerobic vial			
Gram-positive bacteria	7 (43.8%)	9 (56.3%)	
Gram-negative bacteria	11 (78.6%)	3 (21.4%)	0.051 α
Yeast	4 (100%)	0 (0.0%)	
Anaerobic vial			
Gram-positive bacteria	8 (61.5%)	5 (38.5%)	
Gram-negative bacteria	9 (90.0%)	1 (10.0%)	0.384 α
Yeast	1 (100%)	0 (0.0%)	
α Fisher's Exact test was applied where one or more cells had an expected cell count of less than 5			

4.4 COMPARATIVE ANALYSIS OF POSITIVE AND NEGATIVE BLOOD CULTURE WITH BLOOD VOLUME IN AEROBIC AND ANAEROBIC VIALS

The mean blood volume in aerobic vials was 6.3 ± 3.7 ml, and in the anaerobic vials was 5.9 ± 3.0 ml. The association of blood volume in aerobic and anaerobic vials with culture positivity (positive/negative culture) was assessed. In the aerobic vials, the highest percentage of positive cases (37.5%) was observed when the blood volume in the vial was optimal (8-10 ml). Over-filled vials (>10 ml) resulted in the lowest percentage of positive cases in both aerobic and anaerobic vials, 16.7% and 10.0%, respectively. Table 4.5 depicts the relationship of blood volume in aerobic and anaerobic vials with BC positivity.

Table 4.5 Association of Blood Volume with Blood Culture Positivityin Aerobic and Anaerobic Vials

Blood volume	Blood culture (N=276)		p-value
	Positive	Negative	
Aerobic vial			
Inadequate (<3 ml)	2 (14.3%)	12 (85.7%)	0.348 α
Sub-optimum (3 - 7 ml)	20 (24.2%)	62 (75.6%)	
Optimal (8 - 10 ml)	9 (37.5%)	15 (62.5%)	
Over-filled (>10 ml)	3 (16.7%)	15 (83.3%)	
Anaerobic vial			
Inadequate (<3 ml)	4 (21.1%)	15 (78.9%)	0.930 α
Sub-optimum (3 - 7 ml)	15 (18.1%)	68 (81.9%)	
Optimal (8 - 10 ml)	4 (15.4%)	22 (84.6%)	
Over-filled (>10 ml)	1 (10.0%)	9 (90.0%)	
α Fisher's Exact test was applied where one or more cells had an expected cell count of less than 5			

4.5 COMPARATIVE ANALYSIS OF BLOOD VOLUME WITH TIME TO POSITIVITY (TTP) IN AEROBIC AND ANAEROBIC VIALS

The volume of blood in aerobic and anaerobic vials was compared with TTP in the cases where BC was positive. It was observed that, in the aerobic vials, the longest TTP of 18.5 + 15.8 hours was recorded when the vials were sub-optimally filled (3-7 ml). The difference in the TTP with regard to blood volume in the aerobic vials was not statistically significant ($p = 0.452$). In the anaerobic vials, the longest TTP of 37.3 + 28.7 was recorded when the vials were inadequately filled (< 3ml). The difference in the TTP with regard to blood volume in the anaerobic vials was statistically significant ($p = 0.009$). The association of blood volume with TTP in aerobic and anaerobic vials in given in Table 4.6.

The statistically significant difference in TTP among different blood volumes in anaerobic vials was further subjected to post-hoc analysis. The results from the post-hoc Tukey Honest Significant Difference (HSD) test showed that a significant difference in mean TTP of 28.8 hours was observed between Inadequate blood volume (< 3 ml) and Sub-optimum blood volume (3-7 ml) in anaerobic vials (p = 0.001). A post-hoc comparison of the mean difference in TTP among various blood volumes in anaerobic vials is given in Table 4.7.

Table 4.6 Association of Blood Volume with Time to Positivity (TTP) in Aerobic and Anaerobic Vials with Positive Blood Cultures

Blood volume	**Blood culture Positivity**	**Time to positivity (TTP) (h)**	**p-value**
	Positive/total blood cultures N (%)	**Mean ± S.D**	
Aerobic vial (N=34)			
Inadequate (<3 ml)	2/14 (14.3%)	11.1 ± 5.0	0.452 **α**
Sub-optimum (3 - 7 ml)	20/82 (24.4%)	18.5 ± 15.8	
Optimal (8 - 10 ml)	9/24 (37.5%)	18.3 ± 7.2	
Over-filled (>10 ml)	3/18 (16.7%)	6.4 ± 1.3	
Anaerobic vial (N =24)			
Inadequate (<3 ml)	4/19 (21.1%)	37.3 ± 28.7	**0.009 α**
Sub-optimum (3 - 7 ml)	15/83 (18.1%)	8.5 ± 6.6	
Optimal (8 - 10 ml)	4/26 (15.4%)	18.8 ± 14.0	
Over-filled (>10 ml)	1/10 (10.0%)	4.3	
α One-Way ANOVA was applied **p-value in bold shows statistical significance**			

Table 4.7 Post Hoc Comparison of Mean Difference in Time to Positivity (TTP) among Various Blood Volumes in Anaerobic Vials with Positive Blood Cultures (post-hoc Tukey HSD Test)

