Causes of bloodstream infections in adult intensive care patients at eThekwini KwaZulu-Natal hospitals.

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Preface

This study aimed to identify and analyse the common bloodstream isolates in ICUs of the biggest operating hospitals in the district of eThekwini. This may further help describe the prevalence of commonly encountered pathogenic organisms in ICUs, as well as their antimicrobial profiles, signalling the demand for initiation or improvement of antimicrobial stewardship and infection control measures for the healthcare system in eThekwini. In addition, it would help improve and update hospital-based guidelines to optimize patient care management; providing updated knowledge to intensivists about antimicrobial susceptibility patterns. This would assume greater importance in initiating empiric antimicrobial therapy for bloodstream infections.

Declaration

I, **Ms. Sindiswa C. Selane**, declare that the work described in this thesis has not been submitted to UKZN or other tertiary institutions for purposes of obtaining an academic qualification, whether by myself or any other party.

The contributions of others to this project were as follows:

Dr. Masemola Kgaogelo (Microbiology Pathologist) - Extraction of adult ICU-BSI data and classification of organism profile (e.g., CRE, ESBL, MDR, MRSA, XDR) at Prince Mshiyeni Reginal Hospital.

Dr. Ramjathan Praksha (Microbiology Pathologist) - Extraction of adult ICU-BSI data and classification of organism profile (e.g., CRE, ESBL, MDR, MRSA, XDR) at King Edward VIII Tertiary Hospital.

Dr. Mahabeer Yesholata (Microbiology Pathologist) - Extraction of adult ICU-BSI data and classification of organism profile (e.g., CRE, ESBL, MDR, MRSA, XDR) at Inkosi Albert Luthuli Central Hospital.

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Abbreviations

1GC/2GC - First and second-generation cephalosporins
3GC/4GC – Third and fourth-generation cephalosporins
aac - Aminoglycoside N-acetyltransferase gene
AbaI - Acyl-homoserine-lactone synthase
AbaR - Acyl-homoserine-lactone synthase transcriptional regulator
AcrAB - Acridine resistance protein AB
AcrAB-TolC - Acridine resistance AB – "Tolerance" to colicin protein
Ada2 - Adenosine aminohydrolase 2
AIM - Adelaide Imipenemase
AMEs - Aminoglycoside-modifying enzymes
AmpC - ampicillinase C (the gene are italicized ^{bla}*AmpC*)
amp - *N*-acetyl-anhydromuramyl-L-alanine amidase (e.g., ampD and ampE – the genes are italicized e.g., *ampD, ampE*)
AMR - antimicrobial resistance
ant - Aminoglycoside adenylyltransferase gene (or aminoglycoside O-nucleotidyltransferase)
aph - Aminoglycoside phosphotransferase gene (or aminoglycoside O-phosphotransferase)
armA - *Aminoglycoside resistance methylase A*
armZ - Anti-repressor MexZ
AST - Antimicrobial susceptibility test
bap - Biofilm-associated proteins
Bck1 - Bypass of C kinase 1
BES – Brazil extended-spectrum β-lactamase
BICU - Burn Intensive Care Unit
bla - β-lactamase gene
BLICs - β-lactam/β-lactamase inhibitor combinations
BSIs – Bloodstream infections
CA-BSIs - Community-associated bloodstream infections
CAM - Canada MBL
CarO - Carbapenem-associated outer membrane
Cas5 - Caspofungin sensitivity protein 5
CA-UTIs - Catheter-associated urinary tract infections
CCT - Chaperonin complex TCP
CDC - Centers for Disease Control
Cdr- *Candida* drug resistance transporter (e.g., cdr1, cdr2 - the genes are italicized e.g., *cdr1*, *cdr2*)
cfr - Chloramphenicol–florfenicol resistance gene
CG258 - Clonal group 258
CICU - Coronary Intensive Care Unit
CLA-BSIs - Central line-associated bloodstream infections
CLSI - Clinical and Laboratory Standards Institute
CK2 - Casein kinase 2
^{bla}*CMY* – Cephamycinases gene
ColRS - Colistin resistance response regulator/sensor kinase protein
CoNS - Coagulase-negative *Staphylococcus*
COVID-19 – Corona Virus Disease of 2019
CP-Kp - Carbapenemase-producing *K. pneumoniae*
CprRS - Cationic peptide resistance regulator/sensor
CPE - Carbapenemase-producing Enterobacterales
CR - Catheter-related
CRE - Carbapenem-resistant Enterobacterales
CRP - C-reactive protein
crpP - Ciprofloxacin **r**esistance protein, plasmid-encoded gene

Crz1 - Calcineurin-responsive zinc finger protein 1
csuE - chaperone-usher pathway
CTX-M – Cefotaximase – Munich (the gene is italicized e.g., ^{bla}*CTX-M*)
CVCs - Central venous catheters
D-Ala-D-Ala - D-alanyl-D-alanine
D-Ala-D-Lac - D-alanine-D-lactate
D-Ala-D-Ser - D-alanine-D-serine
dfr - dihydrofolate reductase
^{bla}*DHA* - Docosahexaenoic acid gene
DIM - Dutch Imipenemase
DNA - Deoxyribonucleic acid
EARSS - European Antimicrobial Resistance Surveillance System
EARS-Net - European Antimicrobial Resistance Surveillance Network
ECDC - European Centre for Disease Prevention and Control
Erg – Ergosterol gene (e.g., *Erg1, Erg2, etc.* – the enzymes/proteins are not italicized e.g., Erg1, Erg2)
ELISA - Enzyme-linked immunosorbent assay
EnvZ–OmpR - Environmental sensor Z - outer membrane protein regulator component systems
erm - Erythromycin ribosomal methylase gene **(**e.g., *ermA and ermB)*
ESBL – Extended spectrum β-lactamase
EU - Europe Union
EUCAST - European Committee on Antimicrobial Susceptibility Testing
EUROBACT-1/2 - Epidemiology & determinants of outcomes of hospital-acquired bloodstream infections in ICU-1/2
FBC - Full blood count
fim **–** fimbriae gene cluster
Fks - 1,3-β-D-glucan synthase component genes (e.g., *Fks1*, *Fks2*, *Fks3*)
Flr1 - Fluconazole resistance 1 gene
Fos - fosfomycin-resistant glutathione transferase gene (the protein is not italicized e.g., Fos)
FosA - fosfomycin resistance protein A
^{bla}*FOX* – Cefoxitin gene
FUR1 - Uracil phosphoribosyltransferase1
fusA1 - Elongation factor gene A1
GC1 and GC2 – Global clone 1/2
GES - Guiana extended spectrum β-lactamase (the gene is italicized e.g., ^{bla}*GES*)
GIM - German Imipenemase
GlpT - Glycerol-3-phosphate transporter
GSTT - Guy's and St. Thomas' NHS Foundation Trust
Gyr – Gyrase genes (e.g., *gyrA* and *gyrB*)
HA - Hospital-associated/hospital-acquired
HAI – Healthcare-associated infection
HCAIs - Healthcare-acquired infections
HCA-BSIs - Healthcare-acquired bloodstream infections
HCU - High Care Unit
HGT - Horizontal gene transfer
HICs - High-income countries
HMB - Hambourg MBL
Hog1 - High-osmolarity glycerol 1
Hsp90 - Heat shock protein 90
h-VISA - hetero-resistant vancomycin-intermediate *S. aureus*
i5 - isochromosome 5
IALCH - Inkosi Albert Luthuli Central Hospital
ICUs - Intensive Care Units
IMP – Imipenemase (the gene is italicized e.g., ^{bla}*IMP*)
IncFII and IncI1 -Incompatibility groups FII and I1
IPC - Infection prevention and control

ISAba1 - Insertion sequence Aba1
KATs - Lysine acetyl-transferases
KDACs - Lysine deacetylases
KdeA - *Klebsiella* drug efflux A
KEH – King Edward (VIII) Tertiary Hospital
^{bla}*KLUC* - Kluyvera cryocrescens β-lactamase gene
KPC - *K. pneumoniae* carbapenemase (the gene is italicized e.g., ^{bla}*KPC*)
KpgABC - K. pneumoniae gene ABC
KpnEF - *K. pneumoniae* efflux
KpnO - *K. pneumoniae* novel outer membrane porin
LamB - lambda
L-Ara4N - 4-amino-4-deoxy-L-arabinose
LMICs - Low-income and middle-income counties
LOH - Loss of heterozygosity
LPSs - lipopolysaccharides
lsa - Lincosamide and streptogramin A gene
MLSB - macrolide lincosamide-streptogramin B
MAPK - Mitogen-activated protein kinase
MATE - multi-antimicrobial and toxic compound extrusion
MBL - Metallo-β-lactamases
mcr - *Mobilized* colistin resistance gene (e.g., *mcr-1*)
MDR - Multidrug-resistant
Mdr - Multidrug resistance protein (e.g., Mdr1 - the gene is italicized e.g., *Mdr1*)
MDROs - Multidrug-resistant organisms
MexAB-OprM - Multidrug export AB and Outer protorein M
MF - Major facilitator
MGEs - mobile genetic elements
mgrB - Magnesium-responsive B gene
MICU - Medical Intensive Care Unit
MIC - Middle-income countries
MICs - Minimum-inhibitory concentrations
Mkc1 - Map kinase from *C. albicans 1*
Mkk1/2 - Mitogen-activated protein kinase 1/2
MLSB - macrolides, lincosamides, and streptogramin B
^{bla}*MOX* – Moxalactam gene
MR-CoNS - Methicillin-resistant coagulase-negative staphylococci
mrk - Mannose-resistant *Klebsiella*-like gene cluster
Mrr1 - Multidrug resistance regulator 1 gene (the enzyme/protein is not italicized e.g., Mrr1)
MRSA - Methicillin-resistant *Staphylococcus aureus*
MS - Microsoft
MurA - UDP-N-acetylglucosamine enolpyruvyl transferase A (the gene is italicized e.g., *MurA*)
NDM-1 - New Delhi Metallo-β-lactamase 1 (the gene is italicized e.g., *NDM-1*)
NHLS - National Health Laboratory Services
NICD - National Institute for Communicable Diseases
NICU - Neonatal ICU
nlpD - New lipoprotein D gene
npmA - novel plasmid-mediated methyltransferase A
NorA/B/C - Norfloxacin resistance protein A/B/C
Omps - Outer membrane porin/proteins (e.g., OmpA, OmpK35, OprH etc.)
OprD - Outer membrane porin D (the gene is italicized e.g., *OprD*)
OqxAB - Olaquindox and quinolone AB efflux pump
OXA – oxacillinase (the gene is italicized e.g., ^{bla}*OXA*)
PAPI - *P. aeruginosa* pathogenicity islands (e.g., PAPI-1, PAPI-2)
parRS - Partitioning protein RS
Partition C gene – *parC*

Partition E gene - *ParE*
PBP - penicillin-binding protein
PCR - Polymerase chain reaction
PDR - Pandrug-resistant
Pdh1 - Pleiomorphic drug resistance homolog 1
Pdr1 - Pleiotropic drug resistance 1
PER – *Pseudomonas* Extended resistance (the gene is italicized e.g., ^{bla}*PER*)
PEtN - Phosphoethanolamine
pgaB - Poly- β-1,6-N-acetyl-D-glucosamine N-deacetylase
PhoP/PhoQ - Phosphatase-regulated P/Q
PKC - Protein kinase C
Pkc1 - Protein kinase C-like 1
PK/PD - pharmacokinetics/pharmacodynamics
PMMH - Prince Mshiyeni Memorial Regional Hospital
PMQR - Plasmid-mediated quinolone resistance
PmrA/PmrB - Polymyxin modification regulator A/B
PoxB - *Pseudomonas* oxacillinase B
Psr- PBP5 synthesis repressor gene
qnr - quinolone resistance gene (e.g., *qnrA*, *qnrB*, and *qnrS*)
qnrVC1 - Quinolone resistance variant C1
QRDRs - Quinolone resistance-determining regions
RND - Resistance nodulation cell division
rRNA - ribosomal RNA
Rmt - rRNA methyltransferase (e.g., RmtA, RmtB)
SAGA - Spt-Ada-Gcn5-acetyltransferase
SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2
sat - Streptothricin acetyltransferase gene
SCCmec - Staphylococcal cassette chromosome *mec*
SFO – Sulbactam-fixing Oxacillinase (the gene is italicized e.g., ^{bla}*SFO*)
SHV-1 - Sulfhydryl variable lactamase-1 (the gene is italicized e.g., ^{bla}*SHV*, ^{bla}*SHV-2*)
SICSAG - Scottish Intensive Care Society Audit Group
SICU - Surgical Intensive Care Unit
SIM – Seoul imipenemase
Snf2 - Sucrose non-fermenting 2 gene
Snq2 - Sensitivity to 4-Nitroquinoline-N-Oxide 2 gene
SOPs - Standard operating procedures
SPM - Sao Paulo MBL
Spp. - Species
SSIs - Surgical site infections
SSTI - Skin/soft tissue infection
ST1 and ST2 - sequence type 1/2
ST258 - Sequence type
sul – Sulphonamide gene (e.g., *sul1* and *sul2*)
Swi/Snf - Switching defective/sucrose non-fermenting
Tac - Transcription activator gene (e.g., *Tac1*, *Tac1B* - the protein is not italicized e.g., Tac1)
TB – Tuberculosis
TEM - Temoneira (the gene is not italicized e.g., ^{bla}*TEM*, ^{bla}*TEM-3*)
tet - Tetracycline resistance gene (e.g., t*etA*, *tetB* etc. – the proteins are not italicized e.g., tet-A, tet-B)
^{bla}TLA - Tetrahydrolipstatin-like lactamases gene
TolC – Tolerance to colistin
TOR - Target of rapamycin
T1SS-T6SS - Type I-VI secretion systems
UDP-MurNAc - UDP-N-acetylmuramic acid
UhpT - Hexose-6-phosphate transporter
U.K. - United Kingdoms

Upc2A - Uptake control 2 protein A **(**the gene is italicized e.g., *Upc2A***)**
U.S. - United States
USA - United States of America
UTI – Urinary tract infection
Van-type – Vancomycin-type resistance enzyme (e.g., VanA, VanB etc.)
VAP - Ventilator-associated pneumonia
VEB – Vietnam extended-spectrum β-lactamase (the gene is italicized e.g., ^{bla}VEB)
VIM - Verona integron–encoded MBL **(**the gene is italicized e.g., ^{bla}*VIM***)**
VISA - Vancomycin-intermediate *S. aureus*
VRE - Vancomycin-resistant *Enterococcus*
VRSA - Vancomycin-resistant *S. aureus*
WHO - World Health Organization
XDR – Extremely or extensively drug-resistant
Yor1 - Yeast oligomycin resistance protein 1

Abstract

Background: Bloodstream infections (BSIs) are a major cause of morbidity and mortality among critically ill patients, particularly in intensive care units (ICUs). The rise of multidrug-resistant organisms (CRE, ESBL, MRSA, and XDR strains), poses serious treatment challenges, making timely identification and susceptibility profiling essential for effective management.

Aim: This study aimed to describe the distribution of pathogenic organisms and their antimicrobial resistance patterns in adult ICU patients diagnosed with BSIs at three public-sector hospitals in KwaZulu-Natal over a three-year period (2020–2022).

Methods: A retrospective, quantitative, descriptive study was conducted using data from the National Health Laboratory Services (NHLS) TrakCare system. Positive blood cultures from patients aged 18 years and above, admitted to ICUs at regional, tertiary and quaternary hospitals; Prince Mshiyeni Memorial Hospital (PMMH), King Edward VIII Hospital (KEH), and Inkosi Albert Luthuli Central Hospital (IALCH), respectively, were analysed. Organism distribution, antimicrobial susceptibility, and resistance trends were assessed using STATA software.

Results: A total of 739 clinically significant isolates were analyzed. Gram-negative organisms accounted for the majority (65.09%), followed by Gram-positive organisms (25.17%), while fungal isolates, mainly *Candida* spp., comprised 9.47%. *Acinetobacter baumannii* (26.25%), *Klebsiella pneumoniae* (14.34%), and *Pseudomonas aeruginosa* (6.49%) were the common Gram-negative pathogens. Coagulase-negative *Staphylococcus* (8.93%) and *Staphylococcus aureus* (7.16%) were the common Gram-positive isolates. High rates of antimicrobial resistance were observed, with 66.11% of *A. baumannii* isolates classified as possible XDR and 19.09% of *K. pneumoniae* isolates identified as CRE. Multidrug resistant (13.89%) and ESBL-producing organisms (23.68%) were high at PMMH, and surprisingly no MRSA was detected. High CRE rates were noted at KEH and IALCH (24.19% and 20.83%, respectively). Also, XDR (73.12%) was high at KEH, while MRSA detection was high at both facilities (23.08% - IALCH; 21.05% - KEH).

Conclusion: The findings highlight a concerning burden of multidrug-resistant organisms in ICU settings across all levels of care, including regional facilities, which are traditionally assumed to have lower resistance rates. These results necessitate urgent improvements in antimicrobial stewardship programs, infection prevention, microbiological surveillance to contain the spread of resistant pathogens, guidance in effective treatment and control strategies to improve patient outcomes in KwaZulu-Natal ICUs.

Keywords: ICU-acquired BSIs ● Adult patients ● Antimicrobial resistance ● eThekwini, KwaZulu-Natal hospitals.

CHAPTER 1

1. Introduction

1.1. Bloodstream infections

Bloodstream infections (BSIs) occur when organisms are present in the blood. This includes bacteraemia when the organism is bacterial, and fungemia when the organism is fungal (Viscoli, 2016). Since the blood is expected to be sterile (Ochei *et al*., 2000), the presence of any microorganism in the bloodstream is considered abnormal and possibly pathogenic (Doern, 2016). Bacterial invasion of the bloodstream can be a severe complication of infections such as pneumonia or meningitis, due to insertion of catheters and other foreign objects entering the arteries or veins (Sligl *et al*., 2006).

1.1.2. Definitions of bloodstream infections (BSIs)

Primary BSIs are infections that occur in the absence of a known source and in which a pathogen may have been introduced directly into the bloodstream by contaminated instruments (e.g., catheters and, or injections), (Carroll *et al*., 2016). Positive blood cultures confirm these infections, which may be accompanied by an inflammatory response characterized by changes in clinical, laboratory, and haemodynamic parameters (Dellinger *et al*., 2013; Singer *et al*., 2016).

Secondary BSIs occur when there is a suspected source, and the same strain of microorganism is isolated from both cultures. The microorganism may have entered the body through skin cuts, the respiratory tract, gastrointestinal tract, urinary or genital tract (Carroll *et al*., 2016). Infections can spread from the original site to other parts of the body via the blood (haematogenous spread), causing infections such as endocarditis or osteomyelitis (Yang *et al*., 2016). These infections are more common in patients with severe disease (Van Vught *et al*., 2016; Adrie *et al*., 2017), as evidenced by a positive blood culture or clinical signs of sepsis in the presence of infection elsewhere (Levy *et al*., 2003).

1.1.3. Intensive care unit-acquired bloodstream infections (ICU-acquired BSIs)

Bloodstream infections (BSIs) are a major global health problem representing the third-most common infection in approximately 5.00% to 15.00% of all patients within the first month of hospitalization in the intensive care units (ICUs), (Garrouste-Orgeas *et al*., 2006; Vincent *et al*., 2009; Prowle *et al*., 2011). Intensive care unit–acquired bloodstream infection (ICU-acquired BSI) is defined as an infection occurring after 48 hours of ICU admission, with at least one positive blood culture unrelated to an infection incubating at the time of ICU admission (Kallel *et al*., 2020; Tabah *et al*., 2023).

These infections contribute significantly to prolonged hospitalization and cost, especially if they are due to drug resistant organisms (Barnett *et al*., 2013; Puchter *et al*., 2018; Timsit *et al*., 2020). The acquisition of infections remains significantly higher in critical care units as compared to other units, although they accommodate fewer patients (Curcio *et al*., 2011; Bereket *et al*., 2012; Revelas, 2012).

1.1.4. South Africa's healthcare sector divisions

South Africa has five levels of hospitals regulated by the National Health Act, 2003 (Act No.61 of 2003). The Act mandates hospital reclassification to address equity, affordability, efficiency, and effectiveness. Primary healthcare systems such as clinics, community health centers, mobile clinics, satellite clinics, and state-funded or partially and fully subsidized healthcare facilities provide free services to the public (Mayosi *et al*., 2014).

Level 1: District hospitals. Small district hospitals with no fewer than 50 beds and no more than 150 beds; medium size district hospitals with more than 150 beds but no more than 300 beds; and large district hospitals with no fewer than 300 beds but no more than 600 beds fall into this category. Trauma and emergency treatment, in-patient care, outpatient visits, and paediatric and obstetric care are all examples of these healthcare settings. Family physicians, general practitioners, and clinical nurse practitioners provide the services. Only family physicians, paediatricians, obstetrician/gynaecologists, and general surgeons may be employed as specialists (Mayosi *et al*., 2014). (**Table 1**).

Level 2: Regional hospitals. These are also known as referral hospitals that provide general specialty services such as, general surgery, orthopaedics, internal medicine, paediatrics, obstetrics and gynaecology, family medicine, radiology, and anaesthetics, led by experienced specialists to various district hospitals (Mayosi *et al*., 2014). (**Table 1**).

Level 3: Tertiary hospitals. They function at specialist and sub-specialist levels, providing cardiology, cardiothoracic surgery, craniofacial surgery, diagnostic radiology, ear, nose, and throat doctor (ENT), endocrinology, geriatrics, haematology, human genetics, infectious diseases, general surgery, orthopaedics, general medicine, paediatrics, obstetrics, gynaecology, radiology, and anaesthesia services to regional hospitals. They also serve as training institutions and research facilities (Mayosi *et al*., 2014). (**Table 1**).

Level 4: Central hospitals. These hospitals provide a high level of specialized tertiary and quaternary care on a national scale, as well as a platform for health professional training and research. They also serve as highly specialized referral units for other hospitals and offer both high and low volume services. These hospitals use cutting-edge technology and highly skilled personnel (Mayosi *et al*., 2014). (**Table 1**).

Level 5: Specialized hospitals. These are psychiatric hospitals where psychiatrists serve people who are mentally ill/unstable or intellectually challenged. These are also training facilities for medical personnel and research centers. Tuberculosis (TB) hospitals treat patients with acute and complex tuberculosis, including patients with multidrug-resistant (MDR), an extremely drug-resistant (XDR) TB. Finally, Rehabilitation Centers provide specialized rehabilitation services for physically challenged individuals, as well as orthotic and prosthetic services (Mayosi *et al*., 2014). (**Table 1**).

Table 1: Summary of South Africa's healthcare sector divisions (Mayosi *et al*., 2014).

Level and healthcare category/division	Level of professionalism	Services provided
Level 1: District	General practitioners - Family physicians and clinical nurse practitioners.	Surgical interventions under anaesthesia.
Level 2: Regional	General specialists - Anaesthesiologists, general surgeons, internists, obstetricians, gynaecologists, orthopedists, paediatricians, psychiatrist, radiologists, pathologists, and gynaecologists.	Anaesthesiology, general surgery, internal medicine, obstetrics, gynaecology, orthopaedics, paediatrics, psychiatry, radiography diagnostic pathology, as well as allied health services.
Level 3: Tertiary	General specialists and sub-specialists.	Sophisticated treatment and diagnostics of the services offered at regional hospitals. Training and research institutions.
Level 4: Central	Sub-specialists and super-specialists	Sub-specialist and super-specialist services. Training and research institutions.
Level 5: Specialized hospitals	General specialists - psychiatrists	Specialised rehabilitation, orthotic and prosthetic services. Training and research facilities.

1.2. Background

1.2.1. Risk factors of BSIs in the ICUs

Various invasive therapeutic or diagnostic interventions, such as mechanical ventilation, and catheterization (central venous and urinary catheterization), predispose patients to nosocomial infections in ICUs (Akalın, 2001; Çağatay *et al*., 2001; Eggiman *et al*., 2001; Barnett *et al*., 2013; Puchter *et al*., 2018). The risk factors for these infections are immunosuppression, co-morbidities, chronic disease, or illness (Prowle *et al*., 2011), poor infection prevention and control (IPC) practices, and typically, old age (Ramirez-Barba *et al*., 2006). However, regardless of age, critically ill patients often develop healthcare-acquired infections (HCAIs) (Helder *et al*., 2009), which significantly increase the morbidity and mortality rates (Laupland *et al*., 2002; Vincent, 2003; Garrouste-Orgeas *et al*., 2006; Vincent *et al*., 2009).

1.2.2. Sources of BSIs

Device-associated infections such as ventilator-associated pneumonia (VAP), central line-associated bloodstream infections (CLA-BSIs), catheter-associated urinary tract infections (CA-UTIs), and surgical site infections (SSIs) are the main sources of BSIs in ICUs (Rosenthal *et al*., 2006; Durlach *et al*., 2012; Kollef [(a)], 2012; Salgado *et al*., 2017).

Three hundred and ninety-three BSI cases were recorded in a recent investigation of cancer patients in the ICU at Gustave Roussy Hospital in Paris. Catheter-related/central venous catheters (CR-BSIs/CVCs) were responsible for 19.59% (77/393) of all BSIs, primary BSIs accounted for 26.20% (103/393) and 54.19% (213/393) were secondary BSIs (Stoclin *et al*., 2020). Amongst secondary BSIs, the most common was of abdominal origin at 58.68% (125/213), while 15.96% (34/213) were due to VAP, and 10.79% (23/213) were to UTIs (Stoclin *et al*., 2020). The Epidemiology & determinants of outcomes of hospital-acquired bloodstream infections in ICU-1 (EUROBACT-1) international study, reported that most ICU-acquired BSIs resulted from catheter-related infections (21.00%), nosocomial pneumonia (21.00%), and intra-abdominal infections (12.00%) with 24.00% episodes without a definite source (Tabah *et al*., 2012).

According to studies from Iran and the United States (U.S.), the most common types of ICU-acquired infections worldwide are, CA-UTIs estimated at 31.00%, SSIs at 17.00%, intravascular device-associated primary BSIs at 14.00%, and VAP at 13.00% (Shoaei *et al*., 2017; Wenzel, 2007). A Korean study amongst 129 ICU-acquired BSIs identified 87 sources of infection to be from CR-BSIs (38; 29.04%), VAP (27; 20.09%), UTIs (7; 5.04%), wound infection or surgical site related BSI (10; 7.07%), intra-abdominal infection (3; 2.03%), and other infections (2; 1.05%), (Lim *et al*., 2014).

1.2.3. The incidence and prevalence of ICU-acquired BSIs

The incidence of BSIs has escalated substantially over the past two decades, with significant impact on patient outcomes (Vincent *et al*., 2009; Laupland [(a)] *et al*., 2004). A study from the Calgary Health Region ICU in Canada estimated the incidence rate of ICU-acquired BSIs to be between five and nineteen per 1000 patient days (Laupland *et al*., 2002), while studies from the French ICUs have estimated ICU-acquired BSIs to complicate between 1.02% to 6.07% of all ICU admissions, and 4.04% to 6.08% of admissions of longer than 48 hours to 72 hours (Renaud *et al*., 2001; Laupland *et al*., 2002; Garrouste-Orgeas *et al*., 2006).

1.2.4. The morbidity and attributable mortality of BSIs

Despite the emergence of new antimicrobial agents, measures undertaken to contain, manage and treat BSIs, they remain the major cause of high morbidity and mortality rates with adverse outcomes in ICUs (Vallés *et al*., 2003). Approximately 15.00% to 20.00% case fatalities are associated with these infections, which increase to 35.00%, to 50.00% if there is organ failure (Vallés *et al*., 2003; Tabah *et al*., 2012; Timsit *et al*., 2012). European and North American studies reported attributable mortality rates of BSIs ranging from 21.00% to 56.00%, particularly if associated with severe sepsis or septic shock (Renaud *et al*., 2001; Corona *et al*., 2004; Sligl *et al*., 2006). In South Africa, a significantly higher risk of 45.00% deaths occurred in patients who had a BSI compared to 21.00% of those without (Mer, 2005). The most recent study from China highlighted the clinical significance of BSIs to an estimated annual incidence of 204 cases per 100 000 patients, and documented mortality rates from 15.00% to 60.00% (Long *et al*., 2024).

1.2.5. Diagnosis of BSIs

The early warning signs and symptoms of septic shock may vary from patient to patient. The clinical symptoms of sepsis are high fever (> 38°C), rigors, tachycardia (heart rate > 98/min), tachypnea (respiratory rate > 24/min), hypotension (systolic BP < 90mmHg), and hypothermia (< 36 °C), (Hagel *et al*., 2013). (**Table 2**). Fever and typical symptoms and signs associated with bacterial infections observed in younger patients may be absent or may present atypically in older patients (Gbinigie *et al*., 2018).

Blood tests may show increased white blood cell count (Chou *et al*., 2016), increased values (≥ 50 mg/L) of C-reactive protein (CRP), and increased values (≥ 0.2 ng/mL) of procalcitonin (Caterino *et al*., 2004; Dwolatzky *et al*., 2005; Lai *et al*., 2010). (**Table 2**). Molecular tests such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) may aid in making a definitive diagnosis associated with bloodstream bacterial infections (Lee, 2014; Jangale *et al*., 2017). (**Table 2**).

Table 2: Summary of clinical and laboratory features of BSIs.

Clinical features	Laboratory features
• Fever > 38°C • Rigors • Tachycardia (heart rate > 98/min) • Tachypnea (respiratory rate > 24/min) • Hypotension (systolic BP < 90mmHg) • Hypothermia (< 36 °C) In case of progressed infection (sepsis), or another critical bacterial infection. • Sweating, confusion, and extreme pain.	• Full blood count (FBC) → Increased white blood cell count (leukocytosis > 12,000/µL, and neutrophilia) • C-reactive protein (CRP) → ≥ 50 mg/L • Procalcitonin → ≥ 0.2 ng/mL
Molecular tests such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) may be done for the identification of bacteria.	

1.2.6. Blood cultures

Blood cultures are the most definitive laboratory-based test to diagnose BSI (Kenyon *et al*., 2012; Lochan *et al*., 2013). They can detect a wide spectrum of organisms causing BSIs and subsequently susceptibility testing can be performed (Abrahams *et al*., 2015; McKay *et al*., 2015; Lamy *et al*., 2016). Although, contamination of cultures is common, they can still detect pathogens in a significant percentage of patients who are clinically suspected of having sepsis (Shafazand *et al*., 2002).

Coagulase-negative *Staphylococcus* (CoNS), viridans group streptococci, *Bacillus* spp. (excluding *Bacillus anthracis*), *Corynebacterium* spp., *Cutibacterium* spp., and *Micrococcus* spp. represent contamination in a significant proportion of cases (Weinstein, 2003). However, these probable contaminants may be considered significant when detected from two or more blood cultures with the same antimicrobial susceptibility profile, or when the microbiological report supports the clinical context (Tabah *et al*., 2023). Contaminants in blood cultures may lead to inappropriate therapy, resulting in patient complications, longer hospital stays and selection of resistant microorganisms (Siddique *et al*., 2012; Tabah *et al*., 2023).

In 2010, South African clinicians and microbiologists published guidelines aimed at optimizing blood culture yield and further help reduce contamination rates (Ntusi *et al*., 2010). The National Health Laboratory Services (NHLS) has developed a handbook detailing standard operating procedures (SOPs) for blood culture sample collection that would help minimize the risk of contamination (National Health Laboratory Services (NHLS) handbook technical working group, 2015). However, available data indicates that blood cultures are not being employed to their full potential, meaning that their advantages are unlikely to materialize (Grace *et al*., 2001). Additional research involving both adults and children revealed inconsistent blood culture methods and a lack of widespread adoption to the provided standards (Kenyon *et al*., 2012; Lochan *et al*., 2013).

CHAPTER 2

2. Literature review

Bloodstream infections (BSIs) in adult ICUs are a significant cause of morbidity and mortality, primarily caused by a range of Gram-negative and Gram-positive bacteria, as well as fungal pathogens (Wisplinghoff *et al*., 2004; Verway *et al*., 2022). The epidemiology of these infections is dynamic and varies by region, but the most frequently isolated organisms are consistently the multidrug-resistant (MDR) variants of species such as *Acinetobacter* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (including MRSA), coagulase-negative staphylococci (CoNS), and *Candida* spp., (Laupland *et al*., 2002; Garrouste-Orgeas *et al*., 2006; Vincent *et al*., 2009; Prowle *et al*., 2011; Magill *et al*., 2014). The prevalence of these pathogens is often linked to the use of invasive devices and prior antibiotic exposure, which contribute to the selection of resistant strains (Bassetti [(c)] *et al*., 2012**;** Kunac *et al*., 2014**;** Lim *et al*., 2014).

2.1. Common microbes causing BSIs

The most significant ICU-BSI causing microorganisms are Enterobacterales, non-fermenters like *A. baumannii* and *Pseudomonas* spp., *S. aureus*, CoNS, *Enterococcus* spp., streptococci (including group A or group B), anaerobes like *Bacteroides* spp., or fungi such as *Candida* spp., (Siddique *et al*., 2012; Davoudi *et al*., 2014; Bassetti *et al*., 2016). (**Table 3**).

2.1.1. *Escherichia coli*

Escherichia coli ranks first and is the second most common cause of community-associated (CA)-BSIs and HCA-BSIs, respectively. BSIs caused by *E. coli* usually arise as a complication of focal infections of the urinary or gastrointestinal tracts, although they occasionally cause primary bacteraemia without a defined source (Berkley *et al*., 2005; Uslan *et al*., 2007). (**Table 3**). In neonates, it is responsible for invasive infections, including bacteraemic sepsis and meningitis (Diekema *et al*., 2003; May *et al*., 2005; Laupland *et al*., 2007; Skogberg *et al*., 2008). High rates of BSIs have also been reported in the elderly population (De Kraker *et al*., 2013; Abernethy *et al*., 2017).

2.1.2. *Klebsiella pneumoniae*

Klebsiella pneumoniae is the second most common invasive pathogen causing CA-BSIs and HCA-BSIs in studies (Uslan *et al*., 2007; Skogberg *et al*., 2008). BSIs typically develop as a side effect of respiratory, gastrointestinal, or urinary tract infections, rarely without a definable source (Chung *et al*., 2007). In the ICU, it causes ventilator-associated pneumonia, CA-UTIs, SSIs as well as meningitis (Siegel *et al*., 2007; Dorman *et al*., 2017). (**Table 3**).

2.1.3. *Acinetobacter baumannii*

Acinetobacter baumannii is one of the opportunistic pathogens in ICUs accounting for about 2.00% to 10.00% of Gram-negative hospital-acquired infections (HAIs), (Joly-Guillou, 2005; Lynch *et al*., 2017). The organism is commonly isolated from contaminated hospital surfaces, and typically infects organs with high fluid content (the respiratory and urinary tracts, cerebrospinal and peritoneal fluid), and the skin of hospitalised patients. *A. baumannii* is adaptable for its ability to colonize and produce biofilms to survive longer on medical devices and in hospital settings (Howard *et al*., 2012; Jahani-Sherafat *et al*., 2015; Yang [(a)] *et al*., 2019). The risk factors include the use of a CVCs, mechanical ventilation or haemodialysis, ICU admission, and previous antibiotic exposure (Krol *et al*., 2009; Ntusi *et al*., 2012; Saliba *et al*., 2020).

