

**DETERMINATION OF THE DIAGNOSTIC ACCURACY OF RAPID MOLECULAR
ASSAYS FOR DIAGNOSIS OF BLOODSTREAM INFECTIONS IN PIETERSBURG
HOSPITAL, LIMPOPO PROVINCE IN SOUTH AFRICA**

By

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DISSERTATION

Submitted in fulfilment of the requirements for the degree of

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UNIVERSITY OF LIMPOPO

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DEDICATION

I dedicate this work to all the people who are going to benefit from the results that were obtained from this research, to my family that have been very supportive, to my supervisor Dr. I. Rukasha and my co-supervisor Prof. R.M Lekalakala-Mokaba, who have been very helpful and supportive throughout this research, as well as to myself because I worked very hard to ensure that this project is successful.

DECLARATION

I the undersigned, hereby declare that the work done in this dissertation under the topic: Determination of the diagnostic accuracy of rapid molecular assays for diagnosis of bloodstream infections from Pietersburg Hospital, Limpopo Province in South Africa, submitted for the degree of Master of Science in Medical Sciences (Medical microbiology) at the University of Limpopo (Turfloop campus) is my own work, it has not been previously submitted for any other degree at any other institution and that all sources that I have used have been indicated and acknowledged by means of complete references.

Surname and initials

Signature

Date

Miss Maswanganyi N.A



2025/05/05

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ABSTRACT

Background

Bloodstream infections (BSIs) are infections caused by the presence of viable microorganisms in the bloodstream and are an essential cause of morbidity and mortality, especially among immunocompromised individuals. Blood culture is still considered the gold standard for diagnosing BSI; however, prolonged turnaround times limit its use for rapid clinical decision-making. Rapid molecular techniques such as BioFire FilmArray Blood Culture Identification 2 (BCID2) and metagenomics sequencing offer faster turnaround time for pathogen detection than blood cultures, in which bacteria, fungi, and their resistance profiles are detected.

Aim

This study aimed to evaluate the diagnostic accuracy of rapid molecular assays for the identification of pathogens that causes bloodstream infections at Pietersburg Hospital in Limpopo Province, South Africa.

Objectives

- i. Compare the effectiveness of VITEK2 and BCID2 in identifying pathogens present in positive blood culture samples. Metagenomics was used to resolve the discrepant results between the VITEK2 and BCID2.
- ii. To assess the sensitivity, specificity, and overall diagnostic accuracy of the BCID2 and metagenomics in relation to the VITEK2.

Methods

This prospective diagnostic cross-sectional study was conducted at Pietersburg Hospital, Polokwane, Limpopo Province, South Africa. Participants included patients suspected of BSI from various regional hospitals and clinics of Limpopo Province.

Blood samples sent to the National Health Laboratory Service (NHLS) from May to December 2023 were included.

Blood culture bottles were incubated in a BacT/ALERT 3D system. Positive cultures were processed using VITEK 2 and BCID2, with discrepancies resolved by 16s metagenomics sequencing. Data were analyzed using SPSS (v29.0.0.0) and MedCalc (v22.026).

Results

Of the 247 samples, 78% (193/247) were Gram-negative bacteria, 17% (43/247) Gram-positive bacteria, and 4% (11/247) yeast. The ICU and paediatric units contributed 34% (83/247) and 29% (72/247) of samples, respectively. A total of 72% (179/247) were monomicrobial, while 28% (68/247) were polymicrobial. A total of nine off-panel organisms were detected in monomicrobial while seven were detected in polymicrobial samples. BCID2 achieved sensitivity, specificity, and accuracy rates of 95.7%, 95.5%, and 96%, respectively. CTX-M was the most common resistance gene (40%), while van A/B and VIM were rare (0.5% each). Metagenomics confirmed BCID2 in 72% of cases and VITEK 2 in 48%, detecting additional off-panel pathogens.

Conclusions

The BioFire FilmArray Blood Culture Identification 2 test gave a very good diagnostic performance with 96% and a faster detection of pathogens in 60 minutes, making it appropriate in emergency cases. VITEK 2 had a better diagnostic performance but relatively slower and surpassed BCID2 in some pathogens. Metagenomics sequencing, being the most comprehensive technique, confirmed 72% of results from BCID2 and identified novel ones.

These findings highlight the complementary roles of BCID2, VITEK 2 and metagenomics in optimizing bloodstream infection diagnosis in poor resource settings.

Keywords

Bloodstream infections, molecular diagnostics, rapid assays, Blood culture Identification 2 (BCID2), VITEK 2, metagenomics sequencing, Pietersburg Hospital.

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LIST OF PUBLICATIONS AND CONFERENCE CONTRIBUTIONS

PUBLICATIONS

Maswanganyi NA, Lekalakala R.M, Rukasha I: Diagnostic Accuracy of BioFire Blood Culture Identification 2 for Bloodstream Infections in Limpopo, South Africa, 2024. Submitted for publication to the *Southern African Journal of Infectious Diseases* (2025)

CONFERENCE PRESENTATIONS

- i. **Maswanganyi N.A., Lekalakala-Mokaba R.M, Mukavhanyedzi D., Rukasha I** (2022): Blood culture contamination associated pathogens at a general hospital in Limpopo Province, South Africa. The study was presented at the 9th Federation of Infectious Diseases Societies of Southern Africa (FIDSSA), held on 3 to 5 November 2022 at the Elangeni and Maharani Hotel, Durban, South Africa (oral presentation).
- ii. **Maswanganyi N.A, Lekalakala-Mokaba R.M, Rukasha I** (2023): Diagnostic accuracy of rapid assays for diagnosis of bloodstream infections and antimicrobial profiling of pathogens that cause bloodstream infections in a tertiary hospital in Polokwane, Limpopo Province, South Africa. The study was presented at the 5th edition of World Congress on Infectious Diseases conference (WCID), October 23-25, 2023, at Boston, Massachusetts, USA (Oral presentation).
- iii. **Maswanganyi N.A, Lekalakala-Mokaba R.M, Rukasha I** (2023): Blood culture contamination associated pathogens at a general hospital in Limpopo Province, South Africa. The study was presented at the 2023 Pathology Research and Development Congress (PATHRED), 31 August-3 September 2023 at the Raddison Hotel, Johannesburg, South Africa (Poster presentation).
- iv. **Maswanganyi N.A, Lekalakala-Moraba R.M, Rukasha I** (2024): Evaluating the diagnostic accuracy of the BioFire FilmArray Blood Culture Identification 2 Panel for the Diagnosis of Bloodstream Infections at a Tertiary Hospital in Limpopo Province, South Africa. The study was presented at the 20th International Congress on Infectious Diseases (ICID) 2024, 3-6 December 2024 at Cape Town International Convention Centre (CTICC), Cape Town, South Africa (Poster presentation).

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LIST OF ABBREVIATIONS

AARMS	Academic Affairs and Research Management System
AMR	Antimicrobial Resistance
AST	Antimicrobial susceptibility testing
BCID2	Blood Culture Identification 2
BSI	Bloodstream Infections
CA	Community-acquired
CFU	Colony-forming unit
CLSI	Clinical and laboratory standard institute
CMg	Clinical Metagenomics
CO₂	Carbon Dioxide
CRE	Carbapenem-resistant Enterobacteriales
DNA	Deoxyribonucleic acid
DoH	Department of Health
ESBL	Extended-Spectrum Beta Lactamases
FIDSSA	Federation of Infectious Diseases Societies of Southern Africa
GNB	Gram-negative bacteria/ Gram-negative bacilli
GPB	Gram-positive bacteria
GPC	Gram-positive cocci
HAI	Healthcare-associated infections
ICID	International Congress on Infectious Diseases
ICU	Intensive care unit
ID	Identification

MALDI-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MDR	Multidrug resistance
mNGS	Metagenomic Next-Generation Sequencing
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NGS	Next-generation sequencing
NHLS	National Health Laboratory Services
NHRD	National Health Research Database
PATHRED	Pathology Research and Development Congress
PBC	Positive blood culture
PCR	Polymerase chain reaction
PMBs	Polymicrobials
rRNA	Ribosomal Ribonucleic Acid
SOPs	Standard Operating Procedures
TREC	Turfloop Research Ethics Committee
UTI	Urinary tract infections
VRE	Vancomycin-resistant <i>enterococci</i>
WCID	World Congress on Infectious Diseases

DEFINITION OF CONCEPTS

The terms below were used as defined unless stated otherwise.

Antimicrobial resistance: is when bacteria, fungi, viruses, and parasites do not react to antimicrobial treatments, allowing the microorganism to survive inside the host (Tang et al., 2023).

BioFire FilmArray Blood Culture Identification 2 (BCID2): is a rapid molecular diagnostic test that identifies multiple pathogens and resistance genes from positive blood cultures (Chayed et al., 2024; Senok et al., 2023).

Bloodstream infections: Bloodstream infections (BSIs) are characterized by the presence of bacterial or fungal organisms that are viable and capable of inducing an inflammatory response. These infections are typically accompanied by changes in laboratory, clinical, and hemodynamic parameters (Deku et al., 2019; Viscoli, 2016). In this study, bloodstream infections refer to the presence of viable bacteria and/or fungi in the bloodstream.

Diagnostic Accuracy: refers to the test's ability to correctly identify whether a disease is present or not (Faizi & Alvi, 2023). In this study, accuracy was used to determine whether the assays correctly identified the presence or absence of the microorganisms and antimicrobial resistance genes that causes BSIs.

Diagnostic test accuracy study: Research on diagnostic accuracy uses a gold standard to compare the performance of a test and determine how well it can accurately identify a particular disease (Pavlou et al., 2021). In this study, diagnostic accuracy referred to a study that involves comparing at least one diagnostic test to a standard measure that is considered the reference standard for the target condition to be studied.

Metagenomics: is a method that is used to sequence microbial genetic material directly from environmental samples without the need to culture them first (Thomas et al., 2012).

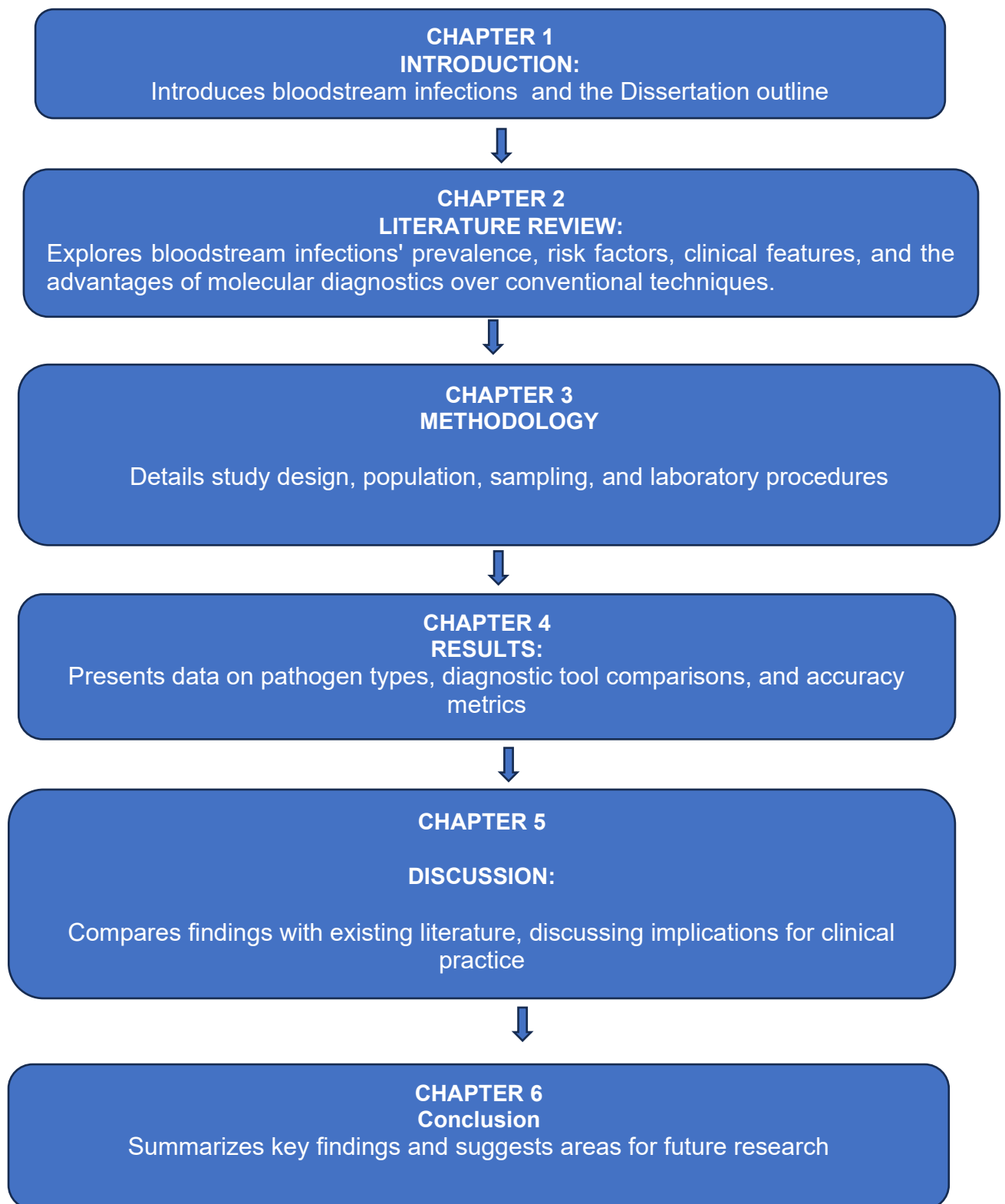
Molecular assays: Molecular assays are based on the detection of the nucleic acid of the targeted pathogen (Das et al., 2017). In this study, molecular assays were used to detect the nucleic acids of the microorganisms of interest.

Polymicrobial infections: these are infections that are caused by multiple microbial species (Brogden et al., 2005). This is particularly important in bloodstream infections, where polymicrobial cases may indicate complex underlying health conditions or contamination,

Sensitivity: is capacity of a test to accurately detect individuals who have the illness (true positives) while reducing the quantity of false negative results (Racehl West, 2020). In this study, sensitivity was used to determine whether a test was able to accurately detect the presence of BSI, by correctly identifying the true positives.

Specificity: is the capacity of a test to accurately identify people who are without illness (true negatives) while reducing false positive results (Racehl West, 2020). In this study, specificity was used to determine whether a test was able to accurately detect the absence of microorganisms in the blood samples.

DISSERTATION OUTLINE



CHAPTER 1

1.1. INTRODUCTION

Bloodstream infections (BSI) defined as the presence of viable microorganisms in the bloodstream is a major cause of illness and death, especially among those with weakened immune systems (Deku et al., 2019). It is estimated that there were 49 million cases of sepsis, and 11 million deaths related to BSI worldwide, accounting for nearly 20% of all deaths globally (Rudd et al., 2020; WHO, 2020). Low- and middle-income nations account for about 85% of all cases (Keeley & Nsutebu, 2021; WHO, 2020). Africa accounts for more than half of the world's cases of BSI, with an estimated 17 million cases and 3.5 million fatalities (Keeley & Nsutebu, 2021; WHO, 2020).

The conventional gold standard for diagnosis of BSI remains blood culture analysis followed by Gram staining and other culture-related methods (Holma et al., 2022; Lamy et al., 2016; Ye et al., 2023). The benefit of employing conventional techniques is that they provide precise species identification as well as a comprehensive profile of antimicrobial susceptibility patterns (Holma et al., 2022). The conventional blood culture methods are often fraught with long turn-around time taking up to 72 hours to get results after a blood culture signals positive. This delay often makes the findings clinically irrelevant for timely treatment of patients (Cendejas-Bueno et al., 2019; Holma et al., 2022; Peri, Ling, et al., 2022). An ideal diagnostic system would quickly identify the organisms that are responsible for infections and the factors that contribute to their antibiotic resistance. This would enable the commencement of appropriate pathogen-targeted treatments, ultimately improving patient outcomes (Paolucci, Paola Landini, et al., 2010).

Pathogenic-specific polymerase chain reaction (PCR), matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS), and fluorescence hybridization probe are some of the methods that have been developed in recent years to maximize the identification of the etiological agent of BSI (Holma et al., 2022). Despite the benefits of these techniques, many of them require numerous test steps to purify and extract their target protein or deoxyribonucleic acid (DNA) (Holma et al., 2022). Furthermore, most of these assays provide minimal information regarding polymicrobial blood cultures (Holma et al., 2022).

Molecular technology and contemporary automation have resulted in the development of molecular-based multiplex platforms, which include Verigene (Luminex, USA), FilmArray (bioMérieux Inc, France), and LightCycler SeptiFast (Roche, Germany), for rapid pathogen detection directly from positive blood culture bottles, to accelerate the detection of pathogens and resistance (Holma et al., 2022). These assays operate exceptionally well even with polymicrobial samples and give a noticeably faster time to detection than traditional methods (Holma et al., 2022). However, molecular assays differ in turnaround time to results and identification of pathogen and antimicrobial resistance markers. One of the most promising molecular assays is the BioFire FilmArray Blood Culture Identification 2 (BCID2) panel, which offers significantly faster pathogen detection time and provides great performance even with polymicrobial samples (Holma et al., 2022).

The BCID2 panel (bioMérieux Inc, France) is a fully automated *in vitro* microbiological diagnostic assay that uses nested multiplex PCR analysis to identify 15 Gram-negative bacteria, 11 Gram-positive bacteria, seven fungal pathogens, and 10 antimicrobial resistance genes in about an hour (Caméléna et al., 2023). The BCID2 has been evaluated in developed and developing countries and showed high sensitivity and specificity (Berinson et al., 2021; El Sherif et al., 2022; Harboe-Sjåvik et al., 2024).

The performance and utility of the BCID2 assay (bioMérieux Inc, France), a recently released updated version of the FilmArray BCID panel, has not been evaluated in a rural setting. Therefore, we evaluated the performance of BCID2 assays in a routine rural laboratory in Limpopo, South Africa, where alternative assays are necessary to overcome resource inaccessibility in areas located further away from major laboratories.

The clinical metagenomics sequencing technique is the process of identifying pathogens and determining their susceptibility to antimicrobials by sequencing the nucleic acids found in a clinical sample (Timsit et al., 2020). Within eight hours before the second dose of broad-spectrum antibiotics, a single metagenomic test can identify bacteria, fungi, viruses, parasites, and determinants of AMR, enabling focused therapy and minimizing unwanted and inappropriate antimicrobial use (Trotter et al., 2019). Metagenomics have shown a clinical sensitivity of 97% and a specificity of 94% in species identification, surpassing conventional blood cultures when evaluated against

established diagnostic techniques (Babady, 2021; Govender and Eyre, 2025) . However, metagenomics and BCID2 have not been validated in Limpopo for their potential diagnostic value.

Therefore, the purpose of this research was to assess BCID2 (bioMérieux Inc, France) and Metagenomics in comparison to conventional blood cultures to ascertain their accuracy, sensitivity and specificity, potentially providing quicker and more reliable methods for diagnosing BSI in Limpopo's healthcare system, thus enhancing patient care and decreasing AMR.

1.2. PROBLEM STATEMENT

Bloodstream infections are serious conditions that can result in significant death and illness if not correctly diagnosed or treated, especially in resource-limited areas like the Limpopo Province in South Africa. In this context, prompt and precise diagnosis of BSI is crucial for improving patient outcomes, as healthcare facilities frequently face limited resources while handling a high number of patients. Blood culture, the traditional "gold standard" method, can require days or weeks to yield results, causing delays in initiating appropriate treatment and often necessitating the use of broad-spectrum antibiotics. This approach might inadvertently raise antimicrobial resistance (AMR) while failing to effectively address the underlying infection.

Recent advancements in diagnostic technologies have demonstrated potential in delivering quicker and more accurate BSI diagnoses by employing rapid molecular assays like clinical metagenomics (CMg) sequencing and the BioFire FilmArray Blood Culture Identification 2 (BCID2) panel (bioMérieux Inc, France). However, these methods (Metagenomics and BCID2) have not been extensively validated in real-world settings with limited resources. Thus, the study sought to assess BCID2 (bioMérieux Inc, France) and Metagenomics in comparison to conventional blood culture to ascertain their accuracy, sensitivity, and specificity, potentially providing quicker and more dependable methods for identification of BSI in the healthcare system of Limpopo, ultimately enhancing patient care and reducing AMR.

1.3. PURPOSE OF THE STUDY

1.3.1 Aim:

To determine the diagnostic accuracy of rapid molecular assays (BioFire FilmArray Blood Culture Identification 2 (BCID2) and metagenomics) for the identification of pathogens that causes bloodstream infections in Pietersburg Hospital, Limpopo Province, South Africa.

1.3.2. Objectives:

- Compare the effectiveness of VITEK 2 and BCID2, in identifying pathogens present in positive blood culture samples. Metagenomics was used to resolve the discrepant results between the VITEK2 and BCID2.
- To assess the sensitivity, specificity, and overall diagnostic accuracy of the BCID2 and Metagenomics in relation to the VITEK2.
-

1.3.3. Research Question

What is the diagnostic accuracy of rapid molecular assays (BioFire FilmArray Blood Culture Identification 2 (BCID2) and metagenomics) for bloodstream infections in Pietersburg Hospital, Limpopo Province, South Africa?

1.4. SIGNIFICANCE OF THE STUDY:

Enhanced BSI diagnostics have huge possibilities for cutting down healthcare costs, reducing rates of infection-related mortality, and averting the occurrence of antimicrobial resistance through targeted therapies. With quicker pathogen identification, clinicians may get the opportunity to initiate specific antibiotic regimens sooner to improve patient outcomes and prevent the unnecessary use of broad-spectrum antibiotics that could lead to resistance.

Furthermore, these results might be used by the South African National Department of Health to guide diagnostic procedures and establish optimal practices for the treatment of BSI, especially in a low-resource context such as Limpopo. The results of this study may provide informed decision-making and ensure successful implementation of new diagnostic technologies in the healthcare system where common blood-culture techniques alone can no longer provide treatment on time.

In addition, the results of this study will help researchers and laboratory workers to better understand newer diagnostic assays and help in determining facets for improvements.

THEORETICAL FRAMEWORK

A theoretical framework establishes the research question, guides data collection and analysis methods, provides background information for discussing the findings, and draws attention to the subjectivities of the researcher (Luft et al., 2022).

The theoretical framework for this study is grounded in diagnostic accuracy principles, germ theory, and Koch's postulates, providing a robust basis for evaluating rapid molecular assays. Diagnostic accuracy measures how well a test can determine whether individuals have a disease or not (Faizi & Alvi, 2023). A diagnostic test's performance is assessed using key metrics like sensitivity, specificity, and predictive values (Buehler et al., 2019). Higher sensitivity leads to fewer false negatives, whereas greater specificity helps to minimize false positives. These metrics are crucial for making clinical decisions, particularly in bloodstream infections (BSI), where inaccurate diagnoses may lead to delayed treatment, increased mortality, and antimicrobial resistance.

The germ theory of disease and Koch's postulates establish the basis for understanding the causes of bloodstream infections (BSIs). Koch's principles demonstrated that certain microbes cause diseases, resulting in the creation of diagnostic tools for detecting pathogens. Advanced methods such as BioFire FilmArray BCID2 and metagenomics improve these core theories by allowing precise detection of pathogens and resistance profiling straight from blood samples. This signifies a notable change from conventional techniques that, despite being regarded as the gold standard, frequently encounter issues like prolonged turnaround times and restricted sensitivity in cases of polymicrobial infections.

In areas with constrained resources like Limpopo Province, healthcare institutions encounter difficulties such as insufficient diagnostic tools and a large patient influx, highlighting the necessity for swift molecular diagnostics. These tools enhance diagnostic precision by minimizing delays and facilitating targeted treatment, essential for tackling the elevated mortality rates linked to bloodstream infections in these environments. For instance, the BCID2 panel detects pathogens and resistance genes in under an hour, much quicker than conventional blood culture techniques.

This study aims to validate the effectiveness of BCID2 and metagenomics in

enhancing BSI management in Limpopo by assessing their sensitivity, specificity, and diagnostic accuracy compared to the gold standard. The framework highlights the importance of innovative diagnostics in enhancing patient results and decreasing AMR, in line with worldwide healthcare objectives.

CHAPTER 2

LITERATURE REVIEW

This section offers a comprehensive review of the existing literature on bloodstream infections. To identify relevant studies, a comprehensive search was performed on multiple databases, including Google Scholar, Internet search engines, and medical databases such as PubMed and ScienceDirect. The search criteria were specifically designed to encompass all peer-reviewed articles published in English that focused on bloodstream infections.