Blood volume in anaerobic vials		**Mean difference in TTP (h)**	**Std. error**	**95% CI for mean difference**	**p-value**
Inadequate (<3 ml)	Sub-optimum (3-7 ml)	28.8	7.6	12.9 - 44.8	**0.001**
	Optimal (8-10 ml)	18.5	9.6	-1.5 - 38.5	0.068
Sub-optimum (3-7 ml)	Inadequate (<3 ml)	-28.8	7.6	-44.8 - (-12.9)	**0.001**
	Optimal (8-10 ml)	-10.3	7.6	-26.3 - 5.6	0.191
Optimal (8-10 ml)	Inadequate (<3 ml)	-18.5	9.6	-38.5 - 1.5	0.068
	Sub-optimum (3-7 ml)	10.3	7.6	-5.6 - 26.3	0.191
p-value in bold shows statistical significance **Blood volume category (overfilled) with fewer than two cases was excluded from the analysis**					

4.6 INVESTIGATING THE ASSOCIATION OF PRE-ANALYTICAL FACTORS WITH BLOOD CULTURE POSITIVITY

The association of various pre-analytical factors with true and false positive cultures was studied. Factors such as age, gender, ward/location of presentation, diagnosis, site of sample collection, pre-analysis time, and volume of blood sample were examined. False positive cases were higher in females compared to males (41.7% vs. 32.1%, p = 0.720). Similarly, patients aged 50 years or older showed higher false positive rates than younger patients (37.1% vs. 20.0%, p = 0.640). Samples drawn from a central line showed higher false positive rates compared to samples drawn from a peripheral vein (50% vs. 30%, p = 0.278). However, no statistically significant association of any of these factors was observed with culture positivity ($p > 0.05$). The details of the association of various demographic and pre-analytical factors with BC positivity are provided in Table 4.8.

Table 4.8 Association of Various Demographic and Pre-Analytical Factors with Blood Culture Positivity (N = 40 Patients)

Factors	Positivity		p-value
	True Positive N (%)	False Positive N (%)	
Gender			
Female	7 (58.3%)	5 (41.7%)	0.720 α
Male	19 (67.9%)	9 (32.1%)	
Age			
< 50 years	4 (80.0%)	1 (20.0%)	0.640 α
> 50 years	22 (62.9%)	13 (37.1%)	
Ward / Location			
Emergency Department	13 (68.4%)	6 (31.6%)	1.000 α
Internal Medicine	5 (71.4%)	2 (28.6%)	
General Surgery	1 (50.0%)	1 (50.0%)	
ICU	5 (62.5%)	3 (37.5%)	
Orthopedics	1 (50.0%)	1 (50.0%)	
Others	1 (50.0%)	1 (50.0%)	
Diagnosis			
Non-infectious	9 (64.3%)	5 (35.7%)	1.000 α
Infectious	17 (65.4%)	9 (34.6%)	
Collection Site			
Central	5 (50.0%)	5 (50.0%)	0.278 α
Peripheral	21 (70.0%)	9 (30.0%)	
pre-analytical time			
< 6 hours	12 (66.7%)	6 (33.3%)	1.000 α
6 - 12 hours	4 (66.7%)	2 (33.3%)	
> 12 hours	10 (62.5%)	6 (37.5%)	
Volume of Blood			
Optimal (8 -10ml)	8 (66.7%)	4 (33.3%)	1.000 α
Sub optimum (<8 ml)	18 (64.3%)	10 (35.7%)	
α Fisher's Exact test was applied where one or more cells had an expected cell count of less than 5			

4.7 MULTIVARIATE ANALYSIS OF VARIOUS DEMOGRAPHIC AND PRE-ANALYTICAL FACTORS PREDICTING BLOOD CULTURE POSITIVITY

Multivariate analysis of various demographic and pre-analytical factors for predicting the positivity of BCs showed that age, gender, ward/location of presentation, diagnosis, site of sample collection, pre-analysis time, and volume of blood sample did not significantly predict true or false positive cultures ($p > 0.05$). Cases aged 50 years or older had a higher odds ratio of 3.357 (95% CI: 0.144 - 78.228, $p = 0.451$) for predicting positivity compared to those younger than 50 years. Patients observed in the General Surgery Department had an odds ratio of 8.015 (95% CI: 0.216 - 298.005, $p = 0.259$) for predicting culture positivity. A delay in analysis time of 6 – 12 hours predicted culture positivity with an odds ratio of 2.045 (95% CI: 0.144 - 29.127, $p = 0.598$), and a delay of more than 12 hours showed an odds ratio of 1.734 (95% CI: 0.269 - 11.157, $p = 0.562$). However, none of these factors significantly predicted culture positivity ($p > 0.05$). Detailed results of the multivariate analysis of demographic and pre-analytical factors predicting culture positivity are presented in Table 4.9.