Acinetobacter baumannii is known to cause bacteremia, and other severe infections such as VAP, SSTI/SSIs, CA-UTIs, meningitis, peritonitis, and endocarditis (Doi *et al*., 2015; Nasr, 2020). (**Table 3**). According to Gramatniece *et al*., (2019), 56.20% of bacteraemic patients with multidrug-resistant *A. baumannii* infections (MDR-*A. baumannii*) are at a higher mortality risk than those infected by non-resistant strains. On average, 10.60% patients die due to MDR- *A. baumannii*, and it has previously resulted in global outbreaks amongst critically ill patients (Corbella *et al*., 2000; Fridkin, 2001; Alberti *et al*., 2002; Zhou *et al*., 2019).

2.1.4. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa accounts for approximately 3.00% to 7.00% of all BSIs, and 23.00% to 26.00% of Gram-negative bacteraemias (Kang *et al*., 2003; Gaynes *et al*., 2005). Pneumonia, biliary tract infection, CA-UTI, SSTI and intravascular foci have been identified as potential sources of infection (Wisplinghoff *et al*., 2004; Micek *et al*., 2005). (**Table 3**). ICU studies from the University of Udine in Italy and the University Hospital of Lausanne in Switzerland, discovered that *P. aeruginosa* infections in immunocompromised and critically ill patients were caused by the pervasive nature of the organism to colonize and survive in hospital reservoirs including medical ventilators, oxygen respirators, sinks, taps, toilets, and dialysis machines (Bassetti *et al*., 2018). Despite recent advances in critical-care management, fatality rates from *P. aeruginosa* BSIs remain high, ranging from 27.00% to 48.00% (Wisplinghoff *et al*., 2004; Kang *et al*., 2005).

2.1.5. *Enterobacter* species

Enterobacter species have ranked fifth most common pathogen isolated in ICU, and eighth-most common in non-ICU wards causing bacteraemia (Wisplinghoff *et al*., 2004). These species are known to cause a wide variety of clinical infections in humans including those of the lower respiratory tract, urinary tract, bone, eye, and skin, and are a common cause of bacteraemia among adults and neonates in ICUs (Wisplinghoff *et al*., 2004; Chen *et al*., 2009). (**Table 3**). Previous studies have reported an increasing annual incidence of bacteraemia in some parts of the world caused by *Enterobacter* spp., (Al-Hasan *et al*., 2011).

2.1.6. *Proteus mirabilis*

Proteus mirabilis is one of the most prevalent Gram-negative bacteria found in clinical specimens and can cause a variety of community- or hospital-acquired infections, particularly in complex or CA-UTIs, wound infections, and BSIs (O'Hara *et al*., 2000; Chen [(a)] *et al*., 2012). (**Table 3**).

2.1.7. *Staphylococcus aureus*

Staphylococcus aureus is by far the most clinically relevant Gram-positive bacterium (Feltham, 2008). It has an extensive arsenal of virulence factors, such as adhesion ability, host-cell damaging and immunomodulatory molecules, which differ in their presence or specificity between clones, as seen by the variety of infections it causes (Laabei *et al*., 2014; Boyle-Vavra *et al*., 2015; Thammavongsa *et al*., 2015; Spaan *et al*., 2017). It has the ability to form and survive in biofilms and is often associated with invasive devices (intravascular catheters, endotracheal tubes, and urinary catheters) that are commonly used in ICUs (García-Vázquez *et al*., 2013; Lim *et al*., 2014). (**Table 3**).

CR-BSIs, which are a common source of *S. aureus* infections, are characterized by better clinical outcomes compared with other sources of BSIs and primary bacteraemia (Garrouste-Orgeas *et al*., 2006; Prowle *et al*., 2011; Bassetti [(a)] *et al*., 2012). VAP is also a common source of BSIs. (Kunac *et al*., 2014). Many studies have also reported on the predominance of this organism in wound infections leading to secondary bacteraemia (Blomberg *et al*., 2004; Lilani *et al*., 2005; Fehr *et al*., 2006; De *et al*., 2013).

2.1.8. Coagulase-negative *Staphylococcus* (CoNS)

CoNS is opportunistic in that it can readily adhere and proliferate on the surface of medical devices through a slime layer with a muco-polysaccharide structure (Becker *et al*., 2014; Shrestha *et al*., 2017). It results in bacteraemia when it reaches the bloodstream through the penetration of the skin and the mucous membrane barrier by medical devices (Sabate *et al*., 2017; Rampelotto *et al*., 2018). The increasing use of invasive medical devices gives opportunity to the rising incidence of CoNS-bacteraemia (Spelman *et al*., 2017; Lourtet-Hascoet *et al*., 2018; Zhu *et al*., 2018; Gao *et al*., 2019). Most CoNS-positive blood cultures are false-positives because they are usually due to blood culture contamination rather than true infection (Asaad *et al*., 2016). Previous studies detected false-positive rates of up to 90.09%, if only one single CoNS-positive blood culture was present within 48 hours (Richter *et al*., 2002).

However, CoNS bacteraemia was defined by Tabah *et al*., (2012) as two blood cultures showing the same phenotype on separate occasions within 48-hours or at least one positive blood culture for clinical sepsis, with no other infectious process, and antimicrobial treatment initiated. This pathogen may be significant when isolated from immunocompromised patients and those with indwelling urinary catheters or central intravenous lines (Rubin *et al*., 2002; Tokars, 2004). (**Table 3**).

2.1.9. *Enterococcus* species

Enterococcus species are an important cause of healthcare-associated bacteraemia (Fisher *et al*., 2009). *Enterococcus faecalis* followed by *Enterococcus faecium* are the most prevalent species accounting for more than 90.00% of clinical enterococcal isolates causing bacteraemia (Fisher *et al*., 2009). Intravenous catheters, surgical wounds, as well as urinary tract infections (UTIs) are risk factors for developing bacteraemia from *Enterococcus* spp., (Arias, 2015). (**Table 3**). These species are common nosocomial pathogens in the USA, responsible for three to four cases of BSIs per 10 000-hospital discharges, and the third most common cause of BSIs reported in combined medical-surgical ICUs (Richards *et al*., 2000; Chiang *et al*., 2017; Ministry of Health, Labour, and Welfare, 2020). Resistant *Enterococcus* spp. can cause bacteraemia in patients who have had long hospital stays or frequent antibiotic use in the past (Kasper, 2015).

2.1.10. *Candida* species

Candida species were reported to be the third and fourth most common pathogen isolated from blood cultures in USA, accounting for 8.00% to 10.00% of BSIs, whereas in Europe it is the sixth to tenth most identified pathogen responsible for 2.00% to 3.00% of BSIs in the ICU (Méan *et al*., 2008; Kollef [(b)] *et al*., 2012). Other studies have reported 8.00% to 15.00% of *Candida* spp. BSIs in the ICU (Prowle *et al*., 2011; Tabah *et al*., 2012). *Candida* infections occur five to ten times more often (2.00 to 6.07 in 1000 admitted patients) in ICUs than in medical or surgical wards (Marchetti *et al*., 2004). (**Table 3**).

Table 3: Summary of common ICU-BSI isolates (Tabah *et al*., 2022).

Source of infection	Urinary	Respiratory	Intra-Abdominal	Intra Vascular Catheter	SSIs/Wounds
Pathogens (GNB/ GPC)	• *E. coli* • *K. pneumoniae* • *Acinetobacter* spp. • *P. aeruginosa* • *Enterobacter* spp. • *P. mirabilis* • *S. aureus* • CoNS • *Enterococcus* spp. • *Candida* spp.	• *K. pneumoniae* • *Acinetobacter* spp. • *P. aeruginosa* • *Enterobacter* spp. • *S. aureus*	• *E. coli* • *K. pneumoniae* • *P. aeruginosa* • Methicillin-resistant *Staphylococcus aureus* (MRSA) • *Enterococcus* spp. • *Candida* spp. • Anaerobes Polymicrobial	• *K. pneumoniae* • *Acinetobacter* spp. • *P. aeruginosa* • *S. aureus* • CoNS	• *E. coli* • *K. pneumoniae* • *Acinetobacter* spp. • *P. aeruginosa* • *Enterobacter* spp. • *P. mirabilis* • *S. aureus*/ Methicillin-resistant *S. aureus* (MRSA) • *Enterococcus* spp.

2.2. Bacteriological profile of ICU-BSI causative agents in low-income and middle-income, and high-income countries

High prevalence of HCAIs is found in low-income and middle-income counties (LMICs) rather than in high-income countries (Allegranzi *et al*., 2011; Rosenthal *et al*., 2016). This is due to limited resources, and lack of strategic planning amongst many other reasons to prevent and control healthcare infections (Allegranzi *et al*., 2007; Pittet *et al*., 2008; McFee, 2009). A European study that was conducted from 2011 to 2012, reported high ICU-acquired infection rates in LMICs with an average point prevalence rate of 22.04%. This was somewhat higher than the average point prevalence rate of 19.05% in high-income countries during that period (European Centre for Disease Prevention and Control (ECDC), 2013). Gram-negative bacteria were recorded for more than 50.00% of the total number of ICU-acquired infections in LMICs (Vincent *et al*., 2009; Saharman *et al*., 2021).

2.2.1. Lower-middle income countries (LMIC)

An ICU study conducted in an urban tertiary-care teaching hospital in India reported *P. aeruginosa* and *A. baumannii* (both 18.18%), followed by *K. pneumoniae* (15.15%) and *E. coli* (9.09%) as common isolates (Jangale *et al*., 2017). (**Table 4**). These findings were similar to other Indian studies conducted by Girg *et al*., (2007), and Mehata *et al*., (2007), which reported *Acinetobacter* spp., *K. pneumoniae*, *P. aeruginosa* and *E. coli* as common causes of BSIs in Indian ICUs. (**Table 4**). In another Indian study conducted in four ICUs of Karnataka, *Klebsiella* spp. (30.00%), *E. coli* (18.00%) and *Acinetobacter* spp. (8.00%) were amongst the most frequent Gram-negative isolates (Banerjee *et al*., 2020). (**Table 4**). *Enterococcus* spp. (6.00%) were the major Gram-positive isolates, while *Candida* spp. (*albicans, tropicalis, and parapsilosis*) were identified in 8.00% of the isolates (Banerjee *et al*., 2020). (**Table 4**).

In an Egyptian study, *Enterobacter* spp. (36.08%) were the most common agents in the Surgical ICU (SICU), while *P. aeruginosa* (21.01%), *E. faecalis* (15.08%), *A. baumannii* (10.05%), *K. pneumoniae* (10.05%) and *S. aureus* (5.03%) were common in the Medical/Coronary ICU (MICU/CICU), (Malek *et al*., 2018). (**Table 4**).

2.2.2. Middle-income countries (MIC)

Gram-negative organisms (*A. baumannii*, *P. aeruginosa, and K. pneumoniae*) were isolated in 83.06% of the cases that were included in a study conducted in the ICU of Mohammed V Military Teaching Hospital of Rabat, Morocco, *A. baumannii* being the most frequent isolate (Lachhab *et al*., 2017). A previous study also reported on the prevalence of Gram-negative bacteria among adult patients with hospital-acquired (HA) BSIs from 333 ICUs worldwide (Tabah *et al*., 2012). (**Table 4**).

The findings of the first nosocomial BSIs study conducted in Uganda, reported both Gram-negatives (*K. pneumoniae*, *E. coli*, *Enterobacter* spp., *P. aeruginosa, and Acinetobacter* spp.), and Gram-positives (*S. aureus,* CoNS, and *Enterococcus* spp.) to be the most common bacterial isolates in the ICU (Agaba *et al*., 2017). (**Table 4**). In contrast, a Nigerian study revealed that *S. aureus* was the most common cause of BSIs, accounting for 18.02% cases, followed by CoNS and *K. pneumoniae* (both 13.06%), (Iwuafor *et al*., 2016). (**Table 4**). The study also revealed isolates of *P. mirabilis* (11.01%) and were identified as one of the most frequently isolated pathogens, although its significance in BSI cases is given insufficient recognition (Endimiani *et al*., 2004; Iwuafor *et al*., 2016).

In a Turkish ICU study, the pathogen distribution was *A. baumannii* (20.00%), followed by *Candida* spp., (19.43%), *P. aeruginosa* (14.29%), CoNS (13.71%), *E. coli* (12.57%), *S. aureus* (7.43%), and *Klebsiella* spp., (6.86%), (Dereli *et al*., 2013). (**Table 4**).

An outbreak of *A. baumannii* in the ICU ward in Suva, Fiji was reported during the study period of August to December 2012 (Naidu *et al*., 2014). The common Gram-negative isolates were, *P. aeruginosa*, *K. pneumoniae* (ESBL), while CoNS was the prevalent Gram-positive isolate (Naidu *et al*., 2014). (**Table 4**). A South African study conducted in the ICU of Groote Schuur Hospital (GSH) Tertiary Academic and Teaching Hospital reported the predominance of *A. baumannii* complex (16.00%), *Klebsiella* spp. (15.08%), *S. aureus* (11.06%) and *E. coli* (9.09%) HCA-BSI isolates (McKay *et al*., 2015). (**Table 4**).

A Brazilian University Hospital ICU study, conducted between 2012 and 2014, reported that BSIs amounted to 33.04% amongst other infections (Sabino *et al*., 2020). It was further reported that in 787 blood isolates, the most prevalent BSI agents were CoNS (45.02%), followed by *A. baumannii* (8.07%) and *P. aeruginosa* (7.09%), (Sabino *et al*., 2020). (**Table 4**). A Colombian study amongst ICU patients from 33 Colombian hospitals reported CoNS (39.06%) as the most prevalent bacteria followed by *S. aureus* (12.03%), *K. pneumoniae* (8.02%), *E. coli* (5.07%), *A. baumannii* (4.00%), and *P. aeruginosa* (3.08%), (Cortes *et al*., 2013). (**Table 4**). In opposition, *E. coli* was the common isolate in the adult ICU of the Tertiary-Care Hospital in Yucatán, Mexico (Uc-Cachón *et a*l., 2019). (**Table 4**).

2.2.3. High-income countries (HIC)

A Korean study reported 129 ICU-acquired BSI episodes amongst 1 545 ICU patients. Common isolates were *S. aureus* (27.13%), *Candida* spp. (24.81%), *Enterococcus* spp. (13.95%), and *Acinetobacter* spp. (13.18%). The most common isolates in 38 CR-BSIs were *Candida* spp. (47.37%) and *S. aureus* (31.58%), while in 27 VAP-related BSIs, *Acinetobacter* spp. (44.44%), *S. aureus* (25.93%), and *K. pneumoniae* (11.11%) were commonly isolated (Lim *et al*., 2014). (**Table 4**).

A study in the United Kingdom (U.K.), conducted in a large mixed medical and surgical ICUs at Guy's and St. Thomas' NHS Foundation Trust (GSTT) Hospital, reported *S. aureus* and *Enterococcus* spp. as the common Gram-positive isolates, while *E. coli*, *Klebsiella* and *P. aeruginosa* spp. were the common Gram-negative isolates (Culshaw *et al*., 2014). These findings correspond to other previously published ICU studies (Laupland [(a)] *et al*., 2004; Laupland [(b)] *et al*., 2004; Blot *et al*., 2009; Vincent *et al*., 2009). Approximately, 11.00% of isolates were fungi, most often *Candida* spp., (Culshaw *et al*., 2014). This was similarly reported in a large multicentre study of nosocomial BSIs from the U.S., (Wisplinghoff *et al*., 2004). (**Table 4**).

Findings in 14 ICUs across Canada, amongst critically ill patients with BSIs, revealed that 39.00% were due to Gram-negative bacilli, 47.00% were Gram-positive cocci and 6.00% were yeast. *E. coli* (15.03%), *S. aureus* (12.04%), CoNS (8.03%), *K. pneumoniae* (6.01%) and *S. pneumoniae* (6.00%) were the common isolates (Savage *et al*., 2016). (**Table 4**). In another large Canadian study, *S. aureus* (18.00%), followed by CoNS (11.00%) and *E. faecalis* (8.00%) were the most isolated organisms causing ICU-acquired BSIs.

Approximately 12.00% of these infections were due to antimicrobial-resistant bacteria (Laupland [(a)] *et al.*, 2004). (**Table 4**).

Table 4: An overview of the bacteriological profile of ICU-BSIs.

SES	Country	Author/Ref	Most common isolates
LMIC	India	Jangale *et al.*, 2017.	**GNB**: *P. aeruginosa* (18.18%), *A. baumannii* (18.18%), *K. pneumoniae* (15.15%), and *E. coli* (9.09%).
		Girg *et al.*, 2017.	**GNB**: *Acinetobacter* spp., *K. pneumoniae*, *P. aeruginosa* and *E. coli*.
		Mehata *et al.*, 2017.	**GNB**: *Acinetobacter* spp., *K. pneumoniae*, *P. aeruginosa,* and *E. coli*.
		Banerjee *et al.*, 2020.	**GNB**: *Klebsiella* spp. (30.00%), *E. coli* (18.00%), and *Acinetobacter* spp. (8.00%). **GPC**: *Enterococcus* spp. (6.00%). **Fungi**: *Candida* spp. (*albicans, tropicalis*, and *parapsilosis*) (8.00%).
	Egypt	Malek *et al.*, 2018.	**GNB**: *Enterobacter* spp. (36.08%) in SICU, *P. aeruginosa* (21.01%), *A. baumannii* (10.05%), *K. pneumoniae* (10.05%), in MICU/CICU. **GPC**: *E. faecalis* (15.08%), and *S. aureus* (5.03%) in MICU/CICU.
MIC	Morocco	Lachhab *et al.*, 2017.	**GNB**: *A. baumannii*, *P. aeruginosa,* and *K. pneumoniae* in 18.02% of isolated cases.
	Uganda	Agaba *et al.*, 2017.	**GNB**: *K. pneumoniae*, *E. coli, Enterobacter* spp., *P. aeruginosa,* and *Acinetobacter* spp. **GPC**: *S. aureus,* CoNS, and *Enterococcus* spp.
	Nigeria	Iwuafor *et al.*, 2016.	**GPC**: *S. aureus* (18.02%) and CoNS (13.06%). **GNB**: *K. pneumoniae* (13.06%), and *P. mirabilis* (11.01%).
	Turkey	Dereli *et al.*, 2013.	**GNB**: *A. baumannii* (20.00%), *P. aeruginosa* (14.29%), *E. coli* (12.57%), and *Klebsiella* spp. (6.86%). **GPC**: CoNS (13.71%), and *S. aureus* (7.43%). **Fungi**: *Candida* spp. (19.43%).
	Suva, Fiji	Naidu *et al.*, 2014.	**GNB**: *A. baumannii* outbreak *(*August to December 2012*), P. aeruginosa*, *K. pneumoniae,* and ESBL-producing *Klebsiella.* **GPC**: CoNS.
	South Africa	McKay *et al.*, 2015.	**GNB**: *A. baumannii* complex (16.00%), *Klebsiella* spp. (15.08%), and *E. coli* (9.09%). **GPC**: *S. aureus* (11.06%).
	Brazil	Sabino *et al.*, 2020.	**GPC**: CoNS (45.02%). **GNB**: *A. baumannii* (8.07%), and *P. aeruginosa* (16.09%).
	Colombia	Cortes *et al.*, 2013.	**GPC**: CoNS (39.06%), and *S. aureus* (12.03%). **GNB**: *K. pneumoniae* (8.02%), *E. coli* (5.07%), *A. baumannii* (4.00%), and *P. aeruginosa* (3.08%).

	Mexico	Uc-Cachón *et al*., 2019.	**GNB**: *E. coli*.
HIC	Korea	Lim *et al*., 2014.	**GPC**: *S. aureus* (27.13%), *Enterococcus* spp. (13.95%). **Fungi**: *Candida* spp. (24.81%). **GNB**: *Acinetobacter* spp. (13.18%). **CR-BSIs** *Candida* spp. (47.37%). *S. aureus* (31.58%). **VAP-related BSIs** *Acinetobacter* spp. (44.44%). *S. aureus* (25.93%). *K. pneumoniae* (11.11%).
	United Kingdoms (U.K.)	Culshaw *et al*., 2014.	**GNB**: *E. coli*, *Klebsiella* spp., and *P. aeruginosa* spp. **GPC**: *S. aureus* spp., and *Enterococcus* spp. **Fungi**: Commonly *Candida* spp. in 11.01% of ICU-BSI isolates.
	Canada	Savage *et al*., 2016.	**GNB:** Accounted for 39.00% isolates: *E. coli* (15.03%), *K. pneumoniae* (6.01%). **GPC**: Accounted for 47.00% isolates: *S. aureus* (12.04%), CoNS (8.03%), and *S. pneumoniae* (6.00%).
		Laupland [(a)] *et al*., 2004.	**GPC**: *S. aureus* (18.00%), CoNS (11.00%), and *E. faecalis* (8.00%). Antimicrobial resistant bacteria (12.00%).

Note: SES = Socio-Economic Status; **GNB** = Gram-negative bacilli; **GPC** = Gram-positive cocci; **LMIC** = Low-middle-income countries; **MIC** = Middle-income countries; **HIC** = High-income countries; **MICU/CICU** = Medical/Coronary ICU; **SICU** = Surgical ICU.

2.3. Trends in ICU-acquired BSIs during the Coronavirus disease 2019 (COVID-19) pandemic

The Coronavirus disease 2019 (COVID-19) pandemic exerted a profound influence in ICUs worldwide. Data from the Scottish Intensive Care Society Audit Group (SICSAG) demonstrated that the pandemic was associated with a markedly altered ICU case-mix, alongside significant shortages of trained personnel and critical equipment (Scottish Intensive Care Society Audit Group (SICSAG), 2021). Even prior to the pandemic, healthcare-associated infections (HAIs), including BSIs, constituted a major risk for critically ill patients (Antimicrobial Resistance and Healthcare Associated Infection (ARHAI), 2022). Emerging evidence indicates that the incidence of ICU-acquired BSIs increased during the COVID-19 pandemic (Bonazzetti *et al*., 2021; Zhu [(b)] *et al*., 2022; Lepape *et al*., 2023). Nevertheless, the identification of specific risk factors remains challenging due to the complex epidemiology of HAIs and the presence of numerous confounding variables, including shifts in patient demographics and modifications in clinical management practices (Falconer *et al*., 2025).

The pandemic period was further characterized by inappropriate antimicrobial use, including both excessive and insufficient administration, aimed at preventing complications related to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. Such practices likely contributed to the selection and dissemination of multidrug resistant (MDR) organisms. Particular concern has been raised regarding infections (especially BSIs) caused by Gram-negative pathogens, which have been associated with rising global mortality rates. Over the past two decades, one of the most notable changes in BSI epidemiology has been the increasing antimicrobial resistance among causative organisms, particularly Gram-negative bacteria. BSIs caused by MDR Gram-negative pathogens are associated with poor clinical outcomes and pose substantial therapeutic challenges, especially when appropriate antimicrobial therapy and source control are delayed. These adverse outcomes are driven by the diverse resistance mechanisms employed by these pathogens, which contribute to increased mortality risk, prolonged hospital stays, elevated healthcare costs, and limited treatment options (Viscoli, 2016; Bassetti *et al*., 2020; AbdelHadi *et al*., 2024).

During the COVID-19 pandemic, notable shifts were observed in the etiological agents of sepsis. Pathogens such as carbapenem-resistant Enterobacterales (CRE) and *A. baumannii* were frequently associated with high levels of antimicrobial resistance and increased mortality (Munro *et al*., 2024). Gram-negative organisms play a major role in the development of HAIs, particularly among ICU patients who frequently require invasive procedures. Such interventions have been shown to increase the risk of HAI development by 5 to10-fold (Moolchandani *et al*., 2017). Findings from the Epidemiology and determinants of outcomes of hospital-acquired bloodstream infections in ICU-2 (EUROBACT-2) study, which included 2600 patients from 333 ICUs across five continents between June 2019 and January 2021, revealed that Gram-negative bacteria predominated among hospital-acquired BSIs. The most frequently isolated pathogens were *Klebsiella* spp. (27.09%), *Acinetobacter* spp. (20.03%), *E. coli* (15.08%), and *Pseudomonas* spp. (14.03%), (Tabah *et al*., 2023).

Surveillance data from the European Centre for Disease Prevention and Control (ECDC) indicated that resistance to carbapenems among *K. pneumoniae* isolates increased by more than 30.00% in 2020 and by an additional 20.00% in 2021. Furthermore, the number of reported *Acinetobacter* spp. resistant to multiple antimicrobial classes more than doubled compared with the pre-pandemic period (2018–2019), (European Centre for Disease Prevention and Control (ECDC), 2022). In 2022, the highest antimicrobial resistance (AMR) rates and estimated incidence of BSIs caused by resistant bacteria were reported in southern and eastern European countries (European Centre for Disease Prevention and Control (ECDC), 2023).

From a Romanian study, *Klebsiella* spp. represented the most frequently isolated pathogens from blood cultures, accounting for nearly one-third of all isolates. A significantly higher proportion of *Klebsiella* spp. was observed in the post-COVID-19 period compared with the pandemic period ($p = < 0.05$). High resistance (70.00%) profiling to second-, third- and fourth-generation cephalosporins (2/3/4GC) was noted. High resistance rates were also documented for fluoroquinolones (up to 80.00%) and amoxicillin/clavulanic acid

(approximately 80.00%), as well as for aztreonam (over 70.00%). In the post-COVID-19 period, resistance to colistin (p = < 0.001) and tigecycline (p = 0.005) increased significantly. Although resistance rates to most other antimicrobials declined following the pandemic, a statistically significant reduction was observed only for amoxicillin/clavulanic acid (p = < 0.001). Overall, 45.73% of *Klebsiella* spp. were classified as MDR, PDR (4.72%; 10/212), and CRE (64.15%; 136/212), (Golli *et al*., 2025).

Acinetobacter spp. was the second most frequently isolated pathogen, accounting for nearly one-quarter of all Gram-negative isolates, with approximately 80.00% identified during the post-COVID-19 period. Throughout the study period, *Acinetobacter* isolates demonstrated extremely high resistance (over 95.00%) to third- and fourth-generation cephalosporins (3- and 4GC), with universal resistance to cefotaxime and aztreonam. Widespread resistance to aminoglycosides (90.00%) carbapenems (95.00%), colistin (over 90.00%), fluoroquinolones (97.00%), and piperacillin-tazobactam (97.00%) was documented. While a modest reduction in resistance to most antimicrobials was observed in the post-COVID-19 period, these changes were not statistically significant. In contrast, resistance to colistin increased significantly, rising from 72.22% to 99.21% (p = < 0.001). The majority of isolates identified as carbapenem-resistant (92.31%; 156/169), MDR (88.76%; 150/169), and pandrug resistance (PDR), (5.33%; 9/169), (Golli *et al*., 2025).

Furthermore, approximately two-thirds of *P. aeruginosa* isolates were recovered during the COVID-19 period. Resistance to colistin and tigecycline exceeded 90.00%, while substantial resistance (60.00%) was also noted to third- and fourth-generation cephalosporins (3- and 4GC), fluoroquinolones (60.00% – 66.00%), aminoglycosides (55.00%), and carbapenems (65.00% – 70.00%). The proportions of MDR and PDR strains were identical, each accounting for 26.31% of isolates (Golli *et al*., 2025).

Findings from a Scottish study assessing the impact of the COVID-19 pandemic on ICU-acquired BSIs demonstrated that *S. aureus* was the most frequently isolated organism during the pandemic period (overall 14.5%), followed by *S. epidermidis* (10.10%). In contrast, during the pre-pandemic comparator period, *E. coli* was the predominant pathogen (13.90%), followed by *S. aureus* (11.50%). With the exception of *E. coli*, which showed a significantly lower proportion during the pandemic compared with the comparator period (7.90% vs. 13.90%; p = < 0.001), no major differences in pathogen distribution were observed between these periods (Falconer *et al*., 2025) .

The incidence of ICU-acquired *S. aureus* BSI per 1000 patient-days was significantly higher during the pandemic than in the pre-pandemic period (1.00 vs. 0.46), corresponding to an unadjusted incidence rate ratio (RR 2.19; 95% CI: 1.63–2.95). However, this difference was not significant when comparing non-COVID-19 admissions during the pandemic with the comparator group. COVID-19 admissions exhibited a substantially higher incidence of *S. aureus* BSI than non-COVID-19 admissions (1.98 vs. 0.63 per 1000 patient-days; unadjusted RR 3.14; 95% CI: 2.27–4.35). Similar trends were observed for several other pathogens, including *S. epidermidis*, *K. pneumoniae*, *E. faecalis*, *S. hominis*, and *S. pneumoniae*. Although

the overall *E. coli* incidence did not differ significantly between the pandemic and pre-pandemic periods, COVID-19 admissions demonstrated higher rates compared with non-COVID-19 admissions (0.90 vs. 0.40 per 1000 patient-days; unadjusted RR 2.23; 95% CI: 1.43–3.47). No pathogens showed higher incidence rates in the comparator period than during the pandemic or among non-COVID-19 admissions (Falconer *et al*., 2025).

In another investigation evaluating BSIs in ICUs across four consecutive SARS-CoV-2 pandemic waves in Italy, Gram-positive bacteria predominated, accounting for 74.60% of BSI episodes. A significant decline in BSIs caused by *Enterococcus* spp. was observed from the first wave (57.40%) to the fourth wave (32.70%) (p = 0.004), whereas the proportion of CoNS increased over time (p = 0.003). Among MDR Gram-positive organisms, vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *S. aureus* (MRSA) accounted for 4.90% and 3.10% of episodes, respectively (Pozza *et al*., 2023).

Gram-negative bacteria were responsible for 40.00% of BSI episodes, with Enterobacterales comprising 21.80%. Of these, 4.70% were due to Extended spectrum β-lactamase (ESBL)-producing organisms and 2.90% to carbapenemase-producing Enterobacterales (CPE). A significant reduction in CPE-associated BSIs was noted from the first to the fourth pandemic wave (p = < 0.001). *P. aeruginosa* accounted for 10.40% of episodes, with 2.90% caused by MDR strains, and demonstrated a significant increase in incidence across successive pandemic waves without a corresponding rise in MDR prevalence. Fungal isolates accounted for 2.00% of BSIs, predominantly *C. albicans*, followed by *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* (Pozza *et al*., 2023).

2.4. Antimicrobial resistance in the ICU

Since the introduction of penicillin, many bacteria have developed antimicrobial resistance with transmission to other species (World Health Organization (WHO), 2012). Antimicrobial resistance is a significant concern worldwide because it increases morbidity, mortality, as well as costs related to ICU infections, particularly in the care of critically ill patients (Evans *et al*., 2007; Gales *et al*., 2012). Some of the major resistant nosocomial pathogens of concern are *E. coli*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *Enterobacter* spp., *P. aeruginosa*, and *Enterococcus* spp., (especially *E. faecium*), (the ESKAPE group of pathogens), (Llaca-Díaz *et al*., 2012; Sani *et al*., 2012; Pendleton *et al*., 2013; Oliphant *et al*., 2015).

2.5. Definitions of antimicrobial resistance

Antimicrobial resistance can be microbiological or clinical in pathogenic bacteria. Microbiological resistance is the presence of an acquired or mutated genetical resistance mechanism that classifies a pathogen as resistant or susceptible based on the application of a set cut-off in a phenotypic laboratory test. For example, the treatment of a pathogen with a drug to which it has tested susceptible produces a better outcome than is

attained with a drug to which the pathogen has tested resistant. On the other hand, clinical resistance is correlated with therapeutic failure (MacGowan, 2008).

Increased bacterial resistance rates within healthcare institutions are reported globally (World Health Organization (WHO), 2002). However, the increasing resistance to antimicrobial agents poses a more significant concern in LMICs and is highly prevalent in ICUs (Sabino *et al*., 2020). More adverse clinical outcomes and higher economic strain have been reported for patients with BSIs caused by resistant pathogens. BSIs involving 3GC-resistant Enterobacterales have shown increased mortality risk as compared to BSIs involving susceptible strains (Prowle *et al*., 2011; Stewardson *et al*., 2016). Nosocomial infections are associated with increased incidence of multidrug-resistant organisms (MDROs), such as methicillin-resistant *S. aureus* (MRSA), Extended spectrum β-lactamase (ESBL) - producing Enterobacterales, multidrug-resistant (MDR) *A. baumannii*, and *P. aeruginosa*, which present a much complex approach for appropriate empiric treatment, especially in ICU patients (Leone *et al*., 2003; Zahar *et al*., 2011).

Multidrug resistance (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Horan *et al*., 2008; Magiorakos *et al*., 2012). They may delay the initiation of effective antimicrobial therapy, and in turn become more expensive to treat than susceptible bacterial infections (Ammerlaan *et al*., 2013; Liu *et al*., 2018). Extreme/extensive drug-resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories - bacterial isolates remain susceptible to one or two categories. Pandrug resistance (PDR) is non-susceptibility to all tested or approved antimicrobial agents (Magiorakos *et al*., 2012).

Extended spectrum β-lactamases (ESBLs) are defined as enzymes produced by bacteria that are capable of hydrolyzing penicillins, extended spectrum cephalosporin, and β-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime, and the monobactam aztreonam (oxyimino-monobactam), (Bradford, 2001; Paterson *et al*., 2005). Carbapenem-resistant Enterobacterales (CRE) are defined by their resistance to at least one carbapenem (ertapenem, imipenem, meropenem) or the presence of a carbapenemase (Smith *et al*., 2024).

The ampicillinase C (AmpC) type β-lactamases resistance appeared in numerous Gram-negative species in the past decades (Sanders, 1992). This class C enzyme conferred resistance to oxyimino-cephalosporins and monobactams (Jacoby, 1994; Medeiros, 1997).

Methicillin-resistant *S. aureus* (MRSA) is the resistance of *S. aureus* to β-lactams such as penicillin (methicillin and oxacillin/amoxicillin) including cephalosporin and carbapenems. It is also defined as an oxacillin minimum inhibitory concentration (MIC) of greater than or equal to four micrograms/mL (Kırmusaoğlu *et al*., 2017; Lakhundi *et al*., 2018; Vestergaard *et al*., 2019).

Enterococcus spp. has acquired resistance mechanisms to several antibiotics, including aminoglycosides, β-lactams, tetracyclines, quinolones, and vancomycin (glycopeptide). Vancomycin-resistant *Enterococcus* (VRE) resistance is caused by alterations in the synthesis of the peptidoglycan that makes up the bacterial cell wall (Cetinkaya *et al*., 2000; Wassilew *et al*., 2018; Kreidl *et al*., 2018).

2.5.1. Resistance mechanisms of Gram-negative multidrug-resistant organisms (MDROs)

The epidemiology and determinants of outcomes of hospital-acquired bloodstream infections in ICU (EUROBACT) study discovered that MDR Gram-negative pathogens infect more than half of ICU cases (Tabah *et al*., 2012). A high percentage of MDR, especially carbapenem-resistance, may be due to inappropriate usage of antimicrobials in the wrong dosage, failure of antimicrobial de-escalation, and longer usage of broad-spectrum antimicrobials (Uc-Cachón *et al*., 2019; Banerjee *et al*., 2020).