2.1. Background of Bloodstream Infections

Bloodstream infections are defined by the presence of viable bacterial or fungal microbes that cause inflammatory response and are frequently linked to changes in hemodynamic, laboratory, and clinical parameters (Deku et al., 2019; Viscoli, 2016). BSIs are associated with a significant rate of morbidity and fatality rate (Schöneweck et al., 2021). Bloodstream infection (BSI), which can be primary, meaning it has no known origin, or secondary to a known source, is indicated when a patient has positive blood cultures and systemic symptoms (Timsit et al., 2020).

Blood cultures are usually used to diagnose bloodstream infections, and broad-spectrum antimicrobials are generally used to treat them. Contrary to bacterial infections, which are detected through blood cultures and treated with antibiotics, fungal and viral infections, despite being linked to higher mortality rates in cancer patients, typically go unreported in BSI instances (Gyarmati et al., 2016). When BSI is connected to admission to the ICU, the case-fatality rate is 35–50% (Timsit et al., 2014). Among clinical infections, BSIs are among the most severe and can affect people of all ages. Due to a number of innate factors, including the immune system and mucocutaneous barrier that do not mature to their full potential, children are more susceptible to BSI than adults (Nan et al., 2022).

2.2. Epidemiology of Bloodstream Infections

The epidemiology of BSI needs to be updated in order to guide protocols for empirical therapy, as well as for healthcare planning and prevention, as effective empirical treatment of BSI is linked to lower mortality worldwide. Bloodstream infections affect 30 million people and cause 6 million deaths annually (Schöneweck et al., 2021). Three million babies and 1.2 million children also get sepsis as a result of these

infections (Schöneweck et al., 2021). Statistics for Africa are limited, especially for the adult population, and are estimated to range from 7 to 15% (Alex et al., 2021). In terms of AMR, more than 40% of African countries do not have data (Alex et al., 2021).

According to epidemiological studies on the bacterial distribution, *Klebsiella pneumoniae* (8, 7%), *Escherichia coli* (13%), and *Salmonella spp.* (4,3%) are the second most common bacteria in adults after *Staphylococcus aureus*, with a 14:9 (60.8%: 39.1%) ratio between Gram-positive and Gram-negative bacteria and there were no much differences between their ratios (Figure 1.)(Kolesnichenko et al., 2021).

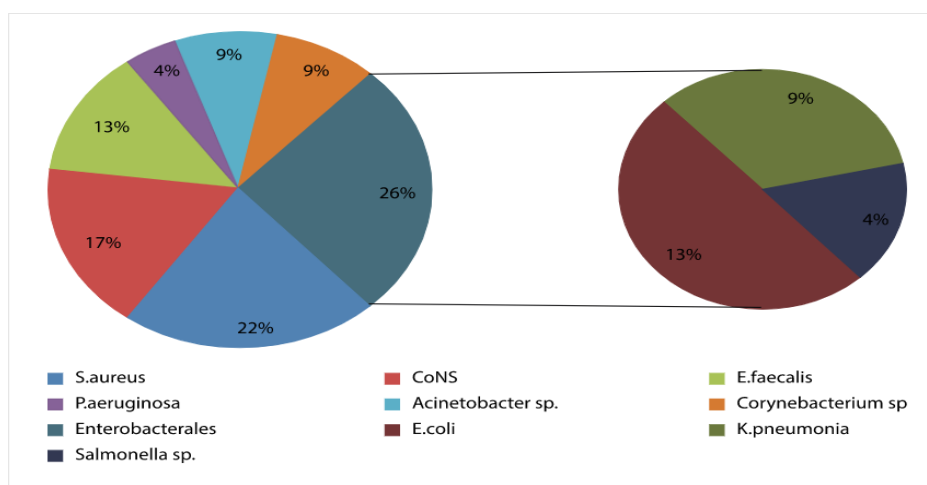


Figure 1: Gram-positive and Gram-negative microbial ratio in adults (Kolesnichenko et al., 2021)

However, there was a notable difference between the proportion of Gram-positive and Gram-negative microorganisms in children: Gram-positive bacteria were statistically found in children more frequently at 37: 5 (86%: 11.6%) (Kolesnichenko et al., 2021). *Enterococcus* species constitute 52% of all microorganisms in children (Figure 2).

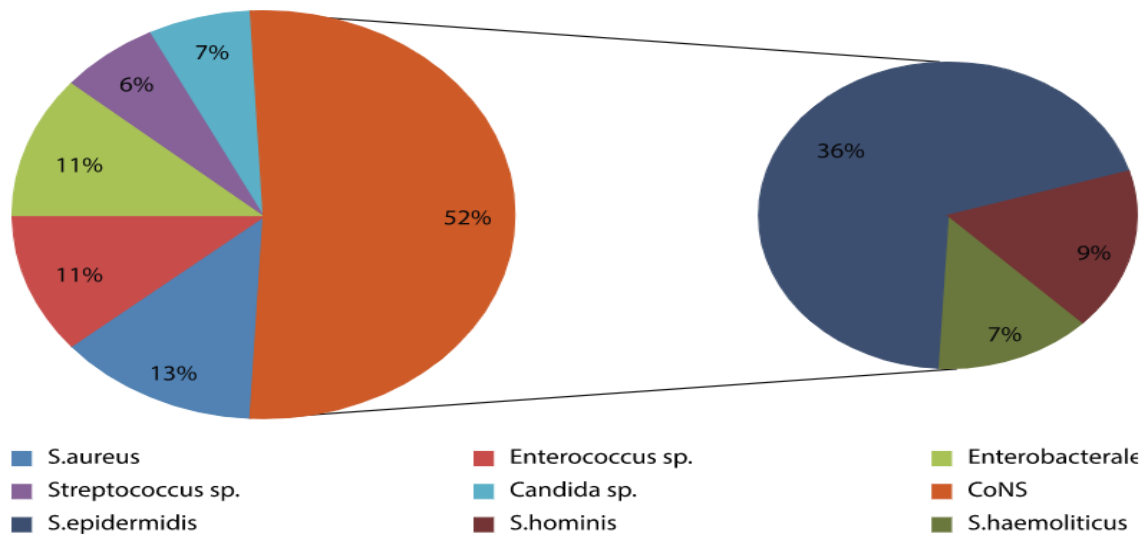


Figure 2: Gram-positive and Gram-negative microbial ratio in children (Kolesnichenko et al., 2021).

Several studies conducted in South Africa indicated that *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* are the most predominant Gram-negative causative agents of bloodstream infections in South Africa, whilst among the Gram-positive bacteria, *Staphylococcus aureus*, *Enterococcus species*, and *Streptococcus* species were the most prevalent (Alex et al., 2021; Dramowski et al., 2024; Glover et al., 2022; Meiring et al., 2025).

2.3. Risk Factors of Bloodstream Infections

The risk factors for bloodstream infections include but are not limited to mechanical ventilation, renal substitutive therapy, surgical procedure, HIV acquisition risk factors, such as injectable drug use, and the degree of immunosuppression of a patient (Lang et al., 2022; Rodrigues Pires de Campos et al., 2022). Additional risk factors include intravenous chemotherapy or therapy, receiving wound care, receiving enteric nutrition, being admitted to an acute care hospital, visiting a hospital or dialysis clinic, and residing in a nursing home or long-term care facility (Cardoso et al., 2014).

2.4. Clinical Symptoms of Bloodstream Infections

Fever, chills, low blood pressure, altered mental state, hypothermia, excessive perspiration, decreased vascular tone, and increased risk of organ failure are all examples of mild symptoms of bloodstream infections (Deku et al., 2019). In critical

situations, clinical signs include tachycardia, tachypnoea, leukopenia and death (Santella et al., 2020).

2.5. Diagnosis of Bloodstream Infections

Prompt identification of pathogens and antimicrobial susceptibility testing (AST) of the pathogen causing bloodstream infections and prompt identification of pathogens are necessary for efficient delivery of antibiotic therapy (Barnini et al., 2016). Blood cultures continue to be the gold standard for the diagnosis of BSI; however, traditional microbiological techniques have a slow turnaround time and low sensitivity (Dubourg et al., 2018). Rapid molecular diagnostics technologies (cartridge-based assays and metagenomics) have been proposed to expedite the diagnosis of microbiological BSI, but there is insufficient information on their application in daily practice.

Understanding of a new diagnostic platform provides new perspectives to advance and speed up the diagnosis and treatment of septic patients.

2.5.1. Diagnosis of blood stream infections using blood cultures

Blood culture is still the "gold standard" for BSI screening, followed by Gram staining and conventional culture-based methods (Briggs et al., 2021). Blood cultures are used to detect, develop, and isolate the bacterium for the diagnosis of BSI, which cannot always be done as early as desired (Pérez-Crespo et al., 2021). Blood should be taken using a sterile needle or syringe through a peripheral vein and/or a central line (Mun et al., 2022). Since more than 50% of patients present with a bacteremia of less than 1 colony-forming unit (CFU)/mL, sufficient volumes (i.e. at least two to three sets, filled with 10 mL per bottle) and an increase in the number of blood cultures are required to achieve a sensitivity of >95% (Peker et al., 2018). In adults, a volume of 5 to 10 mL is required, while in children 2 to 3 mL is required.

Blood cultures can identify as little as 1 colony-forming unit (CFU) of bacteria, fungi, or yeast per 10 ml of blood. Blood cultures are relatively simple to carry out and have a good sensitivity for cultivable pathogens (Afshari et al., 2012). A number of culture techniques which range from manual to automatic assay are currently in use with a mean sensitivity of around 60 (Table 1).

Table 1: Different types of culture-based techniques currently available worldwide for Antimicrobial Susceptibility Testing (AST) (Maugeri et al., 2019)

ID & AST technologies	Example of Assay Manufacturer	Summary of method	Time of AST	Automatic Or Manual	References
Agar Dilution Assays	Oxoid	Bacteria inoculated on agar plates with various antibiotic concentrations	16 to 24 hours	Manual	(Jorgensen & Ferraro, 2009)
Disk diffusion	Oxoid	Bacteria inoculated on agar plates with single antibiotic disks	16 to 24 hours	Manual	(Grant et al., 2017; Jorgensen & Ferraro, 2009)
Gradient test	bioMerieux	Bacteria inoculated on agar plates with graded antibiotic concentration strips	16 to 24 hours	Manual	(Balouiri et al., 2016; Jorgensen & Ferraro, 2009)
Broth dilution assay	Oxoid	Bacteria inoculated in liquid media with different antibiotics to monitor growth	12 to 24 hours	Manual	(Jorgensen & Ferraro, 2009)
MicroScan WalkAway	Beckman Coulter	Measure bacterial growth in the presence of antibiotics by recording bacterial turbidity using a photometer	4.5 to 18 hours	Automatic	(Mcgregor et al., 1995; Wiegand et al., 2008)
Vitek bioMerieux	bioMerieux	Detect bacterial growth in the presence of antibiotics by measuring the turbidity of the bacteria with a photometer.	6 to 11 hours	Automatic	(Syal et al., 2017)
BD phoenix	Becton Dickinson	Record bacterial growth in the presence of antibiotics by recording bacterial turbidity and colorimetric changes	9 to 15 hours	Automatic	(Syal et al., 2017)
Sensititre	ThermoFisher Scientific	Record bacterial growth with antibiotics by measuring fluorescence	18 to 24 hours	Automatic	(Jorgensen & Ferraro, 2009)

However, culture-based approaches continue to have significant limitations, including a relatively long turnaround time, the failure to detect obligatory or slow-growing intracellular microorganisms, and pathogens other than bacteria or yeast (Peker et al., 2018). Additionally, the sensitivity of the test is further decreased by empirical antibiotic use prior to hospital admission, which jeopardizes bacterial growth in culture (Decuypere et al., 2016). Bacterial incubation typically takes 5 days, but it could take longer for slow-growing species (Berinson et al., 2021). Blood culture assays are the only diagnostic assays available routinely used for BSI in South African laboratories, including in Polokwane.

2.5.2. Molecular based assays

To counteract the problems of long turnaround times and limited susceptibility testing abilities of blood culture and VITEK 2 (BioMérieux, Inc., Durham, NC), molecular assays have been introduced (Timsit et al., 2020). Molecular-based technologies utilize quantitative real-time PCR (qPCR) or convectional polymerase chain reaction (PCR) to amplify particular nucleic acid sequences, allowing AST and pathogen identification at the same time. The PCR-based techniques are categorised into two categories, i. PCR performed directly on raw samples and ii. PCR performed on culture-enriched assays, which instead require a previous growth step of the raw sample in an enrichment media. Culture-enriched tests need a DNA extraction step from isolated strains, a lot of cells, and prior knowledge of the sequences to amplify in order to collect enough DNA.

Culture-independent PCR assays are molecular-based methodologies which allow identification of pathogens directly from patient samples namely whole blood, serum, blood cultures, and urine, without the need for culture and extraction steps. The current trend in molecular assays is to perform automatized commercial cartridge-based assays. Cartridge-based assays are commercially available and designed to target different chromosomal regions of pathogens in combination with assays to detect their antimicrobial resistance signatures (Table 2). A recent and highly developed assay that represents major improvements in molecular assays is the BioFire® FilmArray® BCID2 panel (Berinson et al., 2021; Paolucci, Landini, et al., 2010). The BioFire FilmArray BCID2 panel now includes 43 molecular targets linked to BSI, including 10

antimicrobial resistance genes, 7 yeast species, 11 Gram-positive bacteria, and 15 Gram-negative bacteria (Table 3) (<https://www.biomerieux-diagnostics.com/biofire-bcid-panel>). The final targets include genes that encode extended-spectrum β -lactamase (ESBL) (CTX-M), carbapenemases (IMP, KPC, OXA-48-like, NDM, and VIM), resistance to colistin (*mcr-1*), resistance to methicillin (*mecA* / C and, specifically for methicillin-resistant *Staphylococcus aureus* [MRSA], *mecA/C* and MREJ [*mec* right-extremity junction]), or resistance to vancomycin (*vanA/B*) (Cortazzo et al., 2021).

According to a number of studies, the BCID2 considerably shortens the turnaround time for pathogen identification and resistance marker detection when compared to traditional techniques (Caméléna et al., 2023; Peri et al., 2022). Additionally, the BCID2 detects other infections that traditional culture-based techniques might overlook or miss (Berinson et al., 2021). Nevertheless, the BCID2 has drawbacks as well, including the ability to misidentify certain species and produce negative results for samples that contain organisms that are off-panel for BCID2 (Berinson et al., 2021; Caméléna et al., 2023). The BCID2's inability to detect phenotypic resistance markers is one of its other drawbacks.

Table 2: A summary of major molecular-based methods for the detection of bloodstream infections (BSIs) (Jordana-Lluch et al., 2014).

Name of Assay	Genes detected	Unique species identified	Resistance genes identified
SeptiFast (Roche, Mannheim, Germany)	25	<i>Candida</i> species and <i>Aspergillus fumigatus</i>	<i>mecA</i>
VYOO (SIRS-Lab,	34	Six species of <i>Candida</i> and <i>Aspergillus fumigatus</i>	<i>mecA</i> , <i>vanA</i> and <i>vanB</i> , β -lactamase genes <i>blaSHV</i> and <i>blaCTX-M</i>

Jena, Germany)			
Magicplex Sepsis Real- Time Test (Seegene, Seoul, Korea)	21	five species of <i>Candida</i> , and <i>Aspergillus</i> <i>fumigatus</i>	<i>mecA</i> , <i>vanA</i> and <i>vanB</i>
GeneXpert system (Cepheid		MRSA, Methicillin susceptible <i>Staphylococcus aureus</i> (MSSA)	<i>spa</i> , <i>SCCmec</i> , and <i>mecA</i> genes
BioFire 2 FilmArray culture identification (BCID2)	43	7 fungal <i>Candida albicans</i> , <i>Candida auris</i> , <i>Candida krusei</i> , <i>candida</i> <i>parapsilosis</i> , <i>candida tropicalis</i> , <i>cryptococcus neoformans</i> and <i>gatii</i>	<i>MecA/C</i> , <i>mecA/C</i> , MREJ, <i>vanA/B</i> , CTX-M, <i>mcr-1</i> , VIM, NDM, Oxa-48, KPC, IMP

One major limitation of commercially available assays is the limitation of the number of PCR probes, with several of them targeting a limited number of microorganisms and very few targeting a wide range of available resistance genes of interest. Given the overall results, the potential source of infection, and other available bacteriological data, a negative PCR should be interpreted accordingly (Timsit et al., 2020).

To counteract the limitation of identifying limited organisms and DST results, a method based on compressive molecular sequencing is needed.

Table 3: Forty-three (43) molecular targets of the BioFire FilmArray Blood Culture Identification 2 Panel

Gram-negative bacteria (15)	Gram-positive bacteria (11)	Fungi (7)	Antimicrobial resistance genes (10)
<i>Acinetobacter calcoaceticus-baumannii</i> complex	<i>Enterococcus faecalis</i>	<i>Candida albicans</i>	CTX-M
<i>Bacteroides fragilis</i>	<i>Enterococcus faecium</i>	<i>Candida auris</i>	IMP
<i>Enterobacterales</i>	<i>Listeria monocytogenes</i>	<i>Candida parapsilosis</i>	KPC
<i>Enterobacter cloacae</i> complex	<i>Staphylococcus spp.</i>	<i>Candida krusei</i>	<i>mcr-1</i>
<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida glabrata</i>	<i>mecA/C</i>
<i>Klebsiella aerogenes</i>	<i>Staphylococcus epidermidis</i>	<i>Candida tropicalis</i>	<i>mecA/C</i> and MREJ (MRSA)
<i>Klebsiella oxytoca</i>	<i>Staphylococcus lugdunensis</i>	<i>Cryptococcus neoformans/gattii</i>	NDM
<i>Klebsiella pneumoniae</i> group	<i>Streptococcus spp.</i>		OXA-48-like
<i>Proteus spp.</i>	<i>Streptococcus agalactiae</i> (Group B)		<i>vanA/B</i>
<i>Salmonella spp.</i>	<i>Streptococcus pneumoniae</i>		VIM
<i>Serratia marcescens</i>	<i>Streptococcus pyogenes</i> (Group A)		
<i>Haemophilus influenzae</i>			
<i>Neisseria meningitidis</i>			

<i>Pseudomonas aeruginosa</i>			
<i>Stenotrophomonas maltophilia</i>			

2.5.3. Clinical metagenomics

Clinical metagenomics, a culture-independent method, is the process of identifying pathogens and determining their susceptibility to antimicrobials by sequencing the nucleic acids found in a clinical sample (Timsit et al., 2020). This process has become a promising tool in clinical microbiology laboratories due to significant developments in sequencing and computational technology in recent years. Additionally, the prospect of Metagenomics is becoming more feasible as sample costs and turnaround times from specimen reception to clinical care continue to decrease. It can serve as a single assay for subtyping, AMR detection, and virulence profiling in addition to being used for diagnostics (Forbes et al., 2018). It is helpful to use Metagenomics in clinical practice due to its extensive coverage, high throughput, short turnaround time, and precise and accurate sequencing results. Because it examines all the genetic information that may be retrieved from the sample non-specifically, metagenomics is also objective. Additionally, Metagenomics can analyze numerous samples at once, and bioinformatics analysis of the outcomes can produce a large amount of data (Nan et al., 2022). There are numerous benefits to this technique. Firstly, it avoids missed diagnoses of pathogens that are difficult to culture by not requiring sample culture prior to testing. Secondly, this method is impartial, minimizing the possibility of missed diagnosis and allowing the discovery of host genes to aid in diagnosis. All nucleic acid fragments are analyzed immediately and non-specifically without the prior selection of a coverage range. Thirdly, NGS can be repeated for patient follow-up in order to compare quantitative and semi-quantitative data (such as the number of reads for pathogens) before and after treatment. Rapid, precise, and high-throughput detection is the most valuable benefit of metagenomics because it provides evidence for early, tailored anti-infective treatment (Nan et al., 2022).

Although metagenomics has advantages, it also has its shortcomings. Clinical metagenomics demands infrastructure and is expensive to operate and requires well trained laboratory personnels (Marais et al., 2023). Additionally, metagenomics

samples may contain a significant number of contaminants reads from various sources, including the environment, reagents, or manual handling. This can lead to false positives and complicate the interpretation of metagenomics sequencing data (Moragues-Solanas et al., 2024). Clinical metagenomics must be implemented locally to develop pertinent systems, assess cost-effective approaches for its use, and guarantee that reference databases and tools for interpreting results that are suitable for the local epidemiology (Marais et al., 2023). Metagenomics assays are fairly new, and their application has not been used for routine diagnosis in South Africa. Thus, this study will use metagenomics to determine the prevalence of pathogens in Limpopo province.

2.6. Treatment of Bloodstream Infections

Broad-spectrum antibiotics are commonly used as an empirical treatment for BSI; however, they are frequently ineffective against invading microorganisms due to a lack of specificity or resistance mechanisms (Gyarmati et al., 2016). Clinical microbiology laboratories play a critical role in monitoring antimicrobial resistance (AMR) trends, improving patient care for patients with infectious diseases, and guiding public health initiatives by testing the antimicrobial susceptibility of significant bacterial isolates (Wenzler et al., 2023). The objectives of testing are to ensure susceptibility to the preferred medications for specific diseases and to detect potential drug resistance in common pathogens (Jorgensen & Ferraro, 2009). Patients may also need to get surgical treatment for an infection site in addition to their predominant antimicrobial therapy to optimally manage shock or other metastatic suppurative consequences (e.g., debridement, abscess drainage, or removal of intravascular devices). For BSI to be effectively treated, all isolated organisms must be in vitro sensitive to the chosen antimicrobials, administered the right way and at the right time after blood cultures are collected (Timsit et al., 2014). The type of antimicrobials used to treat BSI in critically ill patients depends on a number of overlapping factors, including the empirical or targeted approach of treatment, the suspected or verified source of the infection, the presence of AMR (especially in healthcare settings where MDR pathogens are endemic and/or in patients who have recently been exposed to antimicrobial drugs) and the suspected or proven presence of candidemia (Timsit et al., 2020). The primary risk factors for the development of bacterial resistance in the Gram-negative

microbiota are certainly poor pharmacodynamics and insufficient antimicrobial dosage (Timsit et al., 2014).

2.6.1 Drug resistance mechanisms

The increase in AMR to frequently given antimicrobials has made the BSI situation worse. Antimicrobial resistance (AMR) is a public health issue with significant societal, economic, and health implications (Dhingra et al., 2020). Antimicrobial resistance is the ability of a microorganism (such as bacteria, virus, or parasite) to counteract the effects of an antimicrobial (such as an antibiotic). Multidrug resistant (MDR) organisms have emerged and are spreading throughout the world. Examples include those that produce extended-spectrum β -lactamases (ESBL), carbapenem-resistant Enterobacterales (CRE), *vancomycin-resistant Enterococcus species* (VRE), MDR Gram-negative bacilli (GNB), Methicillin-resistant *Staphylococcus aureus* (MRSA), and MDR non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter spp.* These organisms have become a significant problem (Diekema et al., 2019).

2.6.1.1. Carbapenem-resistant Enterobacterales (CRE)

The most effective drug classes for treating infections caused by Enterobacterales are often those that contain carbapenem antibiotics. However, most Enterobacterales have recently been found to be carbapenem-resistant. The bacteria known as Enterobacterales are resistant to the antibiotic class known as carbapenem (i.e., meropenem, doripenem, imipenem, and ertapenem) (X. Li & Ye, 2017). Majority of the CRE (70%) in retrospective research carried out in South Africa between 2015 and 2018 in patients with bacteraemia were nosocomial, with *Klebsiella pneumoniae* being the most common species (78%). The two most common carbapenemase genes were *bla*-NDM (34%) and *bla*-OXA-48 (52%) (Perovic et al., 2020).

2.6.1.2. Vancomycin resistant enterococci (VRE)

Gram-positive cocci known as enterococci are a typical component of human and animal intestinal flora (NICD, 2022). *Enterococcus faecalis* and *Enterococcus faecium* are two examples of species that are clinically significant. These organisms cause a number of infections, such as cellulitis, endocarditis, wound infections, prostatitis, urinary tract infections (UTIs), intra-abdominal infections, and bloodstream infections (NICD, 2022). Some enterococci are naturally resistant to numerous aminoglycosides

and popular beta-lactam antibiotics (such as cephalosporins, penicillins, and carbapenems) (NICD, 2022).

When first-line antibiotics are ineffective, other antibiotics, such as vancomycin, can be used to treat infections. However, there is an emergence of VRE in medical facilities. Due to their resistance to most antibiotics and the difficulty in treating their diseases, VREs are sometimes referred to as "superbugs" (NICD, 2022). Health care-associated infections (HAIs) can be caused by this pathogen (NICD, 2022). VRE prevalence was reported to be 26.8% (95% CI; 10.7-43.0%) in all Africa, with South Africa having the highest prevalence of 74.8% (Alemayehu & Hailemariam, 2020). However, there is paucity of data on VRE prevalence in Limpopo province.