Table 4.9 Multivariate Analysis of Factors Predicting the Positivity of Blood Cultures (N = 40 Patients)

Factors	Positivity		Odds Ratio	95 % CI	p-value
	True Positive N (%)	False Positive N (%)			
Gender					
Female	7 (58.3%)	5 (41.7%)	1	-	-
Male	19 (67.9%)	9 (32.1%)	0.295	0.044 - 1.984	0.209
Age					
< 50 years	4 (80.0%)	1 (20.0%)	1	-	-
≥ 50 years	22 (62.9%)	13 (37.1%)	3.357	0.144 - 78.228	0.451
Ward / Location					
Emergency Department	13 (68.4%)	6 (31.6%)	1	-	-
Internal Medicine	5 (71.4%)	2 (28.6%)	0.994	0.069 - 14.241	0.997
General Surgery	1 (50.0%)	1 (50.0%)	8.015	0.216 - 298.005	0.259
ICU	5 (62.5%)	3 (37.5%)	2.259	0.169 - 39.739	0.493
Orthopaedics	1 (50.0%)	1 (50.0%)	1.572	0.055 - 44.844	0.791
Others	1 (50.0%)	1 (50.0%)	5.022	0.135 - 186.903	0.382

Factors	Positivity		Odds Ratio	95 % CI	p-value
	True Positive N (%)	False Positive N (%)			
Diagnosis					
Non-infectious	9 (64.3%)	5 (35.7%)	1	-	-
Infectious	17 (65.4%)	9 (34.6%)	0.754	0.118 - 4.810	0.765
Collection Site					
Central Line	5 (50.0%)	5 (50.0%)	1	-	-
Peripheral Vein	21 (70.0%)	9 (30.0%)	0.329	0.052 - 2.069	0.236
pre-analytical time					
< 6 hours	12 (66.7%)	6 (33.3%)	1	-	-
6 - 12 hours	4 (66.7%)	2 (33.3%)	2.045	0.144 - 29.127	0.598
> 12 hours	10 (62.5%)	6 (37.5%)	1.734	0.269 - 11.157	0.562
Volume of Blood					
Optimal (8 -10ml)	8 (66.7%)	4 (33.3%)	1		
Sub optimum (<8 ml)	18 (64.3%)	10 (35.7%)	2.522	0.233 - 27.295	0.447

CHAPTER FIVE

DISCUSSION

5.1 DEMOGRAPHIC AND PRE-ANALYTICAL FACTORS INFLUENCING BLOOD CULTURE POSITIVITY OUTCOMES

This study aimed to evaluate how various demographic and pre-analytical factors influence the outcome of BC positivity. The main findings are discussed here.

5.1.1 Role of Gender in Determining Blood Culture Positivity

Among the 138 blood samples analyzed, 58% were from male patients. This proportion increased to 70% among culture-positive patients, compared to 30% females, thus indicating a gender disparity in infection prevalence. This observation aligns with findings from another research. For example, an Italian study that tracked patients with probable sepsis from 2015 to 2019 consistently reported higher proportions of male patients, ranging from 53% to 65% annually (Santella et al., 2020).

Similarly, a study on Iraqi patients suspected of septicemia found that 62.3% of those with positive BCs were males (Al-Asady et al., 2020). Factors contributing to this disparity may include estrogen-modulated activation of macrophages in females and a higher prevalence of risk factors such as smoking, alcohol consumption, and outdoor activities in males (Zhang et al., 2021; Lakbar et al., 2023).

5.1.2 Role of Age in Determining Blood Culture Positivity

Regarding age demographics, the mean age of all study participants was 61.8 ± 14.4 years, with the majority (84.4%) being aged 50 years or older. This distribution mirrors global trends; for instance, a multinational study involving over 15,000 patients with suspected BSIs reported a mean age of 61.1 ± 17.3 years (Vincent et al., 2020). In a similar study, Koh et al. (2021) recorded a mean age of 57 ± 16.9 years in their study at Hospital Kuala Lumpur, with 34.4% of cases being over 65 years of age. These results corroborate the literature that indicates a higher prevalence of BSIs among older age groups, excluding studies focused on specific populations like pediatrics.

The increased susceptibility in older individuals can be attributed to the downregulation of both adaptive and innate immune responses associated with aging, as well as prolonged exposure to risk factors such as smoking, alcohol and comorbidities like diabetes mellitus (Du et al., 2021; Schoevaerdts et al., 2021).

5.1.3 Distribution of Blood Culture Samples by Departments in Determining Culture Positivity

In the present study, the highest number of blood samples were received from EDs (35.5%), followed by the Internal Medicine Department (27.5%) and the Department of General Surgery (13.0%). These findings are consistent with other studies, though there is some variation depending on the hospital and patient population. For instance, a Malaysian study on the prevalence of pseudomonal bacteremia at Hospital Seri Manjung (HSM) found that the highest number of cases were admitted to the medical ward (72.9%), followed by the Surgery Department and the ICU, each with 10.1% of cases. Notably, more than half of the patients eventually required ICU admission for further management (Tan et al., 2021).

These variations highlight that the origin of blood samples can differ significantly based on the hospital's admission policies and the specific conditions under consideration. Despite this variability, the ED and medical ward are commonly the primary sources of BC samples received in microbiology laboratories. Therefore, it is crucial to follow proper BC collection protocols with a specific focus on these two departments, ensuring targeted training and handling practices in these areas to improve diagnostic accuracy and patient outcomes.

5.1.4 Distribution of Blood Culture Samples by Central or Peripheral Route in Determining Culture Positivity

BC can be drawn from either central or peripheral routes. In the current study, the majority (84.8%) of the BC samples were drawn from peripheral veins, while a smaller proportion (15.2%) were obtained from central lines. This distribution likely reflects that many patients in this study did not have central lines, which are more commonly used in ICU settings.