Enterobacterales producing ESBL represent a great challenge due to their resistance to 3GC (Harris *et al*., 2015). These organisms have been mostly reported in some parts of Europe, Asia, and South America (Bell *et al*., 2002). The effectiveness of antimicrobial regimens including β-lactam/β-lactamase inhibitors has not been fully established in randomized clinical trials in critically ill patients; hence, carbapenems are often used as first choice treatment (Harris *et al*., 2015). As a result, carbapenem-resistance rates in ICU patients with HCA-BSIs in Europe have continuously increased in *Acinetobacter* spp., (69.00%), *Klebsiella* spp., (37.00%), and *Pseudomonas* spp., (5.07%), (Tabah *et al*., 2012). Higher rates were reported in the Southern European ICUs (Dimopoulos *et al*., 2015).

2.5.1.1. Antimicrobial resistance mechanisms in *A. baumannii*

Acinetobacter baumannii has become one of the most multidrug-resistant pathogenic organisms in the ICUs over the past decades (Agarwal *et al*., 2006; Dima *et al*., 2007). This organism has a unique ability to remain in hospital environments for longer periods while acquiring intrinsic resistance to broad-spectrum antimicrobials (Hosein *et al*., 2002; Schreckenberger *et al*., 2007). The discovery of the carbapenem-hydrolysing **Class D** oxacillinase (OXA) enzymes on some of the *A. baumannii* strains has further made infections sustained by this organism difficult to control and treat (Fournier *et al*., 2006; Zander *et al*., 2014).

In previous European studies, nearly 90.00% of *Acinetobacter* isolates from critically ill patients were found to be resistant to aminoglycosides and piperacillin-tazobactam (Falagas *et al*., 2008). Another study from the Burn Intensive Care Unit (BICU) in a university hospital setting in Brazil reported 6.00% to 95.00% of *Acinetobacter* isolates to be resistance to 3GC, while 50.00% demonstrated resistance to ciprofloxacin (Santucci *et al*., 2003). Studies from the U.S. reported the same (Tognim *et al*., 2004). Thorough analysis on the available data on *Acinetobacter* antimicrobial resistance suggested that the proportions of *Acinetobacter*

isolates that were resistant to various antimicrobial agents were higher in the studies originating from Asian and European countries than those from the U.S., (Tognim *et al*., 2004; Falagas *et al*., 2008).

Plasmids in *Acinetobacter* spp. are central to the dissemination of antimicrobial resistance genes and are largely restricted to this genus (not stably maintained in other Gram-negative bacteria, especially Enterobacterales), (Lam *et al*., 2023). Most *A. baumannii* strains harbour at least one resistance plasmid type, which can be classified into R1 (Pfam01446), R3 (Pfam01051), RP (Pfam03090), and rep-less (lacking an identifiable Rep protein) families based on replication genes. While R1-type plasmids are not associated with resistance, R3, RP, and rep-less plasmids play an important role in the spread of carbapenem resistance genes (Lam *et al*., 2024).

A. baumannii Global Clones 1 and 2 (GC1 and GC2) corresponding to sequence types (ST1 and ST2) are the primary lineages driving carbapenem-resistance in *A. baumannii* outbreaks in healthcare settings. Their success is largely attributed to horizontal gene transfer (HGT) mediated by mobile genetic elements (MGEs), which enable rapid acquisition and integration of antimicrobial resistance genes. While early GC1 and GC2 isolates were resistant to older antimicrobials, ongoing evolutionary processes have resulted in resistance to modern agents, including fluoroquinolones, cephalosporins, and carbapenems, leading to significant genetic diversification within these clones (Holt *et al*., 2016; Hamidian [(a)] *et al*., 2019; Hamidian [(b)] *et al*., 2019).

Enzymatic inactivation

Enzymatic inactivation is one of the mechanisms used by *A. baumannii* to confer antimicrobial resistance. **Class A** are the β-lactamases; Temoneira (TEM), sulfhydryl variable lactamase (SHV), Cefotaximase-Munich (CTX-M), Guiana extended-spectrum β-lactamase (GES), *Pseudomonas* extended resistance (PER), *K. pneumoniae* carbapenemase (KPC), Vietnam extended-spectrum β-lactamase (VEB), Brazil extended-spectrum β-lactamase (BES), and Sulbactam-Fixing Oxacillinase (SFO), (Černiauskienė *et al*., 2023). **Class B** consists of Metallo-β-lactamases such as the New Delhi Metallo-β-lactamase (NDM), Verona integrated-encoded Metallo-β-lactamase (VIM), and Imipenemase β-lactamase (IMP), and Seoul imipenemase (SIM), (Chen *et al*., 2017; Ayibieke *et al*., 2018; López *et al*., 2019). **Class C** is ampicillinase C (AmpC), (Liu [(a)] *et al*., 2015). **Class D** is Oxacillinase (OXA), (Donald *et al*., 2000; Lin *et al*., 2014; Huang *et al*., 2015; Liao *et al*., 2015; June *et al*., 2016; Oliveira *et al*., 2019; Zhao *et al*., 2019; Avci *et al*., 2023). (**Table 5**).

The ambler class enzymes (**Class A-D**) are known to hydrolyse penicillins, cephalosporins, and carbapenems, respectively (Ingti *et al*., 2020; Zhu [(a)] *et al*., 2022; Wu *et al*., 2023). The main mechanisms underlying β-lactam antimicrobial resistance in *A. baumannii* are the **Class A** TEM-type ESBLs, while GES ESBLs promote resistance to conventional ESBL inhibitors, such as clavulanate, sulbactam, and tazobactam. Moreover, the GES ESBLs express high potassium clavulanate and sulbactam hydrolysis efficiency (Saral *et al*., 2016).

Class B MBLs are known to hydrolyse most anti-β-lactamase medications, decreasing their bactericidal efficiency. The MBLs are also resistant to conventional enzyme inhibitors (clavulanate, sulbactam, and tazobactam), (López *et al*., 2019). The New Delhi MBL (NDM-1) is highly effective in hydrolysing carbapenems (Ayibieke *et al*., 2018), and is transmissible from one strain to another through plasmids (which play a key role in the pandrug-resistant *A. baumannii* outbreaks), thus bacteria bearing the *NDM -1* gene expresses pandrug resistance (Chen *et al*., 2017). (**Table 5**).

Class C β-lactamases (AmpC enzymes or cephalosporinases), target cephalosporins and are not inhibited by clavulanate. These enzymes promote *A. baumannii* resistance to 3GC, and are expressed when the insertion sequence (ISAba1) is present upstream of their genes (Liu [(a)] *et al*., 2015). On the other hand, **Class D** β-lactamases (oxacillin-OXA) consist of a carboxyl lysine which assists in hydrolysing semisynthetic penicillins (e.g., oxacillin), cephalosporins and carbapenems. The presence of MBLs and OXA enzymes is a main mechanism underlying carbapenem resistance in *A. baumannii* (Lin *et al*., 2014). (**Table 5**).

Target site modification

Aminoglycoside-modifying enzymes (AMEs) such as acetyltransferases, phosphotransferases, and nucleotidyltransferases inactivate aminoglycosides (e.g., amikacin, gentamicin) by acetylation, phosphorylation, or adenylation, preventing them from binding to their bacterial ribosomal target (16S rRNA component of the 30S ribosomal subunit), (Hasani *et al*., 2016). Resistance to fluoroquinolones (e.g., ciprofloxacin), is by mutations in the aminoglycoside transferase (Venkataramana *et al*., 2022). (**Table 5**).

Altered membrane permeability

Modification in the penicillin-binding proteins (target gene PBP-3), permeability defects associated with efflux pump overexpression, reduced expression, and mutation of genes (e.g., *ant(3'')-I, aac(3)-I*) encoding AMEs contribute to resistance and spread by bacterial populations (Nie *et al*., 2014; Lupo *et al*., 2018; Wang *et al*., 2022). Not only do these alterations promote resistance, but they also minimise the binding affinity of β-lactams to their target sites (Hasani *et al*., 2016; Kyriakidis *et al*., 2021). (**Table 5**).

Studies from China and Greece (Palmieri *et al*., 2020; Sun *et al*., 2020), discovered that any alteration on the Lipid A biosynthesis genes (*lpxA*, *lpxC*, and *lpxD*) affects the membrane's permeability, weakens the activity of hydrophilic antimicrobials (i.e., β-lactams, aminoglycosides, and tigecycline) and, also reduces their intracellular concentration. The modifications on the lipopolysaccharides as well as the transposition of insertion sequences (ISAba) contribute to polymyxins (e.g., colistin) resistance (Hamidian *et al*., 2013; Pelletier *et al*., 2013; Novovi´c *et al*., 2023), while mutations in the DNA gyrase subunit (*gyrA* gene) and the topoisomerase IV subunit C (*parC* gene) confers resistance to fluoroquinolone (Roy *et al*., 2021).

Additionally, the downregulation of the outer membrane protein A (OmpA) and carbapenem-associated outer membrane (CarO) porins (33–36 kDa) causes passive diffusion of antimicrobial agents, and is associated with carbapenem resistance (Magnet *et al*., 2001). (**Table 5**).

Efflux pump overexpression

Previously, Magnet *et al*., (2001) reported that the overexpression of the three-resistance nodulation cell division (RND)-family efflux pumps (i.e., AdeABC, AdeFGH, and AdeIJK) as well as the multi-antimicrobial and toxic compound extrusion (MATE)-family in *A. baumannii* are due to amino acid substitutions in their regulatory genes, and their over activity induces resistance to antimicrobials such as aminoglycoside and tigecycline (Jacoby [(a)] *et al*., 2014; Liu *et al*., 2024). These findings were recently supported by Darby *et al*., (2023). (**Table 5**).

Biofilm-associated antimicrobial resistance

The ability of *A. baumannii* to form biofilms for cell adhesion and structural stability giving its strong persistence multidrug resistance character is supported by the biofilm-associated proteins (bap), OmpA, part of the chaperone-usher pathway (csuE), and poly- β-1,6-N-acetyl-D-glucosamine N-deacetylase (pgaB - involved in polysaccharide production), as well as the acyl-homoserine-lactone synthase/transcriptional regulator (AbaI/AbaR) sensing system (for biofilm formation regulation), (Bhargava *et al*., 2010). (**Table 5**).

2.5.1.2 Antimicrobial resistance mechanisms in *K. pneumoniae*

The significant increase of *Klebsiella* spp. has been reported worldwide in ICUs and other high-risk wards following the identification of ESBL (Asensio *et al*., 2000; Babini *et al*., 2000; Rebuck *et al*., 2000; Bradford, 2001); while the efficacy of conventional treatments against *K. pneumoniae* has decreased over the past decades as a result of the pathogen's development of resistance against the main antimicrobial classes including aminoglycosides, carbapenems, cephalosporins, and fosfomycin (Ferreira *et al*., 2019).

Enzymatic antimicrobial inactivation and modification

Drug modification is one of the main antimicrobial resistance mechanisms in *K. pneumoniae*, primarily mediated by the production of β-lactamase enzymes (Santajit *et al*., 2016). These enzymes hydrolyse β-lactams and are categorised into ESBLs, AmpC β-lactamases, and carbapenemases (Yong *et al*., 2009; Liu [(b)] *et al*., 2015; Tooke *et al*., 2020; Theuretzbacher *et al*., 2021). ESBLs, including SHV, TEM, CTX-M, and OXA types, confer resistance to penicillins and cephalosporins. AmpC enzymes, which may be chromosomally or plasmid-mediated, provide resistance to cephalosporins (1-3GC), cephamycins, and β-lactamase inhibitors, with over 40 known genotypes facilitating rapid dissemination via plasmids.

Carbapenemase production further reduces susceptibility to carbapenems, contributing to the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) and complicating treatment (Hall *et al*., 2005). (**Table 6**).

Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated resistance mechanisms encoded within the accessory genome of *K. pneumoniae*. The β-lactamase $^{(bla)}$*SHV* is one of the most common resistance genes naturally found in *K. pneumoniae,* and its presence is directly linked to the organism's natural resistance to ampicillin. The SHV β-lactamase can be transferred between bacteria, accelerating the spread of resistance across different strains and species. Furthermore, due to its adaptability, it contributes to widespread resistance, treatment failures, and hospital outbreaks especially when combined with other resistance mechanisms like TEM or CTX-M β-lactamases (Lee *et al*., 2006; De Jesus *et al*., 2015; Tsang *et al*., 2024).

The first ESBL gene, ^{bla}*SHV-2,* was identified in Germany (Kliebe *et al*., 1985), followed by the detection of ^{bla}*TEM-3* in France (Sirot *et al*., 1987). ESBLs confer resistance to a broad range of β-lactam antimicrobials, although their activity can be inhibited by clavulanic acid (Bush *et al*., 1995). Over time, CTX-M-type replaced TEM and SHV variants as dominant genotypes due to efficient dissemination via plasmids and transposons (Li $^{(a)}$ *et al*., 2018).

Additional ESBL genes, such as ^{bla}*OXA*, ^{bla}*GES*, ^{bla}*SFO*, ^{bla}*VEB*, β-lactamase *Pseudomonas* Extended resistance (^{bla}*PER*), Tetrahydrolipstatin-like lactamases (^{bla}*TLA*), and β-lactamase Kluyvera cryocrescens (^{bla}*KLUC-5*), have also been acquired through horizontal gene transfer (HGT), (Bradford, 2001; Slama *et al*., 2016; Li $^{(b)}$ *et al*., 2018). Globally, the prevalence of ESBL-producing *K. pneumoniae* has increased substantially, reaching endemic rates of up to 50.00% in certain regions (Li $^{(b)}$ *et al*., 2018). (**Table 6**).

Resistance to carbapenems

Carbapenem resistance in *K. pneumoniae* is mainly due to the production of carbapenemases (Van Diun *et al*., 2020). The increasing prevalence of MDR ESBL-producing *K. pneumoniae* has contributed to the emergence of carbapenem resistance due to antimicrobial selective pressure. As a result, the organism has become the most common carbapenem-resistant Enterobacterale (CRE). Plasmid-mediated carbapenemase production is the main mechanism driving this resistance. *K. pneumoniae* carbapenemase (KPC) is the most prevalent and clinically important carbapenemase that is associated with clonal group 258 (CG258) and the globally disseminated sequence type (ST258), (Ørjan *et al*., 2009; Breurec *et al*., 2013). (**Table 6**).

Table 5: An overview of antimicrobial resistance mechanisms in *A. baumannii* (Scoffone *et al.*, 2025).

Resistance mechanism	Target Mechanism	Genes/ Proteins	Target antimicrobial agent and Localization	Author/Ref
Enzymatic inactivation	**Class A** β-lactamases	*^{bla}TEM-92*, *^{bla}SHV*, *^{bla}GES-11*, *^{bla}GES-14*, *^{bla}PER-1/7*, *^{bla}SFO-1*, and *^{bla}VEB-1*	Carbapenems and penicillins – (Chromosomal, plasmid, and mobile genetic elements - MGE)	Wu *et al.*,2023.
	Class B Metallo-β-lactamases	*^{bla}VIM-1*, VIM-1/2/3/4/11, IMP-1/2/4/5/9/10, SIM-1, and NDM-1	Carbapenem, cephalosporins, and penicillins – (Plasmids and integrons)	Zhu [(a)] *et al.*,2022.
	Class C β-lactamases	AmpC	Carbapenems, cephalosporins, and sulbactams – (Chromosomal)	Ingti *et al.*,2020.
	Class D OXA-type (oxacillinase)	*^{bla}OXA-23/24/40/51/58/72/143/235*	Carbapenems – (Chromosomal and plasmid)	Yamano *et al.*,2021; Wang *et al.*,2022.
	Aminoglycoside modifying enzymes (AME)	*aac*, *ant*, *aad*, and *aph* genes	Aminoglycosides- (Chromosomal, integron, transposon, integrative conjugative element, plasmid, and chromosomal genomic island)	Nie *et al.*,2014; Lupo *et al.*,2018; Venkataramana *et al.*,2022.
Target site modification	Penicillin-binding protein (PBP)	ftsI_A515V, and PBP3	β-lactams – (Chromosomal)	Kyriakidis *et al.*,2012.
	16S rRNA of the 30S ribosomal subunit	armA, rmtB, rmtB1, and rmtE	Aminoglycosides – (Chromosomal and plasmid)	Hasani *et al.*,2016.
	Lipid A and Lipopolysaccharides	pmrCAB, *mcr*, *hns-eptA*, *lpxA*, *lpxC*, *and lpxD*	Colistin – (Chromosomal and plasmid)	Palmieri *et al.*,2020; Sun *et al.*,2020; Ilsan *et al.*,2021; Novovi´c *et al.*,2023; Prity *et al.*,2023.

	DNA gyrase and topoisomerase IV	*gyrA* and *parC*	Fluoroquinolones – (Chromosomal)	Roy *et al.*,2021.
Altered membrane permeability	Porins	OmpA and CarO	β -lactams, aminoglycosides, carbapenems, and tigecycline – (Chromosomal)	Magnet *et al.*,2001.
	Lipopolysaccharides	*lpsB*, *lptD*, and *vacJ*	Polymyxins (e.g., colistin) – (Chromosomal)	Pelletier *et al.*,2013.
	Polysaccharide-rich capsule	Capsule biosynthesis and regulatory genes	Aminoglycosides – (Chromosomal)	Akoolo *et al.*,2022.
Active efflux system	RND-family and MATE-family efflux pumps	adeABC, adeRS, adeFGH, adeIJK, abeM, and qepA	Aminoglycoside, carbapenems, cephalosporins, chloramphenicol, erythromycin, fluoroquinolones, tetracycline, and tigecycline – (Chromosomal and plasmid)	Magnet *et al.*,2001; Jacoby [(a)] *et al.*,2014; Darby *et al.*,2023; Liu *et al.*,2024.
Other	Biofilm	bap, ompA, csuE, pgaB, and AbaI/AbaR quorum sensing genes	Multidrug resistance – (Chromosomal)	Bhargava *et al.*,2010; Lysitsas *et al.*,2024.

In addition, *K. pneumoniae* frequently carries AmpC β-lactamase genes, including cephamycinases (^{bla}*CMY*), docosahexaenoic acid (^{bla}*DHA*), cefoxitin (^{bla}*FOX*), and moxalactam (^{bla}*MOX*). These β-lactamase genes, together with porin loss or increased efflux, enhance β-lactam and carbapenem resistance. Plasmid-borne resistance genes can be highly expressed, further increasing carbapenem resistance. Notably, the coexistence of multiple β-lactamase genes, such as ^{bla}*SHV*, ^{bla}*AmpC*, ^{bla}*KPC*, and carbapenemases within the same strain results in synergistic resistance effects; for example, although NDM, IMP, and VIM do not confer resistance to aztreonam alone, resistance may occur when they coexist with ESBL or AmpC enzymes (Jacoby, 2009; Bush, 2010). (**Table 6**).

Target site alteration

Another resistance mechanism is by antimicrobial targets alteration. *K. pneumoniae* produces drug resistance by preventing the matching antimicrobials from attaching to the target site by changing the target gene or methylating certain bases (Li *et al*., 2023). The modification of Lipid A renders resistance of *K. pneumoniae* to polymyxin, while aminoglycoside and fluoroquinolone target the ribosomal RNA (rRNA-16S) and DNA topoisomerase, respectively (Haeili *et al* 2017; Azargun *et al*., 2019). Additionally, the mechanism of resistance to fosfomycin is by modification on the UDP-N-acetylglucosamine enolpyruvyl transferase A (*MurA*) gene which alters the protein structure, minimising the binding affinity of fosfomycin to the target side and decreasing its effectiveness (Liu *et al* 2020). (**Table 6**).

Porin loss and mutation

Trimeric transmembrane proteins also known as outer membrane proteins (Omps) or porins are widely produced on the outer membranes of Gram-negative bacteria, therefore; *K. pneumoniae* gains resistance to antimicrobials by reducing the outer membrane pore protein (Li *et al*., 2014; Ye *et al*., 2021; Liu *et al*., 2022). The outer membrane porin (OmpK35 and OmpK36 - non-specific porins) are the main porins linked to antimicrobial resistance in *K. pneumoniae*. Also, intrinsic resistance is influenced by lambda (LamB - affects the permeability of the bacterial outer membrane to certain antimicrobials), OmpK26, phosphoporin E (PhoE), and *K. pneumoniae* novel outer membrane porin (KpnO), (Pulzova *et al*., 2017; Wu *et al*., 2020). (**Table 6**).

Increased efflux pump expression

One of the main causes of MDR *K. pneumoniae* is the ability of the active acridine resistance A/B – "Tolerance" to colicin protein (AcrAB-TolC) efflux system to expel antimicrobials outside the cell; thus, reducing their intracellular concentrations and the organism's susceptibility to a variety of antimicrobials including β-lactams, fluoroquinolones, and tetracycline (Nielsen *et al*., 2014; Bialek-Davenet *et al*., 2015; Tang *et al*., 2020; Bharatham *et al*., 2021). (**Table 6**).

Biofilm-associated antimicrobial resistance

K. pneumoniae forms biofilms with the assistance of pili and capsular structures, such as the fimbriae (*fim)* and mannose-resistant *Klebsiella*-like (*mrk*) gene clusters. The biofilms exhibit osmotic barriers which facilitate antimicrobial resistance (Desai *et al*., 2019; Tang *et al*., 2020). A Malaysian study discovered that the biofilms in *K. pneumoniae* decreased susceptibility to ampicillin, ciprofloxacin, and gentamicin (Chung, 2016), while another study in Spain reported the presence of biofilms to be linked to colistin resistance (Cepas *et al*., 2019). (**Table 6**).

Resistance to aminoglycoside

Aminoglycosides were widely used between 1940 and 1980 but were later replaced by 3GC, carbapenems, and fluoroquinolones (Krause *et al*., 2016). Since then, *K. pneumoniae* developed key aminoglycoside resistance mechanisms, mainly through plasmid-encoded drug-modifying enzymes (via acetylation, adenylation, and phosphorylation) associated with aminoglycoside N-acetyltransferase, aminoglycoside O-phosphotransferase, and aminoglycoside O-nucleotidyltransferase (*aac*, *aph*, and *ant*) gene families (Opal *et al*., 2015). (**Table 6**).

Moreover, the reduced usage of aminoglycosides initially limited the emergence of new resistance genes until the appearance of aminoglycoside resistance methylase A (armA), which encodes the 16S rRNA methylase (Doi *et al*., 2016). This enzyme confers high-level resistance to nearly all aminoglycosides, including newer agents such as plazomicin (Poulikakos *et al*., 2013), and is plasmid-mediated in *K. pneumoniae* (Galimand [(a)] *et al*., 2003). Chromosomal mechanisms also contribute to resistance by altering cell permeability through changes in the AcrAB-TolC and *K. pneumoniae* EF (KpnEF) efflux pump systems and the loss of the *K. pneumoniae* novel outer membrane porin (KpnO), resulting in variable resistance profiles to different aminoglycosides. In particular, resistance to tobramycin, streptomycin, and spectinomycin has been associated with loss of *KpnO* (Srinivasan *et al*., 2012). (**Table 6**).

Resistance to quinolones

Quinolones act by inhibiting bacterial DNA replication by targeting DNA gyrase and topoisomerase IV. In *K. pneumoniae*, resistance to fluoroquinolones arises mainly from chromosomal mutations in gyrase A (*gyrA*) and partition C (*parC*) genes (which are the most frequently reported target gene alterations), (Nam *et al*., 2013; Redgrave *et al*., 2014). Reduced drug susceptibility is further enhanced by changes in cell permeability, including loss of OmpK36, overexpression of the acridine resistance protein A and B (AcrAB) efflux pump, and altered expression of *Klebsiella* drug efflux A (kdeA), (Mazzariol *et al*., 2002; Ping *et al*., 2007). (**Table 6**).

Plasmid-mediated quinolone resistance (PMQR) also contributes to resistance, particularly through the olaquindox and quinolone AB (OqxAB) efflux pump and other PMQR determinants that protect DNA gyrase and topoisomerase IV from quinolone activity (Ruiz *et al*., 2012; Wong *et al*., 2015; Zheng *et al*., 2018). The PMQR gene (*aac*(6′)-*Ib-cr*) modifies certain quinolones and other antimicrobial agents and has been identified on both plasmids and chromosomes in *K. pneumoniae* (Ruiz *et al*., 2012). Plasmid-mediated quinolone resistance genes confer low to moderate resistance. However, they facilitate the development of higher-level chromosomal resistance, thereby promoting MDR phenotypes (Fabrega *et al*., 2009). (**Table 6**).

Resistance to polymyxins (colistin)

The emergence of CRE has led to the renewed use of polymyxins (i.e., colistin) as last-line therapy (Anastasia *et al*., 2007). In *K. pneumoniae*, polymyxin resistance most commonly arises from mutations in regulatory genes such as magnesium-responsive B gene (*mgrB*), which alters Lipid A and reduces polymyxin binding (Cannatelli *et al*., 2015; Laurent *et al*., 2015; da Silva *et al*., 2020). The first encounter of a plasmid-mediated colistin resistance via mobilized colistin resistance-1 gene (*mcr*-1) was identified in China from an *E. coli* isolate; and it remains uncommon in *K. pneumoniae* bloodstream isolates (Liu [(a)] *et al*., 2016). (**Table 6**).

Nevertheless, the detection of Pan-resistant *K. pneumoniae* reported in 2016 highlights the threat posed by transferable resistance mechanisms, even when colistin resistance is not mediated by *mcr*-1 (Mcgann *et al*., 2016; Lai *et al*., 2017; Lv *et al*., 2017). Additionally, colistin-resistant strains of *K. pneumoniae* have been documented in ICUs across Europe, North America, South America, Asia, and South Africa, and is most likely driven by the selection pressure exerted by colistin (Ah *et al*., 2014; Monaco *et al*., 2014; Giacobbe *et al*., 2015; English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR), 2017; Otter *et al*., 2017). (**Table 6**).

Resistance to tigecycline

Tigecycline is a broad-spectrum tetracycline effective against ESBL-producing *K. pneumoniae* and has been clinically used since 2005 (Guillard *et al*., 2016). However, resistance in *K. pneumoniae* emerged soon after its introduction and is mainly chromosomally mediated through ribosomal target modification and reduced intracellular drug accumulation (Sekyere *et al*., 2016). The predominant resistance mechanisms which actively expel tigecycline from the cell include overexpression of efflux pumps, AcrAB-TolC, OqxAB, *K. pneumoniae* gene ABC (*KpgABC*), and tetracycline resistance gene A and B (*TetA and TetB*) variants. Additionally, mutations in ribosomal protein S10 (encoded by rpsJ) near the tetracycline-binding site of the 30S subunit contribute to tigecycline resistance (Guillard *et al*., 2016; Sekyere *et al*., 2016). (**Table 6**).

Resistance to fosfomycin

Fosfomycin, first discovered in 1969, exhibits broad-spectrum bactericidal activity and has regained clinical importance due to its effectiveness against MDR bacteria (Raz, 2012; Kurabayashi *et al*., 2015). However, increased clinical use has been accompanied by the emergence of fosfomycin-resistant strains (Liu [(a)] *et al*., 2017; Cao *et al*., 2017). Resistance to fosfomycin arises through several mechanisms, including alterations or overexpression of the target enzyme UDP-N-acetylglucosamine enolpyruvyl transferase A (MurA), reduced or absent expression of the Glycerol-3-phosphate transporter (GlpT) and Hexose-6-phosphate transporter (UhpT) transporters that are responsible for drug uptake, and enzymatic inactivation mediated by fosfomycin-resistant glutathione transferase (*Fos*) genes that encode glutathione S-transferase activity

(Falagas *et al*., 2018). Notably, Liu *et al*., (2020) identified the plasmid-mediated *FosA*3 gene as the primary mechanism of fosfomycin resistance in carbapenem-resistant *K. pneumoniae* (CRKP), while mutations in MurA and the GlpT transporter were associated with fosfomycin resistance in *FosA3*-negative CRKP isolates. (**Table 6**).

2.5.1.3. Antimicrobial resistance mechanisms in *E. coli*

Escherichia coli is resistant to various antimicrobial classes, with the emergence of AmpC-type β-lactamases and ESBLs strains being of most concern (Rodríguez- Baño *et al*., 2008; Rodríguez- Baño *et al*., 2009). The production of ESBLs further confers resistance to third- and fourth-generation cephalosporins (3GC/4GC), and monobactams which further complicates treatment (Ewers *et al*., 2012; Walsh *et al*., 2012; Lopez-Cerero *et al*., 2013).

Enzymatic inactivation

Escherichia coli resistance mechanisms involve enzyme inactivation by ^{bla}*CTX-M*, ^{bla}*TEM*, and ^{bla}*SHV* which enable horizontal gene transfer, conveying multiple resistance determinants via integrons and transposons. Mobile genetic elements (MEGs) also play a vital role in the rapid dissemination of β-lactam resistance (Alipour *et al*., 2019). These plasmid-encoded enzymes confer resistance to 3GC and monobactams (aztreonam), (Gniadkowski, 2008; Copur *et al*., 2013; Bajpai *et al*., 2017; Hussain *et al*., 2021). The plasmid-mediated ^{bla}*CMY-2* confers resistance to cephamycins (e.g., cefoxitin) and extended-spectrum cephalosporins, while ^{bla}*NDM*, ^{bla}*KPC*, ^{bla}*OXA*, and ^{bla}*VIM* hydrolyse carbapenems as well as other β-lactams on plasmids that spread between strains (Zhang *et al*., 2014). Plasmid-encoded AmpC β-lactamases hydrolyse cephamycins and penicillins while evading β-lactamase inhibitors such as clavulanic acid (Sadeghi *et al*., 2021).

Altered membrane permeability and expression of the efflux system

Mutations in PBPs (particularly PBP2 and PBP3) result in structural modifications that allow peptidoglycan synthesis which contribute to β-lactam resistance by reducing its binding affinity (Issakhanian *et al*., 2019; Whelan *et al*., 2023). Also, modifications in cell wall integrity and reduction of β-lactam efficiency without degrading the cell viability is linked to mutations in the new lipoprotein D gene (*nlpD* – Murein hydrolase activator), a regulator of peptidoglycan hydrolases (Tsang *et al*., 2017; Choi *et al*., 2024).

Porin downregulation and structural modifications, alongside efflux-mediated drug extrusion, play a critical role in the development of MDR (Rijavec *et al*., 2006; Ganjo *et al*., 2024). Loss or mutation of the outer membrane porins (OmpF and OmpC) reduces antimicrobial influx and lowers intracellular drug concentrations (Choi *et al*., 2019). These alterations are regulated by the environmental sensor Z - outer membrane protein regulator (EnvZ–OmpR) two-component system, which adjusts porin expression in response to environmental stress. The combined effect of enhanced efflux activity and decreased membrane

permeability strengthens bacterial barriers, reinforcing MDR phenotypes and reducing the effectiveness of multiple antimicrobial classes including chloramphenicol, ciprofloxacin, and tetracycline. Moreover, the tripartite resistance-nodulation-division pump actively works to expel β-lactams, fluoroquinolones, and trimethoprim/sulfamethoxazole; this lowers antimicrobial intracellular concentrations and efficacy (Choi *et al*., 2024).

Target site alteration

The alteration of the enzyme structure of DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) to prevent the binding affinity of fluoroquinolone occurs through point mutations in the quinolone resistance-determining regions (QRDRs). The enzymatic changes on the *gyrA* protein occur from serine (S) to leucine (L) at position 83 (Ser83L), and from aspartic acid (D) to asparagine (N) at position 87 (D87N); while the codon change on the *parC* protein occurs from serine (S) to isoleucine (I) at position 80 (S80I), and from glutamic acid (E) to lysine (K) at position 84 (E84K), (Sorlozano *et al*., 2007).

Resistance to quinolones

Plasmid-mediated quinolone resistance (PMQR) genes such as quinolone resistance A, B and S (*qnrA*, *qnrB*, and *qnrS*) encode proteins binding to the DNA gyrase and topoisomerase IV, protecting them from fluoroquinolone inhibition. These mutations not only reduce the effectiveness of fluoroquinolone, but also allow bacterial replication regardless of antimicrobial exposure, which further accelerates resistance (Tran *et al*., 2002; Sorlozano *et al*., 2007; Jacoby *et al*., 2015; Li [(b)] *et al*., 2016; Rezazadeh *et al*., 2016; Recacha *et al*., 2017). Additionally, aminoglycoside acetyltransferase (*aac*(6′)-*Ib-cr*) stimulates resistance by acetylation of other antimicrobials such as ciprofloxacin and norfloxacin (Morgan-Linnell *et al*., 2009; Whelan *et al*., 2024).

Table 6: Overview of antimicrobial resistance mechanisms in *K. pneumoniae* (Li *et al.*, 2023).

Resistance mechanism	Target/action mechanism	Genes/ Proteins	Target antimicrobial agent	Author/Ref
Enzymatic inactivation	ESBLs production - Hydrolysis of extended-spectrum cephalosporins and penicillins	*bla*CTX-M, *bla*SHV, *bla*TEM	Penicillins & cephalosporins	Liu [(b)] *et al.*, 2015
	Class A carbapenemase production - Hydrolyses carbapenems and most β-lactams; associated with outbreaks	*bla*KPC, CG258 ST258	Carbapenems	Ørjan *et al.*, 2009; Breurec *et al.*, 2013; Tooke *et al.*, 2020; Theuretzbacher *et al.*, 2021
	Class B Metallo-β-lactamase production - Zinc-dependent hydrolysis of almost all β-lactams	*bla*NDM, *bla*VIM, *bla*IMP	Carbapenems	Yong *et al.*, 2009
	Class C β-lactamase production - Resistance to cephamycins and reduced susceptibility to inhibitor combinations	AmpC, *bla*CMY, *bla*DHA, *bla*FOX *bla*MOX	Penicillins, cephalosporins & β-lactam/β-lactamase inhibitor combinations	Jacoby, 2009; Bush, 2010
	Class D OXA-48-like carbapenemase production - Weak carbapenem hydrolysis; often phenotypically silent	*bla*OXA-48-like	Carbapenems	Bradford, 2001
	Aminoglycoside-modifying enzymes (AME) - Enzymatic modification prevents ribosomal binding	*aac*, *aph*, and *ant*	Aminoglycosides	Opal *et al.*, 2015
Target site modification	16S rRNA methylation - High-level aminoglycoside resistance	armA, rmtA–F	Aminoglycosides	Doi *et al.*, 2016; Poulikakos *et al.*, 2013
	Reduced binding of fluoroquinolones to DNA gyrase/topoisomerase IV	*gyrA*, *parC*	Fluoroquinolones	Nam *et al.*, 2013; Redgrave *et al.*, 2014
	Protection of DNA gyrase or enzymatic drug modification	*qnrA*, *qnrB*, *qnrS*, *aac(6')-Ib-cr*	Fluoroquinolones	Ruiz *et al.*, 2012
	Lipid A modification - Reduced polymyxin binding to LPS	*mgrB* inactivation, pmrA/pmrB, *mcr-1*	Colistin	Cannatelli *et al.*, 2015; Laurent *et al.*, 2015; da Silva *et al.*, 2020
	Alters the protein structure, minimising the binding affinity	MurA enzyme, *MurA* gene	Fosfomycin	Liu *et al* 2020

Altered membrane permeability	Porin loss with β-lactamase co-production - Reduced permeability enhances resistance in ESBL/AmpC producers	Loss/mutation of OmpK35, OmpK36	Carbapenems & cephalosporins	Mazzariol *et al.*, 2002; Ping *et al.*, 2007.
Active efflux system	Efflux pumps / ribosomal protection - Active efflux or ribosomal shielding	*tetA*, *tetB*	Tetracyclines	Guillard *et al.*, 2016; Sekyere *et al.*, 2016.
	Efflux pump overexpression - Multidrug extrusion reducing intracellular antibiotic concentrations	AcrAB-TolC, *KpgABC*	Multiple classes	Nielsen *et al.*, 2014; Bialek-Davenet *et al.*, 2015; Tang *et al.*, 2020; Bharatham *et al.*, 2021.
Other	Biofilm formation - Reduced antibiotic penetration and enhanced persistence	*fim*, *mrk* gene clusters	Multiple classes	Chung, 2016; Cepas *et al.*, 2019; Desai *et al.*, 2019; Tang *et al.*, 2020.