2.6.1.3. Extended spectrum beta-lactamase

Except for cephamycins and carbapenems, ESBLs have the ability to be resistant to the majority of β -lactam antibiotics (Castanheira et al., 2021). Genes encoding ESBLs and resistance to various antimicrobial medications, such as trimethoprim, aminoglycosides, sulfonamides, chloramphenicol, and tetracyclines, are commonly found on large plasmids (Rawat & Nair, 2010). The prevalence of ESBL has been found to be 38% (95% confidence interval [CI] = 24-53 %), with Congo having the highest prevalence at 92% (95% CI = 90–94%) (Onduru et al., 2021). However, there is paucity of data on the prevalence of ESBL in Limpopo province.

CHAPTER 3

METHODOLOGY

3.1. Study Design

This was a prospective diagnostic accuracy cross sectional study.

A diagnostic accuracy study compares at least one diagnostic test to a comparator that is considered the reference standard for the target condition to be researched (Mallett et al., 2012). This study was a diagnostic accuracy study because we compared the accuracy, sensitivity and specificity of BCID2 (BioMérieux, Inc., France) and metagenomics to the chosen gold standard, VITEK2, for the identification of pathogenic microorganisms that causes BSI.

In prospective studies, individuals are followed up over time to determine the occurrence of outcomes even when the outcome (and perhaps even the exposure or intervention) has not yet occurred when the study begins (Ranganathan and Aggarwal, 2018). This was a prospective study because the BSI was not yet identified from the sample before the study started.

In a cross-sectional study, the investigator measures the result and exposures in study participants at the same time. They are used for population-based surveys and to assess the prevalence of diseases in clinic-based samples (Setia, 2016). This was a cross-sectional study because the blood culture bottles were used once to determine the prevalence of BSIs in Limpopo province, and the two sets of blood culture bottles were collected once.

3.2. Study Area

The study was carried out in the Limpopo Province of South Africa at Pietersburg Hospital, which is in the city of Polokwane.

3.3. Study Population and Setting

The study targeted samples of blood culture bottles from patients suspected of having BSI in wards and outpatients of all regional hospitals and clinics in Limpopo Province whose samples were sent to the NHLS microbiology laboratory at Pietersburg Hospital.

3.4. Sampling Method

Convenience non-probability sampling was used in this study.

Convenience sampling is a non-probability sampling method in which units are selected for inclusion in the sample because they are the easiest for the researcher to access (Nikolopoulou, 2022). This was a convenience non-probability study because blood culture samples that were available, ready, and not collected from a single venipuncture were used.

3.5. Sample Size Calculation

The level of significance considered was 95%, which gave an alpha value of 0.05 and, therefore, a $Z_{\alpha/2}$ of 1.96. The prevalence of BSIs used in this study was 15% based on local unpublished data from Limpopo Province, South Africa. The design effect for convenient non-probability sampling is 10%. With this information, the sample size was calculated using the following formula:

Formula for sample size calculation by (Suresh & Chandrashekara, 2012)

$$N = \frac{Z_{\alpha/2}^2 * P(1-P) * D}{E^2}$$

N= is the sample size.

$Z_{\alpha/2}$ = Level of Significance, where $Z_{\alpha/2}$ = 1.96 for 95% confidence interval

P= is the prevalence or proportion of events of interest for the study

D =is the design effect, which reflects the sampling design used in the survey type of study.

E= Precision/ Margin of Error multiplied by prevalence, where 10% is the given margin of error/ precision

$$N = \frac{(1.96)^2 \times (0.15) \times (1-0.15) \times 0.1}{(0.015)^2}$$
$$= 218$$

$$\text{Added 10\% sample attrition} = 218 + 22$$
$$= 240$$

Therefore, a minimum of 240 blood culture samples were needed for this study.

3.6. Inclusion Criteria

All blood culture bottles that were collected from patients in the defined study period were included in the study (from May 2023 to December 2023).

3.7. Exclusion Criteria

Duplicate blood culture samples that were collected from the same patient simultaneously and samples that were found to contain only organisms that are regarded as skin contaminants were excluded from the study.

3.8. Data Collection Tool

A request form from the medical practitioner was used to collect demographic characteristics that included age, gender, specimen type (Blood), ward, and the required test, which is blood culture (Appendix B5).

3.9. Laboratory Diagnosis

The following laboratory techniques were used in this study.

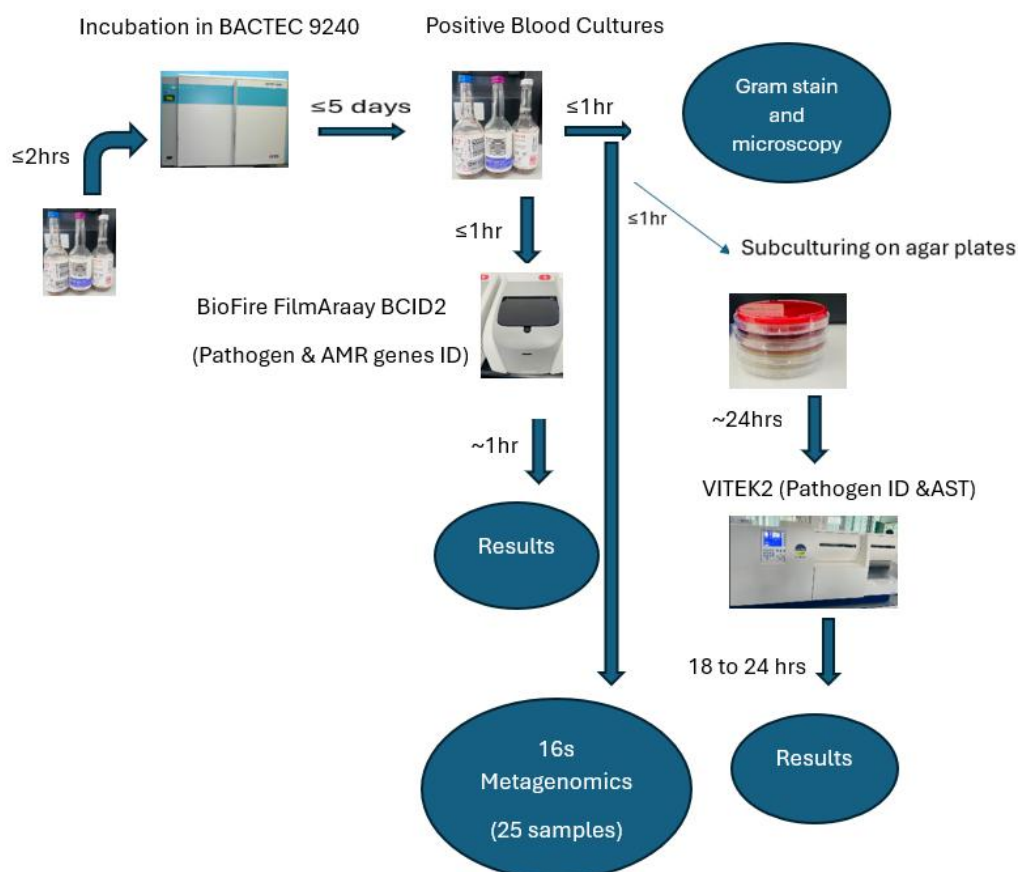


Figure 3. Laboratory workflow for positive blood cultures.

Two sets of blood samples were collected from different venipuncture sites in each patient. In this study, blood culture bottles that were sent to the lab were used. In this study, we took the blood culture bottles and used them after when the laboratory was done using them for diagnostic purposes. One bottle contained a medium for anaerobic growth and the other contained a medium for aerobic growth. Blood culture bottles were incubated in the BD BacTec microbial detection system (BioMérieux, Durham, NC, USA) (Appendix A1) at $36\text{ }^{\circ}\text{C}\pm 0.5\text{ }^{\circ}\text{C}$ for 48 hours. Blood culture bottles that had bacterial or fungal growth in the bottles were considered positive cultures and further processed for identification using conventional microbial techniques, such as Gram staining, seeding in various types of growth medium, as well as using the BioFire FilmArray Blood Culture Identification 2 panel and VITEK 2 (BioMérieux, Inc., Durham, NC). The BCID2 results were compared with those of conventional identification or detection (i.e. VITEK 2 relative to antimicrobial resistance genes, PCR sequencing, both performed on microbial isolates subcultured from positive BC samples). 2 ml of blood was transferred from the blood culture bottles to the EDTA tubes so that they can be stored for the purposes of performing metagenomics on them. From all blood culture bottles, one drop (40 μ l) was streaked onto a MacConkey agar plate and a blood agar plate. A Sabouraud dextrose agar plate (Oxoid, Basingstoke, UK) was added where yeasts were observed in Gram stain. Incubation was carried out at $37\text{ }^{\circ}\text{C}$ and ambient conditions for a maximum of 48 hours. A chocolate agar plate (Oxoid, Basingstoke, UK) was also inoculated with 40 μ l of blood culture medium, which was then streaked and incubated for at least 4 hours at $37\text{ }^{\circ}\text{C}$ with 5% CO₂ (Berinson et al., 2021).

The Vitek2 system (BioMérieux, Inc., Durham, NC) was used to identify all samples after they were re-cultured for 24 hours on blood agar, MacConkey, and chocolate agar plates at $37\text{ }^{\circ}\text{C}$. According to the 2018 recommendations of the Clinical and Laboratory Standards Institute (CLSI), all antimicrobial susceptibility tests (AST) were carried out using a Vitek II automated system (BioMérieux, Inc., Durham, NC) (Appendix A2). According to numerous independent investigations, the automated microbiological identification system VITEK 2 (BioMérieux, Inc., Durham, NC) produces results that are extremely accurate and reproducible. With its colorimetric reagent cards and associated hardware and software advances, the VITEK 2

(BioMérieux, Inc., Durham, NC) offers a cutting-edge technology platform for phenotypic identification approaches.

Biofire FilmArray BCID2 (Appendix A3)

The BCID2 is a diagnostic test that produces results for 26 bacteria, 7 fungi, and 10 genes associated with AMR in about an hour from positive blood culture (PBC) samples. The BioFire FilmArray BCID2 panel testing was carried out in accordance with the manufacturer's recommendations (BioMérieux Diagnostics, 2021). The selected well of the BCID2 pouch received a hydration solution. The next step was to add sample buffer and transfer 200 µl of blood culture broth to the sample injection well. Following the loading of the BCID2 pouch, the BioFire FilmArray instrument (Release Version 2, Software Module Version: BioFire FilmArray FA Link UI 2.1.273.0) performed automated nucleic acid purification, two-stage multiplex PCR, and DNA melting curve analysis to identify the presence of bacterial, fungal, and antimicrobial resistance gene targets in the blood culture sample under investigation. The results were available after about an hour.

Clinical metagenomics (Appendix A4)

From the samples that had discrepant results between the VITEK2 and BCID2, only 25 samples were selected. These included samples that were identified as polymicrobial by the BCID2 but monomicrobial by the VITEK2, those that yielded negative by the BCID2 but VITEK2 identified the organisms, and those that had misidentifications by the BCID2. From these samples, plasma was extracted from blood that was kept in EDTA tubes by centrifuging it at 1600 g for 10 minutes at 4 ° C. With the help of glass beads, the pellets were resuspended and vortexed at 3000 rpm for 30 min in lysis buffer to break down the cell walls. 300 µl of specimens was used for DNA extraction. The preparation of a library using a transposase-based method was then applied to 100 ng of DNA, as determined by a Qubit Fluorometer (Invitrogen, Carlsbad, CA). The pooled libraries were sequenced using a 75 bp single-end sequencing kit on a Nextseq 550 sequencing system (Illumina, San Diego, CA).

3.10. Data Analysis

Data was loaded into a Microsoft Excel sheet, version 2402 for categorization and cleaning. SPSS version 29.0.0.0 (241) and MedCalc version 22.026 were used to analyze the data.

3.11. Reliability and Validity

Reliability is the ability to precisely replicate procedures and outcomes (Leung, 2015). In this study, reliability was ensured by repeating a random selection of 10% of tests more than twice, to check if the machines will produce the same results. Maintenance of the machines was done before we started using them, following the routine laboratory plan.

Validity refers to the degree to which a measurement achieves what it is intended to capture (Bolarinwa, 2015). To ensure validity in this study, more than one blood culture bottle was used to rule out contamination. All the equipment that was used in this study was used according to the manufacturer's instructions. All reagents crucial for the study methodology were in good condition and were validated. Laboratory standard operating procedures (SOPs) were followed to ensure validity and reliability. All machines and reagents were validated first before being used.

3.12. Bias

Bias is a type of systematic inaccuracy that can skew measuring results and affect scientific investigations. A study's design, execution, or analysis could all incorporate systematic errors (Jamieson, 2020).

Selection bias was minimized by collecting consecutive specimens during the study period. Methodological bias was minimized using validated data collection or laboratory methods. Statistical bias was minimized by calculating the sample size and using appropriate statistical tests. Analysis bias was minimized by analysing the data obtained quantitatively without any addition or views from researchers.

3.13. ETHICAL CONSIDERATIONS

3.13.1. Ethical considerations and permission to conduct the study

The main principles of ethics include respect for people, beneficence, and justice.

For approval, the research proposal was submitted to Turfloop Research Ethics Committee (TREC) (Appendix B1). Permission to conduct the study was obtained from the Limpopo Provincial Department of Health via its online National Health Research Database (NHRD) (Appendix B3) and the Academic Affairs and Research Management System (AARMS) (NHLS) to use their facilities and samples from the NHLS laboratory (Appendix B2).

3.13.2. Anonymity and confidentiality

Anonymity entails not collecting information that could be used to identify or track down a specific person or entity (Badampudi et al., 2022). This was ensured by making sure that no personal information of the real patient was written on the reports obtained. The clinical isolates obtained are identified using a unique identifier instead of using the patient's identity, making sure that they are not identifiable from the real patient. Confidentiality refers to the duty of researchers to protect the information that has been entrusted to them (Turcotte-Tremblay & Mc Sween-Cadieux, 2018). According to National Health Act of South Africa section 14 Act no 61, 2003; all information concerning users or participants involving information related to their health status and treatment should remain confidential. The clinical, laboratory, and health status of the participants are not linked to their real names and therefore cannot be disclosed to maintain confidentiality. The laptop used to store participant's information is encrypted by means of a password and the information is only known by the researcher. The research records will be kept for 15 years. Researchers signed confidentiality forms to ensure the discretion of use of information regarding the results.

3.13.3. Consent form

It is not applicable in this research because the samples were not collected directly from the patients. Ethics waiver was applied (Appendix B4).

3.13.4. Waste and disposal

The waste material and glass bottles were disposed into appropriate biohazard waste containers.

3.13.5. Harm

Refers to any pain or discomfort to which participants or researchers may be exposed to during the study, particularly during sample collection. In this study, participant harm

was not applicable since blood culture samples were collected from the stored NHLS microbiology laboratory.

For researchers, no harm was experienced by the researchers.

Handling of blood samples

Blood samples were handled with care and used only for the purpose of this research.

Act governing blood and collection of participants.

According to the national health Act 61 of 2003 chapter 8, Section 58 subsection 1, blood should be collected for intended medical or dental purposes only. According to the Mental Health Act 17 of 2002, mentally ill people should not participate in this study. Based on these two Acts, blood collected from participants in this study was used for research purposes and the investigation of variables stated in the information leaflet that are agreed upon by participants and researchers.

3.13.6. Financial support

Financial support for the study was sought from the National Health Laboratory Service Academic Affairs and Research Management Systems (NHLS AARMS) and BioMérieux Inc., France.

CHAPTER 4

RESULTS

This chapter consists of three sections: Sections A, B, and C. Section A provides demographic data, as well as the prevalence of pathogens included in the study based on gram staining, and the prevalence of AMR genes. Section B compares the diagnostic performance of VITEK2 and BCID2 for pathogen identification. Section C provides information on the sequenced samples. It compares the diagnostic performance of the VITEK2, BCID2, and sequencing for pathogen identification.

SECTION A

4.1. DEMOGRAPHICS AND PREVALENCE OF PATHOGENS AND AMR GENES

4.1.1 Introduction

Section A of the results examines the demographic characteristics and prevalence of pathogens in bloodstream infections. The study analyzed 247 positive blood culture samples collected from patients with suspected bloodstream infections at Pietersburg Hospital. Of the 247 positive blood cultures, 289 clinical isolates were identified in our study.

4.1.2 Gender distribution of participants included in the study

Out of 247 blood culture samples, demographic analysis revealed a slightly higher prevalence of cases among males, comprising 53% (132) samples, while females represented 43% (107) of the samples (Figure 4.1). The gender of the participants was not indicated in 3% (8) of the samples.

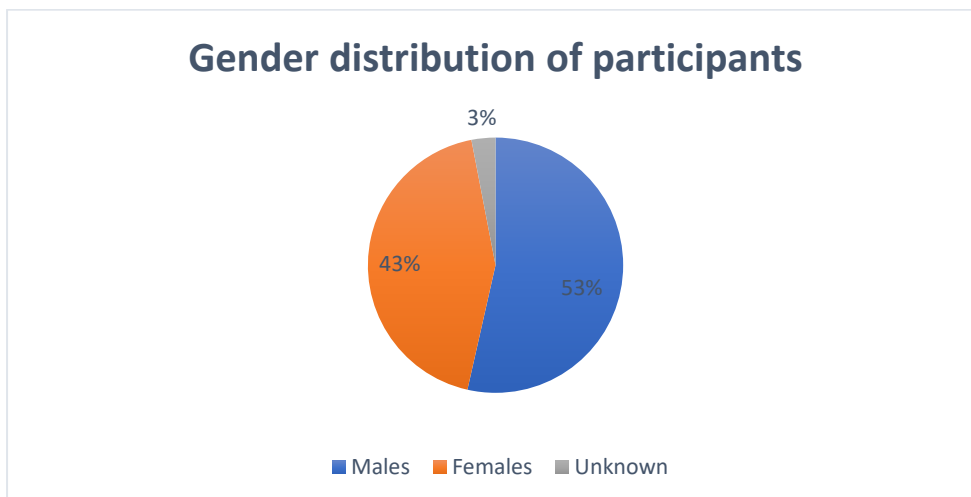


Figure 4.1. Gender distribution of participants included in the study

4.1.3. Age distribution of participants included in the study

Out of the 247 samples, the majority were infant (under 1 year), which represented 49% (121) samples, indicating that younger patients, particularly neonates and infants, are more susceptible to bloodstream infections (Figure 4.2). Adults aged 21 to 40 years represented 19% (48) samples, making it the second most prevalent age group, followed by patients aged 1 to 20 years with 11% (28) samples (Figure 4.2). While, the least number of samples were adults aged 41 to 59 years, 10% (24) samples and seniors aged 60 and older, 9% (22) samples (Figure 4.2).

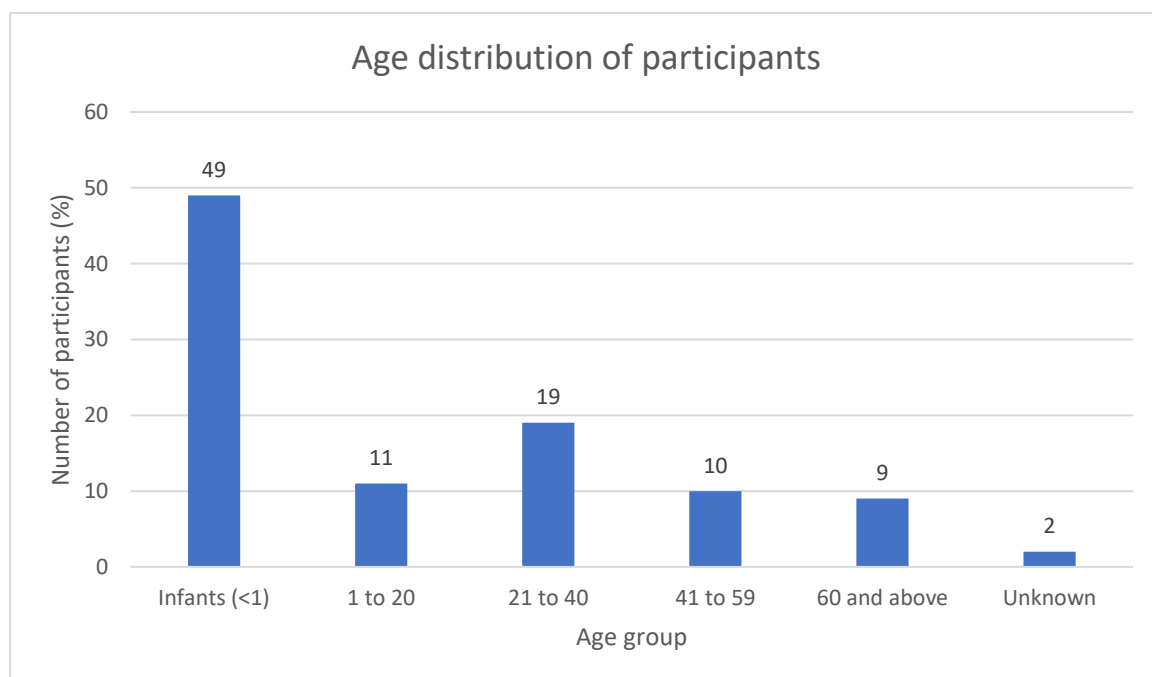
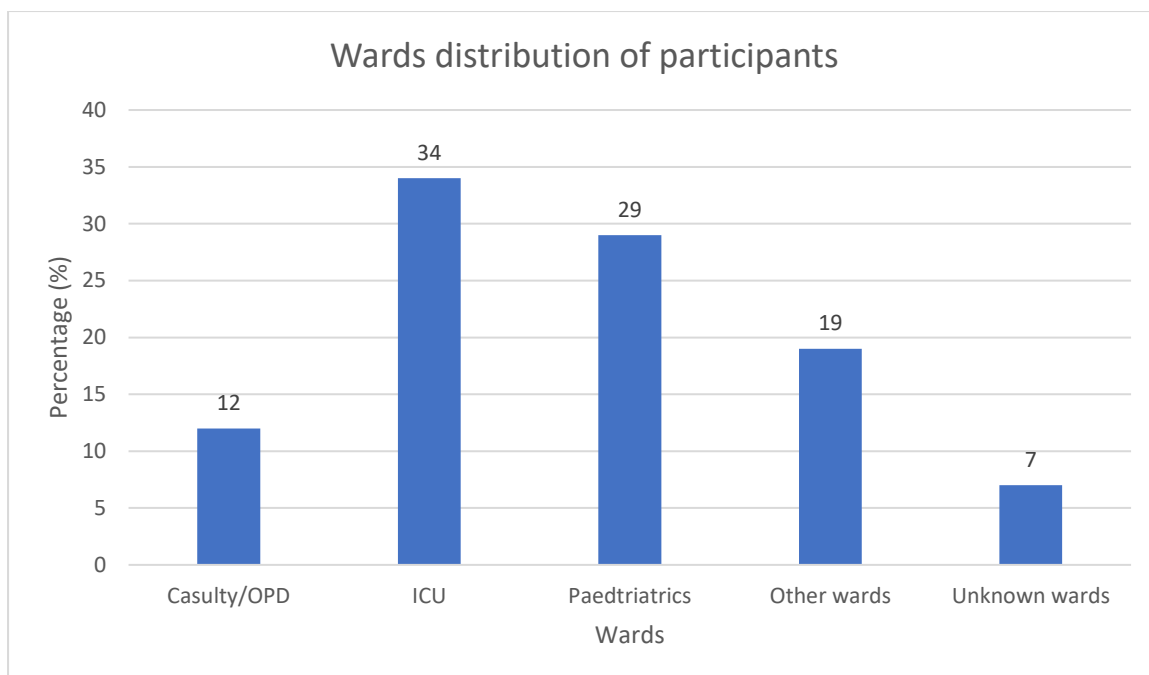


Figure 4.2. Age distribution of participants included in the study

4.1.4. Wards distribution of participants included in the study

The distribution of the samples on the hospital wards showed the highest number of patients were found in the high care unit. Intensive care unit (ICU) patients comprised 34% (83) of all samples, followed by the paediatric wards which accounted for 29% (72) samples (Figure 4.3). While, the remaining 19% (46) samples distributed across a variety of units, such as orthopaedic, neurology, and general medical wards.



OPD-Outpatient Department, ICU – Intensive Care Unit

Figure 4.3. Wards distribution of participants included in the study

4.1.5. Pathogen distribution based on Gram stain

Of the 247 samples, pathogen analysis revealed that Gram-negative bacteria (GNB) dominated the infection landscape, representing 78% (193) of all isolates (Figure 4.4). This is consistent with other studies, which often report Gram-negative organisms as the leading causes of bloodstream infections, particularly in healthcare settings. Gram-positive bacteria (GPB) made up 17% (43) of isolates (Figure 4.4), with yeasts representing a minority at 4% (11) (Figure 4.4), reflecting a smaller, but clinically significant portion of bloodstream pathogens.