Although peripheral veins are usually preferred because they are easier to access and have a lower risk of contamination, the clinical setting and the patient's condition can greatly affect the choice of BC collection method. This finding aligns with results from the University of Nebraska Medical Center, where 89.8% of samples were drawn from peripheral veins (Liaquat et al., 2022). Drawing BCs through peripheral veins is technically less demanding and is associated with lower contamination rates compared to central catheters and is thus often the preferred site for drawing BCs where possible (Doern et al., 2019; Ombelet et al., 2019).

However, in a study conducted at King Fahad Medical City, Riyadh, Saudi Arabia, the proportion of BC samples taken from peripheral and central routes was almost equal (50.7% vs. 49.3%) (Hafiz et al., 2023). A plausible reason for the higher proportion of blood samples drawn from the central route may be that more than 40% of the samples were drawn from ICU patients, who are usually critical and have central lines placed. These differences highlight that, while peripheral veins are generally preferred for BC collection due to ease and lower contamination risk, the clinical setting and patient condition, such as ICU admission and the presence of central lines, can significantly influence the route of BC collection.

5.1.5 Recommended Pre-Analysis Time and Delays in Processing of Blood Culture Samples

In this study, the pre-analytical time was less than 12 hours for 61.4% of the BC samples, while the remaining 38.6% exceeded this duration. Only 52 (37.7%) samples were analyzed within 6 hours. Although recommended guidelines advocate for the incubation of BC samples within 2 to 4 hours of collection, delays are commonly observed (De Plato et al., 2019).

A study by Nannan et al. (2019) found no significant differences in BC positivity rates when comparing various pre-analytical time cut-offs. The study observed a median pre-analytical time of 15 hours, which is longer than the recommended pre-analysis time. The yield of BCs tends to be inversely proportional to pre-analytical time. Delays are often due to increased transport times from hospital wards to the laboratory and human or material resources constraints. Laboratory operating hours also influence these times, with delays more prevalent during weekends or holidays, thus significantly impacting the effectiveness of BCs, reducing the quality of patient care, and incurring unnecessary healthcare costs (Simundic et al., 2020; Kufa et al., 2020; Skusa et al., 2021).

Addressing challenges related to pre-analytical delays can significantly enhance pathogen recovery rates, thereby improving the efficiency and accuracy of BCs. Moreover, understanding the mean collection time is vital in medical diagnostics, as it offers insights into the typical duration between sample collection and processing. Shorter collection times may be associated with specific clinical conditions or the urgency of testing, whereas longer times could reflect delays in sample processing or transportation (Skusa et al., 2021; Simundic et al., 2020). Therefore, minimizing turnaround times is essential, as exceeding recommended durations can negatively impact BC yield, limit microbial growth, and delay the initiation of appropriate antimicrobial therapy (Doern et al., 2019; Skusa et al., 2021).

5.1.6 Role of Blood Volume in Aerobic and Anaerobic Vials in Determining Blood Culture Positivity

The volume of blood in aerobic and anaerobic vials is a crucial factor in determining BC positivity. Although various guidelines recommend 10 mL of blood in both vial types, 40-85% of BC samples are estimated to contain less than the optimal amount (Fabre et al., 2022). In this study, optimal blood volume (8-10 mL) was present in only 17.4% of the aerobic vials and 18.8% of the anaerobic vials. The remaining vials were either underfilled or overfilled. The highest positivity rate was observed in the aerobic vials when the blood volume was optimal.

The mean blood volume in aerobic vials was 6.3 ± 3.7 mL, and in anaerobic vials, it was 5.9 ± 3.0 mL. Although these volumes fall within the manufacturer's recommended range of 5–10 mL, they are still considered suboptimal. From the perspective of the literature, some studies regard the range of 8-10 mL volume as optimal.

Furthermore, the current findings are consistent with studies in Taiwan and South Korea, where most vials contained less than the recommended 8 to 10 mL (Lin et al., 2022). Additionally, the study showed that the median sampling volume was higher in anaerobic vials (5.7 mL) compared to aerobic vials (4.7 mL) (Lin et al., 2022). To address the low rates of optimally filled samples, interventions are necessary.

At the Birmingham Public Health Laboratory (PHL), an intervention based on a plan-do-study-act (PDSA) cycle of user education, real-time feedback about the volume of samples received and reporting of laboratory data increased the rate of optimal volume samples from 16% to 29% (Birkhamshaw & Winzor, 2019). The lower rates of optimal samples in the current study similarly indicate the need for such interventions to improve BC collection practices in hospitals. Therefore, it is recommended that training programs and standardized protocols be implemented to ensure optimal blood volume collection.

5.1.7 Influence of Number of Aerobic and Anaerobic Vials on Blood Culture Positivity

In the present study, BCs were taken from both central and peripheral sites using one or two sets of both aerobic and anaerobic vials, depending on BC requests. Culture positivity was observed in 24.6% of the samples stored in aerobic vials and 17.4% of the samples stored in anaerobic vials. Overall, BC positivity (either aerobic or anaerobic vial) was observed in 29.0% of the cases. Current guidelines recommend taking at least two sets of BCs inoculated into both aerobic and anaerobic vials for optimal detection of blood pathogens (Azem & Ransom, 2022). Clinical trials supporting this approach suggest that relying solely on two sets may underestimate BSIs by approximately 15% (Kovoor et al., 2020).