Resistance to aminoglycosides

The aminoglycoside resistance methyltransferase A (armA) was found as a new determinant conferring high-level resistance to aminoglycosides in a French clinical isolate of *K. pneumoniae* in 2003 (Galimand [(a)] *et al.*, 2003); subsequently, it was found in human isolates of *Enterobacteriaceae* from various countries in Europe (Galimand [(b)] *et al.*, 2003). The novel plasmid-mediated methyltransferase A (npmA) was the only one acquired 16S rRNA methyltransferase (16S-RMTase) isolated in *E. coli* from a clinical human strain in Japan in 2007. The *armA* and *npmA* genes are not only responsible for high-level resistance to amikacin, gentamicin and tobramycin, but also to a broader spectrum of resistance to this class, including apramycin and neomycin (Wachino *et al.*, 2007; Poirel *et al.*, 2018).

Resistance to polymyxins (colistin)

The modification of lipid A of lipopolysaccharides in *E. coli* through the addition of phosphoethanolamine (PEtN) and/or 4-amino-4-deoxy-L arabinose (L-Ara4N) reduces the affinity of polymyxins (i.e., colistin) to the bacterial cell. This resistance mechanism further activates the polymyxin modification regulator A/B and phosphatase-regulated P/Q (PmrA/PmrB and PhoP/PhoQ) protein component systems by specific gene mutations of *pmrA*, *pmrB*, *phoP*, and *phoQ* leading to overexpression of genes modifying lipopolysaccharides (Rhouma *et al.*, 2016). In 2015, a plasmid-designated *mcr-1* gene that confers colistin resistance was found in human and pig *E. coli* strains in China (Liu [(a)] *et al.*, 2016). In 2016, another *mcr-1* gene was detected in a human *E. coli* isolate in the U.S., (Doumith *et al.*, 2016).

Furthermore, an *in vitro* study demonstrated self-transfer of the *mcr-1* gene from conjugative plasmids (Wang *et al*., 2018). Rapid spreading of the *mcr-1* gene plasmids occurred shortly after its first detection, and were found worldwide in the environment, animals, food, infected patients as well as in asymptomatic human carriers (Doumith *et al*., 2016; Skov *et al*., 2016). Xavier *et al*., (2016) later discovered the *mcr-2* gene, and that it shared about 77.00% colistin resistance as the *mcr-1* gene. Thus far several *mcr* genes and their variants have been identified in animal *E. coli* strains (García-Meniño *et al*., 2019; Khine *et al*., 2020). However, it is hypothesized that the *mcr-1* gene long existed and has been silently transmitted throughout the years among Enterobacterales, although it was not identified (Dalmolin *et al*., 2018).

Resistance to tetracycline

The most prevalent genes encoding the efflux systems that cause tetracycline resistance in *E. coli* are class A-C tetracycline resistance proteins (tet-A, tet-B, and indirectly tet-C), (Sheikh *et al*., 2012; Seifi *et al*., 2016). In addition, *tet-M* and *tet-W* genes bind to the ribosomal units to reduce the binding affinity of tetracyclines, while *tet-X* encodes the oxidoreductase that inactivates first- and second-generation tetracyclines (Roberts, 2005). Furthermore, *E. coli*'s resistance to sulphonamides is carried out by incompatibility groups FII and I1 (IncFII and IncI1) plasmids carrying sulphonamide 1and 2 (*sul1* and *sul2*) genes (Wu *et al*., 2010; Li *et al*., 2022). The presence of the dihydrofolate reductase (*dfr*) gene encoding dihydrofolate reductases confers resistance to trimethoprim (Jiang *et al*., 2019; Nowaczek *et al*., 2021).

2.5.1.4. Antimicrobial resistance mechanisms in *P. aeruginosa*

Pseudomonas aeruginosa is one of the resistant bacteria with multiple intrinsic or acquired mechanisms of resistance to various antimicrobial classes (European Centre for Disease Prevention and Control (ECDC), 2018; Dey *et al*., 2019). Infections caused by MDR- *P. aeruginosa* are commonly reported in ICUs worldwide, especially when carbapenems (treatment of choice) have been rendered inefficient due to the production of carbapenemases (Ramprasad *et al*., 2010; Breidenstein *et al*., 2011; Zahra *et al*., 2011; Alhazmi, 2015; Pillai *et al*., 2015).

P. aeruginosa establishes its inherent resistance (intrinsic, acquired, and adaptive resistance mechanisms) through a variety of genetically encoded mechanisms, additional genes or mutations under selection pressure, and the development of biofilms on infection sites (Lee *et al*., 2017). This organism can acquire horizontally transferable genetic elements and use molecular pathways for environmental adaption to thrive under adverse conditions (Meletis *et al*., 2012). The outer membrane proteins (Omps) assist in adherence, antimicrobial resistance, and nutrient uptake (Sabnis *et al*., 2021).

Biofilm-associated antimicrobial resistance

Biofilm-associated drug resistance is supported by pili, flagella, and exopolysaccharides, which promote surface attachment, colonization, and minimise bacterial clearance by the host (Ozer *et al*., 2021). Type I-VI secretion systems (T1SS-T6SS) facilitate motility (swarming and twitching), evasion of immune responses, and host colonization (Alatraktchi *et al*., 2020; Chadha *et al*., 2022). Additionally, *P. aeruginosa* survives within phagocytic cells by using antioxidant enzymes such as alkyl hydroperoxide reductases, catalases and superoxide dismutases to neutralize reactive oxygen species (Dar *et al*., 2021).

Acquired resistance mechanism

The acquired resistance mechanism is achieved through plasmids, transposons, and integrons (mobile genetic elements - MGEs) encoding resistance determinants, mutations altering drug targeted sites, and by over-expression or down-regulation of pre-existing genes. *P. aeruginosa* acquires the ^{bla}*CTX-M* gene from other microorganisms through genetic transfer via self-transmissible mega-plasmids or insertion of DNA fragments into genomic regions (PA0069- high plasticity genomic region), (Subedi *et al*., 2018). The downregulation of specific porin proteins and overexpression of efflux pumps destroys the outer membrane's permeability, affecting the influx of β-lactams, aminoglycosides, quinolones, cephalosporins, and antipseudomonal penicillins (Poole, 2000; Chevalier *et al*., 2017; Wu *et al*., 2024).

Class 1 and **2** integrons facilitate horizontal spreading through expression of resistance genes on plasmids (Goudarzi *et al*., 2015; Heidarzadeh *et al*., 2019). *P. aeruginosa* pathogenicity islands (PAPI-1 and PAPI-2) harbour high-risk clones and essential virulence determinants, and are frequently associated with epidemics (Harrison *et al*., 2010). Host damage and immune evasion in *P. aeruginosa* is carried out by mobile elements which carry genes encoding biofilm-related polysaccharides, elastases, exotoxins, and proteases. Resistance genes such as β-lactamases, aminoglycoside-modifying enzymes, and multidrug efflux pumps are located on these elements (Qin *et al*., 2022). The ability of *P. aeruginosa* to combine all three mechanisms (intrinsic, acquired, and adaptive resistance mechanisms) has spiked the emergence of resistance to first-line antipseudomonal agents. This has led to decreased efficacy of drugs that were once effective (Meletis *et al*., 2025).

Enzymatic inactivation

Enzymatic inactivation in *P. aeruginosa* involves some **Class C** and **D** β-lactamases as well as aminoglycoside modifying enzymes (AME), which hydrolyse susceptible bonds like amides and esters in certain β-lactams and aminoglycosides (Meletis *et al*., 2025). Traditional β-lactamase inhibitors like clavulanic acid and sulbactam do not impact AmpC β-lactamases, while more recent β-lactamase inhibitors like avibactam and relebactam are stable against AmpC hydrolysis (Lob *et al*., 2017; Pang *et al*., 2019; Lee *et al*., 2023).

However, the *ampC* gene which encodes **Class C** β-lactamases can be activated by cefoxitin, clavulanic acid, and imipenem (Philippon *et al*., 2022).

The **Class A** carbapenem-hydrolyzing enzymes found in *P. aeruginosa* include the plasmid-encoded KPC and GES that hydrolyse carbapenems, cephalosporins, monobactams, and penicillins, and are moderately inhibited by boronic acid, clavulanic acid, and tazobactam (Diene *et al*., 2014; Naas *et al*., 2016). *P. aeruginosa* harbours the most **Class B** MBLs that have been detected worldwide than other species, namely: IMP, VIM, NDM, SIM, Adelaide Imipenemase (AIM), Canada MBL (CAM), Dutch Imipenemase (DIM), German Imipenemase (GIM), Hambourg MBL (HMB), and Sao Paulo MBL (SPM), (Halat *et al*., 2022). **Class D** OXA-48 and OXA-198 found in *P. aeruginosa* hydrolyse oxacillin and cloxacillin and are resistant to various former β-lactamase inhibitors, but are susceptible to novel inhibitors such as avibactam (Pfennigwerth *et al*., 2020).

The KPC, IMP, VIM, NDM, and OXA-48 type carbapenemases detected in *P. aeruginosa* have spread to several different bacterial species and nations. While IMP, VIM, GES, and OXA types are globally distributed, KPC-producing isolates are primarily found in Latin America. NDM-producers are dispersed in India, Australia, and are rapidly spreading in Greece. All other carbapenemases detected in *P. aeruginosa* remain in their initial specific geographic areas of discovery, as designated by their nomenclature (Halat *et al*., 2022; Protonotariou *et al*., 2024).

Class D, *Pseudomonas* oxacillinase B (PoxB) hydrolyses oxacillin, methicillin, and cloxacillin (Zincke *et al*., 2015). *Pseudomonas* oxacillinase B (PoxB) has a lower hydrolytic activity than that of the clinically relevant carbapenemases, and rarely overexpressed in clinical isolates; it is not an alarming contributor to the widespread clinical resistance seen in *P. aeruginosa* infections. Although, its overexpression may lead to increased meropenem MICs without any concurrent increase in imipenem (Kong *et al*., 2005).

Target site alterations

Mutations around the active site of PBP3 have shown reduced binding affinity for most β-lactams, including aztreonam, cefepime, ceftazidime, meropenem, and ticarcillin (Glen *et al*., 2024). The PBP5 due to its structure resembling that of also the **Class A** β-lactamases contributes to the ability of *P. aeruginosa* to resist the activity of some β-lactams (Smith *et al*., 2013).

Resistance to quinolones

The overexpression of the Multidrug export AB and Outer protorein M (MexAB-OprM), Multidrug export CD and Outer protorein J (MexCD-OprJ), Multidrug export EF and Outer protein N (MexEF-OprN) efflux systems, mutations in the DNA gyrase (*gyrA* and *gyrB* genes), as well as the topoisomerase IV (*ParC* and *ParE* genes) are responsible for the resistance against fluoroquinolones in *P. aeruginosa* (Rubin *et al*., 2008; Langendonk *et al*., 2021). In addition, fluoroquinolone resistance was also observed in strains carrying ciprofloxacin resistance protein, plasmid-encoded gene (*crpP*), quinolone resistance variant C1 (qnrVC1), and quinolone resistance-determining regions (QRDRs), (Khan *et al*., 2020).

Resistance to aminoglycosides

Mutations in the anti-repressor MexZ (armZ), elongation factor gene A1 (abbreviated as *fusA1*), multidrug export Z (MexZ), and the partitioning protein RS (parRS) genes facilitate acquired resistance against aminoglycosides (Thacharodi *et al*., 2022; Atassi *et al*., 2023). Nucleotidyl transferases like *ant(2')-I, ant(3")* together with acetyl transferases like *aac(3)* genes are associated with a chromosomal transposon (Tn801), which also carries the ^{bla}*TEM-21* gene; drive the resistance against gentamicin, streptomycin, and tobramycin (Dubois *et al*., 2002; Poole, 2005; Zárate *et al*., 2018; Atassi *et al*., 2023). Additionally, Phosphotransferases like *aph(2")*, *aph(3')-IIb*-like, and *aph(3')-VI* inactivate anti-pseudomonal aminoglycosides and other less frequently used aminoglycosides like kanamycin and neomycin. The 16S rRNA methylases (RmtA, RmtB), and ArmA prevent aminoglycoside binding, leading to high-level resistance to all aminoglycosides (Poole, 2005; Wachino *et al*., 2012).

Resistance to β-lactams

The presence of 1,6-anhydro-N-acetylmuramyl-L-alanine amidase D and E (ampD and ampE) regulatory gene induces the hyperproduction of AmpC β-lactamase, which confers resistance to monobactams in *P. aeruginosa* (Philippon *et al*., 2002; Jacoby, 2009). It was previously discovered that another regulatory gene, *ampE* with other unknown genes, had an indirect contribution to β-lactam resistance (Juan *et al*., 2005). The CTX-M variants (CTX-M1/2/43) located on the β-lactam-binding sites, are effective in hydrolysing narrow-spectrum cephalosporins, cefotaxime, and penicillin (Rossolini *et al*., 2008). These variants are commonly identified in MDR isolates (Zhao *et al*., 2013). Additionally, *Pseudomonas* Extended Resistance (PER) enzyme confers resistance to penicillins and cephalosporins (3- and 4GC) including oxyimino-β-lactams, and aztreonam (Laudy *et al*., 2017).

Resistance to carbapenems

Carbapenem resistance in *P. aeruginosa* is mediated by the compromised or loss of the OprD porin, the production of carbapenemases, and the overexpression of the AmpC β-lactamases and efflux pumps demonstrated in a single strain (Meletis *et al*., 2014). Once the outer membrane's permeability is compromised, carbapenems are prevented from reaching the PBPs located in the periplasmic space (Biggel *et al*., 2023). The loss or downregulation of the *oprD* gene not only results to imipenem resistance, but also facilitates pseudomonal colonization to the mucosal surfaces. This was demonstrated in mouse infection models (Skurnik *et al*., 2013; Richardot *et al*., 2015; Atrissi *et al*., 2013).

Furthermore, the Overexpression of the MexAB-OprM efflux pumps lead to an increased expulsion of meropenem. Imipenem is less affected, unless if the Overexpression co-exists with the loss of the OprD (Chalhoub *et al*., 2016; Pan *et al*., 2016; Abdallah *et al*., 2021). Even though the AmpC β-lactamases are not considered carbapenemases due to their low hydrolytic activity against carbapenems, their overexpression in *P. aeruginosa* induces carbapenem resistance when combined with one or more other carbapenem resistance mechanisms (Philippon *et al*., 2022).

Resistance to polymyxin (colistin)

Polymyxin B (in the form of polymyxin B/colistin sulfate) and polymyxin E (in the form of colistin methanesulfonate) are the only therapeutic options available against MDR and XDR *P. aeruginosa* isolates (Morita *et al*., 2014). *P. aeruginosa* does not express intrinsic resistance against polymyxins; however, it has the potential to develop acquired resistance against them through chromosomal mutations and plasmid-mediated resistance. chromosomal mutations on regulatory genes causes the malfunctioning of the pmrA-pmrB, phoP-phoQ, cationic peptide resistance response regulator and sensor kinase (CprRS), as well as the colistin resistance regulator protein and sensor kinase protein (ColRS) component regulatory systems (Gutu *et al*., 2016; Huang *et al*., 2020). These systems modify the lipopolysaccharides by adding 4-amino-4-deoxy-L-arabinose (L-ara4N), phosphoethanolamine (PEtN), or galactosamine to the Lipid A of the lipopolysaccharides' core. As a result, the decreased negative charge of the phosphate groups reduces the binding affinity of polymyxins to the lipopolysaccharides (Abd El-Baky *et al*., 2020).

The horizontally transferable *mcr* gene facilitates plasmid-mediated resistance. This gene has been detected in polymyxin-resistant *P. aeruginosa* strains worldwide (Hameed *et al*., 2019). A PEtN transferase encoded by the *mcr-1* gene and its variants binds PEtN to Lipid A, decreasing the effectiveness of colistin (Osei *et al*., 2019; Aboelsuod *et al*., 2023). It was previously reported that the overexpression of the outer membrane protein H (OprH) reinforces the acquired resistance of *P. aeruginosa* to polymyxins by occupying putative polymyxin B binding sites on lipopolysaccharides (Macfarlane *et al*., 1999).

Resistance to fosfomycin

Fosfomycin is an old antimicrobial that was revived in the past decade due to the persistent emergence of MDR isolates. This agent is administered orally and intravenously as calcium or trometamol salt and disodium salt, respectively. Oral fosfomycin is used for the treatment of lower UTIs, while intravenous fosfomycin is used for bacteremia/sepsis, respiratory tract, joint, intra-abdominal, and skin/soft tissue infections (Falagas *et al*., 2016).

There are three different ways that *P. aeruginosa* can counteract the effects of fosfomycin. Firstly, by producing Fos enzymes, which alter the drug's structure and render fosfomycin inactive. Secondly, by peptidoglycan recycling enzymes that avoid the *de novo* production of peptidoglycan by restoring the UDP-N-acetylmuramic acid (UDP-MurNAc); and thirdly, by mutations of the glucose-3-phosphate transporter (GlpT) encoding gene that decrease the permeability of fosfomycin (Castañeda-García *et al*., 2009; Zheng *et al*., 2022).

According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), most *P. aeruginosa* strains produce fosfomycin resistance protein A (FosA) enzymes. Breakpoints for *P. aeruginosa* are not available for both EUCAST and Clinical and Laboratory Standards Institute (CLSI) documents (European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2024; Clinical and Laboratory Standards Institute (CLSI), 2025; European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2025). Therefore, fosfomycin monotherapy against *P. aeruginosa* infections is not advisable, and it is inappropriate to assume fosfomycin breakpoints from Enterobacterales to *P. aeruginosa* because the resistance mechanisms involved are significantly different (Zheng *et al*., 2022).

2.5.2. Resistance mechanisms of Gram-positive multidrug-resistant organisms (MDROs)

Interventional and invasive monitoring procedures create a corresponding rise in Gram-positive bacteraemia rates (Niederman, 2006; Wiesen *et al*., 2006). Gram-positive bacteria develop resistance either through the production of β-lactamases, acquisition of exogenous deoxyribonucleic acid (DNA), or by altering the native penicillin-binding protein (PBP) genes to reduce the affinity and susceptibility of their target site (Berger-Bächi, 2002; Munita *et al*., 2015).

2.5.2.1. Antimicrobial resistance mechanisms in *S. aureus* and *Staphylococcus* spp.

Methicillin-resistant *S. aureus* (MRSA) is one of the problems encountered in the clinical practice (European Antimicrobial Resistance Surveillance Network (EARS-Net), 2013). It was previously hypothesized that the existing clones derived from a common parent, may have evolved into MRSA strains by horizontal genetic transfer of staphylococcal cassette chromosome *mec* (*SCCmec*), which is a mobile genetic element encoding the *mecA* or *mecC* genes (Tenover *et al*., 1995). This element is responsible for the organism's high-level of resistance to methicillin, and to most β-lactam antibiotics where PBP2 is altered to PBP2a (Tenover *et al*., 1995). The *SCCmec* also contains Tn554, pUB110, and pT181 genetic structures, which confer resistance to non-β-lactam antimicrobials (Chambers, 1997; Ito *et al*., 1998; Ito *et al*., 1999).

In South African tertiary public hospitals, hospital-associated MRSA (HA-MRSA) infections previously accounted for 28.30% of patients with *S. aureus* bacteraemia, while resistance rates of up to 64.05% were reported between different countries (European Antimicrobial Resistance Surveillance Network (EARS-Net), 2013; Shuping *et al*., 2017). Higher resistance rates have also been reported in Greece, Italy, and Portugal (Southern Europe), whereas less than 1.00% of all *S. aureus* isolates displayed resistance to methicillin in the Northern countries (European Antimicrobial Resistance Surveillance Network (EARS-Net), 2013).

The Epidemiology and determinants of outcomes of hospital-acquired bloodstream infections in ICU (EUROBACT) reported methicillin resistance in approximately 50.00% of isolates in ICU settings, hence the increase in vancomycin prescriptions over its efficacy against MRSA in critical patients (Bassetti [(a)] *et al*., 2012; De Waele *et al*., 2014). Global increases in vancomycin minimum-inhibitory concentrations (MICs) have been reported, although the "MIC-creep" phenomena vary greatly between nations and institutions (Kehrmann *et al*., 2011; Sancak *et al*., 2013).

CoNS are usually multidrug-resistant organisms (Singh *et al*., 2016). The antimicrobial susceptibility of these isolates is critical for clinical empirical antibiotic therapy (Shah *et al*., 2014). Some studies have previously reported CoNS to be highly resistant to penicillin, oxacillin and erythromycin, with more than 50.00% being resistant to cephalosporins, aminoglycosides and quinolones (Shah *et al*., 2014; Singh *et al*., 2016; Xu *et al*., 2018).

Staphylococcus spp. is naturally susceptible to penicillins; however, the emergence of resistance has necessitated the use of semi-synthetic penicillins (e.g., cloxacillin, oxacillin, nafcillin) and glycopeptides such as vancomycin, which remains the standard treatment for methicillin-resistant *Staphylococcus* infections despite risks of nephrotoxicity and the persistent emerging resistance. Several alternative agents—including ceftaroline, daptomycin, linezolid, trimethoprim–sulfamethoxazole, and lipoglycopeptides are the alternatives to vancomycin as they demonstrate good activity against resistant *Staphylococcus* strains (John, 2020; Shariati *et al*., 2020; Szymanek-Majchrzak *et al*., 2021).

Resistance to β-Lactams

Resistance to β-lactam primarily results from the production of β-lactamase (confers resistance to penicillins), and production of PBP2a (has a low affinity for β-lactams). The *mecA* and *mecC* genes within the *SCCmec* element are responsible for the synthesis of an altered PBP2a leading to the resistance of nearly all β-lactams (except newer agents such as ceftaroline), carbapenems or monobactams. The *mecC* homologue (*mecALGA251*) has been identified in isolates from humans and a wide range of animals across Europe, with cattle recognized as a major reservoir. On estimation, 80.00% - 90.00% *Staphylococcus* isolates in humans associated with hospital-acquired infections are methicillin-resistant coagulase-negative staphylococci (MR-CoNS), particularly *S. epidermidis*, and may act as a reservoir of resistant genes for *S. aureus*. It was noted that MR-CoNS are mostly isolated from food than MRSA (Podkowik *et al*., 2012; Gómez *et al*., 2016).

Resistance to aminoglycoside

Aminoglycoside resistance in *S. aureus* is largely mediated by clinically significant AMEs (ant(4')-I, acc(6')/aph(2"), and aph(3')-III), encoded by genes such as *aac(6')/aph(2")*, *aph(3')-IIIa*, *ant(4')-Ia*, and *ant(6')-I* (Ramirez *et al*., 2010; Perumal *et al*., 2016). These enzymes inactivate and compromise the activity of therapeutically important aminoglycosides such as gentamicin, kanamycin, and tobramycin by chemical modification, while reduced intracellular transport of these agents may also contribute to resistance (Klingenberg *et al*., 2004).

Resistance to tetracyclines

Resistance to tetracyclines in *Staphylococcus* commonly occurs through reduced intracellular drug accumulation mediated by active efflux systems encoded by the *tetK* and *tetL* genes, which produce membrane transport proteins. Inducible tetracycline resistance is typically plasmid-encoded, whereas constitutive resistance is associated with chromosomal genes such as *tetM* and *tetO*. These latter mechanisms do not involve efflux but instead confer resistance by protecting the ribosome from tetracycline binding. Efflux-mediated resistance also contributes to reduced susceptibility to fluoroquinolones and the trimethoprim–sulfamethoxazole combination (Emaneini *et al*., 2013).

Resistance to quinolones

Fluoroquinolone resistance in *Staphylococcus* primarily arises from mutations in DNA gyrase (topoisomerase II) and topoisomerase IV, enzymes essential for DNA replication. These mutations occur within the QRDR of the *gyrA*, *gyrB*, *parC*, and *parE* genes, resulting in amino acid substitutions that reduce drug binding (Yoshida *et al*., 1990). Resistance may also be acquired via plasmid-mediated gene transfer, allowing dissemination independent of chromosomal inheritance (Jacoby [(b)] *et al*., 2014). Additionally, overexpression of efflux pumps - Norfloxacin resistance protein A (NorA), norfloxacin resistance protein B (NorB), and norfloxacin resistance protein C (NorC), leads to a 4- to 8-fold increase in fluoroquinolone MICs. The NorA

primarily confers resistance to hydrophilic fluoroquinolones such as norfloxacin and ciprofloxacin, whereas NorB and NorC affect both hydrophilic and hydrophobic agents, including moxifloxacin (Truong-Bolduc *et al*., 2006).

Moxifloxacin resistance is generally lower than that to ciprofloxacin, making it a preferred option for treating *Staphylococcus* infections. Fluoroquinolone resistance in hospital-acquired *S. aureus* strains is frequently associated with methicillin resistance. The chloramphenicol–florfenicol resistance gene (*cfr*) gene encodes a methylase that modifies adenine at position 2503 in domain V of the 23S rRNA, resulting in cross-resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramins. Initially identified on plasmid pSCFS1 in *S. sciuri* isolated from cattle, *cfr* gene is often co-located with *erm* genes, contributing to MDR phenotypes (Schwarz *et al*., 2000; Wendlandt *et al*., 2013; Li [(a)] *et al*., 2017).

Resistance to macrolides, lincosamides, and streptogramin B (MLSB)

The resistance to macrolides, lincosamides, and streptogramin B antimicrobials (MLSB) in *Staphylococcus* spp. inhibits protein synthesis by binding to the 23S rRNA subunit at adenine positions 2058 or 2059. Resistance is primarily mediated by *erm* genes encoding rRNA methylases that modify the antimicrobial target site, thereby preventing drug binding (Schwarz *et al*., 2017). Numerous *erm* variants have been identified in *Staphylococcus*, including *ermA*, *ermB*, *ermC*, *ermF*, *ermG*, *ermQ*, *ermT*, *ermY*, *erm33*, *erm43*, and *erm48* (Wendlandt *et al*., 2015). The resistance to MLSBs may be constitutive or inducible, depending on whether methylase expression occurs continuously or only in the presence of an inducing agent. Multiple *erm* genes have been detected in MRSA strains from farm animals, most commonly combinations of *ermA* with *ermC* or *ermB* (Lüthje *et al*., 2007; Sudagidan *et al*., 2012). Methicillin-resistant strains frequently harbour additional resistance determinants, rendering them MDR (Kizerwetter-´Swida *et al*., 2016).

Epidemiological data indicate regional variability in resistance patterns across Europe, with higher resistance rates reported in southern and south-eastern regions. Although MRSA prevalence declined in Europe Union (EU) countries between 2013 and 2016, it remains a major public health concern, with over 25.00% prevalence reported in several countries (European Antimicrobial Resistance Surveillance Network (EARS-Net), 2022). Resistant *Staphylococcus* spp. is also prevalent in livestock, with transmission to humans occurring mainly through contaminated animal products (Pexara *et al*., 2020; Mourabit *et al*., 2021).

2.5.2.2. Antimicrobial resistance mechanisms in *Enterococcus* spp.

Enterococcus spp. poses a significant clinical challenge due to their intrinsic and acquired resistance to multiple antimicrobial classes and their ability to transfer resistance genes. Severe enterococcal infections are typically managed with combination therapy using a β-lactam (ampicillin or penicillin) and a synergistic aminoglycoside; however, vancomycin is often the last-line option when resistance emerges. For vancomycin-resistant enterococci (VRE), alternative agents include linezolid, daptomycin, quinupristin–

dalfopristin, and tigecycline (O'Driscoll *et al*., 2015; St˛epie´n-Py´sniak [(a)] *et al*., 2021). Widespread and irrational antimicrobial use has contributed to increasing multidrug-resistant *Enterococcus* strains (Leclercq, 1997; Urban-Chmiel *et al*., 2022).

Enterococcus spp. exhibit intrinsic resistance to β-lactams, all generations of cephalosporins, and sulphonamides, with relatively lower resistance to aminoglycosides, lincosamides, and quinolones. The resistance to β-Lactam is primarily mediated by low-affinity penicillin-binding proteins (PBP5 in *E. faecium* and PBP4 in *E. faecalis*), with variability in susceptibility among different β-lactams. Additional mechanisms include overproduction of PBP5, regulated by the PBP5 synthesis repressor (*psr*) gene in species such as *E. hirae*, and plasmid-mediated β-lactamase production, often linked with gentamicin resistance genes (Murray, 2000; Sifaoui *et al*., 2001; European Centre for Disease Prevention and Control (ECDC) Surveillance Atlas, 2018; Vincent *et al*., 2020; European Centre for Disease Prevention and Control (ECDC) Surveillance Atlas, 2021).

Resistance to glycopeptides

Resistance to glycopeptides arises from structural modification of peptidoglycan precursors, reducing vancomycin binding. Replacement of the terminal amino acid D-alanyl-D-alanine (D-Ala-D-Ala) with D-alanine-D-serine (D-Ala-D-Ser) or D-alanine, D-lactate (D-Ala-D-Lac) leads to varying resistance levels. Based on these mechanisms, seven vancomycin resistance phenotypes (VanA–VanL) have been described (Courvalin, 2006; Boyd *et al*., 2008). Clinically, VanA and VanB phenotypes are most significant. VanA confers high-level resistance to vancomycin and teicoplanin via D-Ala-D-Lac synthesis and is transferable through transposon Tn1546 present on a plasmid or chromosome (Courvalin, 2005; Courvalin, 2006, Kawalec *et al*., 2007). The VanB phenotype also involves D-Ala-D-Lac substitution but retains susceptibility to teicoplanin (Courvalin, 2005; Werner *et al*., 2008). VanC represents intrinsic low-level vancomycin resistance in motile species such as *E. gallinarum* and *E. casseliflavus* (Navarro *et al*., 1994). VanD, VanE, VanG, and VanL phenotypes are rare and associated with distinct genetic mechanisms and resistance profiles (McKessar *et al*., 2000; Patino *et al*., 2002; Depardieu *et al*., 2003; Boyd *et al*., 2008).

Resistance to aminoglycosides

Aminoglycoside resistance in *Enterococcus* spp. includes intrinsic low-level resistance due to reduced membrane permeability and acquired high-level resistance mediated by aminoglycoside-modifying enzymes (Leclercq *et al*., 1992; Ramirez *et al*., 2010). The most prevalent enzyme, *aac(6')-aph(2")*, encoded by *aac(6')-Ie-aph(2")-Ia*, confers resistance to most aminoglycosides (e.g., amikacin, kanamycin, netilmicin, and tobramycin) except streptomycin, and is responsible for high-level gentamicin resistance in over 90.00% of clinical isolates (Chow, 2000). High-level streptomycin resistance results from enzymatic modification or mutations in ribosomal protein S12, reducing drug binding (The European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2019).

Resistance to macrolides, lincosamides, and streptogramin B (MLSB)

Resistance to macrolides, lincosamides, and streptogramins is predominantly mediated by methylation of 23S rRNA through the erythromycin ribosomal methylase (*erm*) genes, particularly *ermB* and less frequently *erm-A*, producing the macrolide lincosamide-streptogramin B (MLSB) phenotype (Roberts, 2008). Additional mechanisms include enzymatic drug inactivation and ATP-dependent efflux. *E. faecalis* exhibits intrinsic resistance to clindamycin (lincosamides), dalfopristin and quinupristin (streptogramins A and B, respectively) due to expression of the lincosamide and streptogramin A (*lsa*) gene, which encodes an ABC transporter–like efflux system. Some *Enterococcus* spp., have the streptothricin acetyltransferase (*sat*) gene encoding acetyltransferases which inactivates streptogramin A. Previously in Texas, the *lsa* gene was detected in 180 (100%) *E. faecalis* strains and in none of 189 other *Enterococcus* spp., (Singh *et al*., 2002).

Resistance to tetracycline

Tetracycline resistance occurs via active efflux pumps resulting from the expression of *tetK* and *tetL* genes, and ribosomal protection proteins encoded by *tetM*, *tetO*, and *tetS* genes. The *tetM* gene, located on the chromosome is the most prevalent and is commonly transferred via conjugative transposons such as Tn916 (Bentorcha *et al*., 1991). The resistance to sulphonamides and trimethoprim involves reduced affinity of dihydropteroate synthase, overproduction of p-aminobenzoic acid, and bypass metabolic pathways.

Resistance to trimethoprim–sulfamethoxazole and quinolones

Notably, *Enterococcus* spp. can absorb exogenous folic acid, rendering trimethoprim–sulfamethoxazole ineffective (Chenoweth *et al*., 1990). On the other hand, resistance to fluoroquinolone results from chromosomal point mutations in genes encoding DNA gyrase (*gyrA*, *gyrB*) and topoisomerase IV (*parC*, *parE*), with resistance levels increasing when mutations occur in both targets. These mutations are non-transferable and correlate with antimicrobial exposure intensity (Jacoby, 2005; Boccella *et al*., 2021; St˛epie´n-Py´sniak [(b)] *et al*., 2021).

2.5.3. Resistance mechanisms of *Candida* spp.

The incidence of fungal infections has increased markedly since the 1980s, particularly among immunocompromised patients (Low *et al*., 2011; Puebla, 2012; Bongomin *et al*., 2017; Rodrigues *et al*., 2020). Although more than 17 *Candida* spp. can cause human infection, approximately 90.00% of invasive candidiasis cases are attributed to *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* (Bhattacharjee, 2016; Bongomin *et al*., 2017; Musa *et al*., 2017). *C. albicans* remains the leading cause of invasive disease, with clinical manifestations ranging from superficial mucosal infections to life-threatening systemic involvement (Gullo, 2009). Despite advances in antifungal therapy, the global rise in antifungal-resistant *Candida* isolates presents a significant clinical challenge and underscores the need for novel

therapeutic strategies (Alexander *et al*., 2013; Lockhart *et al*., 2017; Rodriguez *et al*., 2017; Maheronnaghsh *et al*., 2020).

The ICU is a high-risk environment for invasive candidiasis due to factors such as mucocutaneous colonization, disruption of host barriers through surgery or wounds, and the use of indwelling intravascular devices (Blumberg *et al*., 2001; Dimopoulos *et al*., 2007; Costa-de-Oliveira [(a)] *et al*., 2008). Currently, azoles/triazoles, polyenes, and echinocandins constitute the main classes of antifungal agents used as first-line therapy for invasive candidiasis (Wiederhold *et al*., 2003; Park *et al*., 2005; Pappas *et al*., 2009; Pappas *et al*., 2016).