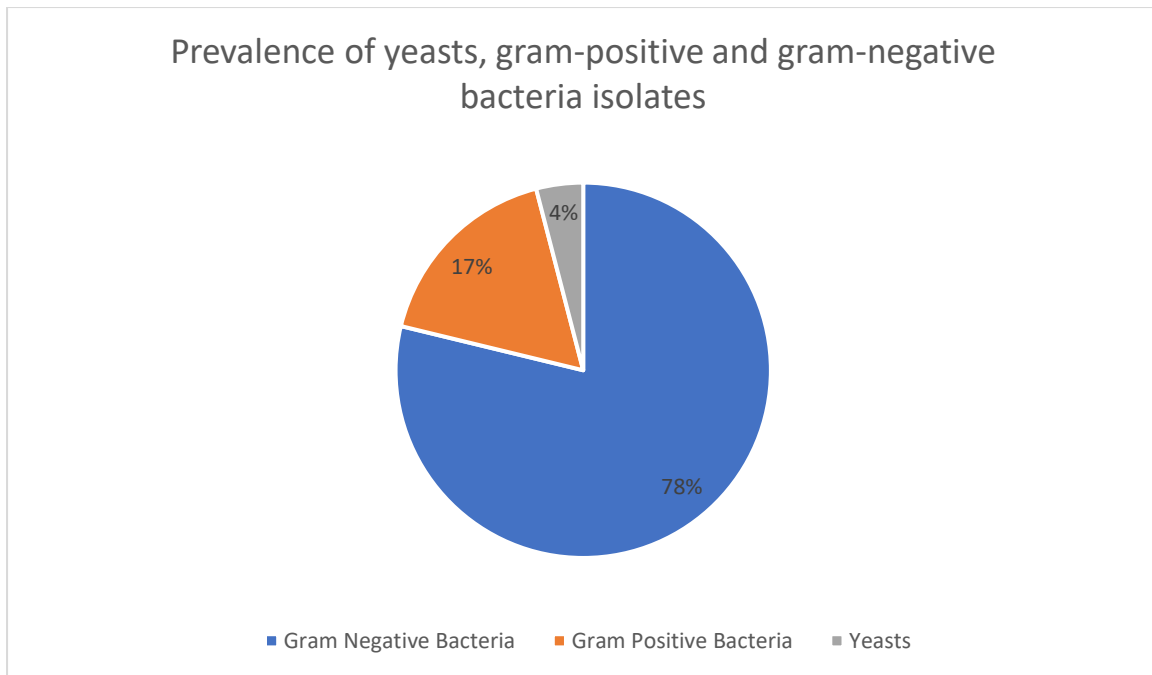


Figure 4.4. Prevalence of Gram-positive, Gram-negative, and yeast isolates

4.1.6. Distribution of antimicrobial resistance genes by BCID2

In addition to the pathogen type distribution, the antimicrobial resistance (AMR) gene profiling identified a total of 200 AMR genes in 144 samples, indicating substantial resistance within the bacterial population. The CTX-M gene, which confers resistance to extended-spectrum beta-lactams, was the most prevalent, appearing in 40% of isolates (Figure 4.5). On the contrary, the vancomycin resistance gene van A/B and the carbapenem resistance gene VIM were the least common, each present in only 0.5% of the isolates (Figure 4.5). From BCID2 on-panel genes, the IMP, KPC, and mcr-1 genes were not identified in our study.

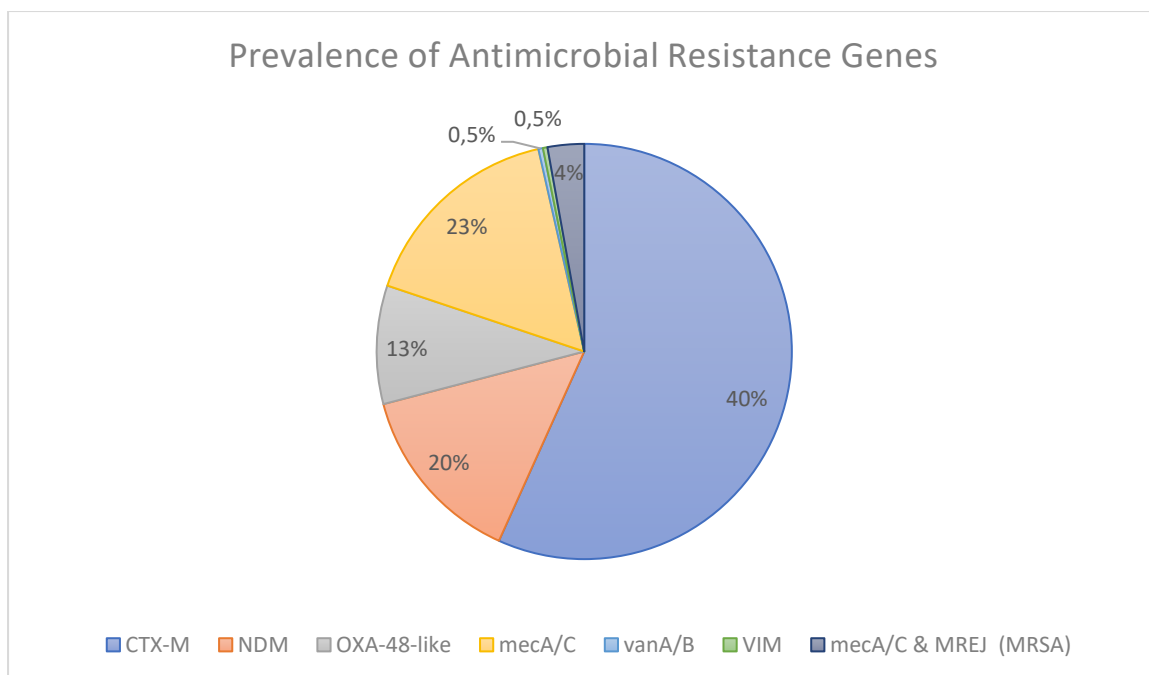


Figure 4.5. Prevalence of antimicrobial resistance genes

4.1.7. Distribution of the AMR among the wards

The distribution of resistance genes varied between wards. Of the 19 genes identified in the casualty outpatient department (OPD), there was a high number of CTX-M genes isolated 42% (8), the least isolated being the OXA-48-like gene 11% (2) (Figure 4.6). Of the 72 genes identified in the ICU, there was a high number of CTX-M genes 31% (22), the least isolated being the *mecA/C* and MREJ (MRSA) gene 7% (5), which is a gene that confers resistance to antimicrobial methicillin in *Staphylococcus aureus* (Figure 4.6). Of the 51 genes identified in the paediatric ward, the most prevalent gene was CTX-M 40% (20), with the *vanA/B* gene 1.96% (1), which is the gene that confers resistance to antimicrobial vancomycin, being the least isolated. 'Other wards' included orthopaedics, neurology, renal unit, mixed medical ward for adults, male medical, female medical, gynaecology, labour, maternity, and oncology wards. Of the 45 genes that were identified in 'other wards', CTX-M 52% (23) was the most prevalent gene, with VIM 2.22% (1) being the least isolated gene (Figure 4.3).

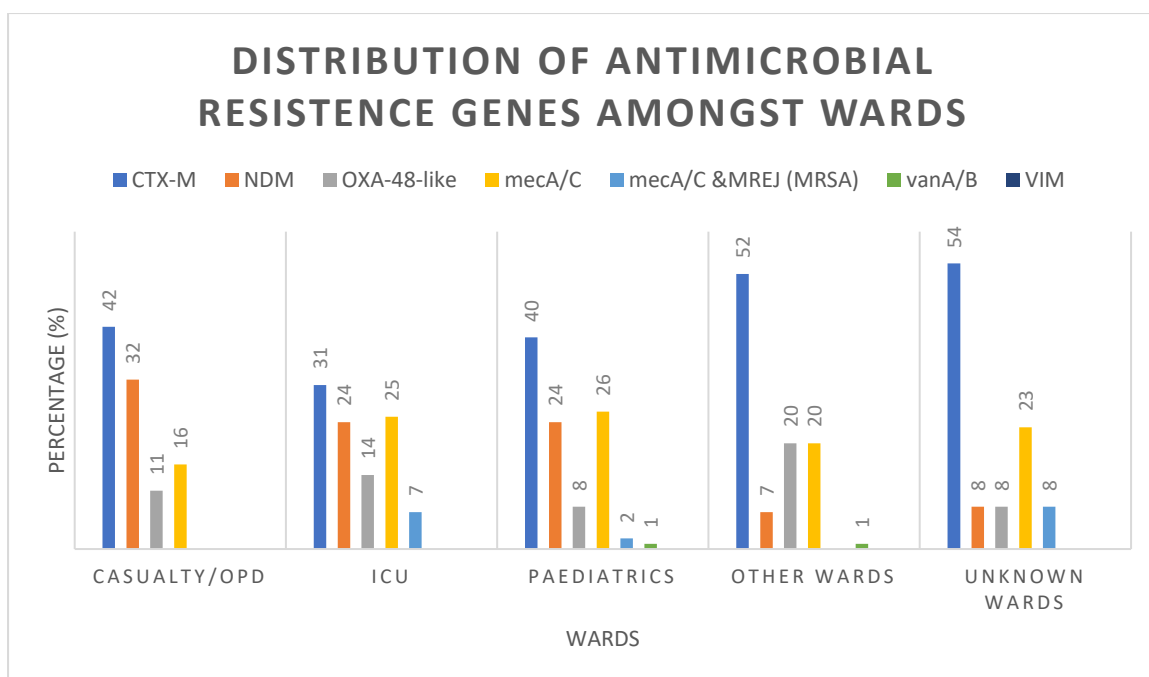


Figure 4.6. Distribution of antimicrobial resistance genes among wards

4.1.8. Summary of Section A

The table below summarizes the demographics of the patients, including age, gender, and wards. It also includes the distribution of pathogens based on Gram stain, as well as their antimicrobial resistance genes (Table 4.1)

Table 4.1. Age, gender, wards, Gram stain and AMR gene distribution

	Category	Percentage
Gender (n=247)		
	Females	(107/247) 43.3%
	Males	(132/247) 53.4%
	Unknown	(8/247) 3.2%
Age (n=247)		
	Infants (<1)	(121/247) 49%
	1 to 20	(28/247) 11.3%
	21 to 40	(48/247) 19.4%
	41 to 59	(24/247) 9.7%
	60 and above	(22/247) 8.9%
	Unknown	(4/247) 1.6%
Wards (n=247)		
	Casualty/OPD	(29/247) 11.7%
	ICU	(83/247) 33.6%
	Paediatrics	(72/247) 29.1%
	Other wards	(46/247) 18.6%

	Unknown wards	(17/247) 6.9%
Gram Stain		
	Gram Negative Bacteria	(193/247) 78.1%
	Gram Positive Bacteria	(43/247) 17.4%
	Yeasts	(11/247) 4.5%
AMR genes		
	CTX-M	(80/200) 40%
	NDM	(39/200) 19.5%
	OXA-48-like	(26/200) 13%
	VanA/B	(1/200) 0.5%
	VIM	(1/200) 0.5%
	MecA/C & MREJ (MRSA)	(4/200) 3.5%
	MecA/C	(46/200) 23%

SECTION B

4.2. DIAGNOSTIC PERFORMANCE OF VITEK2 AND THE BCID2

(An article for this section was submitted to the *Southern African Journal of Infectious Diseases* for publication)

The study compared the diagnostic accuracy of BCID2 (BioMérieux, Inc., France) against the gold standard, the VITEK2 (BioMérieux, Inc., Durham, NC), with a focus on sensitivity, specificity, and overall accuracy in pathogen identification. BCID2 demonstrated high accuracy, with minimum sensitivity, specificity, and accuracy metrics of 95.7%, 95.5%, and 96%, respectively (Table 4.2.1). Despite the strong overall performance, some organisms, such as *Klebsiella oxytoca*, presented challenges, with a significantly lower sensitivity of 66.7% (Table 4.2.1). The monomicrobial and polymicrobial BSIs found in our study were 72% (179/247) and 28% (68/247) respectively. A total of 21% (52/247) with polymicrobial infections were detected by the BCID2 assay but were missed by the VITEK2 assay, which indicated that they were monomicrobial samples.

Table 4.2.1. BCID2 Organism Identification Performance Compared to VITEK2

Organism	T P	F P	TN	F N	SS (TP/TP+F N) (95% CI)	SP (TN/TN+F P) (95% CI)	Accuracy (TP+TN/TP+TN+FN +FP) (95% CI)
Gram Negative Bacteria							
<i>Acinetobacter calcoaceticus-baumannii</i> complex	4 5	9	19 2	1	97.8% (88.5- 99.9%)	95.5% (91.7- 97.9%)	96% (92.7-98%)
<i>Bacteroides fragilis</i>	0	4	24 3	0	-	98.2 % (95.5- 99.5%)	-
<i>Enterobacter cloacae</i> complex	2 4	4	21 9	0	100% (85.8- 100%)	98.2% (95.5- 99.5%)	98.4% (95.9-99.6%)
<i>Escherichia coli</i>	2 2	4	22 0	1	95.7% (78.1- 99.9%)	98.2% (95.5- 99.5%)	98% (95.3-99.3%)
<i>Klebsiella aerogenes</i>	1	2	24 4	0	100% (2.5- 100%)	98.2% (97.1- 99.9%)	99.2% (97.1-99.9%)

<i>Klebsiella oxytoca</i>	2	0	24 4	1	66.7% (9.43- 99.16)	100% (98.5- 100%)	99.6% (97.77- 99.99%)
<i>Klebsiella pneumoniae</i> group	5 5	4	18 8	0	100% (93.5- 100%)	97.9% (94.8- 99.4%)	98.4% (96-99.6%)
<i>Proteus spp.</i>	5	1	24 1	0	100% (47.8- 100%)	99.6% (97.7- 99.9%)	99.6% (97.8-99.9%)
<i>Salmonella</i> group	4	0	24 3	0	100% (39.8- 100%)	100% (98.5- 100%)	100% (98.5-100%)
<i>Serratia marcescens</i>	6	2	23 9	0	100% (54.1- 100%)	99.2% (97- 99.9%)	99.2% (97.1-99.9%)
<i>Haemophilus influenzae</i>	1	0	24 6	0	100% (2.5- 100%)	100% (98.5- 100%)	100% (98.6-100%)
<i>Neisseria meningitidis</i>	0	0	24 7	0	-	100% (98.5- 100%)	-
<i>Pseudomonas aeruginosa</i>	3 3	4	21 0	0	100% (89.4- 100%)	98.1% (95.3- 99.5%)	98.4% (95.9-99.6%)
<i>Stenotrophomonas maltophilia</i>	2	1 4	23 1	0	100 (15.8- 100%)	94.3 (90.6- 96.8%)	94.3% (90.7-96.9%)
Gram-Positive Bacteria							
<i>Enterococcus faecalis</i>	2 4	9	21 4	0	100% (85.8- 100%)	96% (92.5- 98.1%)	96.4% (98.3-100%)
<i>Enterococcus faecium</i>	1 4	1 0	22 3	0	100% (76.8- 100%)	96.1% (92.8- 98.2%)	96.3% (93.2-98.3%)
<i>Listeria monocytogenes</i>	1	0	24 6	0	100% (2.5- 100%)	100% (98.5- 100%)	100% (98.5-100%)
<i>Staphylococcus aureus</i>	2 0	3	22 4	0	100% (83.2- 100%)	98.7% (96.2- 99.7%)	98.8% (96.5-99.7%)
<i>Staphylococcus epidermidis</i>	5	4 3	19 9	0	100% (47.8- 100%)	82.2% (76.8- 86.8%)	82.6% (77.3-87.1%)
<i>Staphylococcus lugdunensis</i>	0	2	24 5	0	-	99.2% (97.1- 99.9%)	-

<i>Streptococcus agalactiae</i>	4	2	24 1	0	100% (39.8-100%)	97.2% (97.1-99.9%)	99.2% (97.1-99.9%)
<i>Streptococcus pneumoniae</i>	1	1	24 5	0	100% (2.5-100%)	99.6% (97.8-99.9%)	99.6% (97.8-99.9%)
<i>Streptococcus pyogenes</i>	0	0	24 7	0	-	100% (98.6-100%)	-
Yeasts							
<i>Candida albicans</i>	6	4	23 7	0	100% (54.1-100%)	98.3% (95.8-99.5%)	98.4% (95.9-99.6%)
<i>Candida glabrata</i>	1	0	24 6	0	100% (2.5-100%)	100% (98.5-100%)	100% (98.5-100%)
<i>Candida krusei</i>	0	2	24 5	0	-	99.2% (97.1-99.9%)	-
<i>Candida parapsilosis</i>	4	5	24 0	0	100% (39.8-100%)	98% (95.3-99.3%)	98% (95.4-99.3%)
<i>Candida tropicalis</i>	0	1	24 6	0	-	99.6% (97.8-99.9%)	-
<i>Cryptococcus neoformans</i>	-	-	24 7	0	-	100% (98.5-100%)	-

TP=True Positive; FP=False Positive; TN=True Negative; FN=False Negative;
SS=Sensitivity; SP=Specificity

The BCID2 could not identify *Salmonella spp.*, *Proteus spp.*, and some *Streptococcus spp.*, to their species level.

The BCID2 assay did not detect certain organisms identified by VITEK2. *BCID2* missed *Escherichia coli* in one sample, while VITEK2 identified it. In another sample, BCID2 misidentified *Acinetobacter baumannii* as *Serratia marcescens*. Furthermore, BCID2 did not fully identify polymicrobial samples containing off-panel or rare organisms due to its limited panel coverage of 43 targeted pathogens. In this study, a total of 16 BCID2 off-panel microorganisms were identified (Table 4.3).

Table 4.2.2 BCID2 off-panel pathogens

Gram-negative Bacteria (N)	Gram-positive Bacteria (N)
---------------------------------------	---------------------------------------

<i>Cronobacter sakazakii</i> group (1)	<i>Aerococcus viridans</i> (1)
<i>Pluralibacter gergoviae</i> (1)	<i>Kocuria kristinae</i> (1)
<i>Pseudomonas fluorescens</i> (1)	<i>Leuconostoc mesenteroides</i> ssp. <i>dextranicum</i> (1)
<i>Sphingomonas poucimobilis</i> (2)	
<i>Pseudomonas putida</i> (1)	
<i>Aeromonas hydrophila</i> (2)	
<i>Acinetobacter</i> spp. (4)	
<i>Kluyvera ascorbata</i> (1)	
Total= 13	Total= 3

SECTION C

4.3. SEQUENCING RESULTS

This section presents the comparison of pathogen identification by the BioFire FilmArray Blood Culture Identification 2 (BCID2) panel (BioMérieux, Inc., France), VITEK-2 (BioMérieux, Inc., Durham, NC), and metagenomics.

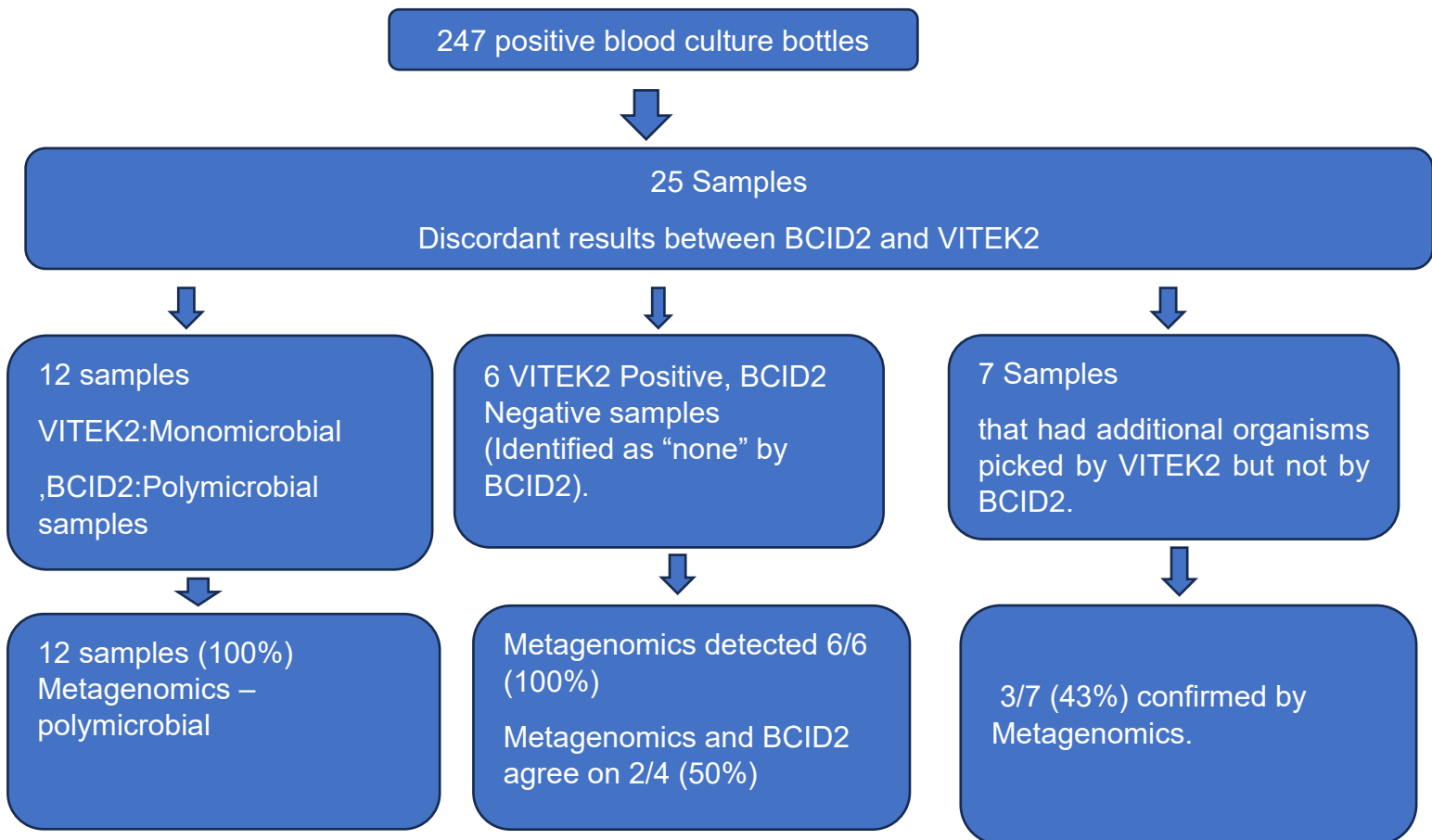


Figure 4.7. Flow diagram depicting metagenomics results

4.3.1. Pathogen detection and percentage agreement for sequenced samples

Metagenomics, BCID2, and VITEK-2 showed high accuracy in pathogen detection, although with some differences in scope. Metagenomics confirmed the pathogens identified by BCID2 in 72% of cases (18 out of 25) and VITEK-2 in 48% of cases (12 out of 25). Additionally, Metagenomics detected some pathogens that were not identified by both BCID2 and VITEK-2.

Metagenomics detected a total of 43 pathogens that were not detected by BCID2 and VITEK2, while VITEK2 detected a total of 6 pathogens that were not detected by both metagenomics and BCID2. BCID2 did not detect any unique pathogens. These 43 pathogens that were detected by metagenomics only included *Klebsiella quasipneumoniae* (2), *Acinetobacter junii* (1), *Serratia ureilytica* (1), *Negativicoccus succinicivorans* (1), *Sphingomonas aquatilis* (1), *Alistipes onderdonkii* (1), *Butyrivibrio fibrisolens* (1), *Butyrivibrio paravirosa* (1), *Streptococcus koreensis* (1), *Enterococcus lactis* (1), *Tissierella carlieri* (1), *Cellulomonas hominis* (2), *Frankia coriariae* (3), *Lysobacter soli* (1), *Acinetobacter radioresistens* (6), *Pseudomonas nosocomialis* (3), *Pseudomonas juntendi* (4), *Klebsiella planticola* (3), *Enterobacter kobei* (3), *Klebsiella michiganensis* (1), *Enterobacter roggenkampii* (1), *Citrobacter freundii* (2), and *Leptotrichia hofstandii* (1). The four organisms that were only detected by VITEK2 included *Kocuria kristinae* (1), *Sphingomonas paucimobilis* (2), and *Aerococcus viridans* (1). The six pathogens that were detected by the VITEK2 only but missed by the BCID2 and metagenomics included *Kocuria kristinae* (1), *Sphingomonas paucimobilis* (2), *Leuconostoc mesenteroides ssp dextranicum* (1), *Aerococcus viridans* (1), and *Pseudomonas putida* (1).