Using a single set results in up to a 40% decrease in the detection rate of bacteria and also lacks the ability to distinguish contamination from true bacteremia (Fabre et al., 2022). These findings emphasize the importance of following recommended guidelines for BC collection. Utilizing multiple sets and sites enhances the likelihood of detecting pathogens and differentiating true bacteremia from contamination, thereby improving diagnostic accuracy and patient outcomes.

5.1.8 Common Bacterial Isolates in Aerobic and Anaerobic Blood Culture Samples

Gram-positive bacteria were more common in both aerobic and anaerobic vials compared to Gram-negative bacteria and yeast. Specifically, Gram-positive bacteria were isolated from 47.1% of the positive cultures in aerobic vials and 54.2% of the positive cultures in anaerobic vials. *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CONS) were the most common Gram-positive bacteria, whereas *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli* were the most common Gram-negative bacteria.

These findings are consistent with other studies. For example, Santella et al. (2020) studied 3,949 positive BCs from the University Hospital San Giovanni di Dio e Ruggi d'Aragona in Salerno, Italy. They found that *Staphylococcus aureus* and CONS were the most isolated Gram-positive bacteria, whereas *Klebsiella pneumoniae* and *Escherichia coli* were the most common Gram-negative bacteria.

Although the microorganisms isolated from cultures can depend on the condition being studied and the age group of the patients, these bacteria are frequently reported as the most commonly isolated microorganisms in many studies. The consistency recorded in the analysis underscores the importance of these pathogens in clinical settings and highlights the need for targeted approaches in their detection and management.

5.1.9 TTP of Positive Blood Cultures in Aerobic and Anaerobic Vials

In the present study, the mean TTP for samples taken in aerobic vials was 16.9 ± 13.1 hours, and in anaerobic vials, it was 14.8 ± 16.8 hours. A similar study by Lambregts et al. (2019) at Leiden University Medical Center, Netherlands, reported a median TTP of 15.7 hours. They found that anaerobic bacteremia was more common in blood cultures with prolonged TTP. However, there was no significant difference in TTP between Gram-negative and Gram-positive bacteremia. Additionally, in more than 85% of the cases, the TTP was less than 24 hours (Lambregts et al., 2019).

Understanding TTP patterns is important because it can aid in the timely discontinuation of empirical antibiotics, preventing their non-judicious use, especially in neonatal and critically ill patients when blood cultures do not show growth within the expected time range (De Rose et al., 2021). Having knowledge of estimated TTP can be helpful in the clinical management of the patients as clinicians and microbiologists can relate to the expected TTP before labeling cultures as positive or negative. This is particularly important in conditions with shorter TTP as this may enable early discontinuation of empirical antibiotic therapy. Furthermore, adjusting the timing for stopping antibiotics can help healthcare providers limit unnecessary antibiotic use and lower the chances of developing resistance. (Lambregts et al., 2019).

5.1.10 True/False Positive Blood Cultures in Aerobic and Anaerobic Vials

This study analyzed the true and false positive BCs in aerobic and anaerobic vials. The reasons for ordering it are important for understanding the results of this study. BCs are usually requested when there is a clinical suspicion of infection, with healthcare providers taking into account symptoms like fever, chills, and an increased heart rate. In this study, out of the 40 positive samples, 26 (65.0%) were true positives that matched the clinical signs of infection, while 14 (35.0%) were false positives, showing no clinical symptoms for BSIs. This led to an overall true positive rate of 18.8% and a false positive rate of 10.1% among the 138 cases, which seems higher than most other studies reported. This discrepancy raises concerns about the criteria for ordering these tests. Factors such as the local prevalence of infections, patient history, and clinical presentation may affect these rates (Timsit et al., 2020). Moreover, some healthcare providers might order BCs as a precaution for patients with unusual symptoms to rule

out serious infections (Fabre et al., 2020). Recognizing these factors highlights the need for clearer guidelines on when to order BCs.

The distribution of microorganisms in BC vials revealed that Gram-negative bacteria had the highest percentage of true positive cultures, with 78.6% in aerobic vials and 90% in anaerobic The Gram-positive bacteria showed a lower true positivity rate of 43.8% in aerobic and 61.5% in anaerobic vials. The p-value of 0.051 for aerobic vials indicates a trend towards significance, implying that with a larger sample size, Gram-negative bacteria might show a statistically significantly higher true positive rate. These findings support the general understanding that Gram-positive bacteria exhibited a lower true positive rate (higher contamination rate), likely due to contamination from commensal Gram-positive rods present commonly in skin flora (Palavecino et al., 2024). A 100% true positive rate for yeast was observed in both aerobic and anaerobic vials, which may signify more accurate detection of yeast as compared to bacteria. However, considering the smaller number of yeast cultures isolated in the current study, it may not be possible to draw logical conclusions about the accuracy of yeast detection in aerobic and anaerobic vials.