Azoles remain widely used because of their efficacy, affordability, oral availability, and relatively low toxicity (Wiederhold *et al*., 2003; Hospenthal *et al*., 2004; Rodrigues *et al*., 2005; Costa-de-Oliveira [(b)] *et al*., 2008; Pappas *et al*., 2016). These agents inhibit ergosterol biosynthesis, leading to toxic accumulation of sterol precursors and impaired membrane integrity (Pedrosa *et al*., 2011). While azoles are fungistatic against *Candida* species, but fungicidal against *Aspergillus*, prolonged or repeated exposure has contributed to the emergence of resistance, particularly with fluconazole, the most commonly prescribed agent for invasive candidiasis (Blumberg *et al*., 2001; Wiederhold *et al*., 2003; Brion *et al*., 2007; Meyer *et al*., 2007; Kanafani *et al*., 2008; Peman *et al*., 2009). Newer triazoles such as voriconazole and posaconazole exhibit broader antifungal spectra, with structural differences contributing to enhanced activity against diverse fungal species (Xiao *et al*., 2004; Nagappan *et al*., 2007; Espinel-Ingroff *et al*., 2010).

Polyenes, including nystatin and amphotericin B, exert fungicidal activity by binding ergosterol and forming membrane pores that disrupt cellular integrity (Peman *et al*., 2009). Nystatin is primarily used topically and plays an important prophylactic role in vulnerable populations (Gotzsche *et al*., 2002; Howell *et al*., 2009; Chandrasekar, 2011). Amphotericin B remains the gold standard for severe invasive fungal infections, although its clinical utility is limited by nephrotoxicity. Lipid formulations improve tolerability but are costly, restricting their use to second-line or salvage therapy (Wingard *et al*., 2000; Lacerda *et al*., 2013).

5-Flucytosine, a pyrimidine analogue, is active against pathogenic yeasts through intracellular conversion to 5-fluorouracil, which disrupts DNA and RNA synthesis. Due to rapid resistance development, 5-fluorouracil is used in combination with amphotericin B rather than as monotherapy. Resistance in *Candida* spp. is commonly associated with mutations in uracil phosphoribosyltransferase (FUR1), impairing conversion to active metabolites (Akins *et al*., 2005; Peman *et al*., 2009).

Alterations in drug targets

Azole resistance in *C. albicans* commonly arises from amino acid substitutions in the drug target ergosterol 11 (Erg11), which reduce azole binding to the lanosterol demethylase enzyme (Revie *et al*., 2018).

More than 140 Erg11 substitutions have been linked to azole resistance, predominantly clustered within hotspot regions (amino acids 105–165, 266–287, and 405–488), although only a limited number have been experimentally confirmed to confer resistance (Morio *et al*., 2010; Xiang *et al*., 2013; Wu *et al*., 2017). Structural mapping shows that these mutations occur in critical functional regions of the enzyme, including the catalytic site, the fungus-specific external loop, and areas near the heme group (Flowers *et al*., 2015).

Similar mechanisms found in *C. albicans* have been identified in *Candida auris*. Whole-genome sequencing studies revealed key *Erg11* substitutions on the amino acid tyrosine (Y) with phenylalanine (F) at position 132 (Y132F), lysine (K) to arginine (R) substitution at position 143 (K143R), and phenylalanine (F) to leucine (L) at position 126 (F126L) have been associated with fluconazole resistance; with Y132 and K143 mutations detected in the majority of resistant isolates (Lockhart *et al*., 2017; Chowdhary *et al*., 2018; Lockhart *et al*., 2019). Functional studies using heterologous expression in *Saccharomyces cerevisiae* confirmed that the Y132F and K143R substitutions increase resistance to fluconazole and voriconazole (Healey *et al*., 2018). These *Erg11* mutations also show strong geographic clustering, with Y132F and K143R linked to South Asian and South American clades, and F126L associated with the African clade (Lockhart *et al*., 2017). In contrast, Erg11 mutations play a minimal role in azole resistance in *Candida glabrata*, with only a single resistant isolate reported to harbour an *Erg11* substitution (Brun *et al*., 2004; Hull *et al*., 2012; Salazar *et al*., 2018; Spettel *et al*., 2019).

In addition to structural alterations, *Erg11* overexpression contributes significantly to azole resistance in *C. albicans* by increasing target abundance (Robbins *et al*., 2017; Revie *et al*., 2018). This overexpression is largely driven by gain-of-function mutations in the transcriptional regulator uptake control 2 protein (Upc2), which lead to constitutive activation of ergosterol biosynthesis genes, elevated ergosterol levels, and reduced fluconazole susceptibility (Silver *et al*., 2004; MacPherson *et al*., 2005; Dunkel [(a)] *et al*., 2008; Heilmann *et al*., 2010; Hoot *et al*., 2011; Flowers *et al*., 2012; Revie *et al*., 2018). Disruption of *Upc2* reverses *Erg11* overexpression and restores azole susceptibility, underscoring its central role in resistance (Vasicek *et al*., 2014). A similar regulatory mechanism has been observed in *C. glabrata*, where disruption of *Upc2A* decreases ergosterol content and increases susceptibility to sterol biosynthesis inhibitors (Whaley *et al*., 2014).

Overexpression of efflux pumps in azoles

Overexpression of plasma membrane efflux pumps is a major contributor to azole resistance across multiple fungal pathogens. Two main efflux pump families are implicated: ATP-binding cassette (ABC) transporters, which use ATP hydrolysis to export antifungal agents, and major facilitator (MF) transporters, which rely on the proton gradient for drug efflux (Coleman *et al*., 2009).

In *Candida albicans*, azole resistance is frequently associated with the overexpression of the ABC transporters *Candida* drug resistance 1 and 2 (Cdr1 and Cdr2), particularly in patients undergoing prolonged antifungal therapy (Robbins *et al*., 2017; Revie *et al*., 2018). The deletion of *Cdr1*gene leads to a 4–8-fold reduction in azole resistance, whereas *Cdr2* gene deletion has a weaker effect (Tsao *et al*., 2009). Expression of both genes is regulated by the transcriptional activator 1 (Tac1), which binds drug-response elements in their promoters. Gain-of-function mutations in *Tac1* result in constitutive overexpression of *Cdr1* and *Cdr2*, thereby conferring azole resistance (Coste *et al*., 2006). A similar mechanism operates in *C. glabrata*, where upregulation of *Cdr1* and *Cdr2* contributes to azole resistance, and combined disruption of these transporters markedly increases susceptibility (Sanglard *et al*., 2001).

In *C. glabrata*, the ABC transporter Snq2 also plays a significant role in azole resistance (Torelli *et al*., 2008). Regulation of these transporters is mediated by the zinc cluster transcription factor pleiotropic drug resistance 1 (Pdr1), which binds pleiotropic drug-response elements in target gene promoters (Paul *et al*., 2011; Paul *et al*., 2014). Gain-of-function mutations in *Pdr1* represent the most common mechanism of clinical azole resistance in *C. glabrata*, leading to increased expression of multiple efflux pumps (Tsai *et al*., 2006; Vermitsky *et al*., 2006; Caudle *et al*., 2011). The mediator complex, particularly interaction with the Gal11A/Med15A subunit, is essential for *Pdr1*-driven transcription, while in *C. albicans*, mediator tail module components are required for full Tac1-mediated *Cdr1* expression and azole resistance (Thakur *et al*., 2008; Liu [(b)] *et al*., 2017).

Among MF transporters, fluconazole resistance in *C. albicans* is primarily associated with multidrug resistance protein 1 (Mdr1), (Wirsching *et al*., 2000; Gaur *et al*., 2008). The Mdr1 expression is controlled by the transcription factor Mrr1, with activating mutations in multidrug resistance regulator 1 gene (*Mrr1)* leading to increased azole resistance, while *Mrr1* deletion restores fluconazole susceptibility (Morschhauser *et al*., 2007; Dunkel [(b)] *et al*., 2008). The switching defective/sucrose non-fermenting (Swi/Snf) chromatin remodelling complex further modulates this pathway, as deletion of sucrose non-fermenting 2 (*Snf2)* in *Mrr1* gain-of-function strains significantly reduces *Mdr1* expression and fluconazole resistance (Liu [(c)] *et al*., 2017). In contrast, the *C. glabrata Mdr1* homologue, fluconazole resistance 1 gene (*Flr1*), is not strongly linked to azole resistance, a pattern observed in several non-*albicans Candida* spp., (Chen *et al*., 2007; Coleman *et al*., 2009).

In *Candida auris*, genomic analyses have identified numerous ABC and MF transporters homologous to those in *C. albicans*, including *Cdr1*, *Cdr2*, *Cdr4*, *Mdr1*, and two copies of sensitivity to 4-Nitroquinoline-N-Oxide 2 (*Snq2*), (Chatterjee *et al*., 2015; Munoz *et al*., 2018). Functional studies demonstrate a central role for *Cdr1* in azole resistance, as deletion of *Cdr1* significantly reduces azole MICs and increases fluconazole sensitivity by up to eight-fold (Kim *et al*., 2019; Rybak *et al*., 2019). Efflux-mediated resistance is also prominent in *C. auris* biofilms, where transcriptomic analyses revealed upregulation of both ABC and MF transporters, and the use of efflux pump inhibitors enhanced fluconazole activity by 4- to16-fold (Kean *et al*., 2018).

The *C. auris* genome also encodes multiple transcription factors involved in efflux regulation, including two copies of *Tac1* and *Mrr1*, suggesting enhanced or altered regulatory control of efflux pump expression. Notably, resistance-associated mutations in *Tac1B* emerge rapidly under fluconazole exposure and have been detected in clinical isolates. Additionally, removal of one *Mrr1* allele raises the possibility of species-specific regulatory differences that may contribute to constitutive efflux pump overexpression and azole resistance in *C. auris* (Munoz *et al*., 2018; Montoya *et al*., 2019; Rybak *et al*., 2020).

Modulation of stress responses in azoles

Azole resistance in *Candida albicans* can arise through modulation of cellular stress responses, particularly via alterations in the ergosterol biosynthesis pathway. Loss-of-function mutations in *Erg3*, which encodes the Δ-5,6-desaturase, prevent accumulation of the toxic sterol intermediate 14-α-methyl-3,6-diol produced following Erg11 inhibition by azoles, thereby allowing fungal survival through incorporation of alternative sterols into the cell membrane. Several missense and nonsense mutations in *Erg3* have been associated with azole resistance in *C. albicans* clinical isolates (Morio *et al*., 2012; Spettel *et al*., 2019).

A central regulator of stress-induced azole tolerance and resistance is the molecular chaperone heat shock protein 90 (Hsp90), which stabilizes a wide range of client proteins involved in signal transduction and transcriptional regulation (Jarosz *et al*., 2010; Taipale *et al*., 2010; Taipale *et al*., 2012; Cowen *et al*., 2013; O'Meara *et al*., 2017). The Hsp90 promotes the rapid evolution of azole resistance across diverse fungi, as its genetic or pharmacological inhibition reduces azole tolerance and abolishes resistance (Cowen *et al*., 2005; Cowen *et al*., 2009). In both *C. albicans* and *Saccharomyces cerevisiae*, azole resistance arising from *Erg3* loss-of-function is Hsp90-dependent, while in *Candida auris*, Hsp90 governs azole tolerance in susceptible strains exposed to membrane stress. However, resistance mediated by efflux pump overexpression in *C. albicans* and *C. auris* occurs independently of Hsp90 (Cowen *et al*., 2005; Kim *et al*., 2019).

The Hsp90 exerts its effects largely through regulation of key signaling pathways, notably via stabilization of the calcium-calmodulin-activated phosphatase calcineurin (Cowen *et al*., 2005; Singh *et al*., 2009). Azole exposure activates calcineurin-dependent stress responses, and disruption of either calcineurin or Hsp90 results in heightened azole sensitivity, demonstrating their functional interdependence in resistance regulation (Cruz *et al*., 2002; Singh *et al*., 2009). In addition, the protein kinase C (PKC)– mitogen-activated protein kinase (MAPK) pathway, involving Pkc1, bypass of C kinase 1 (Bck1), mitogen-activated protein kinase 1/2 (Mkk1/2), and map kinase from *C. albicans* 1 (Mkc1), contributes to azole stress responses in *C. albicans*. Disruption of this signaling cascade phenocopies Hsp90 or calcineurin inhibition, while Hsp90 depletion destabilizes multiple PKC-MAPK components, underscoring its central regulatory role (LaFayette *et al*., 2010).

Other kinases further modulate azole stress responses. Casein kinase 2 (CK2) contributes to fluconazole resistance in a calcineurin-dependent manner, and directly regulates Hsp90 activity through phosphorylation (Bruno *et al*., 2005; Mollapour *et al*., 2011; Diezmann *et al*., 2012). Target of rapamycin (TOR) signaling also influences azole resistance, as pharmacological inhibition of TOR abrogates *Erg3*-mediated resistance (Robbins *et al*., 2010). The natural product beauvericin potentiates azole activity by simultaneously inhibiting efflux, suppressing TOR signaling, activating CK2, and impairing Hsp90 function, highlighting the interconnected nature of kinase-mediated stress pathways (Bruno *et al*., 2005; Mollapour *et al*., 2011; Diezmann *et al*., 2012; Shekhar-Guturja *et al*., 2016; Khandelwal *et al*., 2018).

Post-translational modifications further regulate azole tolerance. Lysine acetyl-transferases (KATs) and lysine deacetylases (KDACs) modulate chromatin structure, transcription, and protein function, including Hsp90 activity (Revie *et al*., 2018; Robbins *et al*., 2016; Robbins *et al*., 2017). In *C. albicans*, multiple KDACs contribute redundantly to azole resistance, and their inhibition increases drug susceptibility (Robbins *et al*., 2012; Li [(b)] *et al*., 2017). The KDAC inhibitor MGCD290 exhibits synergistic activity with azoles against resistant *C. albicans* isolates and has demonstrated efficacy in in vivo and early clinical studies when combined with fluconazole (Pfaller *et al*., 2009; Besterman *et al*., 2012; Pfaller *et al*., 2015). In *C. glabrata*, chromatin remodeling and sirtuin-family KDACs similarly influence azole tolerance, while impairment of the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex component adenosine aminohydrolase 2 (Ada2 - also known as adenosine deaminase) in *C. albicans* reduces efflux pump induction and enhances fluconazole sensitivity (Sellam *et al*., 2009; Orta-Zavalza *et al*., 2013).

Environmental pH also modulates azole tolerance, as acidic conditions reduce *C. albicans* growth at supra-MIC azole concentrations (Cornet *et al*., 2014). The pH sensing is mediated by the conserved Rim signaling pathway, which regulates genes involved in cell wall integrity, morphogenesis, adhesion, and biofilm formation (Shapiro *et al*., 2011). Multiple Rim pathway components contribute to azole tolerance, with Hsp90 acting downstream of Rim signaling to influence antifungal stress responses (Garnaud *et al*., 2018).

Modulation of stress responses in polyenes

Resistance to polyenes in *Candida* spp. is rare but, when present, is primarily driven by alterations in ergosterol biosynthesis that reduce drug binding or deplete membrane ergosterol (Ellis, 2002). In *C. albicans*, reduced susceptibility to amphotericin B is associated with mutations in *Erg2*, *Erg3*, *Erg5*, and *Erg11* (Sanglard *et al*., 2003; Vandeputte *et al*., 2008; Jensen *et al*., 2015), while in *C. glabrata*, resistance has been linked to mutations in *Erg2*, *Erg6*, and *Erg11*(Vandeputte *et al*., 2008; Hull *et al*., 2012). In *C. auris*, resistant isolates exhibit increased expression of ergosterol pathway genes, including *Erg1*, *Erg2*, *Erg6*, and *Erg13*, following amphotericin B exposure. Additionally, prior azole treatment may enhance polyene resistance by lowering cellular ergosterol levels (Munoz *et al*., 2018).

Polyene resistance is further influenced by modulation of cellular stress responses, despite significant fitness costs. Loss-of-function mutations in *Erg3* confer cross-resistance to polyenes and azoles in *C. albicans* due to reduced ergosterol content. Maintenance of amphotericin B resistance is dependent on Hsp90 function, as inhibition of Hsp90 reverses resistance in *C. albicans* and *C. tropicalis*. Resistant strains also show constitutive activation of stress-response pathways regulated by calcineurin, protein kinase C-like 1(Pkc1), and high-osmolarity glycerol 1 (Hog1), alongside enhanced antioxidant defenses that mitigate oxidative damage by reducing reactive oxygen species and increasing catalase production (Gonzalez-Parraga *et al*., 2011; Vincent *et al*., 2013; Mesa-Arango *et al*., 2014).

Modulation of stress responses in echinocandins

Echinocandin resistance in *Candida* species is predominantly mediated by mutations in the 1,3-β-D-glucan synthase component (*Fks*) genes, which encode subunits of the β-1,3-glucan synthase complex. In *C. albicans*, resistance arises from mutations in *Fks1*, particularly within two conserved hot-spot regions (amino acids 641–649 and 1357–1364), leading to reduced glucan synthase sensitivity, elevated MICs, and cross-resistance to echinocandins. Substitutions at S645 are most frequently observed and confer the strongest resistance phenotype (Park *et al*., 2005; Perlin, 2007; Garcia-Effron *et al*., 2008; Garcia-Effron [(a)] *et al*., 2009; Garcia-Effron [(b)] *et al*., 2009). Although the roles of *Fks2* and *Fks3* are less defined in *C. albicans*, their deletion results in increased *Fks1* expression and reduced echinocandin susceptibility (Suwunnakorn *et al*., 2018).

In *C. glabrata*, echinocandin resistance involves mutations in both *Fks1* and *Fks2*, with *Fks2* being more highly expressed and more frequently mutated. The most common substitution occurs at S663 in *Fks*2, corresponding to *C. albicans* S645 (Katiyar *et al*., 2006; Garcia-Effron [(a)] *et al*., 2009). In *C. auris*, resistance has been linked to substitutions at S639 in *Fks1*, including phenylalanine, proline, and tyrosine variants, which have been shown to confer echinocandin resistance and reduce treatment efficacy *in vivo* (Chowdhary *et al*., 2018; Rhodes *et al*., 2018).

Unlike azoles, efflux pump overexpression plays a minimal role in resistance to echinocandins and polyenes, likely due to their extracellular sites of action and poor compatibility with transporter binding pockets (Cannon *et al*., 2009; Robbins *et al*., 2016; Perfect, 2017). However, *C. auris* may exhibit an atypical mechanism of amphotericin B resistance involving mutations in membrane transporters rather than ergosterol biosynthesis genes, though the functional relevance of this mechanism remains unclear (Escandon *et al*., 2018).

Echinocandin exposure also triggers adaptive stress responses that modulate tolerance and resistance. Cell wall damage activates Rho1-mediated signaling pathways, including PKC-MAPK**,** calcineurin, and high-osmolarity glycerol, which promote compensatory cell wall remodeling, notably increased chitin synthesis.

This compensatory response contributes to the paradoxical growth observed at high echinocandin concentrations in *C. albicans* (Levin, 2005; Stevens *et al*., 2006; Munro *et al*., 2007; Walker *et al*., 2008; Lee *et al*., 2012; Walker *et al*., 2013). Similar signaling pathways underpin basal echinocandin tolerance in *C. glabrata* (Cota *et al*., 2008; Singh-Babak *et al*., 2012).

The molecular chaperone Hsp90 plays a central role in echinocandin tolerance and resistance by stabilizing calcineurin and PKC-MAPK pathway components. Genetic or pharmacological inhibition of Hsp90 enhances echinocandin activity and reverses resistance in *C. albicans*, *C. glabrata*, and *Aspergillus fumigatus*, including strains harboring Fks mutations (Cowen *et al*., 2009; Singh *et al*., 2009; Lamoth *et al*., 2012; Singh-Babak *et al*., 2012). Additional regulatory factors, such as the transcription factor caspofungin sensitivity protein 5 (Cas5) and the chaperonin complex TCP (CCT), further link cell wall stress responses, cytoskeletal integrity, and echinocandin resistance, highlighting a complex network of tolerance mechanisms beyond target-site mutations (Bruno *et al*., 2006; Xie *et al*., 2017; Caplan *et al*., 2018).

Genomic modifications

Fungal pathogens exhibit substantial genomic plasticity, enabling rapid adaptation to antifungal pressure through large-scale genomic alterations such as aneuploidy, loss of heterozygosity (LOH), and chromosomal rearrangements that modulate the expression of drug targets and resistance determinants. Aneuploidy provides a reversible mechanism for generating genetic and phenotypic diversity, particularly under antifungal stress (Selmecki *et al*., 2010; Chen [(b)] *et al*., 2012).

Azole exposure is a major driver of genomic instability, promoting aberrant cell cycle events and aneuploidy formation (Harrison *et al*., 2014). In *C. albicans*, azole resistance is frequently associated with a segmental aneuploidy of chromosome 5, specifically the isochromosome 5 (i5), which increases copy numbers of *Erg11* and *Tac1* and is closely linked to the acquisition and loss of azole resistance (Selmecki *et al*., 2006; Coste *et al*., 2007; Selmecki *et al*., 2008). Variant forms of (i5), including those incorporating regions of chromosome 3 containing *Mrr1*, further enhance resistance (Selmecki *et al*., 2009). Additional aneuploidies involving chromosomes 3, 4, and 6 also contribute to azole resistance, with chromosome 4 amplification repeatedly identified during experimental evolution under azole pressure (Hill *et al*., 2013; Anderson *et al*., 2017; Hirakawa *et al*., 2017). Similar adaptive chromosomal changes have been described in *C. glabrata*, where increased *Erg11* copy number arising from aneuploidy is associated with fluconazole resistance (Polakova *et al*., 2009).

The LOH events, occurring over localized regions or entire chromosomes, further potentiate resistance by fixing gain-of-function mutations. In *C. albicans*, LOH involving the transcriptional regulators *Tac1* and *Mrr1* is commonly observed in azole-resistant isolates (Coste *et al*., 2006; Dunkel [(b)] *et al*., 2008).

In contrast, chromosomal alterations contribute less frequently to echinocandin and polyene resistance. However, rare mechanisms have been reported, including stepwise acquisition of *Fks1* hot-spot mutations followed by LOH during the development of echinocandin resistance in *C. albicans* (Niimi *et al*., 2010). The chromosome copy number changes also influence echinocandin susceptibility, with chromosome 5 monosomy reducing β-1,3-glucan levels and increasing chitin content, and chromosome 2 trisomy decreasing caspofungin susceptibility via calcineurin-dependent pathways independent of calcineurin-responsive zinc finger protein 1 (Crz1), (Yang *et al*., 2013; Yang [b] *et al*., 2019).

In *C. auris*, replicative aging is associated with increased tolerance to fluconazole, micafungin, and amphotericin B. This phenotype correlates with transient gene duplications leading to increased expression of resistance determinants such as *Erg11* and *Cdr1*, although the chromosomal basis of these duplications remains unclear (Bhattacharya *et al*., 2019). Further genomic characterization is required to clarify the role of chromosomal rearrangements in antifungal resistance in this emerging pathogen (Lee *et al*., 2021).

Intrinsic antifungal resistance

Intrinsic resistance to antifungal agents is well documented among several *Candida* spp. and specific growth states. Intrinsic azole resistance is prominent in non-*albicans Candida*, particularly *C. glabrata*, *C. krusei*, and *C. auris*. *C. glabrata* exhibits marked intrinsic resistance to fluconazole, rapidly developing stable resistance phenotypes even in the absence of prior azole exposure (Ferrari [a] *et al*., 2001; Borst *et al*., 2005). This resistance is largely mediated by constitutive and inducible overexpression of ABC efflux transporters, including the pleiomorphic drug resistance homolog 1 (Pdh1), yeast oligomycin resistance protein 1 (Yor1), Cdr1 and Snq2) under the control of the transcription factor Pdr1 (Ferrari [a] *et al*., 2001; Sanglard *et al*., 2001; Torelli *et al*., 2008). Mitochondrial dysfunction in *C. glabrata* "petite mutants" further enhances azole resistance through upregulation of efflux pumps (Ferrari [b] *et al*., 2001; Tsai *et al*., 2006). Additionally, *C. glabrata* can alter membrane sterol composition and actively import exogenous sterols, allowing it to bypass fluconazole-induced inhibition of ergosterol biosynthesis (Zavrel *et al*., 2013).

In *C. krusei*, innate azole resistance is less well defined but is thought to involve reduced susceptibility of Erg11 to azole inhibition and constitutive expression of efflux pumps, including Abc1, whose inhibition restores azole sensitivity (Lamping *et al*., 2009). *C. auris* displays exceptionally high rates of azole resistance, with over 90.00% of isolates exceeding clinical MIC breakpoints. Resistance-associated Erg11 substitutions, such as Tyrosine (Y) with Phenylalanine (F) at position 132 (Y132F) and Lysine (K) to Arginine (R) substitution at position 143 (K143R), have been identified, although the evolutionary origins and drivers of widespread resistance in this species remain under investigation (Chowdhary *et al*., 2018; Lockhart *et al*., 2019; Montoya *et al*., 2019; Rhodes *et al*., 2019).

Intrinsic resistance to echinocandins is observed in *Candida parapsilosis* and related species (*C. orthopsilosis* and *C. metapsilosis*), which show naturally reduced susceptibility due to a conserved amino acid change from Proline (P) to Alanine (A) at position 660 (P660A) substitution in *Fks1* (Garcia-Effron *et al*., 2008; Pfaller *et al*., 2008). This mutation decreases glucan synthase affinity for echinocandins and is associated with elevated cell wall chitin content, contributing to reduced drug susceptibility. Clinically, this intrinsic resistance has been implicated in breakthrough *C. parapsilosis* infections during echinocandin therapy (Cheung *et al*., 2006).

Beyond species-level resistance, biofilm-associated intrinsic resistance represents a major clinical challenge. *Candida* biofilms—particularly those formed by *C. albicans* on medical devices—exhibit inherent multidrug resistance despite planktonic cells being largely susceptible (Douglas *et al*., 2003; Mukherjee *et al*., 2003; Blankenship *et al*., 2006; Mayer *et al*., 2013). Biofilm cells overexpress efflux pumps (*Cdr1*, *Mdr1*) and produce a β-1,3-glucan–rich extracellular matrix that sequesters antifungals, limiting drug penetration and conferring resistance to azoles, echinocandins, and polyenes. Regulation of biofilm-associated glucan synthesis involves Hsp90 and the PKC-MAPK pathway, and disruption of Hsp90 restores azole susceptibility in in vivo biofilm infection models, highlighting potential therapeutic targets for combating biofilm-mediated infections (Ramage *et al*., 2002; Mukherjee *et al*., 2003; Nett *et al*., 2007; Nett [(a)] *et al*., 2010; Nett [(b)] *et al*., 2010; Robbins *et al*., 2011; Revie *et al*., 2018).

2.6. Antimicrobial susceptibility and resistance patterns of ICU-BSIs in low-middle, and high-income countries.

Many MDROs originate in low- and middle-income countries where there is insufficient control over the spread of infections. These nations have turned into the focal points of pandemics involving antimicrobial resistance (Saharman *et al*., 2021). According to a 2014 Wellcome Trust assessment, these nations are very vulnerable to increased morbidity and mortality as a result of resistant infections (Wellcome Trust, 2014). Highly resistant carbapenemase-producing Gram-negative bacteria pose a serious threat as they have been spreading worldwide (Castanheira *et al*., 2011; Szekely *et al*., 2013; Linciano *et al*., 2019; Liu *et al*., 2019).

2.6.1. Lower-middle income countries (LMIC)

In an Indian ICU study, all *P. aeruginosa* isolates were MDR (resistant to amikacin and ciprofloxacin combination, cefepime, cefoperazone, cefotaxime, ceftazidime, chloramphenicol, gentamicin, levofloxacin, meropenem, tetracycline, and tobramycin). Comparatively low resistance levels to colistin, piperacillin, and the combination of piperacillin-tazobactam were noted. The predominance of ESBL and Amp C production was observed in all *E. coli* and *K. pneumoniae* isolates, while *P. aeruginosa* (83.33%) and *A. baumannii* (50.00%) produced metallo-β-lactamase. *A. baumannii* isolates (33.33%) expressed pandrug resistance (PDR), while the rest were MDR. Methicillin resistance was seen in 75.00% of *S. epidermidis* isolates, while they were sensitive to vancomycin, linezolid and teicoplanin. Conversely, *S. aureus* strains were susceptible to methicillin and to other antimicrobials (Jangale *et al*., 2017). (**Table 7**).

In another Indian ICU study, a total number of 62.00% MDRs was isolated from BSIs. *K. pneumoniae* (38.07%), *E. coli* (25.85%), and *A. baumannii* complex (16.01%) were the most frequently isolated MDR Gram-negatives. Extreme/extensive drug-resistance (XDR) was identified in *E. coli* (56.00%), *A. baumannii* complex (25.85%), and in all *Pseudomonas* species. The remainder were identified as ESBL producers. All identified XDR strains were susceptible to colistin and aminoglycosides, while ESBL producing isolates were mostly susceptible to carbapenems (88.09%) and, piperacillin-tazobactam (85.70%), (Banerjee *et al*., 2020). (**Table 7**).

2.6.2. Middle-income countries (MIC)

Findings from an observational study in Morocco, reported that *Pseudomonas* isolates were resistant to ciprofloxacin (100%), netilmicin (40.00%), ticarcillin (33.03%), amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin, tobramycin, piperacillin-tazobactam (each 20.00%), (Lachhab *et al*., 2017). All *Acinetobacter* spp. displayed sensitivity to colistin, while all being resistant to imipenem due to carbapenemase production (Lachhab *et al*., 2017). This resistance rate was higher than in other previous studies (Picot-Gueraud *et al*., 2015). Approximately 20.00% of *S. aureus* isolates were MRSA while all Gram-positive isolates displayed susceptibility to glycopeptides (Lachhab *et al*., 2017). In contrast, a Nigerian ICU study reported 80.00% MRSA, while 57.01% of Gram-negative isolates were MDR (Iwuafor *et al*., 2016). (**Table 7**).

In Uganda, high susceptibility rates for *K. pneumoniae* were to amikacin, and imipenem (both 100%), while none were susceptible to other antimicrobials (Agaba *et al*., 2017). These findings were similar in USA amongst ICU patients (Endimiani *et al*., 2004; Paterson *et al*., 2004; Lockhart *et al*., 2007).

S. aureus displayed the highest susceptibility to gentamicin, tetracycline, and vancomycin (all 100%), followed by oxacillin (66.66%). Both chloramphenicol and ciprofloxacin had the lowest susceptibility rate (33.33%), (Agaba *et al*., 2017). (**Table 7**).

An ICU-BSI study in Turkey reported MRSA in 61.54% *S. aureus* isolates. The ESBL rate in *E. coli* and *Klebsiella* spp., was (48.72%), (Dereli *et al*., 2013). In a Brazilian ICU-BSI study, oxacillin-resistant CoNS (87.04%), carbapenem-resistant *A. baumannii* (65.01%), and broad-spectrum cephalosporin-resistant *Klebsiella* spp. (70.00%) were the major phenotypes identified (Sabino *et al*., 2020). (**Table 7**).

A Colombian study (from January 2001 to June 2008) reported carbapenem resistance in *K. pneumoniae* (3.00%) isolates, but the recovery of *K. pneumoniae* susceptibility to 3GC was also noted. *A. baumannii* demonstrated resistance to multiple antimicrobials (imipenem, ampicillin-sulbactam, and ceftazidime); however, an increased rate of *A. baumannii* resistance to carbapenems was reported in 2001 from 10.05% to

49.05% in 2008. Increasing ciprofloxacin resistance was observed in *E. coli* isolates, ranging from 20.00% to 30.00%, whereas *P. aeruginosa* demonstrated resistance to ciprofloxacin, ceftazidime, cefepime, piperacillin-tazobactam, meropenem, and imipenem during the observation period. Over 70.00% resistance to oxacillin was observed in CoNS isolates. Four percent *E. faecalis* were resistant to vancomycin, although, a reduction in high-level gentamicin resistance was recorded from 25.00% (2001 to 2002), to less than 13.00% (2005 to 2007), (Cortes *et al*., 2013). (**Table 7**).

The findings established from a study in the ICU of Groote Schuur Hospital in South Africa reported vancomycin sensitivity on all *Enterococcus* isolates. *S. aureus* was susceptible to clindamycin (55.06%), and cloxacillin (52.04%). Enterobacterales were susceptible to ciprofloxacin (70.00%), gentamicin (64.06%), and amoxicillin/clavulanate (41.04%). In ESBL-producing isolates, 85.04% were susceptible to amikacin. *P. aeruginosa* isolates expressed susceptibility to colistin (91.07%), ceftazidime (72.02%), and relatively low susceptibility rates to imipenem and ciprofloxacin (both 44.04%). *A. baumannii* complex demonstrated high susceptibility levels to colistin (96.06%) and tobramycin (72.01%). Low susceptibility rates were to gentamicin (39.00%), ceftazidime (20.07%), meropenem (18.06%), and imipenem (18.04%), (McKay *et al*., 2015). (**Table 7**).

A Mexican ICU-BSI study reported high resistance rates exhibited by Gram-negative isolates to ampicillin (95.85%), cefuroxime (84.17%), piperacillin (82.93%), cefotaxime (78.07%), ceftriaxone (77.41%), aztreonam (75.23%), cefazolin (75.00%), and ceftazidime (73.19%), (Uc-Cachón *et al*., 2019). (**Table 7**).

2.6.3. High-income countries (HIC)

A Canadian study reported *S. aureus* to be susceptible to trimethoprim–sulfamethoxazole (95.00%), methicillin (76.04%), and fluoroquinolones (73.04%). CoNS was susceptible to methicillin (32.09%). Enterobacterales demonstrated high susceptibility to carbapenems (98.06%), and aminoglycosides (90.09% to 97.08%), while non-Enterobacterales expressed susceptibility to carbapenems (80.09%), (Savage *et al*., 2016). (**Table 7**).

2.7. Antimicrobial therapy

The administration of the appropriate antibiotic therapy in the early stages of infection, more especially after blood culture results is essential in patients with ICU–acquired BSIs (Timsit *et al*., 2012; Nishie, 2013). The early prescription of an effective antimicrobial treatment has proven to reduce the mortality rate, and improve clinical outcomes, particularly in severely ill patients (Kumar *et al*., 2006; Kumar *et al*., 2009; Micek *et al*., 2011; Zilberberg *et al*., 2014). It was previously reported that inadequate antimicrobial treatment significantly increases the mortality rates amongst critically ill ICU patients (Ibrahim *et al*., 2000).

For adequate empiric coverage, a thorough evaluation of the presence of risk factors for the acquisition of a BSI sustained by MDR pathogens, together with the knowledge of the local epidemiology and resistance patterns is important; as resistance rates differ between countries and institutions (Bassetti [(b)] *et al*., 2012; Dimopoulos *et al*., 2015). The colonization history of MDR pathogens should also be considered, as its likelihood increases the risk of acquisition of an infection sustained by the same pathogen (Giannella *et al*., 2014; Nesher *et al*., 2015). Additionally, effective therapy is dependent on precise and timely organism identification, as well as testing for phenotypic and genotypic antimicrobial susceptibility and resistance mechanisms (Ribas *et al*., 2007; Brink *et al*., 2022).

2.7.1. Treatment options for Gram-negative BSIs

Targeted antimicrobials such as aminoglycosides (plazomicin), carbapenem/β-lactamase inhibitors (meropenem-vaborbactam), and cephalosporins (cefiderocol) are used to treat Gram-negative bacterial infections. Combination therapies or specialized antimicrobials like polymyxins (colistin, polymyxin B) are frequently used for treatment due to the rising MDR strains. The particular antimicrobial used depends on the type of bacteria; for resistant cases, ceftazidime-avibactam is a possibility (Bassetti *et al*., 2019). There are limited therapeutic alternatives available since Gram-negative bacteria are becoming more resistant to traditional antimicrobials. Novel medicines, like those based on nanoparticles, are being investigated to fight MDR strains (Bhowmik *et al*., 2023).