CHAPTER 5

DISCUSSION

The study evaluated three advanced diagnostic tools namely: metagenomics sequencing technique, BioFire FilmArray BCID2 (bioMérieux Inc, France), and VITEK2 (BioMérieux, Inc., Durham, NC) in terms of their diagnostic capabilities. It discusses the advantages they present for the detection of bloodstream infections (BSI) and for identifying antimicrobial resistance (AMR) genes.

SECTION A

Demographic analysis plays a crucial role in the understanding of the epidemiology of BSI and AMR gene prevalence. The demographic factors include ward distribution, age distribution, gender distribution, and ward distribution of the participants.

The study showed that infections among males were higher as compared to females. Males in the study accounted for 53% (132) of the cases. The results are in concordance with previous studies which showed that males are more prone to bloodstream infections than females (Cohen et al., 2013) . The results may reflect healthcare-seeking behaviour between males and females. Generally, women tend to seek treatment earlier as compared to males. This results in men developing more serious complications which lead to BSI. The differences between genders may be due to biological influences which include hormonal influences and differences in immune responses (Ruggierii et al., 2016).

The demographic analysis of the study indicated that infants under one year of age comprised nearly half of the sample size and were seen to have the highest number of BSI cases. The age group showed up to 49% (121) of the total cases. Such a statistic is rather common, perhaps due to various growth aspects such as immature immune systems that expose younger patients to a greater risk of infections. This is consistent with studies that have shown that prematurity, congenital anomalies, and surgery put neonates and infants, especially those in the ICU, at a greater risk of infections (Ting et al., 2022).

The research analyzed how participants were spread across various wards. The highest percentage of cases originated from the ICU, accounting for 34% (83) of the total samples. The elevated count of patients in the ICU can be linked to their extended

hospital stays and the probable need for numerous invasive procedures, which can heighten the risk of hospital-acquired infections (HAI) (Blot et al., 2022; Harte et al., 2021; Munro et al., 2024). Paediatric wards represented about 29% (72) of the cases, emphasizing the heightened susceptibility of younger patients to bloodstream infections.

The pathogen profile assessment done in the current study revealed that 78% of all the isolates were Gram-negative bacteria (GNB), and Gram-positive bacteria (GPB) followed by 17%, while very few yeast isolates accounted for 4% of the total. This trend agrees with the findings of other studies, where GNBs are often cited as the common BSI pathogens (Al Dabbagh et al., 2023; K. Li et al., 2023). Treatment becomes exceedingly difficult once gram-negative bacilli possess complex mechanisms of AMR such as ESBLs and carbapenemases (Sawa et al., 2020). In this study, a total of 200 AMR genes from 144 samples were identified, the most prevalent being CTX-M, which is an ESBL gene, found in 40% of the isolates. This high prevalence of ESBL genes emphasizes how difficult it is to treat BSI in areas with few resources, where improper antimicrobial usage is widespread and where antimicrobial stewardship programs are less robust. Other AMR genes, such as *van A/B* (vancomycin resistance) and *VIM* (carbapenem resistance), were present but less frequent. AMR markers were more prevalent in ICU samples, indicating a connection between admission to the ICU and the development of multidrug-resistant infections. The use of broad-spectrum antibiotics in ICUs may be contributing factors to the development of resistant organisms and AMR (Barnsteiner et al., 2021).

SECTION B

The introduction of molecular assays is a game changer in the rapid identification of bloodstream-associated pathogens and allows clinicians to implement appropriate antibiotic therapy which enhanced or supplanted BSI pathogen detection. A plethora of molecular-based multiplex platforms have been introduced, with the BICD2 being one of the assays with an extensive number of pathogen and antimicrobial resistance markers. In the current study, we compared the performance of the BCID2 to the routinely used VITEK2 for pathogen identification.

The current study showed that the BCID2 offers a high identification accuracy, as compared to the conventional method, the VITEK2. Additionally, the BCID2 shortened the identification time from 18 to 24 hours to about an hour, which is similar to what was also reported in studies by Caméléna et al and Senok et al (Caméléna et al., 2023; Senok et al., 2023).

The overall concordance between culture methods and BCID2 for pathogen identification was very good with 93% and it was similar to what was reported in a study by Berison et al, who reported 94% concordance (Berinson et al., 2021). This high concordance rate is encouraging; however, it should be noted that not all BCID2 targets were found in our samples, making it impossible to assess how well they performed. All off-panel organisms yielded a negative result with the BCID2.

In our study, the microbial profile showed that the predominant organism was found to be 193/247 (78%) of GNB, while only 17% (43/247) were GPB and 4% (11/247) were yeasts. These results were similar to those obtained in a study by Li et al, with 76.7% GNBs and 23.3% GPBs (K. Li et al., 2023).

According to our study, 72% were monomicrobial BSIs and 28% were polymicrobial BSIs. Similarly, Sze et al. and Caméléna et al., reported a higher number of monomicrobials, 87.1% and 85.5% respectively as compared to polymicrobial 12.9% and 14.5%, respectively (Caméléna et al., 2023; Sze et al., 2021). A total of 21% (52/247) with polymicrobial infections were detected by the BCID2 assay but were missed by the VITEK2 assay. The isolates were not picked by the VITEK2 which reported the result as monomicrobial. The inability to pick some polymicrobials by the VITEK 2 assay may prevent proper care of patients. According to the study conducted by Manyahi et al, PMB BSIs are related to high mortality rates; about twice that of monomicrobial infections, and patients with polymicrobial infections have a longer duration of stay in hospitals compared to patients with monomicrobial BSI (Manyahi et al., 2024). Thus, an assay must be able to detect polymicrobials. False negative discrepancies between the BCID2 and the VITEK2 were found on 18 (6%) isolates. Of the total of 18 (6%) cases that the BCID2 failed to correctly identify, 16 (5.5%) were off-panel microorganisms, while two were on-panel microorganisms. Of the 16 off-

panel microorganisms identified, *Cronobacter sakazakii*, *Pluralibacter gergoviae*, *Pseudomonas fluorescens*, *Sphingomonas poucimobilis*, *Pseudomonas putida*, *Kocuria kristinae*, and *Leuconostoc mesenteroides* spp *dextranicum*, have been reported as rare causes of clinical human infections (Arega et al., 2017; Chen et al., 2015; Ghobrial et al., 2023; Peter et al., 2017; Weiss et al., 2022). As a result, the BCID2 panel identified clinically important infections with a good degree of agreement with conventional approaches and minimal detection failures overall, which agrees with the study by Harboe-Sjåvik et al (Harboe-Sjåvik et al., 2024). In our study, the organisms are also classified as rare causes of BSIs. The results indicate the crucial limitation of point of care molecular assays, which is dependence on a fixed panel which restricts its coverage, with failed detection of off-panel pathogens.

The discrepancies of the on-panel isolate by the BCID2 included when the BCID2 identified one sample as *Serratia marcescens*, whilst VITEK2 identified it as *Acinetobacter baumannii*. The second discrepancy was when the VITEK2 identified the organism as *Escherichia coli*, whilst the BCID2 did not detect the organism. This may be due to a limited number of bacteria in the sample, of which culture may increase the amount to detectable levels.

Additional discrepancies were due to the additional identification of organisms by the BCID2. It should be noted that colonies of distinct coagulase-negative staphylococcal species can frequently be mistaken for one another (Berinson et al., 2021). As a result, it is not impossible to assume that choosing the incorrect colony for a VITEK2 species analysis could account for at least some discrepant results (Berinson et al., 2021). Furthermore, it is likely that other Gram-positive organisms that were only identified by molecular BCID2 analysis were prevented or concealed from growing due to the increased proliferation of Gram-negative bacteria (Berinson et al., 2021) .

SECTION C

Metagenomics was used to resolve discrepant results between VITEK2 and BCID2 results. Metagenomics confirmed the pathogen in 72% of the cases found by BCID2 and in 48% by VITEK2. Metagenomics also identified additional pathogens in 28% of cases, emphasizing its potential to fill gaps left by other methods.

Metagenomics picked out 43 extra species of pathogens, such as *Acinetobacter radioresistens*, *Frankia coriariae*, and *Leptotrichia hofstandii*, which BCID2 and VITEK2 did not, thus confirming it as a powerful tool for the diagnosis of difficult and rare infections. A total of 25 samples exhibiting discordant results between BCID2 and VITEK-2 were selected. Among those, metagenomics found 12 samples (48%) to be polymicrobial, while they were considered monomicrobial by VITEK-2. This was also true for seven samples that contained other organisms identified by VITEK-2, but not by metagenomics. Metagenomics demonstrated the highest detection rate whereby it was the only method to detect pathogens that were missed by BCID2 and VITEK2 assays. These additional organisms that were picked by metagenomics might have been contaminants, which is one of the disadvantages of using the metagenomics technique.

Among the best diagnostic methods at the moment, metagenomics renders the widest and most comprehensive coverage of all known pathogens, allowing the detection of a spectrum of different organisms, including mixed infections, rare organisms, and unculturable organisms. Metagenomics has been found to be a powerful sequencing technique that enables the direct detection and characterization of microbial communities from clinical samples without the need for prior culturing (Pérez-Cobas et al., 2020). This method brings important observations about the diversity of pathogens and polymicrobial infections. The information given by this research revalidates the role of metagenomics as a leading technology in the identification of a broader variety of pathogens in comparison with BCID2, and VITEK-2. The increased specificity and sensitivity of metagenomics is a vital tool in clinical microbiology, especially in the detection of rare and multidrug-resistant pathogens.

Despite its strong qualities, metagenomics has limitations (its relatively high cost, a longer time to results, and the need for advanced bioinformatics infrastructure) that hinder its routine applicability in low resource settings. Despite its limitations, the potential to backtrack a multitude of pathogens underscores a broad-spectrum diagnostic capability, especially in a setting where rapid molecular assays or culture-based detection methods have limitations.

However, dependence on a fixed panel restricts its coverage, with failed detection of off-panel pathogens, which are now becoming highly important in healthcare-associated infections. For example, the organisms *Alistipes onderdonkii* and *Butyrivibrio virosa* were identified only by metagenomics, underlining once again that other diagnostic approaches complementary to those developed would be needed to reach completeness for the spectrum of pathogens in cases of BSI.

VITEK-2, though an established automated culture-based system, had a much lower concordance with metagenomics (48%), compared to BCID2; however, it did reveal unique pathogens that were missed by both metagenomics and BCID2. For example, some organisms such as *Kocuria kristinae*, *Aerococcus viridans*, and *Sphingomonas paucimobilis* were only found by VITEK2, putting a mark on its worth in the detection of rare or niche pathogens. The other advantage of VITEK2 is the detailed AST, which is of great importance for guiding the targeted therapy when the pathogens have been cultured. However, its longer turnaround time, which typically ranges from hours to days depending on the growth of an organism, diminishes its usefulness compared to BCID2 in the time-sensitive clinical scenario. Despite this limitation, VITEK-2 occupies an almost indispensable position in confirming culture results and providing in-depth resistance profiling in those cases where molecular methods do not provide adequate information.

These findings are of clinical significance. Rapid diagnostics generated by BCID2 are ideal for the identification of pathogens and resistance profiling in the early course of an illness in the acute setting. The results in an hour facilitate timely clinical decision making, particularly in patients with sepsis or other critical infections. With a limited panel list of pathogens, additional testing is necessary for atypical or off-panel organisms. VITEK-2 is slower but provides value in the form of confirmation of culture results, detection of atypical pathogens, and provision of full AST information, all of which are significant in the optimization of antibiotic therapy. Metagenomics provides an unparalleled breadth of detection and, therefore, is an invaluable tool in the resolution of challenging or culture-negative cases, some of which involve polymicrobial infection or unusual pathogens.

A combination of techniques is recommended to optimize these tests. It could begin with BCID2 for quick testing, followed by confirmation and AST using VITEK-2. For

complex or unclear cases, metagenomics might be utilized due to its broad detection capabilities, which can aid in patient management. This approach could be especially advantageous for high-risk groups such as ICU patients, newborns, or individuals with compromised immune systems, where enhancing diagnostic accuracy, treatment timing, and overall outcomes is crucial.

These findings, therefore, call for more investment in developing training and diagnostic facilities, especially in resource-constrained regions such as Limpopo Province. Increasing access to newer diagnostics, including BCID2 and metagenomics, will improve health care disparities and thus enhance timely detection and treatment of BSI. In addition, if such tools were incorporated into routine workflows, then this would further strengthen the activities of AMR surveillance and result in high-quality data to inform public health action and stewardship programs on antimicrobials. Enabling targeted therapy, such assays can prevent the indiscriminate use of broad-spectrum antibiotics and, hence, decrease the selection pressure for resistance.

In summary, the distinctions between BCID2, VITEK-2, and metagenomics underscore their unique advantages and drawbacks, emphasizing the need for a personalized, multi-faceted diagnostic strategy in addressing BSI. The BCID2 may provide a prompt and accurate diagnosis for prevalent pathogens and resistance indicators, while VITEK-2 serves as a useful resource for detailed results regarding cultured microorganisms and AST. Metagenomics, though demanding more resources, delivers unparalleled diagnostic depth and precision and is thus critical in handling complicated or challenging cases. Together, these tools have the potential to significantly enhance BSI diagnostic accuracy and performance, and consequently patient treatment, and reduce the emerging issue of antimicrobial resistance in a range of clinical settings.

CHAPTER 6

CONCLUSIONS

Evaluation of diagnostic tools showed that rapid molecular assays will change the diagnosis of BSI profoundly. The sensitivity and specificity of BioFire FilmArray Blood Culture Identification 2 (BCID2) panel to VITEK2 were equally impressive, with 95.7% and 95.5%, respectively, with an overall accuracy of 96%.

Its quick performance of about sixty minutes enhances its suitability for the early identification of pathogens and the determination of their resistance patterns, especially in cases of emergency. The VITEK2 assay, though a bit more time consuming than the rest, also demonstrated a good level of diagnostic accuracy, surpassing BCID2 in the identification of pathogens such as *Aerococcus viridans*, among others. However, Metagenomics proved to be the all-encompassing diagnostic method, validating 72% BCID2 positives and 48% VITEK2 positives. It reported novel off-panel pathogens when the rest of the techniques were unable to report those.

The results of the study support the synergistic use of these diagnostic approaches. The rapidity and precision of BCID2 makes it suitable for primary diagnosis and directing early medical management; on the other hand, metagenomics offers more breadth and depth of understanding of the pathogen associated with the profiles. Such tools integrated into routine clinical practice may enhance patient outcomes, improve diagnostic accuracy, and shorten the period taken before appropriate therapy is instituted. However, the introduction of such sophisticated diagnostics is very capital intensive, especially in the laboratory and training of personnel, which is mostly a challenge in developing countries.

In summary, this research highlights the importance of developing advanced molecular diagnostics to facilitate the identification and management of BSI. The findings provide a solid basis for the enhancement of clinical practices including diagnostics, optimization of antibiotic use, and ultimately reducing the burden of healthcare-associated infections and drug-resistant strains. More research and resources will be required on these technologies to achieve the expected outcomes of these innovations and improve health in Limpopo province and elsewhere.

LIMITATIONS

The study was conducted at Pietersburg Hospital and only represent referred samples. This may not accurately reflect the larger population in Limpopo province or other areas of South Africa. The pathogens that cause bloodstream infections and their resistance to antimicrobials can vary significantly depending on economic conditions, geographic regions, and hospital practices.

The present study considered blood culture to be the gold standard for the comparison of the diagnostic accuracy of metagenomics, BCID2, and VITEK2. Although blood culture is the main diagnostic method for BSI, it has various drawbacks, such as long turnaround time (up to several days), decreased sensitivity to fastidious or slow-growing organisms, and susceptibility to false negatives, particularly when patients are receiving empirical antibiotic therapy. Blood culture sensitivity may differ among microorganisms; thus certain pathogens may not be detected or under-expressed. Blood culture as a reference standard has been over-reliant, and this could have contributed to the underestimation of the true diagnostic performance that metagenomics and BCID2 exhibit. Future studies must involve the use of alternative or complementary standards, which will enable a more accurate evaluation of the comparison of diagnostic tools.

BCID2 and VITEK2 are the leading validation technologies, yet the panels of their AMR genes and pathogens are fixed. Only 43 pathogens and a few BCID2 AMR genes are recognized, restricting its ability to detect organisms that are not part of this panel, such as some fungal pathogens or rare bacterial strains. Likewise, although VITEK2 is a powerful tool for the identification of a large number of organisms, the major drawback is that it relies on the culture procedure, thus limiting the detection of non-culturable pathogens and those that are present in very low concentrations. Therefore, it emphasizes the need for diagnostic techniques that would encompass a wider variety of pathogens as well as resistance genes that would provide better coverage for polymicrobial or complex infections.

Despite metagenomics promising pathogen detection capabilities, the approach is fraught with limitations for clinical practice. This is due to metagenomics requiring a long period, with results taking about 12 hours to generate, and also costs a lot as it needs an advanced laboratory, trained personnel to perform bioinformatics analysis,

and tools for data interpretation, which most hospitals, especially those in low and middle-income countries (LMICs), do not have. Furthermore, metagenomics produces large amounts of data that require further analysis and so creates reporting lags in a hospital that does not have these computers or personnel capable of performing the analysis. The great sensitivity of the metagenomics technique works against it as well; very rare and unexpected pathogens can be detected, but so can noise or possible contamination, making interpretation of the results troublesome. These factors are responsible for the challenges associated with the practical application of metagenomics technology and point towards the necessity for improved and affordable sequencing technologies.

Implementing metagenomics and BCID2 can be very expensive, especially in developing countries. Metagenomics is much more costly to set up and run than the BCID2 or VITEK2 systems. Although BCID2 is fast and works directly on blood culture to detect the pathogens present, it is still more expensive than culture techniques. Thus, the additional costs incurred in metagenomics and BCID2 amenities for hospitals operating on a tight budget may become impractical for day-to-day running, thus raising issues on the use of these techniques in everyday clinical practices. It is important to evaluate the cost-effectiveness of these two diagnostic tools, BCID2 as well as metagenomics, and to evaluate whether their diagnostic enhancement will be worth the extra costs incurred in the various hospital settings, especially those with limited healthcare resources.

This research was carried out as a cross-sectional study that assessed the diagnostic effectiveness and frequency of AMR at a particular moment. BSIs and AMR patterns can change with time due to seasonal changes in the predominance of the pathogens and new resistance mechanisms that come into play. Looking at the data trends would be impossible without any longitudinal data in place. In particular, it would not be possible to assess the resurgence of resistant pathogens or the efficiency of the metagenomics and BCID2 systems after an extended period of use.

The study utilized a convenience sampling approach, which might lead to possible biases in the sample gathering process.

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STRENGTHS OF THE STUDY

The investigation reports several strengths that support the validity of its findings, thus adding to the growing literature of bloodstream infection (BSI) diagnostic methods. The study informs about the performance of metagenomics, BioFire FilmArray Blood Culture Identification 2 (BCID2), and VITEK2, which are three different diagnostic tools for detection of pathogens and antimicrobial resistance (AMR) profiling, and all being of great relevance to hospitals and clinical microbiology laboratories. Multiple strengths contribute to the validity of the current study.

This study provides clinicians with the advantage of comparing three different diagnostic techniques, metagenomics, VITEK2, and BCID2. The research will provide a comprehensive overview of the suitability of the tests in medical environments. The research demonstrated the pros and cons of every testing method by analyzing various strategies, giving a comprehensive overview of their usefulness in medical settings.

The detailed examination enables healthcare professionals to choose the appropriate diagnostic instrument for their needs. The main advantages of BCID2 are faster processing time and improved pathogen detection. The VITEK2 is valued for precision in pathogen identification, while metagenomics is valued for its high sensitivity and wide detection range. Precision and wide range of metagenomics may easily place it as a gold standard, especially in cases where speed and completeness are critical to patient outcomes.

The research was carried out at Pietersburg Hospital, which serves as the main referral hospital for clinics in Limpopo. Most of the population in Limpopo province resides in rural areas, making up more than 85% of the total. This study mirrors a realistic clinical setting, making its findings relevant to comparable healthcare settings. This study reflects a real clinical environment, so the findings can be applied to similar healthcare settings. Therefore, the results of this study are suitable for application in clinical practice as they capture patient characteristics, workflow, and usual hindrances common in a hospital setting. Examining common pathogens and AMR gene distributions in a hospital population like Pietersburg Hospital enables personalized patient-centred treatment.

The study sample included a special group of patients who are considered high-risk patients and are more susceptible to bloodstream infections in hospitals, that is, infants, patients in the ICU. The study offers an understanding of the distribution of pathogens and their resistance patterns in high-risk patients vulnerable to nosocomial infections due to frequent invasive procedures, shedding light on the selection process for these individuals. Choosing high-risk patients helps ensure that the findings are relevant in critical care settings where quick lab results and high rates of BSI are common.

Another benefit is the in-depth examination of pathogens and AMR genes using the three diagnostic methods. The study not only identified the types and frequencies of common BSI pathogens, but also profiled key resistance genes, such as CTX-M, OXA-48-like, *mecA/C*, and NDM. The study offers a sophisticated understanding of AMR dynamics in BSIs by incorporating data on less prevalent or difficult resistance indicators such as *vanA / B* and VIM, which is crucial for physicians making treatment decisions. The exceptional sensitivity of metagenomics for detecting complex rare microbial species emphasizes its potential as a powerful treatment option for multidrug-resistant infections.

This study highlights the significance of rapid test outcomes, like BCID2, for making clinical choices in situations where delays may be detrimental, particularly in BSI treatment. The study shows that BCID2 demonstrates significant sensitivity and specificity within one hour, establishing it as an essential primary diagnostic resource for physicians to promptly initiate the correct treatment. This corresponds with the best methods to manage sepsis, as prompt detection and intervention are essential for enhancing patient results.

In summary, the study's strength renders it a significant addition to the domain of clinical microbiology and the management of infectious diseases. Its real-world setting, high-risk patient focus, and detailed analysis of pathogen and AMR genes provide a solid foundation for understanding the strengths and limitations of metagenomics, BCID2, and VITEK2, by offering a balanced perspective on rapid and comprehensive diagnostics, the study equips clinicians and healthcare facilities with insights that can improve diagnostic accuracy, inform targeted therapies, and ultimately improve patient care in the management of bloodstream infections.

FUTURE RESEARCH DIRECTIONS

The findings of the current study provide a strong foundation for understanding the advances of new diagnostic technologies. However, several crucial knowledge gaps remain, future studies must fill important knowledge gaps in the treatment and understanding of drug-resistant infections and their prevalence.

The unique detection ability of molecular tests lies in the ability to detect a wide range of pathogens, which includes rare species. However, like all molecular-based techniques, current molecular diagnostic tools have limited detection range. The BICD2 targets 43 molecular targets for fungi, bacteria, and AMR genes; however, the BICD2 still failed to detect off-panel organisms that were only learned through metagenomics and VITEK2. Further research may thus focus on broadening the molecular panels of the assays to encompass a wider range of pathogens, especially the rare species which still possess clinical significance. Improving and expanding the comprehensiveness of current diagnostic assays may reduce the reliance on multiple diagnostic platforms. The development of tests that can detect a greater variety of resistance genes, including new and developing ones should be a top focus for new diagnostic tools.

The present study was undertaken in Limpopo Province, a predominantly rural area, further studies should be conducted to assess BICD2, VITEK2, and metagenomics in other clinical settings in the future, to test the flexibility of modern molecular assays to function as point-of-care tests in constrained community clinics, where healthcare infrastructure may not be adequately developed. In Limpopo Province future studies should focus on sending molecular point of care tests to deep-rural clinics in Sekhukhune, Mopani, and Vhembe districts. Having insight into how well these technologies work at rural facilities where there is limited electricity supply, internet, or access to technicians will help determine the adaptability of the assays, determine how they affect patient outcomes.

Furthermore, future studies could explore the integration of these diagnostic tools into regular clinical protocols. Research could assess the effect of quick diagnostic tests on antimicrobial drug management, the speed of treatment, and medical decision-making in real situations.

The high costs associated with advanced diagnostic technologies such as metagenomics and BCID2 frequently raise concerns for numerous middle and low-income nations. Therefore, future studies should incorporate thorough cost-effectiveness evaluations to assess financial viability of applying these technologies across different healthcare environments. These investigations could evaluate the initial expenses of these diagnostics against their ability to reduce hospital stays, avoid unnecessary antibiotic usage, and enhance patient survival rates. Research on economic health should investigate the long-term benefits of early and accurate diagnosis of AMR to prevent the spread of drug-resistant infections, ultimately alleviating the pressure on the healthcare system.