Comparatively, a study on 4,797 BC samples from a tertiary care hospital in Spain found an overall culture positivity rate of 15.5%. The false positive rates in both aerobic and anaerobic vials were less than 1% (Halperin et al., 2022). Similarly, a study across four hospitals in the United States reported BCC rates ranging from 6 to 9%. After implementing various educational and training tools, the contamination rates improved to an acceptable range of 1 to 2% (Halstead et al., 2020).

Research by Halstead et al. (2020) shows that hospitals implementing detailed educational programs and refining collection techniques significantly reduced contamination rates, consistently maintaining BCC rates between 1% and 2%. These interventions were based on the involvement of multidisciplinary teams, review of the process of blood culturing, “train the trainer” or “one-on-one training” initiatives for ED nurses, distribution of printed and electronic learning resources and using collector codes for phlebotomists to track their BCC rates. These improvements demonstrated the effectiveness of such strategies in four different hospitals that were subjected to these interventions (Halstead et al., 2020). Broadly adopting these practices is expected to enhance the quality and accuracy of BC testing across healthcare environments.

It is imperative to distinguish true from false positives in clinical diagnostics to avoid unnecessary treatments based on false positive results. In the present study, the false positive rate of 10.1% indicates the need for improved diagnostic protocols to minimize the risk of misinterpreting contamination as an infection. The high true positive rate for Gram-negative bacteria underscores their clinical relevance and the effectiveness of current detection methods for these pathogens. These insights are crucial for refining BC practices and improving patient outcomes by ensuring accurate diagnosis and appropriate treatment. Further research is warranted to explore factors influencing false positive rates and to develop strategies to decrease them.

5.1.11 Relationship of Blood Volume with Blood Culture Positivity

This study evaluated the relationship between blood volume and culture positivity. The highest percentage of positive cultures (37.5%) occurred when the blood volume was optimal (8-10 mL) in aerobic vials. However, there was no statistically significant association between blood volume and culture positivity ($p > 0.05$). Overfilled vials (>10 mL) had the lowest positivity rates, with 16.7% in aerobic and 10.0% in anaerobic vials, highlighting the importance of adhering to optimal blood volumes for accurate culture results. In a similar study, vials with less than 3 mL of blood had the lowest positivity rate, and the highest positivity rate (18.1%) was observed with optimal volumes. There was no increase in positivity rates when the volume exceeded 10 mL (Lin et al., 2022).

5.1.12 Relationship of Blood Volume with TTP of Blood Cultures

Blood volume in the culture vials can also affect the TTP of positive cultures. In the present study, the longest TTP of 18.5 ± 15.8 hours was recorded in the aerobic vials when they were sub-optimally filled (3-7 ml). This difference in TTP of positive cultures with regard to blood volume in the aerobic vials was not found to be statistically significant ($p = 0.452$). However, in the anaerobic vials, a statistically significant difference in TTP among various volumes of blood was observed ($p = 0.009$), and the longest TTP of 37.3 ± 28.7 hours was recorded when the vials were inadequately filled (< 3ml). The post-hoc analysis of the mean difference in TTP in anaerobic vials showed that the most statistically significant difference in mean TTP was observed in inadequate (< 3ml) samples and sub-optimal samples (3-7ml) ($p = 0.001$). The mean

TTP was higher in inadequate samples, with a difference of 28.8 hours as compared to sub-optimal samples. Blood volume is a crucial factor in determining the TTP of BC. Inadequate volumes lead to longer TTP, and even a 1 ml addition of blood volume can improve the detection rate by 4.7% and thus reduce the TTP (Huber et al., 2020)

Similar results were reported by Sastry et al. (2020), where the average TTP was lowest in the vials with optimal blood volume (8-10 ml). The average TTP in vials with optimal blood volume was 16.72 hours, which shows similarity to the current study where the mean TTP in the vials with optimal blood volume was 18.3 ± 7.2 in aerobic and 18.8 ± 14.0 in anaerobic vials. Nevertheless, these results indicate a possible linkage between blood volume in the culture vials and TTP in cases with positive BCs.

To improve BC diagnostics, adhering to guidelines by collecting blood volumes within the optimal range of 8-10 mL is recommended. This can be achieved through implementing training programs for healthcare professionals on the importance of optimal blood volume collection, the development and enforcement of standardized protocols for BC collection, and regular monitoring and feedback on collection practices to ensure compliance. Maintaining optimal blood volumes in both aerobic and anaerobic vials is essential for reliable BC results. These findings should inform best practices in clinical settings to improve the accuracy of BC diagnostics. Future research should continue to explore the impact of blood volume on culture positivity to refine guidelines and improve patient care.

5.1.13 Pre-Analytical Factors Affecting Positivity Outcomes of Blood Culture Samples

A clear understanding of the factors influencing BC sample positivity rates is essential for improving diagnostic accuracy and patient outcomes. Knowledge of these factors increases the likelihood of detecting true pathogens and differentiating genuine bacteremia from contamination, which, in turn, supports more precise diagnoses and effective patient care. The current study analyzed the role of various demographic and pre-analysis factors, such as age, gender, ward/location of presentation, diagnosis, site of sample collection, pre-analysis time, and blood sample volume, in predicting BC samples' positivity. While age and gender have not previously been identified as factors influencing BC positivity, the findings of this study confirm that these variables

do not have an impact on the topic of interest. However, diagnosis may directly reflect the reason for obtaining a BC, which can be used to support its inclusion in the analysis.