Treatment options for *A. baumannii*

Carbapenems were primarily the treatment of choice against infections caused by *A. baumannii* (Garnacho-Montero *et al*., 2015). Previous North American and European studies reported markedly higher susceptibility rates to carbapenems and quinolones (Pfaller *et al*., 2001). However, due to high resistance of *A. baumannii* to carbapenems, polymyxins (colistin or polymyxin B) have become an important therapeutic option (Garnacho-Montero *et al*., 2015).

Colistin (polymyxin E) and polymyxin B are the two approved agents for clinical use (Akajagbor *et al*., 2013). Colistin is widely used for the treatment of *A. baumannii* than polymyxin B because of its availability worldwide. In a randomized trial amongst patients infected by polymyxin B susceptible *A. baumannii*, colistin-meropenem combination showed no significant advantage over colistin monotherapy with regards to clinical and microbiological cure, patient survival, and, or development of resistance in severe carbapenem-resistant bacteria infections (Paul *et al*., 2018).

Acinetobacter baumannii as a species is generally susceptible to tigecycline (a glycylcycline antimicrobial derived from minocycline), (Pachon-Ibanez *et al*., 2004; Denys *et al*., 2013). Resistance to this agent is relatively rare, although it may occur through overexpression of efflux pumps upon exposure (Peleg *et al*.,

2007; Ruzin *et al*., 2007; Rumbo *et al*., 2013). Tigecycline along with colistin is more effective *in vitro* against *A. baumannii* isolates, as well as MDR strains, and may be an alternative agent in treating XDR *A. baumannii* infections (Souli *et al*., 2006; Dizbay *et al*., 2008; Souli *et al*., 2008).

Treatment for ESBL-producing Enterobacterales

Numerous international observational studies including Australia, Asia, Africa, Europe, North and South America have suggested that β-lactam/β-lactamase inhibitor combinations (BLICs) such as piperacillin-tazobactam remain clinically active *in vitro* against infections caused by ESBL-producing Gram-negative pathogens, although results have been controversial (Schuetz *et al*., 2018; Harris *et al*., 2018; Hayden *et al*., 2018).

According to the Merino clinical study, which tested the effects of piperacillin-tazobactam against meropenem for the treatment of BSIs in patients with ceftriaxone-resistant *E. coli* or *K. pneumoniae*; treatment with piperacillin-tazobactam became a convenient option, as it did not result in a non-inferior 30-day mortality in those patients (Harris *et al*., 2018; Hayden *et al*., 2018). This was in agreement with previous comparative studies that supported the role of BLICs therapy for the treatment of critical ESBL-infections (Gutiérrez-Gutiérrez [(a)] *et al*., 2016; Tamma *et al*., 2017). Thus, piperacillin-tazobactam may be a direct agent for BSI therapy if the criteria are met, dose is optimized, and response to treatment is well monitored (Brink *et al*., 2022).

Ertapenem remains a suitable agent against ESBL Gram-negative bacilli infections (including BSIs) for its advantage of not inducing increased resistance in *P. aeruginosa* (Brink *et al*., 2004; Nicolau *et al*., 2012; McDougall *et al*., 2013; Lew *et al*., 2015). Comparable results of ertapenem and the other broader spectrum carbapenems were previously noted in empiric and targeted therapy of mono-microbial BSIs due to ESBL-producing pathogens (Gutierrez-Gutierrez [(b)] *et al*., 2016).

The class two carbapenems (imipenem or meropenem), instead of ertapenem have been recommended for severe BSIs caused by ESBL-producing Enterobacterales (Paul *et al*., 2022). This remains controversial with recent findings not showing any difference in therapeutic outcomes, even in septic shock patients (Park *et al*., 2022). As a result, the carbapenem of choice should be determined by multidisciplinary teams or institutional guidelines for usage in critical patients (Brink *et al*., 2022).

Furthermore, imipenem and meropenem do not appear to differ in their effectiveness. The toxicity profiles in particular hosts are the basis for selecting one over the other. Based on clinical data, the effectiveness of doripenem is comparable to that of imipenem and meropenem. Ertapenem is only used to treat susceptible

ESBL-producing pathogens that are not linked to severe sepsis, despite its once-daily dose and strong *in vitro* performance (Smit, 2016).

Table 7: An overview of the antimicrobial resistance and susceptibility patterns in ICU-BSIs

SES	Country	Author/Ref	Organism	Resistance/susceptibility patterns
LMIC	India	Jangale *et al.*, 2017.	*P. aeruginosa*	• **MDR** (all): Resistant to amikacin and ciprofloxacin combination, cefepime, cefoperazone, cefotaxime, ceftazidime, chloramphenicol, gentamicin, levofloxacin, meropenem, tetracycline, and tobramycin. • Low resistance to piperacillin, piperacillin-tazobactam and colistin. • **MBL** production in 83.33% of isolates.
			E. coli	• **ESBL** and **Amp C** production in all isolates.
			K. pneumoniae	• **ESBL** and **Amp C** production in all isolates.
			A. baumannii	• **MBL** production in 50.00% of isolates. • **PDR** in 33.33% of isolates. • The rest were **MDR**.
			S. epidermidis	• Resistant to methicillin (75.00%). • Susceptibility to vancomycin, linezolid and teicoplanin.
			S. aureus	• Susceptible to methicillin and other antimicrobials.
		Banerjee *et al.*, 2020.	*K. pneumoniae*	• **MDR** (38.07%).
			E. coli	• **MDR** (25.85%). • **XDR** (56.00%) - susceptible to colistin and aminoglycosides.
			P. aeruginosa	• **XDR** (all) - susceptible to colistin and aminoglycosides.
			A. baumannii complex	• **MDR** (16.01%). • **XDR** (25.85%) - susceptible to colistin and aminoglycosides.
			ESBL-producers	• Susceptible to carbapenems (88.09%), piperacillin-tazobactam (85.70%).
MIC	Morocco	Lachhab *et al.*, 2017.	*Pseudomonas* spp.	• Resistant to netilmicin (40.00%), ticarcillin (33.03%), amikacin,

				ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin, tobramycin, piperacillin-tazobactam (each 20.00%). • All susceptible to colistin and rifampicin.
			Acinetobacter spp.	• All resistant to imipenem due to carbapenemase production. • All susceptible to colistin.
			S. aureus	• **MRSA** (20.00%).
			Gram-positive isolates	• All susceptible to glycopeptides.
	Nigeria	Iwuafor *et al*., 2016.	*S. aureus*	• **MRSA** (80.00%).
			GNB	• **MDR** (57.01%).
	Uganda	Agaba *et al*., 2017.	*K. pneumoniae*	• Susceptible to amikacin, and imipenem (both 100%).
			S. aureus	• Susceptible to gentamicin, tetracycline, and vancomycin (all 100%). • Susceptible to oxacillin (66.66%). • Susceptible to chloramphenicol and ciprofloxacin (both 33.33%).
	Turkey	Dereli *et al*., 2013.	*S. aureus*	• **MRSA** (61.54%).
			E. coli and *Klebsiella* spp.	• **ESBL** in 48.72% of the isolates.
	Brazil	Sabino *et al*., 2020.	CoNS	• Resistant to oxacillin (87.04%).
			A. baumannii	• Resistant to carbapenems (65.01%).
			Klebsiella spp.	• Resistant to broad-spectrum cephalosporin (70.00%).
	Colombia	Cortes *et al*., 2013.	*K. pneumoniae*	• Resistant to carbapenems (3.00%). • Susceptible to 3GC. (Slight recovery on susceptibility).
			A. baumannii	• **MDR**: Resistant to carbapenems, imipenem, ampicillin-sulbactam, and ceftazidime.
			E. coli	• Increasing resistance rates to ciprofloxacin from 20.00% to 30.00% during the observation period.
			P. aeruginosa	• **MDR**: Resistant to ciprofloxacin, ceftazidime, cefepime, piperacillin-tazobactam, meropenem, and imipenem.
			CoNS	Resistant to oxacillin (above 70.00%).
			E. faecalis	• Resistant to vancomycin (4.00%).

				• Reduction in high-level resistance from 25.00% (2001 to 2002), to below 13.00% (2005 to 2007).
	South Africa	McKay *et al*., 2015.	*S. aureus*	• Susceptible to clindamycin (55.06%) and cloxacillin (52.04%).
			Enterococcus spp.	• All susceptible to vancomycin.
			Enterobacterales	• Susceptible to ciprofloxacin (70.00%), and gentamicin (64.06%). • Susceptible to amoxicillin-clavulanate (41.04%).
			ESBL-producers	• Susceptible to amikacin (85.04%).
			P. aeruginosa	• Susceptible to colistin (91.07%), ceftazidime (72.02%), imipenem and ciprofloxacin (both 44.04%).
			A. baumannii complex	• Susceptible to colistin (96.06%), tobramycin (72.01%), gentamicin (39.00%), ceftazidime (20.07%), meropenem (18.06%), and imipenem (18.04%).
	Mexico	Uc-Cachón *et al*., 2019.	GNB	• Resistant to ampicillin (95.85%), cefuroxime (84.17%), piperacillin (82.93%), cefotaxime (78.07%), ceftriaxone (77.41%), aztreonam (75.23%), cefazolin (75.00%), and ceftazidime (73.19%).
HIC	Canada	Savage *et al*., 2016.	*S. aureus*	• Susceptible to trimethoprim–sulfamethoxazole (95.00%), methicillin (76.04%), and fluoroquinolones (73.04%).
			CoNS	• Susceptible methicillin (32.09%).
			Enterobacterales	• Susceptible to carbapenems (98.06%), and aminoglycosides (90.09% to 97.08%).
			Non-Enterobacterales	• Susceptible to carbapenems (80.09%).

Note: SES = Socio-Economic Status; **GNB** = Gram-negative bacilli; **LMIC** = Low-middle-income countries; **MIC** = Middle-income countries; **HIC** = High-income countries; **Amp C** = ampicillinase C; **ESBL** = Extended-spectrum β-lactamase; **MBL** = Metallo-β-lactamase; **PDR** = Pandrug-resistance; **XDR** = extreme/extensive drug-resistant.

Treatment for AmpC-producing organisms

The empiric therapy for all AmpC producing bacteria (e.g., *E. coli* or *K. pneumoniae*, *Enterobacter* spp.) which have a chromosomal gene encoding inducible resistance to cephalosporins (1GC/2GC/3GC) as well as ampicillin, includes quinolones, 4GC (i.e., cefepime) or the carbapenems (i.e., meropenem, imipenem and ertapenem), (Smit, 2016).

All carbapenems are stable and recommended for the treatment of AmpC-hyper-producing Enterobacterales (Tamma *et al*., 2019). Fluoroquinolones and trimethoprim-sulfamethoxazole are used as alternatives for pathogens that are susceptible (Tamma *et al*., 2021). Piperacillin-tazobactam (at a MIC ≤ 8 mg/L) and cefepime (at a MIC ≤ 2 mg/L) are prospective targeted therapeutic alternatives for susceptible AmpC-producing Enterobacterales (particularly *S. marcescens*, *M. morganii* and *Providencia* spp.) with a low risk for clinically significant over-expression of AmpC (< 5.00%), (Tamma *et al*., 2019; Mizrahi *et al*., 2020). In infections caused by *E. cloacae* and *K. aerogenes*, de-escalation to piperacillin-tazobactam or cefepime may be considered once the MIC is known (Brink *et al*., 2022).

A systematic review and meta-analysis of the effects of different antimicrobials on mortality in patients with BSIs caused by Enterobacterales-producing chromosomal AmpC β-lactamases found no strong evidence that piperacillin-tazobactam, cefepime, or quinolones are inferior to carbapenems (meropenem or imipenem), provided they are susceptible. Notably, no comparable data exist to evaluate the efficacy of ertapenem treatment for infections caused by AmpC-producing Enterobacterales (Harris *et al*., 2016; Brink *et al*., 2022).

Ceftazidime-avibactam and ceftolozane-tazobactam have similar antimicrobial spectra, however the significant difference is that avibactam inhibits carbapenemase (especially, KPC and OXA-48), while tazobactam does not inhibit Amp-C β-lactamases. Previous findings have shown that ceftolozane-tazobactam is active *in vitro* against 99.70% and 94.70% of isolates with moderately and significantly up-regulated efflux pathways, respectively, as well as 96.60% of bacteria with de-repressed AmpC. This supports previous findings about ceftolozane being able to overcome the two most common mechanisms of resistance (up-regulated efflux and de-repressed AmpC) in *P. aeruginosa* (Carmeli *et al*., 2016; Van Duin *et al*., 2016; Livermore *et al*., 2017; Popejoy *et al*., 2017). However, there is limited clinical data on the efficacy of ceftazidime-avibactam and ceftolozane-tazobactam in the treatment of infections caused by AmpC-producing organisms (Bassetti *et al*., 2021).

Treatment for MBL-producing organisms (*A. baumannii and P. aeruginosa*)

In South Africa, MBLs such as VIM, and AmpC enzymes are prevalent in *P. aeruginosa*-BSIs. Additionally, MDR efflux pumps are present in most non-susceptible isolates investigated (Singh-Moodley *et al*., 2018). Recent data supports the preferred stratified and selective use of ceftolozane-tazobactam in managing serious infections with AmpC producers in the absence of MBLs as directed therapy, notably the difficult-to-treat resistant *P. aeruginosa* (DTR-*P. aeruginosa*), (Brink *et al*., 2022).

Ceftazidime-avibactam and ceftolozane-tazobactam are the first novel BLICs registered in South Africa. The limited usage of this combination in this country is due to the high prevalence of MBLs which suggests further investigations for alternative agents (Brink *et al*., 2022).

It is difficult to treat individuals with carbapenem-resistant pathogens, particularly those that produce MBL. Newly available β-lactam/β-lactamase drugs are effective for treating **Class A** and **D** carbapenemases, but not for MBL. Colistin, cefiderocol, tigecycline, and eravacycline are possible treatments for MBL *A. baumannii*. However, these drugs are unsuitable due to their pharmacokinetics/pharmacodynamics (PK/PD) and adverse effect profile (Paul *et al*., 2022). Some promising combinations include aztreonam-avibactam, sulbactam-durlobactam, novel polymyxins, and cefepime-β-lactamase inhibitors, and are still undergoing trials (Brink *et al*., 2022).

Treatment for CRE Enterobacterales and MDR-*P. aeruginosa*

Ceftazidime-avibactam may enhance outcomes among patients with CRE infections and appear superior to conventional therapies, including colistin, against BSIs caused by KPC with clinical cure in most of the difficult-to-treat infections that had failed previous treatments (King *et al*., 2017; Shields *et al*., 2017; Temkin *et al*., 2017; Van Duin *et al*., 2018; Tootla *et al*., 2021). Other studies have reported on the favorable pharmacokinetic (PK) properties, better tolerance, and cost-effectiveness (for CRE-BSIs, VAP, and Hospital-acquired pneumonia or HAP) of ceftazidime-avibactam when compared to colistin. Similar results were found in other countries, (including in LMICs), (Simon *et al*., 2019; Tichy *et al*., 2020; Bolaños-Díaz *et al*., 2021). However, in South Africa, this antimicrobial agent is effective against MBL-producing isolates when combined with aztreonam (aztreonam-avibactam), (Brink *et al*., 2022).

It was previously noted that ceftazidime-avibactam is as clinically effective as carbapenem comparators against MDR Enterobacterales and *P. aeruginosa* (Stone *et al*., 2018). Moreover, in 2021, Soriano *et al*, evaluated the qualitative evidence of the efficient use of ceftazidime-avibactam for the treatment of hospitalized patients with Gram-negative bacterial infections for which there were fewer treatment options based on clinical and microbiological outcomes, mortality, and evidence of efficacy against CRE and MDR-*P. aeruginosa*.

The role of ceftazidime-avibactam in OXA-48-producing bacteria has been studied over the past years, with successful outcomes found in approximately 70.00% patients with infections caused by OXA-48-producing Enterobacterales treated with ceftazidime-avibactam monotherapy (Sousa *et al*., 2018; Stewart *et al*., 2018). However, no significant difference in mortality was seen in the treatment of CRE infections with ceftazidime-avibactam combination compared to monotherapy; rather, monotherapy produced better results (Fiore *et al*., 2020). Thus, this is the ideal agent for severe OXA-48 producing Enterobacterale infections, on bacteriological or clinical failure of other preferred agents (Tamma *et al*., 2021; Brink *et al*., 2022).

Treatment for carbapenem-resistant *P. aeruginosa* (CR-*P. aeruginosa*)

In the most recent global cohort study of carbapenem-resistant *P. aeruginosa*, ceftazidime-avibactam was the most active antimicrobial agent against 87.00% carbapenemase-positive isolates, mostly being MBL, VIM, and Guiana extended spectrum β-lactamase (GES), (Gill *et al*., 2021).

Another recent study suggested the use of ceftolozane-tazobactam (if active *in vitro*) for severe CR-*P. aeruginosa* infections, while old antimicrobials (that are active *in vitro*) were suggested for non-severe or low risk CR-*P. aeruginosa* infections, based on individuals and the source of infection. Limited evidence is available for other therapy combinations such as imipenem-relebactam, cefiderocol and ceftazidime-avibactam (Paul *et al*., 2022).

2.7.2. Treatment options for Gram-positive BSIs

Antimicrobials that target the cell wall, such as cephalosporins, penicillins (e.g., ampicillin and flucloxacillin), or glycopeptides like vancomycin for resistant strains, are the mainstay of treatment for Gram-positive bacterial infections. It may be necessary to utilize drugs such as linezolid, or tigecycline to treat multidrug-resistant bacteria (e.g., MRSA), (Binda *et al*., 2014; Sizar *et al*., 2023; Said *et al*., 2025). Surgical drainage and focused, targeted treatments are commonly included for treatment (Moellering, 2008).

Treatment for *S. aureus* (MRSA)

Vancomycin has been the drug of choice for MRSA. Other options include glycopeptides and linezolid. Existing data from Lancet Laboratories (Johannesburg – South Africa 2014), shows that all reported *S. aureus* cultures were vancomycin, teicoplanin and linezolid susceptible (Smit, 2016). Studies from Europe, North and South America indicated daptomycin as a new lipopeptide with a quick concentration-dependent bactericidal activity against most Gram-positive bacteria including MRSA, hetero-resistant vancomycin-intermediate *S. aureus* (h-VISA), vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *S. aureus* (VRSA), (Barry *et al*., 2001; Streit *et al*., 2004). This lipopeptide agent is well tolerated, with minimal

side effects (Figueroa *et al*., 2009; Moise *et al*., 2009), and is said to have good anti-biofilm activity, which could be useful when catheters or other devices are the source of infection (Bauer *et al*., 2013).

Treatment for CoNS

The empiric therapy for clinically significant CoNS infections would be vancomycin, teicoplanin or linezolid (Smit, 2016). Recommendations for treatment of BSIs sustained by CoNS are supported by limited evidence. However, some experts have suggested that the removal of an infected catheter without antimicrobial treatment could be adequate for resolving bacteremia in some cases (Mermel *et al*., 2009).

Treatment for *Enterococcus* spp.

Ampicillin is the drug of choice for monotherapy of susceptible *E. faecalis* infection, whereas amoxicillin-clavulanate may be used for rare strains that are resistant to ampicillin due to the production of β-lactamase. Vancomycin is often suggested for patients with a penicillin allergy or infections with strains that have high-level penicillin resistance due to altered PBPs. Linezolid, daptomycin, and tigecycline, are the agents of choice for treating VRE infections (Smit, 2016). Previous research has shown that ampicillin is not recommended as the first-line empirical therapy for enterococcal bacteremia, where *E. faecium* is frequently isolated, and vancomycin should be used instead (Fortún *et al*., 2002; McBride *et al*., 2010).

2.7.3. Treatment options for fungal (*Candida* spp.) BSIs

Fluconazole is an appropriate initial therapy in non-neutropenic and clinically stable patients, however; in cases where there is a very high prevalence of other *Candida* spp. (especially *C. glabrata* or *C. krusei*), or if patients have previously received an azole, amphotericin B would be the appropriate empiric agents. Previous exposure to fluconazole and central line remains the major risk factors for non-*albicans* candidemia. Therefore, the removal of central lines in the presence of candidemia in both neutropenic and non-neutropenic patients is highly important (Pappas *et al*., 2004; Spellberg *et al*., 2006).

The management of fungal BSIs or candidemia in ICU involves finding the source of infection and possibly removing it, as most ICU patients (87.00% to 98.00%) have intravascular lines *in situ* (e.g., CVCs, catheters, etc.), (Denning *et al*., 2003; Pappas *et al*., 2004; American Thoracic Society, 2019). Routine susceptibility testing to assist with appropriate therapy is of high importance (American Thoracic Society, 2019).

2.8. Problem statement

Bloodstream infections cause considerable morbidity and mortality in hospitalized patients (Berkley *et al*., 2005; Brink *et al*., 2006; Laupland, 2012). Regular surveillance and reporting of BSIs and antimicrobial susceptibility, including the distinction of community- and healthcare-acquired infections, can aid in the effective management of infections and the adaptation of local antimicrobial stewardship strategies (Levy *et al*., 2004; Grundmann *et al*., 2011).

Increased interventional and invasive monitoring procedures have led to an increase in Gram-positive bacteraemic rates, while widespread and often inappropriate or insufficient antibiotic use has resulted in an increase in MDR microorganisms, *Clostridium difficile* diarrhea, and fungal infections (Renaud *et al*., 2001; Eggimann *et al*., 2003; Corona *et al*., 2004; Niederman, 2006; Sligl *et al*., 2006; Wiesen *et al*., 2006). The rising antimicrobial resistance of blood-borne isolates is geographically and regionally dependent and has become a global concern, particularly in ICUs with a high incidence of invading pathogens, a high transmission rate, and a high antimicrobial consumption (Garg e*t al*., 2007; Costelloe *et al*., 2010).

Many studies in developing countries, including Africa, have shown high antimicrobial resistance in Gram-negative bacteria to commonly used antimicrobials, resulting in lower efficacy in treating common infections (Allegranzi *et al*., 2011; Le Doare *et al*., 2015; Carroll *et al*., 2016; Kumburu *et al*., 2017). Enterobacterales, *Acinetobacter* spp., and *P. aeruginosa* have been identified as the major pathogens producing MDR bacterial infections (Rossolini *et al*., 2007; Sani *et al*., 2012; Oliphant *et al*., 2015; Oduro-Mensah *et al*., 2016). Additionally, infections caused by resistant bacteria are associated with prolonged hospitalization (Tabatabaei *et al*., 2015).

Clinical practice varies widely at the local, national, and global levels (Corona *et al*., 2003). This has also led to the highly diverse resistance patterns seen in ICUs around the world (Ibrahim *et al*., 2000). Timely selection of an appropriate and adequate antibiotic regimen should have a positive effect on patient outcomes (Garnacho-Montero *et al*., 2003).

2.9. Rationale

This study will investigate BSIs in the ICUs of three different levels of hospital care which are: Prince Mshiyeni Memorial Regional Hospital, King Edward VIII Tertiary Hospital, Inkosi Albert Luthuli Central Hospital. The prevalence of commonly encountered pathogenic organisms and the antimicrobial profile will be determined. This information can be used to improve and update hospital-based guidelines on initiation of empiric antibiotic therapy in critically ill patients. This will lead to effective antimicrobial stewardship and infection control measures for the healthcare system in eThekwini.

2.10. Aim

This study aimed to identify the pathogens isolated from blood cultures of adult patients admitted in the intensive care units at the regional, tertiary and quaternary hospitals. These are: Prince Mshiyeni Memorial Regional Hospital, King Edward VIII Tertiary Hospital, and Inkosi Albert Luthuli Central Hospital, respectively, in eThekwini, KwaZulu-Natal, from 01 January 2020 to 31 December 2022.

2.11. Objectives

- To identify the pathogens isolated from blood cultures in patients from the intensive care units of the three hospitals.
- To describe the antimicrobial susceptibility profile of the causative agents in the three hospitals.
- To compare the prevalence of ICU–acquired pathogens among the three hospitals.
- To compare the susceptibility profiles of the isolated pathogens in these hospitals.

2.12. Keywords

ICU-acquired BSIs ● Adult patients ● Antimicrobial resistance ● eThekwini, KwaZulu-Natal hospitals.

CHAPTER 3

3. Methodology

3.1. Study design, location, and period

This was a retrospective, quantitative, and descriptive analysis of pathogenic organisms found in positive blood cultures of adult patients admitted to the ICU at PMMH, KEH, and IALCH. The data was analyzed from 01 January 2020 to 31 December 2022 and was obtained from the NHLS TrakCare database.

3.2. Study population and sample size

The study included all patients aged 18 and above who were admitted to the ICUs at PMMH, KEH, and IALCH. The study sample consisted of all positive blood cultures which yielded pathogenic microorganisms, including CoNS. CoNS was deemed significant if two or more blood culture sets from the same patient were positive. *Bacillus* spp., *Corynebacterium* spp., *Cutibacterium* spp., *Micrococcus* spp., and environmental Gram-negative bacilli were excluded from the study. Duplicate cultures of the same organism from the same patient within 14 days were also omitted. Patients with multiple occurrences of different pathogens were counted as separate cases (Bryan, 1989).

Prince Mshiyeni Memorial Hospital (PMMH) has 1075 to 1200-beds, with one designated adult ICU, with a 100% bed occupancy. King Edward VIII Hospital (KEH) has 852 beds, 12 ICU beds, and 3 High Care Unit (HCU) beds (100% bed occupancy). While Inkosi Albert Luthuli Central Hospital (IALCH) has 846 beds (100% bed occupancy), with specialized ICUs (adults burn ICU, Trauma ICU, Cardiac ICU, and others).

3.3. Laboratory methods and information capturing

The technical laboratory work was carried out by the laboratory technicians/technologists in the microbiology laboratories of the three study hospitals. The automated BacT/ALERT blood culture system was used to detect positive patient blood cultures (culture bottles) (**Appendix 1**). The Kirby-Bauer disk diffusion was used for antimicrobial susceptibility testing of fastidious organisms. (**Appendix 2**). The identification and susceptibility tests were performed using the Vitek 2 Advanced Expert System™ (bioMerieux). (**Appendix 3**). Testing for carbapenemase production was not conducted. Authorized results were logged onto the laboratory TrakCare system.

3.4. Data collection methods, tools and measurements

Patient microbiological results were obtained from the routine diagnostic laboratory testing captured on the National Health Laboratory Services (NHLS) TrakCare system. The data was accessed from the TrakCare system and compiled on Microsoft (MS) Excel spreadsheets for each of the three participating hospitals for the three-year study period. The identified organisms for each hospital were classified into categories: Gram-negative (including Enterobacterales, non-fermenters, and others – infrequent isolates); Gram-positive (*S. aureus*, CoNS, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus* spp., and others – low in frequency); fungal isolates were classified as *Candida albicans*, *Candida* spp. (which were not speciated further), non-*albicans Candida*, and others - low in frequency). Organisms were reported by year of occurrence, frequencies, and percentage. The susceptibility patterns were reported under the commonly selected antimicrobial panels. **Gram-negative panel**: amikacin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin-tazobactam, and tigecycline. Colistin was excluded from the Gram-negative panel due to the unavailability of a reliable method of testing for it. Also, amikacin susceptibility results are not reported on the Vitek Advanced Expert System™ (bioMerieux). (**Study limitations – 5.1.**). **Gram-positive panel**: ampicillin, cloxacillin and vancomycin. **Antifungal panel**: amphotericin B, fluconazole, and voriconazole. (**Table 8**). Carbapenem-resistant Enterobacterales (CRE) were defined by the resistance to at least one carbapenem (ertapenem, imipenem, meropenem), (Smith *et al*., 2024). Enterobacterales were reported as extended-spectrum β-lactamase (ESBL) if the organism conferred resistance to penicillins (e.g., ampicillin), and cephalosporins including 4GC (e.g., cefepime, ceftazidime), (Bradford, 2001; Paterson *et al*., 2005).

Non-fermenters displaying resistance to one agent in three or more antimicrobial categories (e.g., carbapenems – imipenem/meropenem; quinolones – ciprofloxacin; aminoglycosides – amikacin, gentamicin) were classified as multidrug-resistant (MDR), (Horan *et al*., 2008; Magiorakos *et al*., 2012), while possible extreme/extensive drug-resistant (XDR) organisms were those resistant to at least one agent in all but two or fewer antimicrobial categories (e.g., aminoglycosides - amikacin; glycylcycline - tigecycline), (Magiorakos *et al*., 2012). Colistin susceptibility testing was not available. *Staphylococcus aureus* isolates which displayed resistance to cloxacillin were classified as methicillin-resistant *S. aureus* (MRSA), (Kırmusaoğlu *et al*., 2017; Lakhundi *et al*., 2018).

Table 8: Antimicrobial panels for analysis

Gram-negative panel	Gram-positive panel	Antifungal panel
• amikacin[£] • cefepime • cefotaxime • ceftazidime • ciprofloxacin • ertapenem • gentamicin • imipenem • meropenem • piperacillin-tazobactam • tigecycline	• ampicillin • cloxacillin • vancomycin	• amphotericin B • fluconazole • voriconazole

[£]amikacin results are not reported on Vitek 2 Advanced Expert System™ (bioMerieux) for *Acinetobacter baumannii*.

3.5. Statistical analysis

Descriptive analysis was done using the Statistics and Data (STATA) Software for Windows, Version 17.0. Identified organisms were categorised into their respective family/order following their frequency and year of occurrence, in which the number of each organism per year represents the count variable. The antimicrobial susceptibility results were reported by percentage. The chi-square test was used to analyze the differences in organism frequency and susceptibility between the study years. The *p*-value from $<\mathbf{0.05}$ was considered statistically significant.

CHAPTER 4

4. Results

A total of one thousand six hundred and eighty (1680) positive samples were initially included from all the ICUs across the three hospitals for the study duration. Nine hundred and forty-one (941) samples were excluded from the analysis. This included under-age patient samples (158), samples with unspecified age (84), excluded species (43), duplicates (345), and non-pathogenic CoNS samples (311). Consequently, seven hundred and thirty-nine (739) samples remained for the final analysis. One hundred and eighteen samples were analysed at PMMH, while KEH had 247 samples, and IALCH contributed 374 samples. (**Fig. 1**).

4.1. Study demographics

In this study, age was used as a categorical variable for the collection of a controlled study population. Although age and gender were collected as baseline demographic characteristics, they were not treated as primary study variables. This is because age and gender are non-modifiable factors and often act as proxy measures for underlying biological, clinical, and social determinants rather than direct causal contributors to the outcomes of interest. Additionally, these covariates were included to control potential confounding effects, allowing for clearer assessment of the primary clinical and microbiological variables under investigation (Landy, 2001).

The overall total number of males (**M**) in this study was 444/739 (60.08%, while there were 295/739 (39.92%) females (**F**). In PMMH, there was a total of 71 males, and 47 females. From KEH, a total of 149 and 98 males and females were counted, respectively; while IALCH contributed with a total of 224 males and 150 females. (**Fig. 1**).

4.2. Overall organism distribution

The total number of pathogenic isolates from all hospitals over the study period was 739. Gram-negative organisms were the predominant isolates (481/739; 65.09%). Gram-positive organisms represented 25.17% (186/739) and all fungi accounted for 9.88% (73/739). *A. baumannii* (194; 26.25%) was the leading organism in all hospitals, followed by *K. pneumoniae* (106; 14.34%). *Candida* spp. (70; 9.47%) ranked third in this study, followed by CoNS (66; 8.93%), *S. aureus* (53; 7.17%) and lastly *P. aeruginosa* (48; 6.50%). (**Table 9; Fig. 2**).

The number of Gram-negative organisms was similar among the three hospitals: PMMH 76/188 (64.41%) KEH 161/247 (65.18%), IALCH 244/374 (65.24%). This trend was also seen with Gram-positives; PMMH 27/188 (22.88%), KEH 58/247 (23.48%), IALCH 100/374 (27.00%). The highest percentage of fungal isolates was seen at PMMH 12.71% (15/118), followed by KEH 11.34% (28/247), and IALCH 8.02% (30/374). (**Table 10-12**).

Among the Gram-negative bacilli, *A. baumannii* ranked first in all hospitals throughout the study period, 22.88% (27/118) at PMMH, 32.00% (79/247) at KEH, and 23.53% (88/374) at IALCH. *Candida* spp. ranked second at PMMH (13/118; 11.02%), while ranking third at KEH (27/247; 10.93%) and fourth at IALCH (30/374; 8.02%). Only 42 *Candida* isolates were speciated; 28.57% (12/42) were *Candida albicans*, while 71.43% (30/42) were non-*albicans* spp. *K. pneumoniae* was the third common pathogen at PMMH (11/118;9.32%), while being the second most common at KEH and IALCH (30/247; 12.14%) and (65/374;17.38%), respectively. *E. coli* (10/118; 8.47%) was the fourth most isolated at PMMH, while CoNS ranked fourth at KEH (23/247; 9.31%) and third at IALCH (37/374; 9.89%). The fifth common pathogen at PMMH was *P. aeruginosa* (9/118; 7.63%), sixth at both KEH (14/247; 5.67%) and IALCH (25/374; 6.68%). *S. aureus* was sixth at PMMH (8/118; 6.98%), fifth at both KEH (19/247; 7.70%) and IALCH (26/374; 6.95%). (**Table 10-12**).