Integration of diagnostic technologies with personalized medical strategies is regarded as one of the most optimistic areas for future investigative work. Advanced molecular diagnostics such as metagenomics allow for total genetic evaluation of pathogens, including resistance patterns. Subsequently, future studies should use this information in formulating personalized management plans with consideration given to the specific characteristics of the host and the pathogen.

Future development of predictive analytic and machine-learning approaches for large datasets generated from molecular diagnostics will be essential. By characterizing resistance trends and the pattern of prevalence of pathogens, these models might help in predicting patient outcomes, triggering alerts for potential outbreaks, and guiding public health interventions.

While the current study provides a snapshot of dynamics of antimicrobial resistance, antimicrobial resistance dynamics of pathogens is always evolving. Trends tend to change over time, making it crucial to constantly conduct longitudinal studies to track the evolution of resistance genes over time. These investigations could offer important understanding of how resistance strategies disseminate throughout hospitals and communities. By understanding these trends, certain actions could be formulated, including revised empirical treatment protocols and infection management strategies.

Future research should focus more on the relationships between microbes and hosts in the context of bloodstream infections. Investigating how host factors, including immune response and genetic susceptibility, influence infection severity and treatment outcomes could lead to new therapeutic approaches. Integrating host biomarkers with

pathogen diagnostics could enhance the capacity to forecast disease progression and tailor treatment strategies.

In summary, the results of the present studies create many opportunities for further investigation. Enhancing diagnostic capabilities, assessing practical applications, and incorporating personalized medicine strategies are vital aspects that could transform the management of BSI and AMR. Filling these gaps can aid upcoming research in enhancing patient results and reducing the worldwide risk of antimicrobial resistance. To improve the relevance and generalization of these diagnostic instruments in clinical settings, it is essential to expand sample sizes, integrate multiple centers, employ different reference standards, carry out follow-up studies, and evaluate the expenses of the tools

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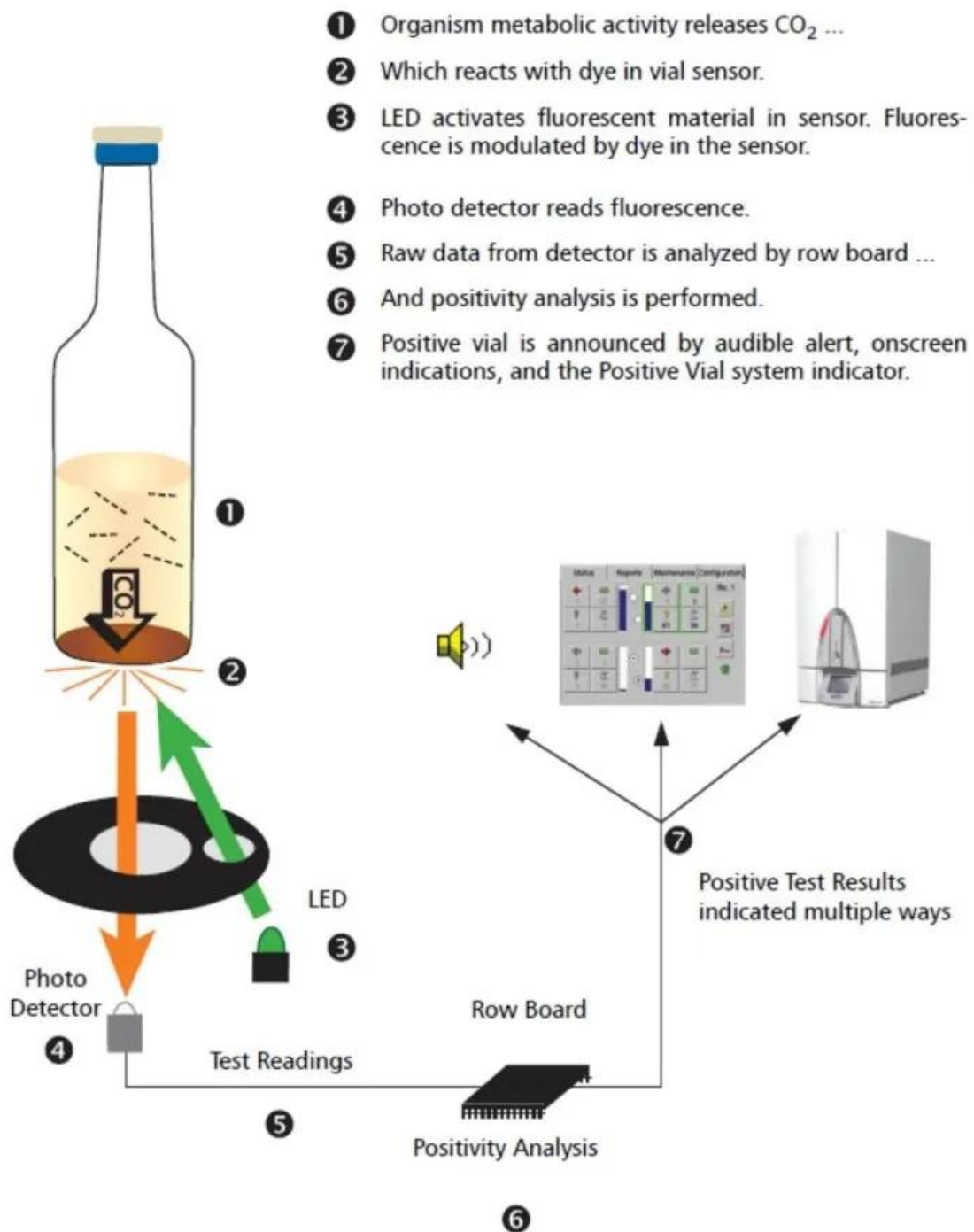
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APPENDICES

APPENDICE A: METHODS

APPENDIX A1: BLOOD CULTURE

Blood culture bottles (Aerobic, Anaerobic, and Paediatric) were incubated in the automated blood culture system, the BacT/ALERT 3D (bioMerieux. Inc. Durham, USA). The positive blood cultures were gram stained, followed by sub-culturing on the Blood, Chocolate, and MacConkey agar plates, and an addition of the Sabouraud Dextrose Agar plate where yeast was observed on gram stain, which were then incubated at 37°C for a maximum of 48 hours.



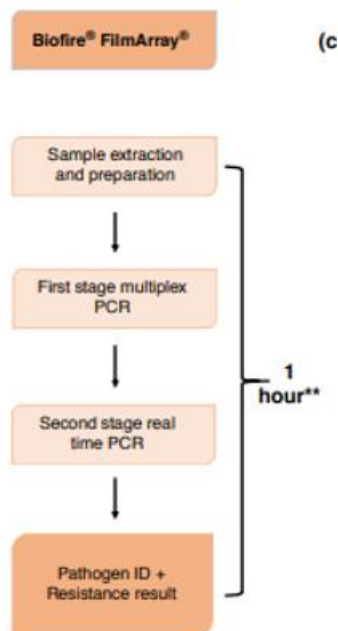
BACTEC Fluorescent Test Technology

APPENDIX A2: VITEK 2

Antimicrobial susceptibility testing (AST) and pathogen identification were conducted using the culture-based VITEK2 instrument (bioMérieux, Marcy l'Etoile, France) following the manufacturer's instructions, which had a turnaround time of 18 to 24 hours. From the solid culture media, pure isolated colonies were selected. Using a clean swab or applicator stick, a suitable number of colonies from a pure culture were transferred. The microorganism was then suspended in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a test tube made of clear polystyrene measuring 12 x 75 mm. Using a VITEK DensiCHEK™ colorimeter (bioMérieux, Marcy l'Etoile, France), the turbidity of the bacterial suspension was adjusted to meet the McFarland 0.5 criterion. Following the standard procedure, the bacterial suspensions were inserted into the Gram-positive cocci (GPC), Gram-negative bacilli (GNB), and yeasts (YST) unique identification cards of the automated VITEK-2 system. The susceptibility testing of the GNB and GPC was conducted using the VITEK2 GN04 and P526 cards, respectively. This was then loaded in the VITEK2 instrument, and results were available within 18 to 24 hours after loading.

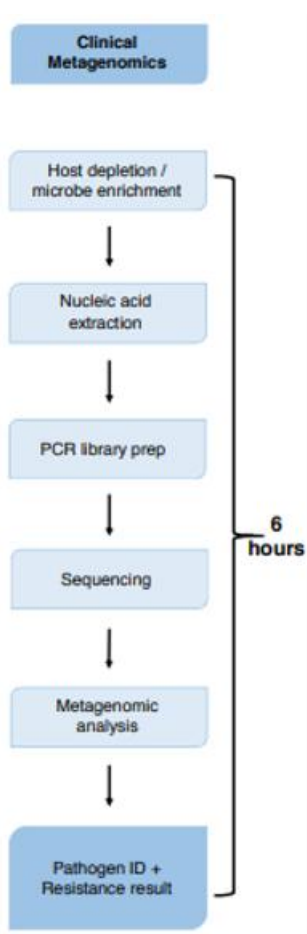
APPENDIX A3: BIOFIRE FILMARRAY BLOOD CULTURE IDENTIFICATION 2 (BCID2):

The BioFire FilmArray BCID2 panel was used according to the manufacturer's instructions. Briefly, a hydration solution was put in the selected well of the BCID2 pouch. Sample buffer and 200µl of blood culture broth were added to the sample injection well. Following the loading of the BCID2 pouch, the BioFire FilmArray instrument performed automated nucleic acid purification, two-stage multiplex PCR, and DNA melting curve analysis to identify the presence of bacterial, fungal, and antimicrobial resistance gene targets in the blood culture specimen under investigation.



APPENDIX A4: CLINICAL METAGENOMICS

Plasma was extracted from blood that has been kept in EDTA tubes by centrifuging it at 1600 g for 10 minutes at 4 °C. With the help of glass beads, pellets were resuspended and vortexed at 3000 rpm for 30 min in lysis buffer to break down cell walls. 300 µl of specimens was used for DNA extraction. The preparation of a library using a transposase-based method was then applied to 100 ng of DNA, as determined by a Qubit Fluorometer (Invitrogen, Carlsbad, CA). Pooled libraries were sequenced using a 75 bp, single-end sequencing kit on a Nextseq 550 sequencing system (Illumina, San Diego, CA).



Bioinformatics pipeline

Before conducting any further studies, raw sequencing data first went through a quality control procedure that eliminated adaptor trimming, low-quality, low-complexity, and short reads (<35 bp). For each sample, at least 10 M reads were produced. The Burrows-Wheeler Alignment (BWA) tool was used to align reads produced from the

human genome to the human reference genome (hg38) before microbial classification. The remaining microbes were categorized by mapping the remaining sequencing reads to a reference microbial database containing the genomes of bacteria, fungi, viruses, and parasites.

APPENDICE B: APPROVALS

APPENDIX B1: APPROVAL BY THE TURFLOOP RESEARCH ETHICS COMMITTEE



University of Limpopo
Department of Research Administration and Development
Private Bag X1106, Sovenga, 0727, South Africa
Tel: (015) 268 4713, Fax: (015) 268 2306, Email: moore.hutamo@ul.ac.za

TURFLOOP RESEARCH ETHICS COMMITTEE
ETHICS CLEARANCE CERTIFICATE

MEETING: 22 May 2023
PROJECT NUMBER: TREC/138/2023: PG
PROJECT:

Title: Determination of diagnostic accuracy of rapid molecular assays for diagnosis of bloodstream infections from Pietersburg Hospital, Limpopo Province in South Africa.
Researcher: NA Maswanganyi
Supervisor: Dr. I Rukasha
Co-Supervisor/s: Prof. R.M Lekalakala-Mokaba
Ms NTC Maguga-Phasha
School: Medicine
Degree: Master of Science in Medical Science

PROF D MAPOSA
CHAIRPERSON: TURFLOOP RESEARCH ETHICS COMMITTEE

The Turfloop Research Ethics Committee (TREC) is registered with the National Health Research Ethics Council, Registration Number: **REC-0310111-031**

Note:

- i) This Ethics Clearance Certificate will be valid for one (1) year, as from the abovementioned date. Application for annual renewal (or annual review) need to be received by TREC one month before lapse of this period.
- ii) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee, together with the Application for Amendment form.
- iii) PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Finding solutions for Africa

APPENDIX B2: APPROVAL FROM THE NHLS-AARMS



Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

06 September 2023

Applicant: Nhlayiso Maswanganyi
Institution: University of Limpopo
E-mail Address: nhlayie2000@gmail.com
Tel : 082 972 4965 **Cell:** 073 865 2801

Cc: Ivy Rukasha

Project Title: DETERMINATION OF DIAGNOSTIC ACCURACY OF RAPID MOLECULAR ASSAYS FOR DISGNOSIS OF BLOODSTREAM INFECTIONS FROM PIETERSBURG HOSPITAL, LIMPOPO PROVINCE IN SOUTH AFRICA.

Reference Number: PR2345776

Research Application Type(s):

1. Request for Samples
2. Permission to use NHLS Facilities

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available **without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)**.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NICD departments and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – <https://aarms.nhls.ac.za>.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

A handwritten signature in black ink, appearing to read "Babatyi", is written over a horizontal line.

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

APPENDIX B3: APPROVAL FROM THE LIMPOPO DEPARTMENT OF HEALTH



LIMPOPO

PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
HEALTH

Ref : LP_2024-10-022
Enquires : Legodi P
Tel : 015-293 6028/6410
Email : Malesela.Legodi@dhsd.limpopo.gov.za

MS MASWANGANYI N. A

CC: UNIVERSITY TO ENSURE COMPLIANCE WITH CLAUSE 2d OF THIS APPROVAL LETTER

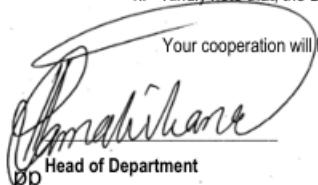
PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;

DETERMINATION OF DIAGNOSTIC ACCURACY OF RAPID MOLECULAR ASSAYS FOR DIAGNOSIS OF BLOODSTREAM INFECTIONS FROM PIETERSBURG HOSPITAL, LIMPOPO PROVINCE IN SOUTH AFRICA.

1. Permission to conduct research study as per your research proposal is hereby Granted.
2. Kindly note the following:
 - a. Present this letter of permission to the office of Clinal Executive Director a week before the study is conducted.
 - b. This Hospital permission is **ONLY** for Pietersburg Hospital.
 - c. In the course of your study, there should be no action that disrupts the routine services or incur any cost on the Department.
 - d. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
 - e. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
 - f. **The approval is only valid for a 1-year period.**
 - g. If the proposal has been amended, a new approval should be sought from the Department of Health
 - h. Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated.



Head of Department

5/11/2024

Date

Private Bag X9302, Polokwane 0700
Fidel Castro Ruz House, 18 College Street, Polokwane 0700
Tel: 015 293 6000. Fax: 015 293 6211. Website: www.doh.limpopo.gov.za

The heartland of Southern Africa - development is about people!

APPENDIX B4: PMREC WAIVERS TO INFORMED CONSENT

PMREC WAIVERS TO INFORMED CONSENT

Section 1. PROTOCOL/PROPOSAL INFORMATION

Protocol/proposal number (for official use ONLY):

Protocol/proposal title:

DETERMINATION OF DIAGNOSTIC OF RAPID MOLECULAR ASSAYS FOR DIAGNOSIS OF BLOODSTREAM INFECTIONS FROM PIETESBURG HOSPITAL, LIMPOPO PROVINCE IN SOUTH AFRICA

Principal investigator:

NHLADISO ATALIA MASWANGANGI

Indicate applicable with an "X"

Independent research	Contract research	~~Post-graduate research~~	Undergraduate research
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Section 2. REQUEST FOR ALTERATION OR WAIVER OF INFORMED CONSENT

Indicate applicable with an "X"

☒	2A	A waiver of informed consent
☐	2B	An alteration of informed consent
☐	2C	A waiver of parent/guardian permission
☐	2D	A waiver of assent because minors are not capable of assent
☐	2E	A waiver of assent because the research holds out a prospect of direct and indirect benefit

Section 3. DECLARATION

I, the principal investigator, declare that:

STATEMENT	DETAIL and/or EXPLANATION
3A The research involves no more than minimal risk to the research participants,	Yes
3B The waiver or alteration will not adversely affect the rights and welfare of the research participants,	Yes
3C The research could not practicably be carried out without the waiver or alteration, and	Yes
3D Whenever appropriate, the research subjects will be provided with additional pertinent information after participation.	No


Signature of principal investigator

06/12/2022
Date

REMARKS (for official use ONLY):

APPENDIX B5: MEDICAL PRACTITIONER'S REQUEST FORM

NATIONAL HEALTH LABORATORY SERVICE				ALL LEVELS OF CARE			
PATIENT Patient I.D. Number: _____ Patient Hospital Number: _____ Surname: _____ Class: _____ First Name: _____ Address: _____ Tel No.: _____ Race: _____ Sex: ☐ M ☐ F D.O.B.: _____ Age: _____ ICD-10 Diagnosis Codes: _____ Medical Aid: _____ Medical Aid Number: _____ Employer: _____ Dep Code: _____ Account To / Principal Member: _____ Member Address: _____ Member Tel. No.: (H) _____ Member I.D. Number: _____				MARK IF URGENT ☐ HOSP Hospital/Clinic: _____ WARD Ward: _____ DIAGN Diagnosis/Reason for Request: _____ MED Medication: _____ Warfarin: ☐ Heparin: ☐ TYPE Type of Specimen: _____ DATE Date Taken: _____ Time: _____ TAKEN Taken By: _____ REQUEST Requesting Health Care Worker: _____ HPCSA HPCSA/SANC Number: _____ CONTACT Contact Numbers: _____ E-MAIL E-mail Address: _____ SIGNATURE Signature: _____			
CHEMICAL PATHOLOGY General: ☐ Blood gases ☐ U+E ☐ LFT ☐ Chl ☐ UA ☐ Sodium ☐ Potassium ☐ Chloride ☐ Urea ☐ Creatinine ☐ Calcium ☐ Magnesium ☐ Inorganic phosphate ☐ Total protein ☐ Albumin ☐ Total bilirubin ☐ Conjugated bilirubin ☐ ALP ☐ GGT ☐ ALT ☐ AST ☐ LDH ☐ Amylase ☐ Lipase ☐ Pseudocholesterase ☐ Red cell cholinesterase Cardiac/Muscle: ☐ Aldolase ☐ Creatine kinase ☐ CK-MB fraction ☐ Troponin Diabetes/Metabolic: ☐ Glucolysis ☐ Glucose fasting ☐ Glucose random ☐ HbA1c ☐ Lactate Lipids: ☐ Lipogram ☐ Triglycerides ☐ Total cholesterol ☐ HDL cholesterol ☐ LDL cholesterol Anaemia: ☐ Iron studies ☐ Iron ☐ Transferrin ☐ Ferritin ☐ Vitamin B12 ☐ Haptoglobin ☐ Foate (red cell)		HAEMATOLOGY General: ☐ FBC + Platelets ☐ Differential count ☐ Morphology ☐ Haemoglobin ☐ White cell count ☐ Platelet count ☐ Reticulocytes ☐ ESR ☐ CD4 count Coagulation: ☐ INR ☐ PTT ☐ Fibrinogen ☐ D Dimer ☐ Anti thrombin ☐ Protein C ☐ Protein S ☐ Lupus anticoagulant ☐ Thrombin time ☐ DIC screen Other: ☐ Coombs ☐ ABO ☐ RH IMMUNOLOGY Inflammation: ☐ CRP ☐ IgG, IgA, IgM ☐ SPEP ☐ C3, C4 Allergy: ☐ Total IgE ☐ IgE RAST Auto Immune: ☐ RF ☐ ANA ☐ ENA ☐ Anti double stranded DNA ☐ Anti cardiolipin ☐ Anti mitochondria ☐ Anti smooth muscle		MICROBIOLOGY Specimen Type: ☐ Blood ☐ CSF ☐ Pus ☐ BAL ☐ TA ☐ Mid stream urine ☐ Catheter urine ☐ SPU ☐ Aspirate ☐ Stool ☐ Rectal swab ☐ Throat swab ☐ Swab ☐ Fluid (specify) _____ ☐ Catheter tip (IV, shunt) _____ Investigation Required: ☐ Parasites ☐ Malaria ☐ Microscopy/Culture/Sensitivity ☐ Anaerobic culture ☐ TB microscopy ☐ NTM ☐ TB PCR direct ☐ TB culture ☐ BCG ☐ TB sensitivity ☐ Fungal microscopy ☐ Fungal culture BACT/ PARA/ FUNGII/ SEROLOGY ☐ Chlamydia PCR (urine/swab) ☐ MF (serum) ☐ Clostridium difficile toxin ELISA ☐ ASOT ☐ Anti-DNAse B ☐ Anti-hyaluronidase ☐ HPR ☐ TPHA ☐ FTA ☐ VDRL ☐ Bihartzia IFA ☐ ELISA ☐ Amoebic IFA ☐ Hyalid IFA ☐ Cysticercosis IgG ☐ Crypto antigen ☐ Pneumocystis IFA ☐ Mycoplasma ELISA ☐ Rickettsia IFA OTHER TESTS ☐ DATE _____ ☐ RECEIVED IN LAB BY _____ ☐ SIGNATURE _____		VIRAL SEROLOGY Hepatitis testing: ☐ Clinical hepatitis: ☐ A ☐ B ☐ C ☐ Hepatitis B ☐ Ab ☐ Ag HIV testing: ☐ ELISA ☐ HIV rapid ☐ HIV Viral load ☐ HIV PCR TORCH screens: IgG IgM ☐ Toxo ☐ Rubella ☐ CMV ☐ Herpes Other Serology: IgG IgM ☐ EBV ☐ VZV ☐ Measles ☐ Mumps ☐ CMV p65 ☐ Rapid RSV ☐ Rota/Adeno Viral Isolation: ☐ Culture (specify) ☐ PCR (specify) SPECIMEN KEY ☐ Yellow (or Red) with gel ☐ Red (without gel) ☐ Green (heparin) ☐ Purple (EDTA) ☐ Blue (citrate) ☐ BL Black (trisodium citrate) ☐ GY Grey (flouride) ☐ BC Blood culture ☐ S Specimen jar ☐ Consult local laboratory ☐ Tan (no additive) ☐ HS Heparinised syringe ☐ O Other	
APPLY BAR CODE LENGTHWISE DO NOT WRAP AROUND 				DESCRIBE WOUND AND SITE 			
 AHCP2641NOF		 AHCP2641NOF		 AHCP2641NOF			

APPENDICE C: RAW DATA

APPENDIX C1: DEMOGRAPHICS, VITEK2 AND BCID2 RESULTS

RESEARCH ID	WARD	AGE (YEARS)	GENER	MICROSCOPY (GRAM STAIN)	BCID2 AMRES	VITEK 2 RESULTS (MICROORGANISMS)	BCID2 RESULTS (MICROORGANISMS)	VITEK2 MONOMICROBIAL/POLYMICROBIAL	BCID2 Monomicrobial/Polymicrobial
OF00217075	Pediatrics	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01447996	Pediatrics	0	F	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01452949	Pediatrics	0	F	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
OM00630940	Pediatrics	0	M	Yeast	None	<i>C. albicans</i>	<i>C. albicans</i>	Monomicrobial	Monomicrobial
OF00215608	Pediatrics	0	F	GPB	mec A/C; mec A/C and MREJ (MRS A)	<i>E. faecalis</i> ; <i>S. aureus</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>S. aureus</i> ; <i>S. epidermidis</i>	Polymicrobial	Polymicrobial
OM00627625	Pediatrics	0	M	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i> ; <i>E. faecium</i> ; <i>C. albicans</i>	Monomicrobial	Polymicrobial
NM01445795	Casualty	0	F	GNB	None	<i>P. aeruginosa</i>	<i>Staphylococcus</i> spp.; <i>Streptococcus</i> spp.; <i>S. pneumoniae</i> ; <i>P. aeruginosa</i>	Monomicrobial	Polymicrobial
NP01155597	Pediatrics	0	M	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial

NM01 4401 49	Pae diatr ics	0	F	Yeast	None	<i>C. albicans</i>	<i>C. albicans</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4438 88	Pae diatr ics	0	F	GNB	None	<i>P. aerugino sa</i>	<i>P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4401 94	ICU	0	F	GNB; GPB	mec A/C; NDM	<i>A. baumann ii; S. epidermi dis</i>	<i>Staphylococcu s spp.; S. epidermidis; A. calcoaceticus- baumannii complex; C. albicans</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NP01 1529 92	ICU	0	M	GPB	None	<i>E. faecium</i>	<i>E. faecium</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OM00 6280 99	Pae diatr ics	0	M	GNB	None	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4245 99	Pae diatr ics	0	F	GNB	None	<i>P. aerugino sa</i>	<i>Staphylococcu s spp., P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4225 21	ICU	0	F	GNB; GPB	mec A/C; NDM	<i>A. baumann ii</i>	<i>Staphylococcu s spp.; S. epidermidis; A. calcoaceticus- baumannii complex; S. maltophilia; C. krusei; C. parapsilosis</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4217 87	ICU	0	F	GNB; GPB	mec A/C	<i>P. aerugino sa</i>	<i>Staphylococcu s spp.; S. epidermidis; P. aeruginosa; C. albicans</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
OQ00 6242 59	Pae diatr ics	0	F	GPB	mec A/C	<i>E. faecium; S. haemolyt icus</i>	<i>E. faecium; Staphylococcu s spp.; S. aureus; S. epidermidis</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4199 39	ICU	0	M	GNB; GPB	mec A/C	<i>A. baumann ii; E. faecium</i>	<i>E. faecium; Staphylococcu s spp.; S. epidermidis; A. calcoaceticus- baumannii complex</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>

NM01 4204 07	ICU	0	M	Yeast	None	<i>C. glabrata</i>	<i>C. glabrata</i>	Monomicrobial	Monomicrobial
NM01 4238 90	ICU	0	F	GNB; GPB	mec A/C; NDM	<i>A. baumannii</i> ; <i>S. epidermidis</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4060 58	Unknown	0	F	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NF01 2663 36	ICU	0	F	GNB	CTX-M; mec A/C; NDM	<i>E. cloacae</i> complex; <i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex; <i>K. pneumoniae</i> group	Polymicrobial	Polymicrobial
N014 0454 4	Pediatrics	0	M	Yeast	None	<i>C. albicans</i>	<i>C. albicans</i> ; <i>C. parapsilosis</i>	Monomicrobial	Polymicrobial
NF01 2732 01	ICU	0	M	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4109 60	ICU	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NK00 2840 50	Unknown	0	F	GNB	None	<i>E. coli</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Enterobacteriales</i> ; <i>E. coli</i>	Monomicrobial	Polymicrobial
NM01 4144 72	Pediatrics	0	M	GNB; GPB	CTX-M; NDM	<i>A. baumannii</i> ; <i>E. cloacae</i> complex	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex; <i>C. krusei</i>	Polymicrobial	Polymicrobial

NM01 4151 12	ICU	0	M	GNB	CTX-M; NDM ; OXA-48-like	<i>Klebsiella pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
OM00 6366 80	Pediatrics	0	F	GNB; GPB	mecA/C	<i>A. baumannii</i> ; CNS	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
OM00 6351 86	ICU	0	F	Yeast	None	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>	Monomicrobial	Monomicrobial
OM00 6356 89	Pediatrics	0	F	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial
NM01 4642 17	Pediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
OA02 6593 06	Pediatrics	0	M	Yeast	None	<i>C. parapsilosis</i>	<i>C. parapsilosis</i>	Monomicrobial	Monomicrobial
NM01 4636 41	Pediatrics	0	F	GNB	CTX-M	<i>K. pneumoniae</i>	<i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
ON00 2482 88	Pediatrics	0	F	GPB	mecA/C	<i>E. faecalis</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp; <i>S. aureus</i> ; <i>S. epidermidis</i> ; <i>S. lugdunensis</i>	Monomicrobial	Polymicrobial
NM01 4642 19	Pediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01 4642 18	Pediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NB00 8212 93	Casualty	0	M	GPB	None	<i>S. agalactiae</i>	<i>Streptococcus</i> spp.; <i>S. agalactiae</i> (Group B)	Monomicrobial	Polymicrobial
NM01 4645 91	ICU	0	M	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomicrobial	Monomicrobial

OV00 1111 17	Pae diatr ics	0	F	GPB	None	<i>E. faecium</i>	<i>E. faecium</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4528 82	ICU	0	Unk no wn	GNB	CTX- M	<i>S. marcesc ens</i>	<i>Enterobacteral es; S. marcescens</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OA02 6600 35	Cas ualty	0	F	GNB	CTX- M; NDM	<i>E. cloacae complex</i>	<i>Enterobacteral es; E. cloacae complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OM00 6341 46	ICU	0	F	GPB	mec A/C	<i>E. faecalis</i>	<i>E. faecalis; Staphylococcu s spp.; S. epidermidis</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4520 66	ICU	0	F	GNB	None	<i>E. cloacae; P. aerugino sa</i>	<i>Staphylococcu s spp.; Enterobacteral es; E. cloacae complex; E. coli; K. pneumoniae group; P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
OA02 6424 79	Cas ualty	0	M	GNB	CTX- M	<i>K. pneumo niae spp. Pneumo niae</i>	<i>Enterobacteral es; K. pneumonie group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4462 57	Pae diatr ics	0	F	GNB; GPB	None	<i>E. coli; E. faecalis</i>	<i>E. faecalis; E. faecium; Staphylococcu s spp.; Streptococcus spp; Enterobacteral es; E. coli; K. oxytoca</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
OA02 6438 86	Pae diatr ics	0	M	GNB; GPB	CTX- M; NDM ; vanA /B	<i>E. cloacae; E. faecium</i>	<i>E. faecium; A. calcoaceticus- baumannii complex; Enterobacteral es; E. cloacae complex; E. coli; K. pneumoniae group; C. parapsilosis</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NM01 4518 04	Pae diatr ics	0	F	GNB	CTX- M	<i>A. baumann ii; K. pneumo</i>	<i>Staphylococcu s spp.; A. calcoaceticus- baumannii</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>

						<i>niae ssp. pneumo niae</i>	<i>complex; Enterobacter al es; K. pneumoniae group</i>		
NM01 4491 00	Pae diatr ics	0	M	GPB	None	<i>Coagulas e Negative Staphylo coccus; E. faecalis</i>	<i>E. faecalis; Staphylococcu s spp.</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OM00 6319 42	ICU	0	M	GNB	CTX-M	<i>K. pneumo niae ssp. pneumo niae</i>	<i>Enterobacter al es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4529 48	Pae diatr ics	0	F	GPB	None	<i>S. aureus</i>	<i>Staphylococcu s spp.; S. aureus</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4529 47	ICU	0	M	GNB	None	<i>P. aerugino sa</i>	<i>P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4582 20	ICU	0	F	GNB	CTX-M	<i>K. pneumo niae ssp pneumo niae</i>	<i>Enterobacter al es; K. pneumoniae group; S. maltophilia</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NP01 1655 98	Pae diatr ics	0	M	GNB	None	<i>E. coli</i>	<i>Enterobacter al es; E. coli</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4580 10	Pae diatr ics	0	F	GNB	None	<i>P. aerugino sa</i>	<i>P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4544 75	ICU	0	F	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus-baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4402 07	ICU	0	M	GNB; GPB	mec A/C	<i>E. coli; P. aerugino sa; P. gergoviae ; S. epidermi dis</i>	<i>Staphylococcu s spp.; S. epidermidis; P. aeruginosa</i>	<i>Polymic robial</i>	<i>Monomicrobi al</i>
NF01 2966 42	ICU	0	M	GNB	CTX-M	<i>K. pneumo niae ssp. pneumo niae</i>	<i>Enterobacter al es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>

OA02 6419 45	Cas ualty	0	M	GNB; GPB	CTX- M; NDM	<i>S. agalactia e; E. cloacae complex</i>	<i>Streptococcus spp.; S. agalactiae (Group B); Enterobacteral es; E. cloacae complex</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NM01 4479 94	Pae diatr ics	0	M	GNB; GPB	CTX- M	<i>K. pneumo niae ssp. pneumo niae; E. faecalis</i>	<i>E. faecalis; E. faecium; Streptococcus spp.; Enterobacteral es; K. pneumoniae group</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
OI008 4393 1	Pae diatr ics	0	F	GNB; GPB	CTX- M	<i>E. faecalis; E. cloacae; K. pneumo niae</i>	<i>E. faecalis; E. faecium; Staphylococcu s spp.; S. aureus; Enterobacteral es; E. cloacae complex; K. pneumoniae group</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NF01 3095 73	Unk now n	0	F	GNB	None	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4695 98	ICU	0	F	GNB	None	<i>S. maltophil ia</i>	<i>S. maltophilia</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OM00 6375 38	Pae diatr ics	0	M	GPB	None	<i>S. agalactia e</i>	<i>Streptococcus spp.; S. agalactiae (Group B)</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4966 44	Pae diatr ics	0	M	GNB; GPB	mec A/C	<i>S. aureus; K. oxytoca</i>	<i>E. faecium; Staphylococcu s spp.; S. aureus; S. epidermidis; Enterobacteral es; K. oxytoca</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NM01 4834 78	Pae diatr ics	0	M	GNB	None	<i>S. maltophil ia</i>	<i>S. maltophilia</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4876 14	Unk now n	0	Unk no wn	GNB	CTX- M	<i>K. pneumo niae; K. pneumo niae ssp</i>	<i>Enterobacteral es; K. pneumoniae group; P. aeruginosa</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>

						<i>pneumoniae</i> ; <i>P. aeruginosa</i>			
NM01 4999 08	Pae diatrics	0	Unk no wn	GNB	CTX-M	<i>K. pneumoniae ssp pneumoniae</i> ; <i>P. aeruginosa</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group; <i>P. aeruginosa</i> ; <i>S. maltophilia</i>	<i>Polymicrobial</i>	<i>Polymicrobial</i>
NM01 4766 07	ICU	0	M	GNB	None	<i>P. aeruginosa</i>	<i>Streptococcus</i> spp.; <i>S. agalactiae</i> (Group B); <i>P. aeruginosa</i> ; <i>S. maltophilia</i>	<i>Monomicrobial</i>	<i>Polymicrobial</i>
OF00 2202 55	Pae diatrics	0	M	GNB	None	<i>K. pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	<i>Monomicrobial</i>	<i>Monomicrobial</i>
NM01 4780 82	ICU	0	F	GNB; GPB	CTX-M	<i>E. faecalis</i> ; <i>K. pneumoniae ssp pneumoniae</i>	<i>E. faecalis</i> ; <i>E. faecium</i> ; <i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>E. coli</i> ; <i>K. pneumoniae</i> group; <i>P. aeruginosa</i> ; <i>S. maltophilia</i>	<i>Polymicrobial</i>	<i>Polymicrobial</i>
NM01 4789 04	Pae diatrics	0	F	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	<i>Monomicrobial</i>	<i>Monomicrobial</i>
NM01 4829 80	ICU	0	F	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	<i>Monomicrobial</i>	<i>Monomicrobial</i>
NM01 4830 61	ICU	0	M	GNB; GPB	mec A/C; NDM	<i>E. faecium</i> ; <i>A. baumannii</i>	<i>E. faecium</i> ; <i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Streptococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex	<i>Polymicrobial</i>	<i>Polymicrobial</i>

NM01 4794 99	Pae diatr ics	0	M	GNB; GPB	NDM	<i>A. baumann ii; E. faecium</i>	<i>E. faecium; Staphylococcu s spp.; A. calcoaceticus- baumannii complex</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NP01 1835 97	Pae diatr ics	0	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo niae</i>	<i>Staphylococcu s spp.; Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4937 03	ICU	0	M	GNB	None	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4942 47	Pae diatr ics	0	M	Yeast	None	<i>C. albicans</i>	<i>C. albicans</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OH00 6919 99	Unk now n	0	F	GNB; GPB	mec A/C	<i>S. paucimo bilis, L. monocyt ogenes</i>	<i>L. monocytogene s; Staphylococcu s spp.; S. epidermidis; C. tropicalis</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4894 57	Pae diatr ics	0	M	GNB	CTX- M; NDM	<i>K. pneumo niae ssp pneumo niae</i>	<i>A. calcoaceticus- baumannii complex; Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4906 08	ICU	0	F	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4916 40	ICU	0	F	GNB; GPB	mec A/C	<i>A. baumann ii; Coagulas e Negative Staphylo coccus</i>	<i>Staphylococcu s spp.; S. epidermidis; A. calcoaceticus- baumannii complex; P. aeruginosa</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 4909 04	Pae diatr ics	0	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo niae; S. marcesc ens</i>	<i>A. calcoaceticus- baumannii complex; Enterobacteral</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>

							es; <i>K. pneumoniae</i> group; <i>S. marcescens</i>		
NM01 4916 25	ICU	0	M	GNB	mec A/C	<i>P. aeruginosa</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01 4914 67	Paediatrics	0	F	GNB	None	<i>S. paucimobilis</i>	None	Monomicrobial	No organism detected
OA02 7035 36	ICU	0	M	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4923 79	ICU	0	M	GPB	None	<i>S. porcinus</i>	<i>E. faecalis</i> ; <i>E. faecium</i> ; <i>Staphylococcus</i> spp.; <i>Streptococcus</i> spp.; <i>S. agalactiae</i> (Group B)	Monomicrobial	Polymicrobial
NM01 4696 24	Paediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Monomicrobial	Polymicrobial
NM01 4697 70	Paediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Monomicrobial	Polymicrobial
OM00 6366 84	Paediatrics	0	M	GNB	CTX-M	<i>K. pneumoniae</i>	<i>Enterobacter</i> spp.; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
NM01 4693 53	Paediatrics	0	F	Yeast	None	<i>C. albicans</i>	<i>C. albicans</i>	Monomicrobial	Monomicrobial
NM01 4700 13	ICU	0	F	GNB	CTX-M	<i>S. marcescens</i> ; <i>P. aeruginosa</i>	<i>E. faecium</i> ; <i>Enterobacter</i> spp.; <i>S. marcescens</i> ; <i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Polymicrobial	Polymicrobial
NM01 4702 77	Paediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Monomicrobial	Polymicrobial
NF01 3095 70	Unknown	0	M	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial

OM00 6372 02	ICU	0	F	GNB	CTX-M; NDM ; OXA-48-like	<i>K. pneumoniae</i> ; <i>E. cloacae</i>	<i>E. faecalis</i> ; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacter</i> es; <i>E. cloacae</i> complex; <i>K. pneumoniae</i> group	Polymicrobial	Polymicrobial
NM01 4692 21	ICU	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4690 95	Pediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NF01 3090 96	ICU	0	F	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4693 55	Pediatrics	0	M	GNB	mecA/c	<i>P. aeruginosa</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Monomicrobial	Polymicrobial
NM01 4693 72	Pediatrics	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
OR00 3762 51	Pediatrics	0	M	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4662 08	Pediatrics	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01 4662 29	ICU	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NM01 4662 30	ICU	0	M	GNB	NDM	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacter</i> es; <i>E. cloacae</i> complex	Monomicrobial	Polymicrobial
NM01 4661 74	ICU	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial

NM01 4662 31	Pae diatr ics	0	M	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4672 88	Pae diatr ics	0	M	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 5149 76	ICU	0	M	GNB	CTX- M	<i>K. ascorbat a; E. cloacae complex</i>	<i>A. calcoaceticus- baumannii complex; Enterobacteral es; E. cloacae complex; E. coli; K. oxytoca; K. pneumoniae group; C. auris</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
NM01 5386 51	Pae diatr ics	0	F	GNB	CTX- M; NDM	<i>P. aerugino sa; S. marcesc ens</i>	<i>Staphylococcu s spp.; Enterobacteral es; S. marcescens; P. aeruginosa; S. maltophilia</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
OA02 6530 15	Unk now n	0	M	GNB	CTX- M	<i>K. pneumo niae</i>	<i>Staphylcoccus spp.; Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OA02 6523 13	Pae diatr ics	0	M	Yeast	None	<i>C. parapsilo sis</i>	<i>C. parapsilosis</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NP01 1647 26	Pae diatr ics	0	M	GNB	None	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4624 42	ICU	0	F	GNB	CTX- M	<i>K. pneumo niae</i>	<i>Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4561 06	ICU	0	M	GNB	mec A/C	<i>P. aerugino sa</i>	<i>Staphylococcu s spp.; S. epidermidis; P. aeruginosa; C. albicans; C. parapsilosis</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>

NM01 4622 26	ICU	0	M	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i> ; <i>S. maltophilia</i>	Monomicrobial	Polymicrobial
ON00 2474 58	Pediatrics	0	F	GPB	mec A/C	<i>E. faecium</i> ; <i>S. haemolyticus</i>	<i>E. faecalis</i> ; <i>E. faecium</i> ; <i>Staphylococcus</i> spp.; <i>S. epidermidis</i>	Monomicrobial	Polymicrobial
NM01 4613 73	Pediatrics	0	F	GNB	CTX-M	<i>S. marcescens</i>	<i>Staphylococcus</i> spp.; <i>Enterobacteriales</i> ; <i>S. marcescens</i>	Monomicrobial	Monomicrobial
NM01 4619 80	ICU	0	M	GNB	None	<i>E. cloacae</i>	<i>E. cloacae</i> complex; <i>Enterobacteriales</i>	Monomicrobial	Monomicrobial
NM01 4594 65	Pediatrics	0	F	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01 4594 62	Pediatrics	0	M	GPB	mec A/C	<i>E. faecium</i>	<i>E. faecium</i> ; <i>Staphylococcus</i> spp.; <i>S. epidermidis</i>	Monomicrobial	Monomicrobial
OM00 6319 77	ICU	0	F	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
NH00 6127 07	Pediatrics	1	M	GPB	None	<i>E. faecalis</i> ; <i>S. aureus</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp., <i>S. aureus</i>	Polymicrobial	Polymicrobial
OV00 1097 12	Pediatrics	1	M	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial
NM01 4036 67	Unknown	1	Unknown	Yeast	None	<i>C. albicans</i>	<i>B. fragilis</i> ; <i>C. albicans</i> ; <i>C. parapsilosis</i>	Monomicrobial	Polymicrobial
NJ00 5453 09	Pediatrics	1	F	GNB; GPB	mec A/C	<i>A. baumannii</i> ; <i>E. faecalis</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Streptococcus</i> spp.; <i>A. calcoaceticus-</i>	Polymicrobial	Polymicrobial

							<i>baumannii</i> complex		
NP01 1730 67	Pae diatr ics	1	F	GNB	CTX- M; OXA- 48- like	<i>A. baumann ii</i>	<i>Enterobacter</i> <i>es</i> ; <i>S. marcescens</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
NM01 4516 12	Othe r ward s	1	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo niae ssp. pneumo niae</i> ; <i>P. aerugino sa</i>	<i>Enterobacter</i> <i>es</i> ; <i>K. pneumoniae</i> group; <i>P. aeruginosa</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
0000 5140 83	Pae diatr ics	1	F	GNB	mec A/C	<i>A. baumann ii</i>	<i>Staphylococcu</i> <i>s spp.</i> ; <i>S. epidermidis</i> ; <i>A. calcoaceticus- baumannii</i> complex	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OH00 6853 48	Unk now n	1	M	GNB; GPB	mec A/C	<i>P. putida</i>	<i>Staphylococcu</i> <i>s spp.</i> ; <i>S. epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4906 12	Pae diatr ics	1	M	GNB; GPB	None	<i>S. enterica</i> <i>ssp</i> <i>enterica</i> ; <i>A. pittii</i> ; <i>S. aureus</i>	<i>Staphylococcu</i> <i>s spp.</i> ; <i>S. aureus</i> ; <i>A. calcoaceticus- baumannii</i> complex; <i>Enterobacter</i> <i>es</i> ; <i>Salmonella</i> <i>spp.</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
OA02 7021 15	Cas ualty	1	M	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii</i> complex	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 7021 16	Cas ualty	1	M	GNB	NDM	<i>A. baumann ii</i>	<i>A. calcoaceticus- baumannii</i> complex	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6704 75	Pae diatr ics	1	M	Yeast	None	<i>C. parapsilo sis</i>	<i>C. parapsilosis</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4593 10	ICU	2	M	GPB	mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcu</i> <i>s spp.</i> ; <i>S. aureus</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>

NM01 4579 35	ICU	2	M	GPB	mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcus</i> <i>spp.</i> ; <i>S.</i> <i>aureus</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6724 68	ICU	2	M	GNB	None	<i>A. baumannii</i>	<i>A. calcoaceticus-</i> <i>baumannii</i> <i>complex</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6805 41	Pae diatr ics	2	F	GNB; GPB	mec A/C	<i>L. mesenteroides</i> ssp <i>dextranum</i> ; <i>C. indologenes</i>	<i>Staphylococcus</i> <i>spp.</i> ; <i>S. epidermidis</i> ; <i>Streptococcus</i> <i>spp.</i>	<i>Polymic</i> <i>robial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4027 12	Pae diatr ics	2	F	GPB	None	<i>E. faecium</i>	<i>E. faecium</i> ; <i>Staphylococcus</i> <i>spp.</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 5016 56	ICU	4	M	GPB	mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcus</i> <i>spp.</i> ; <i>S.</i> <i>aureus</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6910 66	Cas ualty	5	F	GNB	None	<i>Acinetobacter</i> <i>spp.</i>	None	<i>Monomi</i> <i>crobial</i>	No organism detected
OA02 6890 59	Pae diatr ics	5	M	GNB	CTX- M; OXA- 48- like	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4961 71	ICU	5	F	GPB	mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcus</i> <i>spp.</i> ; <i>S.</i> <i>aureus</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6695 54	Pae diatr ics	6	F	GNB; GPB	CTX- M; NDM	<i>E. cloacae</i> complex	<i>E. faecalis</i> ; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
NOM 0018 348	Pae diatr ics	7	M	GPB	mec A/C	<i>E. faecium</i>	<i>E. faecium</i> ; <i>Staphylococcus</i> <i>spp.</i> ; <i>S. epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
OM00 6481 33	Pae diatr ics	7	F	GNB; GPB	mec A/C	<i>P. fluorescens</i> ; CNS	<i>Staphylococcus</i> <i>spp.</i> ; <i>S. epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>

NM01 4494 82	Casualty	10	F	GNB	None	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
NM01 4384 70	Other wards	14	M	GNB; GPB	CTX-M	<i>E. cloacae</i> complex; <i>E. faecalis</i>	<i>E. faecalis</i> ; <i>E. faecium</i> ; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	Polymicrobial	Polymicrobial
OM00 6403 16	ICU	17	Unknown	GNB	CTX-M; OXA-48-like	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
NM01 4910 38	ICU	17	F	GPB	None	<i>A. viridans</i>	None	Monomicrobial	No organism detected
OA02 6392 37	Other wards	25	F	GNB	CTX-M; NDM	<i>E. coli</i>	<i>Enterobacteriales</i> ; <i>E. coli</i>	Monomicrobial	Monomicrobial
OA02 5767 24	Other wards	25	M	GNB	CTX-M; OXA-48-like	<i>E. aerogenes</i>	<i>Enterobacteriales</i> ; <i>E. cloacae</i> complex; <i>S. marcescens</i>	Monomicrobial	Polymicrobial
OA02 6392 36	Other wards	25	F	GNB	CTX-M; NDM	<i>E. coli</i>	<i>Enterobacteriales</i> ; <i>E. coli</i>	Monomicrobial	Monomicrobial
OA02 6052 89	ICU	26	F	GNB	CTX-M; mec A/C; OXA-48-like	<i>E. cloacae</i> complex; <i>A. lwoffii</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	Polymicrobial	Polymicrobial
OA02 6477 20	ICU	26	M	GNB; GPB	None	<i>E. faecalis</i> ; <i>A. baumannii</i>	<i>E. faecalis</i> ; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>K. aerogenes</i>	Polymicrobial	Polymicrobial
NE00 5918 86	Other wards	26	F	GNB	CTX-M	<i>K. pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
OA02 6477 22	ICU	26	M	GNB; GPB	None	<i>A. baumannii</i> complex;	<i>A. calcoaceticus-baumannii</i> complex;	Polymicrobial	Polymicrobial