In both univariate and multivariate analyses, none of these factors seemed to influence the positivity of BC ($p > 0.05$). Although the study indicated higher true positive rates in males (67.9%) compared to females (58.3%), there was no statistically significant association with culture positivity ($p = 0.209$). Similarly, older patients (> 50 years) had higher true positive rates (62.9%) than younger patients (< 50 years), though this was not statistically significant ($p = 0.451$).

Different wards and locations also showed varying true positive rates, with the ED (68.4%) and the Internal Medicine Department (71.4%) having the highest true positive rates. The General Surgery Department had the highest odds ratio (8.015) for predicting culture positivity, although not statistically significant ($p = 0.259$).

Samples collected from a central line exhibited higher false positive rates (50%) compared to peripheral veins (30%), but this was not statistically significant ($p = 0.236$). Pre-analytical time intervals showed no significant impact on culture positivity, with true positive rates of 66.7% for samples processed within 6 to 12 hours and 62.5% for those processed after 12 hours ($p > 0.05$). Additionally, the volume of blood collected did not significantly predict culture positivity, with suboptimal volumes (< 8 mL) showing higher odds (2.522) but no statistical significance ($p = 0.447$).

However, variable results have been reported in the literature. Lin et al. (2022) observed that age, presence of clinical sepsis, samples drawn from the ED, and the sample volume had a statistically significant effect on culture positivity. However, gender and the number of culture sets did not statistically affect culture positivity. In another study conducted at Beijing Chao-Yang Hospital in China on 640 BC samples, age over 65 years was found to be significantly associated with culture positivity ($p < 0.05$), whereas gender did not affect culture positivity ($p = 0.531$) (Yang et al., 2021).

A large-scale study on 3,890 BC samples from VU University Medical Center, Amsterdam, Netherlands, showed that cultures collected during the first 24 hours of hospital admission and samples drawn from the ED were linked with higher positivity rates ($p < 0.01$). However, pre-analysis time ($p = 0.816$) and antibiotic administration ($p = 0.735$) did not influence culture positivity (Nannan Panday et al., 2019).

A thorough literature review shows considerable variation in the association of various demographic and pre-analysis factors with culture positivity. Therefore, there is a dire need for further exploration of these factors to understand their role in predicting cultural outcomes better. In summary, while certain demographic and pre-analytical factors showed trends in predicting BC positivity, none reached statistical significance. BC volume had a significant effect on the TTP of positive cultures. However, this effect was significant only in the anaerobic vials.

This underscores the complexity of predicting BC outcomes and highlights the need for standardized protocols in BC collection and analysis to enhance diagnostic accuracy. Future research with larger sample sizes is needed to validate these findings and explore additional influencing factors. To further improve BC diagnostics, it is recommended to focus on interventions such as training programs for healthcare professionals, standardized protocols for BC collection, and continuous monitoring and feedback on collection practices. These measures can help ensure optimal blood volume collection and reduce the incidence of false positives, ultimately leading to more accurate and reliable BC results.

5.2 STRENGTHS AND LIMITATIONS OF THE STUDY

This study was the first conducted at SASMEC hospital, and it provided important insights into how pre-analytical factors affect BC positivity outcomes. A key strength of the study is the use of clinical validation to distinguish between true positive and contaminated cultures. This was achieved by adhering to the TRF and verification by qualified lab personnel, as documented in the patient's clinical record. This review ensures that the positive results reflect real infections rather than contamination, improving the accuracy of our findings.

However, it is impossible to determine the specific reasons for BC requests as we do not review detailed clinical information. The assessment relies solely on the limited details provided in the test request form. While the reasons for performing BCs in patients without confirmed BSIs were not systematically recorded in this study, some patients had conditions such as healthcare-associated infections (HAIs), community-acquired pneumonia (CAP), and cancer. BCs were likely ordered based on clinical signs or symptoms suggesting infection or in cases of unexplained fever, even when clear signs of infection were absent.

Another strength of the current study is that a wide range of demographic and pre-analytical factors were studied collectively to provide a holistic view of their role in determining cultural outcomes. Previous studies have studied a few variables at a time.

The study focused on specific pre-analytical factors that influence BC positivity outcomes. These factors were selected for their clinical significance and practicality within the framework of this study. They are essential pre-analytical variables that can affect BC positivity outcomes. While the literature review identified several other relevant factors, such as the timing of sample collection in relation to antibiotic administration and sample handling, only these four were examined due to time constraints, resource limitations, and the need to maintain a focused dataset. Nevertheless, several limitations must be acknowledged.

Firstly, the cross-sectional study design limits the ability to establish causality between the identified factors and BC positivity. This design was chosen to capture a broad snapshot of current practices and their immediate outcomes within our resource constraints. Secondly, a relatively smaller sample size and the exclusion of pediatric patients limit the generalizability of the findings. The study did not find a statistically significant association of various demographic and pre-analytical factors with culture positivity, which may partly be due to the smaller sample size.

Thirdly, this is a single-center study, and all the samples were processed in a single laboratory; therefore, a multisite comparison could not be made. Because of the limited scope of our study, we were not able to compare the factors with each other. A comparison of these factors would have provided a better overview of how different factors interact with each other.