Fig. 1: Extraction of the study sample

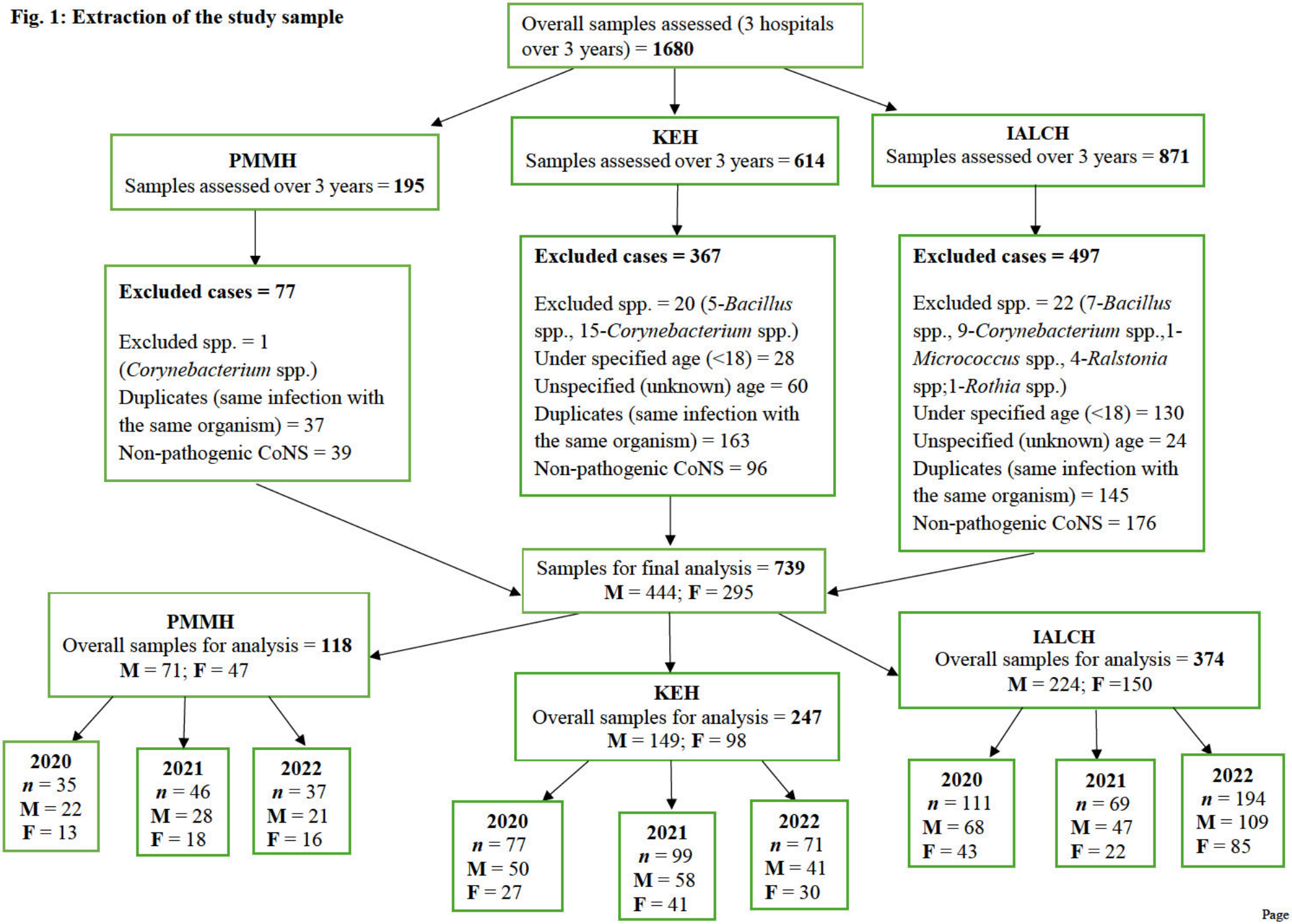
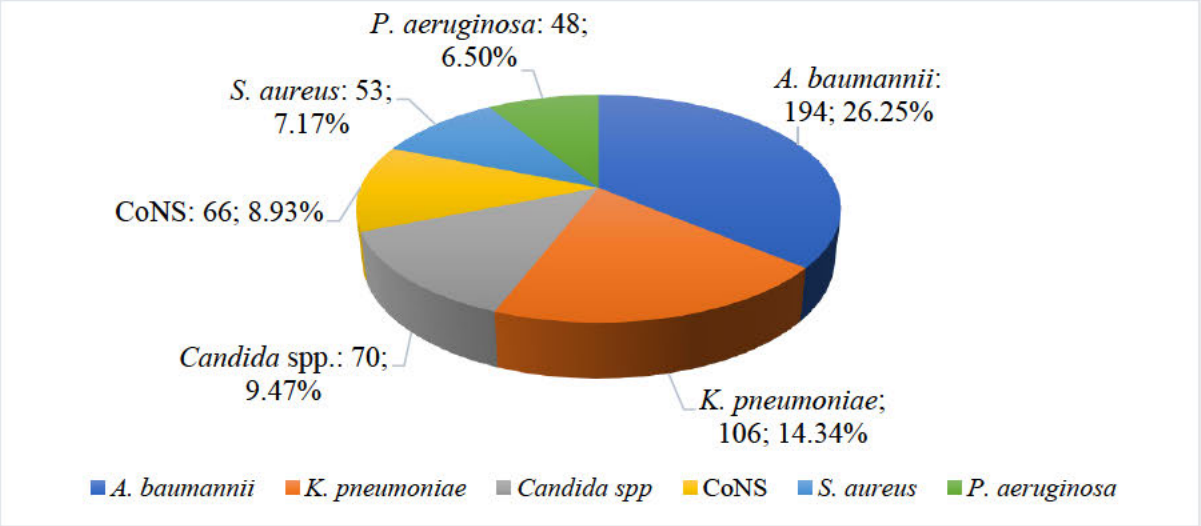


Table 9: The most common isolates in all hospitals over the study period (2020-2022)

Rank	Organisms	*n* (%)
1	*Acinetobacter baumannii*	194 (26.25)
2	*Klebsiella pneumoniae*	106 (14.34)
3	*Candida* spp.	70 (9.47%)
4	CoNS	66 (8.93)
5	*Staphylococcus aureus*	53 (7.17)
6	*Pseudomonas aeruginosa*	48 (6.50)

Fig. 2: Illustration of the most common isolates over the study period (2020-2022)



4.2.1. Organism distribution for PMMH

The total number of pathogens assessed at PMMH over the study period was 118. In 2020, *Candida* spp. were the commonest isolates consisting of 20.00% (7/35) in the overall sample population. A decrease in number of fungal isolates was noted from 2020 (20.00%) to 2021(8.69%), ($p = 0.14$). However, this difference was not of statistical significance because their overall distribution over the three years was low (15/118; 12.71%). *K. pneumoniae*, *A. baumannii* and *S. aureus* (5/35 each; 14.28%) were the common isolates for that year, followed by *P. aeruginosa* (4/35; 11.43%). In both 2021 and 2022, *A. baumannii* was the most common pathogen (12/46; 26.09%) and (10/37; 27.02%), respectively, while other organisms were fewer. (**Table 10; Fig. 3.**).

Table 10: Organism distribution for PMMH (2020-2022)

Year	**2020**	**2021**	**2022**	**Total**
Total no. of isolates/year	**35**	**46**	**37**	**118**
Organisms				
Gram-negatives	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Enterobacterales				
Klebsiella pneumoniae	5 (14.28)	3 (6.52)	3 (8.11)	11 (9.32)
Escherichia coli	3 (8.57)	4 (8.69)	3 (8.11)	10 (8.47)
Enterobacter cloacae	N/A	N/A	4 (10.81)	4 (3.39)
Other*	1 (2.86)	9 (19.57)	3 (8.11)	13 (11.02)
Non-fermenters				
Acinetobacter baumannii	5 (14.28)	12 (26.09)	10 (27.02)	27 (22.88)
Pseudomonas aeruginosa	4 (11.43)	2 (4.35)	3 (8.11)	9 (7.63)
Other†	N/A	1 (2.17)	N/A	1 (0.85)
Other Gram-negatives‡	1 (2.86)	N/A	N/A	1 (0.85)
Total	**19 (54.28)**	**31 (67.39)**	**26 (70.27)**	**76 (64.41)**
Gram-positives				
Staphylococcus aureus	5 (14.28)	2 (4.35)	1 (2.70)	8 (6.78)
CoNS	2 (5.71)	2 (4.35)	2 (5.41)	6 (5.08)
Enterococcus faecium	N/A	N/A	4 (10.81)	4 (3.39)
Enterococcus faecalis	2 (5.71)	4 (8.69)	N/A	6 (5.08)
Streptococcus spp.	N/A	1 (2.17)	N/A	1 (0.85)
Other€	N/A	2 (4.35)	N/A	2 (1.70)
Total	**9 (25.71)**	**11 (23.91)**	**7 (18.92)**	**27 (22.88)**
Fungi/Yeast				
Candida albicans	1 (2.86)	1 (2.17)	N/A	2 (1.70)
Non-*albicans*#	4 (11.43)	3 (6.52)	4 (10.81)	11 (9.32)
Other¥	2 (5.71)	N/A	N/A	2 (1.70)
Total	**7 (20.00)**	**4 (8.69)**	**4 (10.81)**	**15 (12.71)**

*Includes, *Citrobacter freundii*, *Morganella morganii*, *Proteus mirabilis*, *Providencia rettgeri*, *Salmonella* spp., and *Serratia marcescens*.

†Includes, *Chryseobacterium indologenes*.

‡Includes, *Aeromonas hydrophila*.

€Includes, *Gemella morbillorum* and *Gemella sanguinis*.

#Includes, *Candida famata*, *glabrata*, *krusei*, *lipolytica*, *parapsilosis*, and *tropicalis*.

¥Includes, *Cryptococcus* spp., and *Rhodotorula mucilaginosa*.

N/A: Not applicable.

4.2.2. Organism distribution for KEH

Two hundred and forty-seven isolates were analysed at KEH during the course of the study. *A. baumannii* was predominant throughout the study period. The fungal isolates accounted for 11.34% (28/247) of the overall hospital isolates. CoNS (23/247; 9.31%), followed by *S. aureus* (19/247; 7.70%) were the leading Gram-positive pathogens during the investigation period. *P. aeruginosa* (14/247; 5.67%) and *Enterobacter cloacae* (11/247; 4.45%) were the other significant organisms identified at this hospital over the study years. (**Table 11; Fig. 3.**).

Table 11: Organism distribution for KEH (2020-2022)

Year	**2020**	**2021**	**2022**	**Total**
Total no. of isolates/year	**77**	**99**	**71**	**247**
Organisms				
Gram-negatives	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Enterobacterales				
Klebsiella pneumoniae	5 (6.49)	12 (12.12)	13 (18.31)	30 (12.14)
Escherichia coli	2 (2.59)	3 (3.03)	4 (5.63)	9 (3.64)
Enterobacter cloacae	3 (4.00)	7 (7.07)	1 (1.41)	11 (4.45)
Other*	7 (9.01)	4 (4.04)	1 (1.41)	12 (4.86)
Non-fermenters				
Acinetobacter baumannii	17 (22.07)	39 (39.40)	23 (32.39)	79 (32.00)
Pseudomonas aeruginosa	4 (5.19)	6 (6.06)	4 (5.63)	14 (5.67)
Other†	1 (1.30)	2 (2.02)	2 (2.82)	5 (2.02)
Other Gram-negatives‡	1 (1.30)	N/A	N/A	1(0.40)
Total	**40 (51.95)**	**73 (73.74)**	**48 (67.60)**	**161 (65.18)**
Gram-positives				
Staphylococcus aureus	6 (7.80)	5 (5.05)	8 (11.26)	19 (7.70)
CoNS	10 (13.00)	10 (10.10)	3 (4.22)	23 (9.31)
Enterococcus faecium	2 (2.59)	1 (1.01)	2 (2.82)	5 (2.02)
Enterococcus faecalis	2 (2.59)	1 (1.01)	2 (2.82)	5 (2.02)
Streptococcus spp.	2 (2.59)	1 (1.01)	2 (2.82)	5 (2.02)
Other€	1 (1.30)	N/A	N/A	1 (0.40)
Total	**23 (29.87)**	**18 (18.18)**	**17 (23.94)**	**58 (23.48)**
Fungi/Yeast				
Candida albicans	2 (2.59)	2 (2.02)	3 (4.22)	7 (2.83)
Candida spp.±	6 (7.80)	6 (6.06)	2 (2.82)	14 (5.67)
Non-*albicans*#	5 (6.49)	N/A	1 (1.41)	6 (2.43)
Other¥	1 (1.30)	N/A	N/A	1 (0.40)

Total	**14 (18.18)**	**8 (8.08)**	**6 (8.45)**	**28 (11.33)**

*Incudes, *Citrobacter koseri*, *Klebsiella oxytoca*, *Morganella morganii*, *Proteus mirabilis*, *Salmonella* spp., *Serratia ficaria, marcescens*, *odorifera*.
†Includes, *Acinetobacter iwoffii*, *Pseudomonas putida*, *Sphingomonas paucimobilis*, and *Strenotrophomas maltophilia*.
‡Includes, *Haemophilus influenzae*.
€Includes, *Enterococcus avium*.
±Not all *Candida* isolates were speciated.
#Includes, *Candida glabrata* and *krusei*
¥Includes, *Cryptococcus* spp.
N/A: Not applicable.

4.2.3. Organism distribution for IALCH

Three hundred and seventy-four ICU-BSI causing organisms were analyzed at IALCH during the study period. The leading BSI pathogen at this hospital was *A. baumannii* (28/111; 25.00%), (18/69; 26.09%), and (42/194; 21.65%) from 2020 to 2021, and 2022 respectively. The leading Gram-positive organism was CoNS (37/374; 9.87%), followed by *S. aureus* (26/374; 6.93%). *Candida* isolates accounted for (30/374; 8.00%) of the hospital's sample pool. *P. aeruginosa* (25/374; 6.67%) was another significant pathogen identified over the study period. (**Table 12; Fig. 3.**).

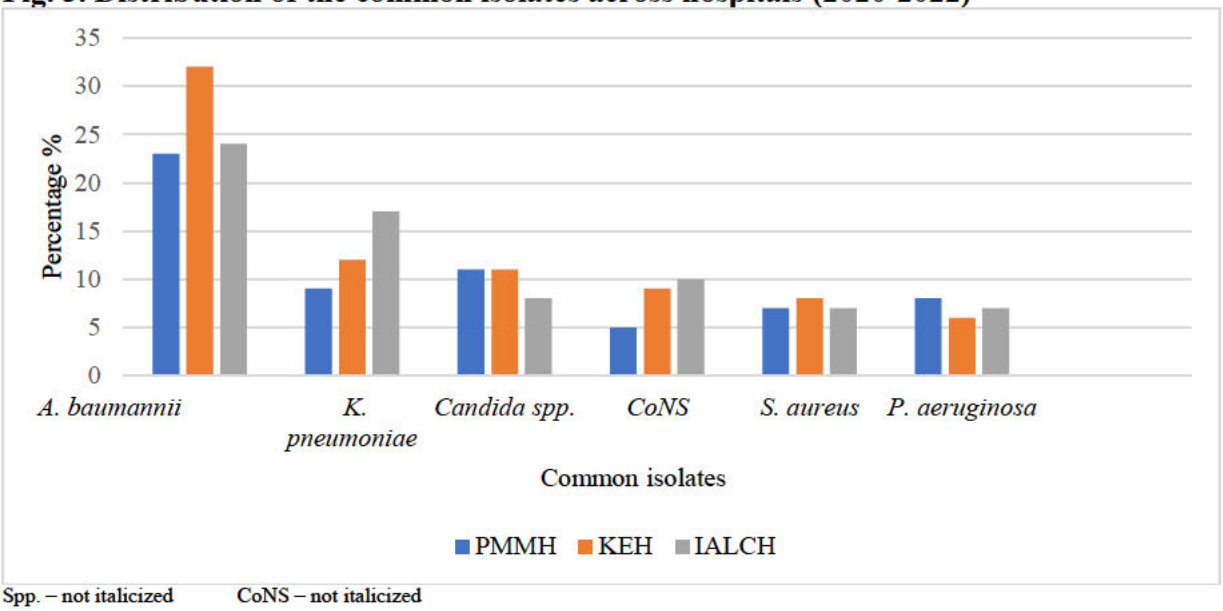
Table 12: Organism distribution for IALCH (2020-2022)

Year	**2020**	**2021**	**2022**	**Total**
Total no. of isolates/year	**111**	**69**	**194**	**374**
Organisms				
Gram-negatives	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Enterobacterales				
Klebsiella pneumoniae	14 (12.61)	7 (10.14)	44 (22.68)	65 (17.38)
Escherichia coli	4 (3.60)	1(1.45)	4 (2.06)	9 (2.41)
Enterobacter cloacae	2 (1.80)	7 (10.14)	6 (3.09)	15 (4.01)
Other*	4 (3.60)	10 (14.50)	17 (8.76)	31 (8.29)
Non-fermenters				
Acinetobacter baumannii	28 (25.23)	18 (26.09)	42 (21.65)	88 (23.53)
Pseudomonas aeruginosa	9 (8.11)	2 (2.90)	14 (7.22)	25 (6.68)
Other†	2 (1.80)	2 (2.90)	5 (2.58)	9 (2.41)
Other Gram-negatives‡	1 (0.90)	N/A	1 (0.52)	2 (0.53)
Total	**64 (57.65)**	**47 (68.12)**	**133 (68.56)**	**244 (65.24)**
Gram-positives				
Staphylococcus aureus	11 (9.91)	6 (8.70)	9 (4.64)	26 (6.95)

CoNS	15 (13.51)	5 (7.25)	17 (8.76)	37 (9.89)
Enterococcus faecium	4 (3.60)	3 (4.34)	7 (3.61)	14 (3.74)
Enterococcus faecalis	7 (6.31)	1 (1.45)	4 (2.06)	12 (3.21)
Streptococcus spp.	2 (1.80)	2 (2.90)	5 (2.58)	9 (2.41)
Other[€]	1 (0.90)	N/A	1 (0.52)	3 (0.80)
Total	**40 (36.03)**	**17 (24.64)**	**43 (22.17)**	**100 (27.00)**
Fungi/Yeast				
Candida albicans	2 (1.80)	N/A	1 (0.52)	3 (0.80)
Candida spp.[±]	4 (3.60)	3 (4.34)	7 (3.61)	14 (3.74)
Non-*albicans*[#]	1 (0.90)	2 (2.90)	10 (5.15)	13 (3.48)
Total	**7 (6.30)**	**5 (7.25)**	**18 (9.28)**	**30 (8.02)**

*Incudes, *Citrobacter koseri*, *Enterobacter aerogenes*, *Enterobacter cancerogenes*, *Klebsiella oxytoca*, *Morganella morganii*, *Proteus mirabilis*, *Proteus penneri*, *Salmonella* spp., *Serratia marcescens/Serratia* spp.
[†]Includes, *Acinetobater junii*, *Acinetobater Iwoffii*, *Acinetobacter haemolyticus*, *Burkholderia cepacia*, *Sphingomonas paucimobilis,* and *Strenotrophomas maltophilia.*
[‡]Includes, *Aeromonas hydrophila.*
[€]Includes, *Aerococcus viridans.*
[±]Not all *Candida* isolates were speciated.
[#]Includes, *Candida auris*, *glabrata*, *krusei*, *parapsilosis*, and *tropicalis*.
N/A: Not applicable.

Fig. 3. Distribution of the common isolates across hospitals (2020-2022)



Spp. – not italicized CoNS – not italicized

4.3. Overall antimicrobial susceptibility patterns in all hospitals combined (2020-2022).

Amikacin (93.03%) was the most effective antimicrobial against Enterobacterales, followed by tigecycline (85.71%), ertapenem (80.61%), meropenem (76.92%), imipenem (72.53%), gentamicin (64.43%), cefepime (62.80%), piperacillin-tazobactam (55.00%), and ciprofloxacin (53.81%). *P. aeruginosa* was highly

susceptible to amikacin (95.45%), ciprofloxacin (89.13%), piperacillin-tazobactam (84.44%) imipenem (82.50%), meropenem (80.95%), cefepime (75.00%), and ceftazidime (69.23%).

Vancomycin was 100% effective against all *S. aureus*, CoNS, *Enterococcus*, and *Streptococcus* isolates. Cloxacillin (80.85%) was highly active against *S. aureus* isolates. Ampicillin susceptibility against *Enterococcus faecalis* and *Streptococcus* isolates was 100% and 66.67%, respectively. *Candida* spp. was highly susceptible to amphotericin B (100%) and voriconazole (100%) with reduced susceptibility to fluconazole (63.04%). (**Table 13**).

Table 13: Overall susceptibility patterns in all hospitals combined (3-years)

Antimicrobials	***n* (%)**
Gram-negative organisms	
Enterobacterales	**220**
amikacin	187/201 (93.03)
cefepime	108/172 (62.80)
cefotaxime	79/191 (41.36)
ceftazidime	79/176 (44.89)
ciprofloxacin	106/197 (53.81)
ertapenem	133/165 (80.61)
gentamicin	125/194 (64.43)
imipenem	132/182 (72.53)
meropenem	150/195 (76.92)
piperaciIlin-tazobactam	99/180 (55.00)
tigecycline	144/168 (85.71)
Non-fermenters	
Acinetobacter baumannii	**194**
cefepime	15/158 (9.50)
cefotaxime	2/151 (1.32)
ceftazidime	16/184 (8.70)
ciprofloxacin	20/174 (11.50)
gentamicin	28/171 (16.37)
imipenem	23/178 (12.92)
meropenem	17/178 (9.55)
piperaciIlin-tazobactam	19/177 (10.73)
tigecycline	143/150 (95.33)
Pseudomonas aeruginosa	**48**
amikacin	42/44 (95.45%)
cefepime	27/36 (75.00%)
ceftazidime	27/39 (69.23%)
ciprofloxacin	41/46 (89.13%)
imipenem	33/40 (82.50%)
meropenem	34/42 (80.95%)
piperaciIlin-tazobactam	38/45 (84.44%)

Gram-positive organisms	
Staphylococcus aureus	**53**
cloxacillin	38/47 (80.85)
vancomycin	43/43 (100)
CoNS∞	**66**
vancomycin	10/10 (100)
Enterococcus faecium	**23**
vancomycin	21/21 (100)
Enterococcus faecalis	**23**
ampicillin	20/20 (100)
vancomycin	17/17 (100)
***Streptococcus* spp.**	**15**
ampicillin	2/3 (66.67)
vancomycin	4/4 (100)
Fungi/Yeast	**73**
amphotericin B	9/9 (100)
fluconazole	29/46 (63.04)
voriconazole	8/8 (100)

∞CoNS isolates are considered to be contaminants; therefore, AST is not conducted routinely.

*Not all antimicrobial susceptibility test (AST) results were available (omitted episodes).

4.4. *A. baumannii* susceptibility patterns in all hospitals over the study period (2020-2022).

It was noted during the investigation period that the number of isolates for organisms other than *A. baumannii* were low per year therefore meaningful conclusions about trends over time could not be drawn. Consequently, susceptibility trends over the three years were only reported for this organism. *A. baumannii* isolates were highly susceptible to tigecycline with an overall susceptibility rate of 95.33%. Other antimicrobials demonstrated poor performance against these isolates throughout the investigation period. The decrease observed in tigecycline susceptibility between 2020 (100%) and 2021 (93.00%), ($p = 0.14$), and the slight increase from 2021 (93.00%) to 2022 (94.44%), ($p = 0.75$), proved to be insignificant. Amikacin results are not reported on Vitek 2 Advanced Expert System™ (bioMerieux) for *A. baumannii.* (**Table 14**).

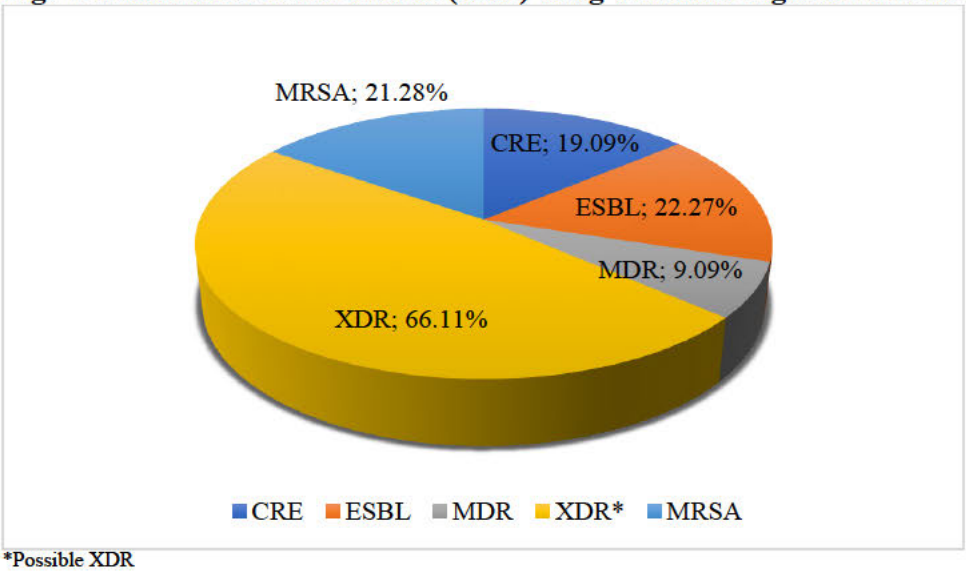
Table 14: *A. baumannii* susceptibility patterns in all hospitals (2020-2022)			
Year	**2020 *n (%)**	**2021 *n (%)**	**2022 *n (%)**
Antimicrobials			
***A. baumannii* [194]**	**[50]**	**[69]**	**[75]**
cefepime	1/40 (2.5)	6/64 (9.38)	8/54 (14.81)
cefotaxime	N/A	N/A	2/54 (3.70)
ceftazidime	1/47 (2.13)	6/66 (9.10)	9/71 (12.90)
ciprofloxacin	3/50 (6.00)	8/57 (14.04)	9/74 (12.16)
gentamicin	5/44 (11.36)	10/58 (17.24)	13/69 (18.84)
imipenem	4/47 (8.51)	7/58 (12.07)	12/73 (16.44)
meropenem	4/48 (8.33)	6/58 (10.34)	7/72 (9.72)
piperacillin-tazobactam	4/48 (8.33)	4/58 (7.00)	11/71 (15.50)
tigecycline	39/39 (100)	53/57 (93.00)	51/54 (94.44)

N/A: Not applicable. *Not all antimicrobial susceptibility test (AST) results were available (omitted episodes).

4.5. Drug-resistant organisms in all hospitals

The total number of drug-resistant organisms during the study period was 285/739 (38.57%); possible XDR (66.11%; 160/242), ESBL (22.27%; 49/220), MRSA (21.28%; 10/47), CRE (19.09%; 42/220), and MDR (9.09%; 22/242). (**Fig. 4.**). The majority of possible XDR (65.70%; 159/242) and MDR (7.02%; 17/242) organisms were *A. baumannii*, while the predominant CRE (19.09%; 42/220) and ESBL (18.64%; 41/220) were *K. pneumoniae* from all hospitals. It was noted during the study that KEH had the highest number of XDR (73.12%; 68/93) and CRE (24.19%; 15/62) organisms. PMMH had the highest ESBL (23.68%; 9/38) and MDR organisms (13.89%; 5/36), whereas MRSA was highest at IALCH (23.08%; 6/26). (**Table 15; Fig. 4.**). Due to the low numbers of drug-resistant organisms in each category per study year in each of the hospitals, analysis of the fluctuations over the three-year study period could not be done.

Fig. 4: Illustration of the overall (total) drug-resistant organisms in all hospitals (2020-2022)

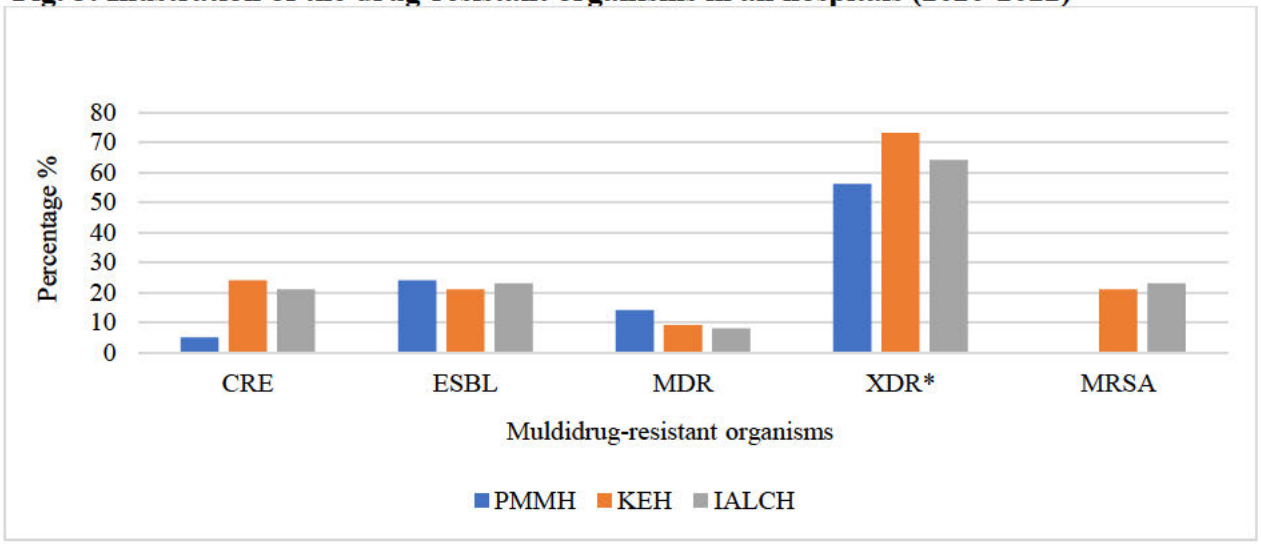


*Possible XDR

Table 15: Drug-resistant organisms in all hospitals (2020-2022)				
Organism profile	**PMMH**	**KEH**	**IALCH**	**Total**
CRE	2/38 (5.26%)	15/62 (24.19%)	25/120 (20.83%)	42/220 **(19.09%)**
ESBL	9/38 (23.68%)	13/62 (21.00%)	27/120 (22.50%)	49/220 **(22.27%)**
MDR	5/36 (13.89%)	8/93 (8.60%)	9/113 (7.96%)	22/242 **(9.09%)**
XDR*	20/36 (55.55%)	68/93 (73.12%)	72/113 (63.71%)	160/242 **(66.11%)**
MRSA	N/A	4/19 (21.05%)	6/26 (23.08%)	10/47 **(21.28%)**

N/A: Not applicable. *Possible XDR - amikacin results were not available for *Acinetobacter baumannii* and colistin was not tested for any organism.

Fig. 5: Illustration of the drug-resistant organisms in all hospitals (2020-2022)



*Possible XDR

A high number of drug-resistant organisms at PMMH were possible XDR (55.55%; 20/36), followed by ESBL (23.68%; 9/38), MDR (13.89%; 5/36), and CRE (5.26%; 2/38). All *A. baumannii* isolates were possible XDR. The MDR consisted of *A. baumannii* (11.11%; 4/36) and only one *P. aeruginosa* (2.78%) isolate. The ESBL isolates were comprised of *K. pneumoniae* (13.16%; 5/38) and *E. coli* (10.53%; 4/38), whereas CRE was identified in *K. pneumoniae* (5.26%; 2/38). There were no MRSA isolates identified in this hospital over the investigation period.

At KEH, all possible XDR (73.12%; 68/93) were *A. baumannii* isolates. All CRE (24.20%; 15/62) were *K. pneumoniae*, while ESBL consisted of 17.74% (11/62) *K. pneumoniae* and 3.23% (2/62) of *E. coli* isolates. The MRSA consisted of 21.05% (4/19) of the sample pool in this hospital.

At IALCH, the possible XDR (63.71%; 72/113) consisted of 62.83% (71/113) *A. baumannii* and one (0.88%) *P. aeruginosa* isolate. The MDR (7.96%; 9/113) comprised 5.31% (6/113) and 2.65% (3/113) of *A. baumannii* and *P. aeruginosa*, respectively. All CRE (20.83%; 25/120) were *K. pneumoniae*. Out of 22.50% (27/120) ESBL-producers, 21.67% (26/120) were *K. pneumoniae* and only one *E. coli* (0.83%). MRSA was found in 23.08% (6/26) isolates at this hospital.

CHAPTER 5

5. Discussion

We investigated the causes and antimicrobial susceptibility of BSIs in ICUs across three different levels of hospital care-regional, tertiary and quaternary, over a three-year period from 2020 to 2022 in KwaZulu-Natal. Gram-negative organisms were the predominant pathogens (65.09%), followed by Gram-positive bacteria (25.17%) and fungi (9.47%). High rates of CRE (19.09%) and XDR *A. baumannii* (66.11%) were detected while 21.28% were MRSA. Additionally, fluconazole susceptibility was only 63.04%.

Age and gender were not analysed as primary predictors in this study, as their influence on clinical outcomes is largely indirect and mediated through factors such as comorbidities, immune status, disease severity, and healthcare exposure. Treating these demographic characteristics as primary variables may obscure the contribution of more proximate and modifiable determinants relevant to antimicrobial resistance and infection outcomes. Consequently, age and gender were considered important contextual and confounding factors rather than direct drivers of the observed findings. This approach allowed the analysis to focus on clinically actionable variables while still accounting for demographic differences within the study population (Landy, 2001).

The organism distribution aligns with several previous South African and international studies that have reported a higher burden of Gram-negative pathogens in critical care settings, particularly in low- and middle-income countries where infection control challenges are common (Brink *et al*., 2016; Rosenthal *et al*., 2020).

Among the Gram-negative isolates, *A. baumannii* emerged as the most frequently isolated pathogen across all three hospitals, accounting for 26.22% of all BSIs. This is in agreement with most previous reports in low- and middle-income countries (Jangale *et al*., 2017; Lachhab *et al*., 2017).

The predominance of *A. baumannii* is consistent with the ability to survive in hospital environments, particularly in ICUs where prolonged hospital stays, invasive procedures, and broad-spectrum antibiotic use are prevalent (Jahani-Sherafat *et al*., 2015; Yang *et al*., 2019). It may also reflect lapses in infection prevention and control (IPC) practices, including hand hygiene compliance, environmental cleaning, and device-associated care (Ntusi *et al*., 2012; Saliba *et al*., 2020).

In this study, *K. pneumoniae* was the second most prevalent pathogen responsible for 14.32% of ICU-BSIs. These findings support previous studies which identified this organism as the second and/or the leading cause of nosocomial BSIs (especially in ICUs), (Liu *et al*., 2014; Jangale *et al*., 2017). Some studies reported lower prevalence rates from 10.00% - 13.06% (Iwuafor *et al*., 2016; Alcántar-Curiel *et al*., 2018; Mohd Asri *et al*., 2021), while others reported rates from 15.15% - 30.00% (Jangale *et al*., 2017; Banerjee *et al*., 2020).

Fungal isolates, primarily *Candida* spp. accounted for 9.47% of all BSIs in this study. These findings are in contrast with other studies that reported lower prevalence rates (2.00% - 3.00%), while others indicated higher prevalence rates (8.00% - 15.00%, 19.43% and 24.08%), (Méan *et al*., 2008; Prowle *et al*., 2011; Kollef [(b)] *et al*., 2012; Dereli *et al*., 2013; Lim *et al*., 2014; Banerjee *et al*., 2020). While relatively lower than bacterial isolates, the presence of *Candida* in ICU-BSIs is clinically significant due to its high associated morbidity and mortality, especially in patients with prolonged ICU stays, broad-spectrum antibiotic exposure, parenteral nutrition, and central venous catheters (Salmanton-García *et al*., 2024; Pyrpasopoulou *et al*., 2025; Tulio *et al*., 2025).

Furthermore, in this study, non-*albicans Candida* spp. (not all *Candida* isolates were speciated) was prominent when compared to *C. albicans* (71.43% vs 28.57%). This is consistent with global findings of a shift from *C. albicans* to non-*albicans* spp., (Lamoth *et al*., 2018). Recently, *C. albicans* was isolated in less than 50.00% of invasive *Candida* infections (Najafzadeh *et al*., 2024). Over the past two decades, a global shift toward an increased prevalence of non-*albicans Candida* spp., including *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* has been documented (Giacobbe *et al*., 2020; Soulountsi *et al*., 2021; Najafzadeh *et al*., 2024). This trend has been partly attributed to the widespread use of antifungal agents, especially fluconazole for prophylaxis (Soulountsi *et al*., 2021; Lass-Flörl *et al*., 2024). Certain non-*albicans* spp. demonstrate higher levels of azole resistance compared to *C. albicans*, azole exposure may exert selective pressure, promoting their predominance (McHugh *et al*., 2025).

Concurrently, increased acquired antifungal resistance has become common among non-*albicans* spp. Of significant concern are reports of clonal outbreaks of fluconazole-resistant *C. parapsilosis* worldwide, with horizontal transmission facilitated by the organism's ability to persist in hospital settings despite routine decontamination measures; notably, transmission has also been observed in patients without prior azole exposure (Thomaz *et al*., 2021; Díaz-García *et al*., 2022; Daneshnia *et al*., 2023).