						<i>E. aerogenes</i>	<i>Enterobacter</i> es; <i>K. aerogenes</i>		
NM01 4893 11	ICU	26	M	GNB	CTX-M; OXA-48-like	<i>K. pneumoniae</i> ; <i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Polymicrobial	Monomicrobial
OC00 5765 57	Other wards	26	F	GNB	CTX-M; NDM; OXA-48-like	<i>K. pneumoniae</i> ssp <i>pneumoniae</i> ; <i>K. oxytoca</i> ; <i>E. coli</i>	<i>Enterobacter</i> es; <i>E. coli</i> ; <i>K. pneumoniae</i> group	Polymicrobial	Polymicrobial
OA02 6904 12	Casualty	26	M	GNB; GPB	CTX-M; OXA-48-like	<i>K. pneumoniae</i> ; CNS	<i>Staphylococcus</i> spp.; <i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
OA02 6904 10	Casualty	26	M	GNB	CTX-M; OXA-48-like	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Staphylococcus</i> spp.; <i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
OA02 6238 94	ICU	27	M	GNB; GPB	mec A/C	<i>A. baumannii</i> ; <i>S. epidermidis</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
OA02 6238 93	ICU	27	M	GNB; GPB	CTX-M; mec A/C	<i>K. pneumoniae</i> ssp <i>pneumoniae</i> ; <i>S. epidermidis</i>	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Enterobacter</i> es; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial
OA02 6724 95	ICU	27	F	GNB	None	<i>E. coli</i>	<i>Enterobacter</i> es; <i>E. coli</i>	Monomicrobial	Monomicrobial
NM01 4964 87	Casualty	29	F	GNB	None	<i>E. coli</i>	<i>Enterobacter</i> es; <i>E. coli</i>	Monomicrobial	Monomicrobial
OO00 5130 83	Other	30	M	GPB	None	<i>S. aureus</i>	<i>Staphylococcus</i> spp.; <i>S. aureus</i>	Monomicrobial	Monomicrobial

	wards								
OA02 6423 92	ICU	31	F	GNB	CTX-M, NDM, OXA-48-like	<i>K. pneumoniae ssp pneumoniae</i> ; <i>E. cloacae</i> complex	<i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex; <i>K. pneumoniae</i> group; <i>S. maltophilia</i>	Polymicrobial	Polymicrobial
OA02 5817 87	Other wards	31	F	GNB; GPB	mecA/C	<i>P. aeruginosa</i> ; CNS	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>P. aeruginosa</i>	Monomicrobial	Monomicrobial
OA02 6423 95	ICU	31	F	GNB	CTX-M; NDM; OXA-48-like	<i>A. baumannii</i> ; <i>E. cloacae</i> complex	<i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex; <i>K. pneumoniae</i> group; <i>S. maltophilia</i>	Polymicrobial	Polymicrobial
OA02 7032 39	ICU	31	M	GNB	CTX-M; NDM; OXA-48-like	<i>K. pneumoniae ssp pneumoniae</i>	<i>A. calcoaceticus-baumannii</i> complex; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial
OA02 7078 50	Other wards	32	F	GNB	None	<i>A. hydrophila</i>	None	Monomicrobial	No organism detected
OA02 6995 48	ICU	32	M	GNB	CTX-M; OXA-48-like	<i>K. pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
OA02 6716 93	Casualty	32	F	GNB	CTX-M	<i>E. coli</i>	<i>Enterobacteriales</i> ; <i>E. coli</i>	Monomicrobial	Monomicrobial
NP01 1512 69	ICU	33	M	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomicrobial	Monomicrobial

OA02 6808 81	Other wards	33	M	GNB; GPB	None	<i>A. baumannii</i> ; CNS	<i>Staphylococcus</i> spp.; <i>A. calcoaceticus-baumannii</i> complex	Monomicrobial	Monomicrobial
OA02 5856 13	Other wards	34	M	GNB	mec A/C	<i>E. cloacae</i> complex	<i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>B. fragilis</i> ; <i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	Monomicrobial	Polymicrobial
OA02 6618 96	ICU	34	M	GPB	None	<i>S. aureus</i>	<i>Staphylococcus</i> spp.; <i>S. aureus</i>	Monomicrobial	Monomicrobial
NM01 4435 10	Unknown	35	F	GNB; GPB	CTX-M; mec A/C	<i>E. coli</i> ; <i>E. faecalis</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>S. epidermidis</i> ; <i>Enterobacteriales</i> ; <i>E. coli</i> ; <i>P. aeruginosa</i>	Polymicrobial	Polymicrobial
NM01 4117 88	Casualty	35	F	GNB	CTX-M	<i>E. coli</i>	<i>Enterobacteriales</i> ; <i>E. coli</i>	Monomicrobial	Monomicrobial
OA02 6469 43	Other wards	35	F	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomicrobial	Monomicrobial
OA02 6639 59	Unknown	35	F	GNB	CTX-M; OXA-48-like	<i>K. pneumoniae</i>	<i>B. fragilis</i> ; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial
OA02 6097 89	ICU	36	M	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
NP01 1398 45	Unknown	36	M	GNB	CTX-M	<i>K. pneumoniae</i> ssp. <i>pneumoniae</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Monomicrobial
NM01 4885 05	Casualty	36	M	GNB; GPB	None	<i>S. enterica</i> ssp <i>enterica</i> ; <i>Streptococcus</i>	<i>Streptococcus</i> spp.; <i>S. pneumoniae</i> ; <i>Enterobacteriales</i> ; <i>Salmonella</i> spp.	Polymicrobial	Polymicrobial

						<i>pneumoniae</i>			
NM01 4950 77	ICU	36	F	GNB	None	<i>Acinetobacter</i> spp.	None	Monomicrobial	No organism detected
NM01 4452 88	Unknown	37	F	GPB	mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcus</i> spp.; <i>S. aureus</i>	Monomicrobial	Monomicrobial
OI008 2577 9	Other wards	37	M	GNB	None	<i>E. cloacae</i> complex	<i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	Monomicrobial	Monomicrobial
OA02 6626 98	ICU	37	M	GNB	None	<i>E. cloacae</i>	<i>Enterobacteriales</i> ; <i>E. cloacae</i> complex	Monomicrobial	Monomicrobial
OA02 6591 50	Other wards	37	F	GNB; GPB	CTX-M; OXA-48-like	<i>K. pneumoniae</i> ; <i>E. faecalis</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i>	Polymicrobial	Polymicrobial
OA02 6385 49	Other wards	38	F	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i> ; <i>S. marcescens</i>	<i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group; <i>S. marcescens</i>	Polymicrobial	Polymicrobial
OA02 6574 28	Casualty	38	M	GNB; GPB	mec A/C	<i>S. aureus</i> ; <i>E. coli</i>	<i>E. faecalis</i> ; <i>Staphylococcus</i> spp.; <i>S. aureus</i> ; <i>S. epidermidis</i> ; <i>Streptococcus</i> spp.; <i>Enterobacteriales</i> ; <i>E. coli</i>	Polymicrobial	Polymicrobial
OA02 6392 56	ICU	38	M	GPB	mec A/C	<i>S. aureus</i>	<i>Staphylococcus</i> spp.; <i>S. aureus</i> ; <i>S. epidermidis</i>	Monomicrobial	Monomicrobial
OA02 6719 72	Unknown	38	F	GNB	CTX-M	<i>K. pneumoniae</i> ssp <i>pneumoniae</i>	<i>Streptococcus</i> spp.; <i>Enterobacteriales</i> ; <i>K. pneumoniae</i> group	Monomicrobial	Polymicrobial

OA02 6566 82	Other wards	39	F	GNB	CTX- M	<i>E. coli</i>	<i>Enterobacter</i> <i>es; E. coli</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4901 31	ICU	39	F	GNB	None	<i>A. baumann</i> <i>ii</i>	<i>A. calcoaceticus-</i> <i>baumannii</i> <i>complex</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 7024 60	Other wards	39	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo</i> <i>niae ssp</i> <i>pneumo</i> <i>niae</i>	<i>Enterobacter</i> <i>es; K. pneumoniae</i> <i>group</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
0000 5130 43	Cas ualty	39	M	GPB	None	<i>S. aureus</i>	<i>Staphylococcu</i> <i>s spp.; S. aureus</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OM00 6169 96	ICU	40	M	GNB	CTX- M; NDM ; OXA- 48- like	<i>K. pneumo</i> <i>niae ssp</i> <i>pneumo</i> <i>niae; K. pneumo</i> <i>niae ssp</i> <i>pneumo</i> <i>niae</i>	<i>E. faecalis; A. calcoaceticus-</i> <i>baumannii</i> <i>complex;</i> <i>Enterobacter</i> <i>es; E. cloacae</i> <i>complex; K pneumoniae</i> <i>group</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
NM01 4994 12	Other wards	41	M	GNB	CTX- M; OXA- 48- like	<i>M. morgani</i> <i>ssp</i> <i>morgani;</i> <i>K. pneumo</i> <i>niae ssp</i> <i>pneumo</i> <i>niae</i>	<i>A. calcoaceticus-</i> <i>baumannii</i> <i>complex;</i> <i>Enterobacter</i> <i>es; K. pneumoniae</i> <i>group; Proteus</i> <i>spp.; P. aeruginosa</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
OA02 6674 57	Other wards	41	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo</i> <i>niae</i>	<i>K. pneumoniae</i> <i>group;</i> <i>Enterobacter</i> <i>es</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6674 56	Other wards	41	F	GNB	CTX- M; OXA- 48- like	<i>K. pneumo</i> <i>niae ssp</i> <i>pneumo</i> <i>niae</i>	<i>Enterobacter</i> <i>es; K. pneumoniae</i> <i>group</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NM01 4923 46	Cas ualty	45	M	GNB	None	<i>Salmonel</i> <i>la group</i>	<i>Enterobacter</i> <i>es; Salmonella</i> <i>spp.</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6296 78	Other	46	F	GNB; GPB	mec A/C	<i>P. mirabilis;</i> <i>CNS</i>	<i>Staphylococcu</i> <i>s spp.; S. epidermidis;</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>

	ward s						<i>Enterobacter</i> <i>es; Proteus</i> <i>spp.</i>		
NM01 4945 81	ICU	46	M	GPB	mec A/C	<i>K.</i> <i>kristinae</i>	<i>Staphylococcu</i> <i>s spp.; S.</i> <i>epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
NM01 4915 39	ICU	46	M	GPB	mec A/C; mec A/C and MREJ (MRS A)	<i>S. aureus</i>	<i>Staphylococcu</i> <i>s spp.; S.</i> <i>aureus; S.</i> <i>epidermidis;</i> <i>Streptococcus</i> <i>spp.</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
NP01 1820 10	Othe r ward s	49	F	GPB	mec A/C	<i>S. aureus</i>	<i>Staphylococcu</i> <i>s spp.; S.</i> <i>aureus; S.</i> <i>epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6438 43	Othe r ward s	52	M	GNB	CTX- M	<i>K.</i> <i>pneumo</i> <i>niae ssp.</i> <i>pneumo</i> <i>niae; P.</i> <i>mirabilis</i>	<i>Enterobacter</i> <i>es; K.</i> <i>pneumoniae</i> <i>group; Proteus</i> <i>spp.</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
OA02 6438 42	Othe r ward s	52	M	GNB	CTX- M	<i>K.</i> <i>pneumo</i> <i>niae ssp.</i> <i>pneumo</i> <i>niae; P.</i> <i>mirabilis</i>	<i>Enterobacter</i> <i>es; K.</i> <i>pneumoniae</i> <i>group; Proteus</i> <i>spp.</i>	<i>Polymic</i> <i>robial</i>	<i>Polymicrobia</i> <i>l</i>
OA02 6548 83	Cas ualty	52	M	GNB	None	<i>H.</i> <i>influenza</i> <i>e</i>	<i>H. influenzae</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NP01 1629 45	ICU	53	M	GNB	None	<i>E.</i> <i>cloacae</i>	<i>Enterobacter</i> <i>es; E. cloacae</i> <i>complex</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OA02 6058 30	Othe r ward s	53	F	GNB	None	<i>E. coli</i>	<i>Enterobacter</i> <i>es; E. coli</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
NP01 1629 46	ICU	53	M	GNB	CTX- M	<i>E.</i> <i>cloacae</i> <i>complex</i>	<i>Enterobacter</i> <i>es; E. cloacae</i> <i>complex</i>	<i>Monomi</i> <i>crobial</i>	<i>Monomicrobi</i> <i>al</i>
OF00 2178 78	Othe r ward s	53	F	GPB	mec A/C	<i>E.</i> <i>faecium</i>	<i>E. faecium;</i> <i>Staphylococcu</i> <i>s spp.; S.</i> <i>epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>
OF00 2178 79	Othe r ward s	53	F	GPB	mec A/C	<i>E.</i> <i>faecium</i>	<i>E. faecium;</i> <i>Staphylococcu</i> <i>s spp.; S.</i> <i>epidermidis</i>	<i>Monomi</i> <i>crobial</i>	<i>Polymicrobia</i> <i>l</i>

NM01 4458 16	Cas ualty	55	F	GNB	None	<i>K. pneumo niae ssp. pneumo niae</i>	<i>Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4645 12	Othe r ward s	55	M	GPB	None	<i>E. faecium</i>	<i>E. faecium</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OA02 7330 92	Othe r ward s	55	M	GNB	None	<i>A. hydrophil a/puncta ta (caviae)</i>	None	<i>Monomi crobial</i>	<i>No organism detected</i>
OI008 3660 0	Othe r ward s	56	M	GNB	CTX- M	<i>E. coli</i>	<i>Enterobacteral es; E. coli</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OI008 3660 1	Othe r ward s	56	M	GNB	CTX- M	<i>E. coli</i>	<i>Enterobacteral es; E. coli</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OA02 7611 77	ICU	56	F	GNB; GPB	None	<i>P. aerugino sa; E. faecalis</i>	<i>E. faecalis; Staphylococcu s spp.; P. aeruginosa</i>	<i>Polymic robial</i>	<i>Polymicrobia l</i>
OM00 6451 97	Othe r ward s	57	F	GNB; GPB	None	<i>E. coli; CNS</i>	<i>Staphylococcu s spp; Enterobacteral es; E. coli</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
NM01 5386 77	ICU	57	M	GNB	None	<i>K. pneumo niae ssp pneumo niae</i>	<i>Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4613 52	Othe r ward s	60	M	GNB	None	<i>E. cloacae complex</i>	<i>Enterobacteral es; E. cloacae complex</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
NM01 4920 92	Cas ualty	60	M	GNB	None	<i>Salmonel la group</i>	<i>Enterobacteral es; Salmonella spp.</i>	<i>Monomi crobial</i>	<i>Monomicrobi al</i>
OA02 6695 73	Othe r ward s	63	M	GNB	CTX- M; OXA- 48- like	<i>K. pneumo niae ssp pneumo niae</i>	<i>E. faecalis; Enterobacteral es; K. pneumoniae group</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>
OA02 7630 03	Cas ualty	64	F	GPB	None	<i>S. agalactia e</i>	<i>Streptococcus spp.; S. agalactiae (Group B)</i>	<i>Monomi crobial</i>	<i>Polymicrobia l</i>

OA02 6403 82	Cas ualty	65	M	GNB	NDM	<i>E. cloacae</i> complex; <i>A. lwoffii</i>	<i>Enterobacter</i> es; <i>E. cloacae</i> complex	Polymic robial	Monomicrobi al
NM01 4416 49	Cas ualty	66	M	GPB	mec A/C	<i>S. aureus</i>	<i>Staphylococcu</i> s spp.; <i>S. aureus</i> ; <i>S. epidermidis</i>	Monomi crobial	Monomicrobi al
OA02 6882 16	Cas ualty	66	M	GNB	NDM	<i>A. baumann</i> <i>ii</i>	<i>A. calcoaceticus</i> - <i>baumannii</i> complex	Monomi crobial	Polymicrobia l
OA02 6910 83	Othe r ward s	66	M	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomi crobial	Monomicrobi al
OA02 6855 04	Cas ualty	66	Unk no wn	GNB	CTX- M	<i>E. coli</i> ; <i>K. pneumo</i> <i>niae ssp pneumo</i> <i>nie</i>	<i>Enterobacter</i> es; <i>E. coli</i> ; <i>K. pneumoniae</i> group	Polymic robial	Polymicrobia l
OA02 6618 12	Othe r ward s	67	M	GNB	CTX- M	<i>E. cloacae</i> complex	<i>Enterobacter</i> es; <i>E. cloacae</i> complex; <i>K. aerogenes</i>	Monomi crobial	Polymicrobia l
NK00 2839 37	Othe r ward s	69	M	GNB	CTX- M	<i>K. pneumo</i> <i>niae ssp pneumo</i> <i>niae</i>	<i>Enterobacter</i> es; <i>E. cloacae</i> complex; <i>K. pneumoniae</i> group	Monomi crobial	Polymicrobia l
OA02 6652 72	Othe r ward s	69	F	GNB	CTX- M	<i>E. coli</i>	<i>Staphylococcu</i> s spp.; <i>S. epidermidis</i> ; <i>Enterobacter</i> es; <i>E. coli</i>	Monomi crobial	Polymicrobia l
OV00 1078 29	Othe r ward s	70	F	GNB	CTX- M	<i>E. coli</i>	<i>Enterobacter</i> es; <i>E. coli</i>	Monomi crobial	Monomicrobi al
NK00 2861 64	Othe r ward s	73	M	GNB; GPB	mec A/C; VIM	<i>P. aeuginos</i> <i>a</i> ; CNS	<i>Staphylococcu</i> s spp.; <i>S. epidermidis</i> ; <i>P. aeruginosa</i>	Monomi crobial	Monomicrobi al
OE00 2655 41	Cas ualty	73	F	GNB; GPB	mec A/C	<i>S. aureus</i> ; <i>P. mirabilis</i>	<i>E. faecalis</i> ; <i>Staphylococcu</i> s spp.; <i>S. epidermidis</i> ; <i>S. lugdunensis</i> ; <i>S. aureus</i> ; <i>B. fragilis</i> ; <i>Enterobacter</i>	Polymic robial	Polymicrobia l

							<i>es; Proteus spp.; P. aeuginosa</i>		
NK00 2918 62	Other wards	74	M	GNB	None	<i>K. oxytoca</i>	<i>Enterobacteriales; K. oxytoca</i>	Monomicrobial	Monomicrobial
OA02 6909 50	Casualty	74	F	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomicrobial	Monomicrobial
NK00 2900 12	Casualty	83	F	GNB	None	<i>E. cloacae</i>	<i>Enterobacteriales; E. cloacae complex</i>	Monomicrobial	Monomicrobial
OA02 6045 34	Unknown	83	M	GNB	None	<i>E. coli</i>	<i>Enterobacteriales; E. coli</i>	Monomicrobial	Monomicrobial
NB00 8182 89	Other wards	83	M	GPB	mec A/C	<i>S. aureus; Enterococcus faecalis</i>	<i>E. faecalis; Staphylococcus spp.; S. aureus; S. epidermidis</i>	Polymicrobial	Polymicrobial
NM01 4704 06	Other wards	90	F	GNB	mec A/C	<i>P. mirabilis</i>	<i>Staphylococcus spp.; S. epidermidis; Enterobacteriales; Proteus spp.</i>	Monomicrobial	Polymicrobial
OA02 6249 20	Other wards	97	F	GNB	None	<i>E. coli</i>	<i>Enterobacteriales; E. coli</i>	Monomicrobial	Monomicrobial
NM01 4227 81	Paediatrics	Unknown	M	GNB	CTX-M; NDM	<i>K. pneumoniae ssp pneumoniae</i>	<i>Enterobacteriales; K. pneumoniae group</i>	Monomicrobial	Monomicrobial
NM01 4126 58	Unknown	Unknown	Unknown	GNB	None	<i>C. sakazakii</i> group	None	Monomicrobial	No organism detected
NM01 4968 27	Unknown	Unknown	Unknown	GNB; GPB	CTX-M	<i>K. pneumoniae spp. Pneumoniae; E. faecalis</i>	<i>E. faecalis; E. faecium; Enterobacteriales; K. pneumoniae group</i>	Polymicrobial	Polymicrobial
OM00 6400 41	Paediatrics	Unknown	F	GPB	None	<i>E. faecalis</i>	<i>E. faecalis</i>	Monomicrobial	Monomicrobial

GNB=Gram-negative bacteria; GPB= Gram-positive bacteria

APPENDIX C2: VITEK2, METAGENOMICS, AND BCID2 RESULTS

Episode number	VITEK2 ORGANISMS	METAGENOMICS ORGANISMS	BCID2 ORGANISMS
OM00645197	E. coli	E. coli; K.quasipnuemoniae; S.hominis;	Staphylococcus spp; Enterobacterales; E. coli
NM01489457	K. pneumoniae ssp pneumoniae	K.pnemoniae; A.baumannii; A. junii; S.koreensis; E.faecalis	A. calcoaceticus-baumannii complex; Enterobacterales; K. pneumoniae group
NM01478082	E. faecalis; K. pneumoniae ssp pneumoniae	E.faecalis; K.pneumoniae; E.coli; E.lactis; K.quasipneumoniae; S.maltophilia	E. faecalis; E. faecium; Staphylococcus spp.; A. calcoaceticus-baumannii complex; Enterobacterales; E. coli; K. pneumoniae group; P. aeruginosa; S. maltophilia
OC00576557	K. pneumoniae ssp pneumoniae; K. oxytoca; E. coli	K.quasipneumoniae; E.coli;	Enterobacterales; E. coli; K. pneumoniae group
NM01476607	P. aeruginosa	P.aeruginosa; S.maltophilia; S.agalactiae	Streptococcus spp.; S. agalactiae (Group B); P aeruginosa; S. maltophilia
NM01491539	S. aureus	Streptococcus spp.; S.aureus; S.haemolyticus; S.epidermidis; S.hominis; C.striatum	Staphylococcus spp.; S. aureus; S. epidermidis; Streptococcus spp.
NM01538651	P. aeruginosa; S. marcescens	S.marcescens; S.ureilytica; P.aeruginosa; N.succinicivorans; S.maltophilia	Staphylococcus spp.; Enterobacterales; S. marcescens; P. aeruginosa; S. maltophilia
OA02763003	S. agalactiae	S.agalactiae; Streptococcus spp; A.baumanii; S.marcescens; S.aquatis;	Streptococcus spp.; S. agalactiae (Group B)
NM01490612	S. enterica ssp enterica; A. pittii; S. aureus	S.enterica; A.pittii; S.agalactiae; A.baumannii; S.aureus; S.haemolyticus; S.hominis; E.faecalis; Streptococcus spp	Staphylococcus spp.; S. aureus; A. calcoaceticus-baumannii complex; Enterobacterales; Salmonella spp.
NM01492379	S. porcinus	S.agalactiae; E.faecalis; E.faecium; Staphylococcus spp	E. faecalis; E. faecium; Staphylococcus spp.; Streptococcus spp.; S. agalactiae (Group B)
OA02688216	A. baumannii	A.baumannii; T.carlieri; Streptococcus spp; S.agalactiae	A. calcoaceticus-baumannii complex
NM01491640	A. baumannii	A.baumannii; P.aeruginosa; S.epidermidis; S.haemolyticus; Streptococcus spp;	Staphylococcus spp.; S. epidermidis; A. calcoaceticus-baumannii complex; P. aeruginosa
OA02703239	K. pneumoniae ssp pneumoniae	A.onderdonkii; B.virosa; B.paravirosa; K.pneumoniae; S.marcescens; S.enterica; A.baumannii;	A. calcoaceticus-baumannii complex; Enterobacterales; K. pneumoniae group
NM01495077	Acinetobacter spp.	Acinetobacter spp.	None
NM01494581	K. kristinae	C.striatum; S.hominis; S.epidermidis; A.radioresistens; P.nosocomialis; P.fluorescens; P.juntendi	Staphylococcus spp.; S. epidermidis

OH00691999	S. paucimobilis, L. monocytogenes	S.epidermidis; L.monocytogenes ; F.corariae; A.radioresistens; P.nosocomialis; P.fluorescens; P.juntendi; C.indologenes; K.planticola; K.ascorbata; E.kobei; A.hydrophila	L. monocytogenes; S. epidermidis; C. tropicalis
OM00648133	P. fluorescens	S.haemolyticus; P.fluorescens; S.epidermidis	Staphylococcus spp.; S. epidermidis
OH00685348	P. putida	P.juntendi; S.epidermidis	Staphylococcus spp.; S. epidermidis
OA02680541	L. mesenteroides ssp dextranicum; C. indologenes	C.indologenes; W. viridescens; L.lactis; S.mitis; S.haemolyticus; A.radioresistens; P.fluorescens; P. juntendi	Staphylococcus spp.; S. epidermidis; Streptococcus spp.
NM01514976	K. ascorbata; E. cloacae complex	K. planticola; K.michiganensis; E.kobei; E.cloacae; E.roggenkampii; C.freundii; A.baumannii; K.ascorbata; A.radioresistens; S.epidermidis; S.haemolyticus	A. calcoaceticus-baumannii complex; Enterobacterales; E. cloacae complex; E. coli; K. oxytoca; K. pneumoniae group; C. auris
OA02691066	Acinetobacter spp.	A. radioresistens; P.nosocomialis; S.haemolyticus; S.epidermidis	None
OA02733092	A. hydrophila/punctata (caviae)	A. hydrophila	None
OA02707850	A. hydrophila	A. hydrophila	None
NM01491467	S. paucimobilis	C.hominis; F.corariae; L.soli; A.hydrophila; K. planticola; K.ascorbata; E.kobei; C.freundii; A.radioresistens	None
NM01491038	A. viridans	L.hofstadii; F. corariae; C.hominis; A.hydrophila	None