Additionally, as this was primarily a laboratory-based study, the lack of access to comprehensive patient clinical information constrains our ability to fully understand the clinical context of the BC results. This limitation highlights the need for more integrated and comprehensive data collection in future research.

5.3 FUTURE RECOMMENDATIONS

Future studies should aim to include larger, more diverse populations to enhance the applicability of the results across different patient groups. In addition, emphasis should be placed on exploring the mechanisms by which pre-analytical factors influence BC outcomes. Other than that, studies employing association and correlational study designs would be particularly suitable for this purpose.

Prospective longitudinal studies will allow for the collection of comprehensive data and the description of trend variations over time. Additionally, research into novel BC technologies and methods to streamline the pre-analytical process could further enhance current diagnostic modalities' diagnostic accuracy and efficiency. These advancements are crucial to improving patient care and clinical outcomes in the field of BC diagnostics.

CHAPTER SIX

CONCLUSION

This study at SASMEC provides important insights into how pre-analytical factors affect BC positivity outcomes. The findings highlight the need to improve BC practices to increase diagnostic accuracy and patient care. Even though no statistically significant associations were found between demographic and pre-analytical factors and BC outcomes, the research offers valuable data for future studies and clinical practices.

In this study, the average age of participants was 61.8 years, with most being 50 years or older. Most blood samples were taken from peripheral veins (84.8%) and mainly collected in the ED (35.5%) and internal medicine department (27.5%). It is important to highlight that the pre-analytical time was longer than the recommended value, as less than 12 hours was recorded for 61.4% of the samples, with an average pre-analytical time of 10.14 hours. Pre-analytical time was less than 12 hours for 61.4% of the samples, with an average of 10.14 hours.

Although guidelines recommend an optimal blood volume of 8 to 10 ml, only 17.4% of cases met this standard, affecting BC positivity. The overall true positive rate was 18.8%, and the false positive rate was 10.1%. The study also found a significant difference in the TTP for anaerobic cultures based on blood volume.

Continuous training programs for healthcare providers on proper BC collection techniques are essential. Following standardized protocols can greatly improve the accuracy of BC results and reduce false-positive and false-negative rates. Future studies should include larger and more diverse populations and research into new BC technologies to further improve diagnostic accuracy and patient care.

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APPENDIX I

SASMEC-PATH
V
R
EFFECTIVE DATE: 03 OCTOBER

SULTAN AHMAD SHAH MEDICAL CENTRE @IIUM
DEPARTMENT OF PATHOLOGY & LABORATORY MEDICINE
MEDICAL MICROBIOLOGY
BACTERIOLOGY, MYCOLOGY & PARASITOLOGY REQUEST FORM

1. PATIENT IDENTIFICATION	
Name	RN
Identification No (NRIC/ passport no.)	Age
Ward/ clinic	Gender □ Male □ Female

2. RELEVANT CLINICAL INFORMATION	**Request by:**
Clinical manifestation & diagnosis:	*Signature & official stamp:*
Recent/ current antibiotic treatment (name, dose, duration):	

3. INFORMATION ON SPECIMEN COLLECTION

Collection date: Collection time:

TYPE OF SPECIMEN COLLECTED

	Abscess/ pus aspirate		Swab (*specify site*):
	Blood; peripheral		Stool
	Blood; central (*specify site*):		Throat swab
	Body fluid (*specify site*):		Tissue (*specify site*):
	Bone		Tracheal aspirate
	Bronchoalveolar lavage		Urethra swab
	Catheter tip (*specify site*):		Urine (midstream)
	Cerebrospinal fluid		Urine (straight catheterization)
	Cervical swab		Urine (suprapubic aspirate)
	Endometrial aspirate		Vagina swab
	Eye (specify site):		Sterility sample (*please specify*):
	Gastric aspirate		
	Genital ulcer		Environmental sample (*please specify*):
	Nasal swab		
	Nasopharyngeal aspirate (NPA)		Others (*specify site*):
	Nasopharyngeal swab		
	Sputum		

4. TYPE OF TEST REQUESTED

□ Gram stain only □ Bacterial culture & sensitivity □ Fungal culture
□ Blood culture vials: □ Aerobe □ Anaerobe □ Paediatric □ Fungal/ TB

SPECIAL TEST

	CSF antigen detection		Rotavirus antigen detection
	CSF India ink		BFMP: □ X1 □ X2 □ X3
	Clostridium difficile toxin detection		Stool ova & cyst
	Corynebacterium diphtheriae identification		MDRO screening (*please specify*):
	Neisseria gonorrhoea identification (cervix/ urethra)		Others (*please specify*):

5. FOR LABORATORY USE ONLY	
Lab result:	Validated by: Date:

Sultan Ahmad Shah Medical Centre @IIUM, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang Darul Makmur.

Appendix I presents the BCs request form, which includes essential details such as patient demographics (name, age, gender, lab barcode, request number, and diagnosis), collection site (peripheral vein or central line), location (Emergency Department, Internal Medicine, General Surgery, ICU, etc.), transportation time (within 6 hours or more than 6 hours), and the types of vials used for BCs (Aerobic and Anaerobic) for each request. These factors are crucial for evaluating the pre-analytical variables that may influence blood culture positivity outcomes. All patient information will remain confidential and will not be disclosed. The data will be anonymized and used solely for this study.