Although less frequently encountered, resistance to echinocandins has also been described in *C. glabrata* and *C. krusei*, which may further restrict available treatment options (Coste *et al*., 2020; Khalifa *et al*., 2020; Nguyen *et al*., 2024).

CoNS were among the top five isolates (8.93%) and the most common Gram-positive organisms overall. These findings are in contradiction with studies that reported higher prevalence rates; Nigeria (13.06%), Turkey (13.17%), Colombia (39.06%), and Brazil (42.02%), (Cortes *et al*., 2013; Dereli *et al*., 2013; Iwuafor *et al*., 2016; Sabino *et al*., 2020). Although frequently dismissed as contaminants, CoNS were only included in this study when isolated from multiple positive blood culture sets, indicating clinical relevance (Bryan, 1989; Tabah *et al*., 2023). The high prevalence in our study may be due to the common utility of indwelling catheters and other invasive devices in the ICU (Zhu *et al*., 2018; Gao *et al*., 2019).

In this study, *S. aureus* was the second most common Gram-positive organism (7.17%). This is in disagreement with previous South African and international findings which reported *S. aureus* as the most common cause of BSIs in the ICU with prevalence rates from 11.06% to 27.13% (Cortes *et al*., 2013; Dereli *et al*., 2013; Lim *et al*., 2014; McKay *et al*., 2015; Iwuafor *et al*., 2016; Savage *et al*., 2016). This difference may be due to the definition of the significance of CoNS in these studies.

Pseudomonas aeruginosa was one of the significant organisms, ranking sixth in this study (6.50%). This is in contrast to previous reports where it ranked as one in the top three causative ICU-BSI agents (Culshaw *et al*., 2014; Naidu *et al*., 2014; Jangale *et al*., 2017). Other global studies have also reported higher ICU-BSI prevalence rates (i.e., 14.29%,18.18%, 21.01%), (Dereli *et al*., 2013; Jangale *et al*., 2017; Malek *et al*., 2018).

In this study, the high prevalence of XDR *A. baumannii* strains (66.11%) raises concern. Tigecycline demonstrated the highest overall *in vitro* susceptibility of 95.33%, confirming its role as one of the few remaining options for the treatment of MDR/XDR *A. baumannii* strains (Souli *et al*., 2008; Denys *et al*., 2013). These findings are supported by previous investigations stating tigecycline as an alternative agent in treating XDR *A. baumannii* infections (Souli *et al*., 2006; Dizbay *et al*., 2008; Souli *et al*., 2008).

Previous studies of BSIs caused by XDR *A. baumannii* found that standard-dose tigecycline (50 mg every 12 hours) was linked to a significantly increased mortality rate compared to alternative antimicrobial therapies (Liou *et al*., 2015; Niu *et al*., 2018; Niu *et al*., 2019). The effectiveness of tigecycline is primarily dependent on the PK/PD parameter represented by the ratio of the area under the plasma concentration–time curve to the MIC (Barbour *et al*., 2009).

Evidence indicates that administering a high-dose tigecycline regimen is associated with improved clinical outcomes across various infections, including ventilator-associated pneumonia, skin and soft tissue infections, and complicated intra-abdominal infections (Ramirez *et al*., 2013; De Pascale *et al*., 2014; Baron *et al*., 2018; Ibrahim *et al*., 2018). Although limited clinical data is available regarding the use of high-dose tigecycline in patients with secondary *A. baumannii* BSIs, its pharmacokinetic and pharmacodynamic characteristics suggest that dose escalation may lead to a higher tigecycline concentrations and prolong the duration during which drug levels remain above the MIC (Meagher *et al*., 2005).

Resistance to most available antibiotics severely limits treatment options, which may lead to a high mortality risk, and, or outbreaks as reported by previous studies (McKay *et al*., 2015; Gramatniece *et al*., 2019; Zhou *et al*., 2019). The high proportion of *A. baumannii* may also reflect lapses in IPC practices, including hand hygiene compliance, environmental cleaning, and device-associated care (Ntusi *et al*., 2012; Saliba *et al*., 2020).

Klebsiella pneumoniae presented with high rates of ESBL production (22.27%) and carbapenem resistance (19.09%). In contrast, a recent study reported 34.90% (61/175) ESBL and 7.43% (13/175) CRE strains causing BSIs (Tofarides *et al*., 2023). The rising resistance trends among *K. pneumoniae* isolates internationally and in South African hospitals was previously documented by the National Institute for Communicable Diseases (Perovic *et al*., 2014; Jangale *et al*., 2017). Higher rates of CRE (24.20%) and ESBL (23.68%) were identified at KEH and PMMH, respectively. These findings were concerning as these strains are often associated with poor clinical outcomes and increased mortality (Tadese *et al*., 2022; Moloto *et al*., 2023).

The sustained susceptibility to amikacin (87.00%) and tigecycline (88.10%) against *K. pneumoniae* isolates in this study is encouraging and has been previously reported (McKay *et al*., 2015; Agaba *et al*., 2017). However, amikacin and tigecycline may not be suitable for all patients or infections due to their PK/PD and adverse effect profile (Paul *et al*., 2022).

For fungal isolates, fluconazole susceptibility was only 63.04%, while amphotericin B and voriconazole remained 100% effective. This is consistent with a shift reported globally and in South Africa, where *Candida* spp. often exhibited reduced susceptibility to fluconazole. This supports the need for routine antifungal susceptibility testing and consideration of echinocandins or azoles with broader activity for empirical therapy, particularly in settings with high non-*albicans* prevalence (Oxman *et al*., 2010; Playford *et al*., 2010; Pfaller *et al*., 2011; Bassetti *et al*., 2013; American Thoracic Society, 2019).

Although the results of susceptibility testing for CoNS were low, as they are often deemed as contaminants, susceptibility to vancomycin was 100%, yet their rising resistance to other agents and biofilm-forming ability complicates management (Becker *et al*., 2014). In this investigation CoNS displayed high resistance to cloxacillin. This is in agreement with most studies that have observed the multidrug resistance trend amongst CoNS isolates (Singh *et al*., 2016; Xu *et al*., 2018).

In our study, methicillin resistance was displayed in 21.28% of *S. aureus* isolates, as opposed to studies where resistance rates reached 64.05% and 80.00% (European Antimicrobial Resistance Surveillance Network (EARS-Net), 2013; Iwuafor *et al*., 2016; Shuping *et al*., 2017). Despite the notable resistance, all *S. aureus* isolates remained fully susceptible to vancomycin (100%), reinforcing its role as the treatment of choice for MRSA, as reported by other local research findings (Smit, 2016).

Cloxacillin also displayed a high susceptibility rate of 80.85%, supporting its continued use for methicillin-sensitive *S. aureus* (MSSA), (Nissen *et al*., 2013; Henderson *et al*., 2019; Malin *et al*., 2022). The stable presence of *S. aureus* across all hospitals and years suggests endemic circulation rather than outbreak dynamics.

Pseudomonas aeruginosa displayed more favourable susceptibility profiles, with high susceptibility to amikacin (95.45%), ciprofloxacin (89.13%), and piperacillin-tazobactam (84.44%). These results are in contradiction with previous findings where *P. aeruginosa* demonstrated resistance to ciprofloxacin and piperacillin-tazobactam (Cortes *et al*., 2013). Other studies reported lower susceptibility rates to amikacin and piperacillin-tazobactam (20.00% each), while ciprofloxacin maintained a 100% susceptibility rate (Lachhab *et al*., 2017). Our study findings also contradict the global trend of rising MDR *P. aeruginosa*, and may reflect more conservative antipseudomonal antimicrobial use or effective IPC in local settings (Alhazmi, 2015; Stone *et al*., 2018; Soriano *et al*., 2021). Nonetheless, vigilance is necessary, given its intrinsic resistance and ability to develop resistance during therapy.

This study was conducted across three public-sector hospitals in eThekwini, KwaZulu-Natal, each representing a different tier of the healthcare system: Prince Mshiyeni Memorial Hospital (PMMH) as a regional hospital, serves approximately 2 million people of the surrounding area, including part of the Eastern Cape. King Edward VIII Hospital (KEH) attends to approximately 22 000 out-patients monthly, providing tertiary services to the whole of KZN and part of Eastern Cape. Inkosi Albert Luthuli Central Hospital (IALCH) as a major tertiary referral institution provides highly specialized services, serving a population exceeding 10 to 11.5 million people across the KZN and parts of the Eastern Cape.

Similar patterns of BSIs were observed across all hospitals, while subtle differences in pathogen distribution and antimicrobial resistance highlight important institutional variations. These hospitals differ in their levels of specialization, patient demographics, ICU capacity, infrastructure, and infection control resources, antimicrobial use and stewardship practices, which inevitably influenced the antimicrobial resistance patterns observed. The analysis revealed both similarities and differences in the distribution of CRE, ESBL, MDR, XDR, and MRSA across all hospitals.

Multidrug-resistant organisms were present in all three hospitals, underlining the widespread nature of AMR in adult ICU settings. Gram-negative pathogens, particularly *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, were commonly isolated. CREs were identified in all facilities, as were ESBL-producing Enterobacterales (*K. pneumoniae* and *E. coli*), reflecting common challenges related to the overuse of antimicrobials and IPC breaches in critical care units. *A. baumannii* (possible XDR) strains were also isolated at all three hospitals—further emphasizing the growing complexity of treating ICU-related infections.

Despite these shared features, important differences in the resistance profiles were noted.

PMMH (Regional hospital): This hospital was the lowest contributor in terms of sample size (118/739; 15.97%). A surprisingly high proportion of fungal isolates (12.71%) was observed despite it being a district hospital, which was similar to the other hospitals. This may be due to prior broad-spectrum antimicrobial exposure, invasive device usage in immunocompromised patients, and patient demographics (e.g., age -

elderly patients), underlying conditions (solid tumors, pulmonary, cardiovascular diseases, diabetes mellitus etc.), improper infection control practices (hand hygiene, catheter care etc.), and, or higher rates of invasive fungal infections or heightened clinical suspicion leading to more frequent testing (Pappas *et al*., 2018; Mete *et al*., 2021; Papadimitriou-Olivgeris *et al*., 2022; Aydın *et al*., 2024; Kazancioglu *et al*., 2024; Meneghello *et al*., 2024; Tulio *et al*., 2025).

Despite its lower level of care, it also had a high proportion of ESBL-producing organisms (23.68%). This unexpected finding may reflect possible empirical use of broad-spectrum antimicrobials or delayed de-escalation practices. This aligns with findings from other South African hospitals where ESBLs are widespread (McKay *et al*., 2015). The high burden of ESBL-producing organisms at PMMH suggests that resistance is no longer confined to higher-level facilities and may be circulating broadly in the community or due to inadequate IPC measures, as similarly reported in Indian and Nigerian ICU studies (Iwuafor *et al*., 2016; Banerjee *et al*., 2020). These findings may reflect empirical broad-spectrum antimicrobial use, delayed de-escalation, or limited access to rapid diagnostic support, all of which can promote selection pressure even in lower-level facilities.

XDR *A. baumannii* and CRE, although in lower numbers, were also reported. This may suggest increasing XDR and CRE circulation at community or district level, possibly driven by excessive empirical use of carbapenems without confirmed resistance testing. The presence of these isolates at PMMH is notable and may indicate a shift in the local epidemiology or unrecognized nosocomial transmission (Iwuafor *et al*., 2016; Lachhab *et al*., 2017).

However, the absence of MRSA was surprising, particularly given its high MDR burden. This could reflect either limited transmission, detection gaps due to lack of confirmatory molecular diagnostics, differences in colonization pressure or patient complexity. This finding is contrary to expectations, as lower-level facilities often report higher MRSA prevalence due to limited isolation capacity, overcrowding, and insufficient IPC measures (European Centre for Disease Prevention and Control (ECDC), 2013; Shuping *et al*., 2017).

Additionally, sustained bed occupancy, limited ICU capacity, and constraints in isolation facilities may facilitate transmission of resistant organisms. The detection of XDR *A. baumannii* and CRE at PMMH further suggests either increasing community-level circulation or unrecognized nosocomial transmission, highlighting the need for strengthened stewardship and IPC measures at regional hospitals.

KEH (Tertiary hospital): A broader range of resistance mechanisms, including both Gram-negative and Gram-positive resistant organisms was displayed at this facility. A hight burden of XDR was demonstrated, particularly in *A. baumannii* (73.12%) isolates. This may reflect outbreak-like dynamics or challenges in infection control measures such as cohorting and environmental disinfection. *K. pneumoniae* emerged as a prominent CRE producer at KEH (24.20%), further complicating treatment.

High MRSA (21.50%) and ESBL-production (21.00%) rates were also detected. As a referral centre for surrounding districts, KEH is more likely to manage critically ill patients with longer hospital stays and exposure to multiple antimicrobials. These findings were expected, and align with literature suggesting that tertiary hospitals managing referred cases from regional hospitals, are most vulnerable to nosocomial spread of infectious microorganisms which may contribute to the diversity of resistant isolates (Harris *et al*., 2015; Magiorakos *et al*., 2012). Despite these trends, the hospital maintained a moderate rate of fungal BSIs (11.33%), and vancomycin susceptibility remained excellent across all Gram-positive organisms.

As a major referral hospital for KwaZulu-Natal, KEH is likely to receive patients with prolonged prior hospitalisation, extensive antimicrobial exposure, and advanced disease severity. These factors, combined with longer ICU stays and increased use of invasive devices, create an environment conducive to the persistence and spread of multidrug-resistant organisms. The high XDR burden observed at KEH may also indicate outbreak-prone dynamics within ICUs, particularly in settings where cohorting and environmental decontamination are challenged by patient volume and infrastructure limitations.

IALCH (Central/quaternary hospital): This hospital was the largest contributor to the study sample (374/739; 50.61%). A consistent prevalence of XDR (63.71%), mostly being *A. baumannii* (62.83%) was noted. *K. pneumoniae* emerged as the prominent CRE and ESBL-producer (20.83% and 21.67%, respectively).

Although worrisome, these results are expected, given the hospital's role as a referral centre managing complex cases with high long-term antimicrobial exposure, use of invasive devices, colonization by patients transferred from other facilities (Tabah *et al*., 2012; McKay *et al*., 2015). These trends are consistent with high-level ICU resistance profiles reported in quaternary facilities worldwide (Zander *et al*., 2014; McKay *et al*., 2015; Van Diun *et al*., 2020).

These inter-hospital variations emphasise the importance of localized antibiograms and infection control audits. While all three facilities face similar BSI threats, the patterns of resistance and organism prevalence differ sufficiently to warrant tailored approaches to empirical therapy and infection control strategies.

The high prevalence of drug-resistant organisms observed in this study is a critical concern, with 38.57% (285/739) of all isolates classified as resistant (possible XDR, MDR, ESBL, CRE, or MRSA). This figure reflects a growing trend of antimicrobial resistance in intensive care settings and mirrors reports from both South Africa and other LMICs where antibiotic stewardship is often challenged by resource constraints, high empirical antimicrobial use, and limited access to rapid diagnostics (Essack *et al*., 2016; World Health Organization (WHO), 2019).

The most striking resistance pattern was the XDR observed in *A. baumannii* isolates, particularly at KEH (73.12%) and IALCH (63.71%). These isolates were resistant to nearly all tested antimicrobials, with tigecycline emerging as the only reliably effective agent (95.33% overall susceptibility). However, as previously mentioned, the use of tigecycline in BSIs is limited by its pharmacokinetics, raising serious concerns about treatment options (Paul *et al*., 2022).

Klebsiella pneumoniae presented a complex resistance profile, with ESBL production in 22.27% and carbapenem resistance (CRE) in 19.09% of isolates. The carbapenem resistance at IALCH is concerning and indicative of ongoing selection pressure, likely driven by frequent carbapenem use in the ICU setting.

Resistance among Gram-positive organisms was relatively low. MRSA represented 21.28% of *S. aureus* isolates, which remains within acceptable thresholds but nonetheless necessitates continued surveillance. Notably, vancomycin retained 100% efficacy against all Gram-positive isolates, affirming its role as the first-line treatment for resistant *Staphylococcus* and *Enterococcus* infections.

The susceptibility patterns of *Candida* spp. further support ongoing antifungal stewardship. Reduced fluconazole susceptibility (63.04%) and the presence of non-*albicans* spp. necessitate consideration of broad-spectrum antifungals in critically ill patients, particularly in units with high rates of invasive candidiasis.

Patients transferred to IALCH from lower-level facilities may already be colonised with resistant organisms, further increasing colonisation pressure within ICUs. The persistence of resistance despite advanced diagnostic and stewardship resources suggests that the intensity of care and antimicrobial exposure at quaternary centres remains a dominant driver of resistance selection.

Overall, these resistance patterns underline the urgent need for targeted antimicrobial stewardship interventions. Hospital-specific antibiograms should guide empirical therapy, while regular IPC audits, microbiology feedback loops, and clinical education are essential for reducing the burden of antimicrobial resistance. Given the evidence of resistance escalation in *A. baumannii* and *K. pneumoniae*, coordinated regional responses may be required, including outbreak investigations and resistance gene surveillance.

A detailed overview of the pathogens responsible for BSIs in adult ICU patients across different levels of hospital care in KwaZulu-Natal was represented in the study findings, with particular focus on the distribution of organisms and their antimicrobial resistance profiles. The predominance of Gram-negative organisms, especially *A. baumannii* and *K. pneumoniae*, and their associated resistance patterns, highlights the growing threat of multidrug-resistant infections in critical care settings.

The emergence of XDR *A. baumannii* and increasing carbapenem-resistant *K. pneumoniae* isolates across all three hospitals raises serious concerns about treatment limitations and clinical outcomes. The antimicrobial agents such as amikacin and tigecycline remain effective against many resistant isolates; the narrowing of therapeutic options underscores the importance of strategic antimicrobial stewardship and robust IPC programs.

Hospital-specific differences in pathogen distribution and resistance patterns suggest that tailored interventions are necessary. Local antibiograms, timely microbiological surveillance, and adherence to IPC standards should form the backbone of clinical decision-making. In addition, ongoing education of healthcare workers and responsible antibiotic prescribing are essential to curb the spread of resistant organisms.

Collectively, these findings demonstrate that while Gram-negative pathogens and multidrug resistance were common to all hospitals, the magnitude and complexity of resistance patterns increased with ascending levels of care. Regional hospitals showed early penetration of ESBLs and emerging CRE, tertiary hospitals exhibited the greatest diversity of resistance mechanisms, and quaternary facilities carried the highest sustained burden of XDR organisms. These trends point out the importance of hospital-specific antibiograms, tiered stewardship strategies, and IPC interventions tailored to institutional capacity and patient population. A uniform approach to empirical therapy across different levels of care may therefore be inappropriate, and locally informed strategies are essential to mitigate further resistance escalation.

Additionally, these findings emphasize the urgent need for a coordinated, multidisciplinary response to manage and mitigate antimicrobial resistance in ICU settings. Strengthening diagnostic capacity, promoting stewardship, and aligning treatment protocols with local resistance trends will be critical in preserving the efficacy of available antimicrobials and improving patient outcomes.

Although the present study excluded COVID-19-associated ICU-BSIs, the study period (2020–2022) overlapped with successive SARS-CoV-2 waves in South Africa. It is therefore plausible that the broader systemic effects of the pandemic— including altered ICU case-mix, increased use of invasive ventilation, expanded critical care capacity, staff redeployment, and heightened empirical broad-spectrum antimicrobial prescribing — may have indirectly influenced the organism distribution and resistance patterns observed across PMMH, KEH and IALCH.

The high burden of MDR and carbapenem-resistant Gram-negative organisms identified in this cohort may reflect, at least in part, the intensified antimicrobial selection pressure and infection prevention challenges documented internationally during the pandemic period. However, as COVID-19 status was not analysed in this dataset, the extent to which pandemic-related factors directly contributed to the observed resistance profiles cannot be determined.

5.1. Study limitations

While this study provides important insights into BSIs and antimicrobial resistance patterns among ICU patients in KwaZulu-Natal, several limitations must be acknowledged:

Retrospective design: As a retrospective analysis, the study relied on existing laboratory data. Disease-correlated causes (confounding variables) were excluded, primarily to reduce bias, ensure the validity of findings, and isolate the specific effect of a particular exposure. This approach may have limited the ability to collect clinical variables such as comorbidities, antimicrobial exposure history, patient source of infection (e.g., primary or secondary, community-acquired or hospital- (nosocomial) acquired), or patient outcomes (e.g., mortality rates). Therefore, determining clinically relevant significance from contamination was challenging.

Specific study population: The study only concentrated on adult (from 18 years old) ICU patients which limited the sample size.

Age and gender covariates (Confounding and collinearity issues): Age and gender are often strongly associated with many other variables (disease severity, treatment choice, outcomes). Making them primary variables can complicate interpretation because it becomes hard to separate their effects from those of correlated factors.

Incomplete or missing patient, speciation and susceptibility data: Not all fungal isolates were speciated, and some antimicrobial susceptibility test (AST) results were missing or not tested e.g., amikacin susceptibility results for *A. baumannii* are not reported on Vitek 2 Advanced Expert System™ (bioMerieux). This may have led to under- or over-estimation of resistance rates for certain organisms. Cases with missing patient data (e.g., Identification number and, or age) were excluded.

Exclusion of colistin (Gram-negative panel): Colistin susceptibility was not analysed in this study because the Microbiology laboratories in the participating hospitals had not yet (during the study period) obtained a reliable method to test for it.

Pre- and post-COVID-19 associated ICU-BSIs: ICU-BSIs that could have surfaced due to COVID-19 were not analysed in this study. Additionally, the COVID-19 cases were excluded to ensure that the studied cohort is homogeneous and to avoid making false conclusions based on missing data initially recorded on patient admission to the ICU.

Lack of molecular characterization: Due to lack of funding, molecular typing or resistance gene detection studies were not performed, which could have provided deeper insight into the mechanisms of resistance and possible outbreak strains.

Limited generalizability: The findings, although valuable, are specific to the three participating hospitals and may not reflect the broader ICU population across non-tertiary care settings in KZN, or in South Africa as a whole.

Potential for contamination or misclassification: Although efforts were made to exclude likely contaminants (e.g., CoNS included only if isolated from ≥2 blood cultures), the possibility of misclassification cannot be completely ruled out.

All these factors may have hindered the accurate analysis to determine the rates of change in the prevalence of common isolates, the rate of change in antimicrobial susceptibility patterns, as well as the prevalence of specific MDROs during the investigation period. This investigation may be insufficient to assess the appropriate importance of temporal changes across organisms/species that occur less frequently. The elimination of several cases resulted in a considerable reduction in the sample size. The organisms without AST data were nonetheless included in the investigation for aetiological purposes.

5.2. Ethical consideration

This study was submitted to the Biomedical Research Ethics Committee (BREC) (BREC/00004902/2022) at University of KwaZulu Natal for review and approval. Consent was sought and obtained from the NHLS Microbiology HOD and Academic Affairs and Research Management System (AARMS) (PR2232923), for use of patient data. Patients' confidentiality was maintained by excluding names and surnames from the data collection process. Patients were identified through hospital numbers. Samples were identified using episode numbers. Work with clinical specimens and live culture do not form part of this study. Therefore, no biosafety hazards were caused. Collected data was secured using a password protected device. The information collected was handled only by the principal investigator and supervisors and destroyed upon completion of the project and, or publication.

5.3. Recommendations

Based on the findings and identified gaps, the following recommendations are proposed:

Strengthen antimicrobial stewardship programs: Hospitals should reinforce stewardship strategies, including regular audit-and-feedback, to optimize antibiotic prescribing and minimize unnecessary broad-spectrum use.

Implement routine local surveillance and reporting: Regular generation and dissemination of hospital-specific antibiograms will support evidence-based empirical treatment decisions.

Enhance Infection Prevention and Control (IPC): Strict adherence to IPC protocols, including hand hygiene, environmental cleaning, and device care bundles, should be prioritized to reduce nosocomial transmission of resistant organisms.

Improve diagnostic and laboratory capacity: Expansion of fungal species identification and routine susceptibility testing—especially for non-*albicans Candida*—is needed. Investment in molecular diagnostic tools can support early detection of resistance mechanisms.

Conduct prospective and outcome-based research: Future studies should include clinical outcomes, risk factors, and resistance gene profiling to provide a more holistic understanding of ICU-related BSIs.

Policy-level support: The provincial and national health departments should prioritize antimicrobial resistance surveillance in ICUs and consider regional strategies to respond to emerging resistance trends such as CRE and XDR *A. baumannii*

CHAPTER 6

Conclusion

This study set out to investigate the causes of BSIs in adult ICU patients across three levels of hospitals in eThekwini, KwaZulu-Natal-regional, tertiary and quaternary, with a particular focus on the distribution of pathogenic organisms and their antimicrobial susceptibility patterns. By analysing 739 clinically significant isolates collected over a three-year period (2020–2022), the study has provided a comprehensive overview of the epidemiology of ICU-related BSIs in this regional context.

The findings revealed that Gram-negative organisms, particularly *A. baumannii* and *K. pneumoniae*, were the predominant causes of BSIs. These pathogens not only exhibited high frequency but were also associated with alarming levels of antimicrobial resistance, XDR, CRE and ESBL phenotypes.

Gram-positive organisms such as *S. aureus* and CoNS were also identified, although at lower prevalence, with vancomycin retaining full efficacy against all tested isolates. Fungal pathogens, particularly non-*albicans Candida* spp. contributed a smaller but clinically relevant proportion of infections, with reduced fluconazole susceptibility observed.

Hospital-specific differences in pathogen distribution and resistance trends were noted, emphasizing the importance of local microbiological surveillance in guiding empirical therapy. The study also highlighted the increasing threat of antimicrobial resistance in critical care settings and the need for urgent action to prevent further erosion of effective treatment options.

Additionally, The findings of this study must be interpreted within the temporal context of the COVID-19 pandemic, a period characterised by substantial disruptions in ICU practice and antimicrobial stewardship globally. Although COVID-associated cases were excluded, the indirect systemic impact of the pandemic may have influenced the observed patterns of antimicrobial resistance across the study hospitals.

Overall, this study demonstrates that BSIs epidemiology and antimicrobial resistance patterns in adult ICUs are strongly shaped by institutional context. While all three hospitals were affected by a predominance of Gram-negative pathogens and high levels of multidrug resistance, the observed inter-hospital variations reflect differences in level of care, referral patterns, ICU capacity, patient complexity, and antimicrobial exposure rather than isolated microbiological trends.

The presence of ESBL-producing organisms at regional level, the broad resistance diversity at tertiary level, and the sustained burden of XDR pathogens at quaternary level collectively illustrate a continuum of antimicrobial resistance across the healthcare system. These findings highlight that antimicrobial resistance in ICUs is not confined to individual institutions but is propagated across interconnected levels of care, emphasizing the need for coordinated, hospital-specific stewardship, strengthened infection prevention and control practices, and integrated regional surveillance to effectively inform empirical therapy and improve patient outcomes.

In conclusion, the study underlines the complexity of managing BSIs in ICU settings, particularly in the context of rising drug resistance. It calls for coordinated efforts to strengthen antimicrobial stewardship, enhance infection prevention and control practices, and invest in diagnostic and laboratory infrastructure. By translating these findings into targeted interventions, healthcare institutions can improve patient outcomes and contribute to a broader effort to combat antimicrobial resistance at the local, provincial, and national levels.

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Appendix 1: BacT/ALERT ®3D Microbial Detection System – Operation, Processing and Maintenance SOP

This method was adopted from the NHLS Q-Pulse website (Q-Pulse7/docs/active/MIC1506v17)

Purpose:
To provide a uniform procedure for the operation and maintenance of the BacT/ALERT®3D Microbial Detection System. To serve as a guide to all technical staff members as to how to remove bottles from the system and to process positive samples.

Abbreviations:
AER - Aerobic
AN - Anaerobic
BA - Blood Agar (2%)
BBA - Boiled Blood Agar (Chocolate Agar)
BC - Blood Culture
BEA - Bile Aesculin
CO_2 - Carbon Dioxide
COLGEN - Columbia with Gentamicin
HTM - Haemophilus Test Medium
LIS - Laboratory Information System
MCC - McConkey
MH - Mueller Hinton Sens
NHLS - National Health Laboratory Services
NYC - New York City
SABS - Sabouraud Dextrose
SPN - MH + Sheep Blood
TBA - Tryptose Blood Agar

Principle:

The BacT/ALERT® Microbial Detection Systems utilize a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide dissolved in the culture medium. If microorganisms are present in the test sample, carbon dioxide (CO_2) is produced as the organisms metabolize the substrates in the culture medium. As CO_2 is produced, the concentration of hydrogen ions increases and the pH falls causing the sensor to change from blue to light green or yellow. The lighter colour results in an increase in reflectance units as monitored by the system. Bottle reflectance is monitored and recorded by the instrument every 10 minutes.

Frequency and turnaround time:

- Blood culture samples are entered into the system as soon as possible after receipt in the laboratory.
- Samples flagging as positive are processed daily, including Sundays and Public Holidays up to 19:15. Any sample that flags positive after 19:15 will be processed in the morning of the next working day.
- Cultures which do not flag positive remain in the system for 5 days for all routine BacT/ALERT bottles.

Responsibility:

- All technical staff members working in this section are to familiarize themselves with the procedures described in this document.
- All students will work under supervision of a competent, qualified staff member.

Health and Safety:

- All samples should be treated as potentially infectious so all general laboratory safety rules as set out in the NHLS Safety Manual should be observed.
- Syringes are used to aspirate blood from the bottles. Great care should be taken to avoid a needle stick injury. Remember never to recap needles and to discard them into the sharp's container.

Materials and reagents:

- Various types of BacT/ALERT routine blood culture bottles: aerobic, anaerobic, and paediatric.
- Sterile syringes and needles
- Various types of culture media
- Biochemical and confirmatory tests
- Gram stain reagents
- Gauze swabs
- 70% alcohol
- Sharps container and biohazard waste container
- Glass slides
- Wire loops

Equipment:

- BacT/ALERT® Microbial Detection System
- Thermometer
- Biological safety cabinet
- LIS system
- Aerobic and CO_2 incubators at 35°C
- Microscope
- Plate rotator
- Densitometer
- Vitek 2

Sampling

- Blood and Fluid specimens may be received in blood culture bottles.
- As much as possible but not exceeding 10ml of whole blood should be added to aerobic and anaerobic routine bottles.
- As much as possible but not exceeding 4ml of whole blood should be added to paediatric routine bottles.

Storage instructions

- Store uninoculated BacT/ALERT culture bottles at room temperature (15 - 30°C). Protect from direct sunlight.

Procedure:

1) **Maintenance schedule for BacT/ALERT:**
   - Perform daily.
   - Read and record the temperature of each incubation module using the digital thermometer (reference thermometer). The temperature must read 35°C ± 0,5°C.

2) **Calibrating an Incubation Module's Temperature:**
   Temperature calibration is required:
   -anytime the optimal (setpoint) temperature is changed
   -anytime the periodic check of the Reference Thermometer reveals that the temperature is $>0.5^0$C above or below the module's setpoint.
   - Check the Incubation Module's temperature as indicated in operation manual.
   - If temperature is $>0.5^0$C above or below the module's setpoint, return thermometer to module, wait 30 minutes and recheck temperature.
   - If still out of range enter the temperature from the thermometer on the **Actual Temp** scroll buttons. (See calibrate module temperature screen).
   - Press the **Check** button to initiate the calibration, or press the **Cancel** button to retain the previous calibration.
   - Press the **Previous screen** button to return to Setup screen.
   - Check the temperature when stable to verify the calibration was successful.
   - Log problem and resolution on "Temperature, Preventative Maintenance and Problem Record" log sheet.

3) **Calibrating an Instrument Cell:**
   It is not necessary to perform a routine cell calibration. This may be required when a cell fails its automatic internal cell calibration and an error 60 is displayed.
   - Locate the cell which failed calibration.
     - View the Main screen to locate the Instrument Fault Code 60 in the **Instrument** icon.
     - Touch the Incubation Module on the **Instrument** icon that contains the fault code.
     - The View Cell Status screen appears
     - Cell faults appear in the cell display
   - If a bottle is located in the cell, relocate the bottle before calibrating the cell.
   - Access the Setup screen and press the **Calibrate cell** button.
   - Choose the appropriate Incubation Module (1-3) with the **Incubation Module** scroll button.
   - Choose the appropriate drawer (A-D) with the **Drawer** scroll button.
   - Choose the appropriate cell (1-60) with the **Cell** scroll buttons.
   - The first screen displays an **X** at the base of the **Staircase** icon.
   - Press the check button at the bottom of the screen to begin the calibration function.
   - After the #1 shows on the first step, open the drawer that has the green drawer light.
   - Insert Standard #1 into the cell that has the green light.
   - Press the Check button.
   - Remove Standard #1 and insert Standard #2.
   - The procedure is repeated as for Standard #1.
   - The procedure repeated for Standard #3 and #4.
   - If a check mark appears at the top of the **Calibration Staircase** icon, then the calibration of the cell was successful.
   - Remove Standard #4 and press the **Check** button to save the new calibration values.

- If Instrument Fault Code 60 appears at the top of the **Calibration Staircase** icon, then the calibration of the cell is unsuccessful. Press the **Cancel** button and follow the above steps to recalibrate the cell. **NB: The calibration standards should not be used beyond their expiry date.**

4) **What to do if the LIS interface is down:**
   - This will be indicated by an alarm and an error code 12 appearing in the instrument icon.
   - Log onto LIS system
   - Click on 'Instrument Control' icon.
   - Select/Highlight instrument (SA BacT/Alert, BioMerieux).
   - Click on 'Machine Details'.
   - Select 'Start' or 'Stop and then Start'.
   - The link should now be re – established.

5) **Fault reporting:**
   - Should you be unable to re – establish the LIS interface, contact the IT technician.
   - Instrument Fault and Status Codes: Refer to user manual for detailed error description and troubleshooting instructions.
   - Should a problem arise with the instrument inform the equipment officer who will contact the service provider representative. The representative will in turn contact the service technician who will make arrangements with the laboratory.

Preparing specimens for loading:
- On receipt, the bottles and request forms are checked that the patient information correlates. If the information does not correlate, the blood culture bottle must be treated as an unlabeled/ mislabeled specimen.
- Ensure that the specimen is labeled. Unlabeled specimens should not be summarily discarded before consultation.
- Remove barcode sticker from bottle and attach to corresponding request form (bottom right hand corner). Write barcode on form if sticker is not available.
- Send forms for registration.

Vitreous and aqueous humour specimens:
- These specimens consist of fluid aspirated out of the patient's eye and thus only small volumes will be received. Please take great care not to waste/lose the specimen etc. They are normally received in Insulin syringes. If both microbiology and virology tests are requested on a small volume of specimen, the registration will be done by the microbiology staff and a registrar will be asked to sort out with the clinician about which test should be done as a priority.
- Both Gram stain and culture are being performed on these specimens. Gram stains are performed by doing a cytospin on a small amount of the fluid. If there is insufficient fluid for both cytospin and culture, **DO NOT PERFORM** a Gram stain. Only culture needs to be done in these cases. **Please indicate this on the computer.**
- The fluids are cultured in Paediatric blood culture bottles (yellow tops). A supply is kept in the blood culture laboratory and is obtained from Red Cross Hospital laboratories.
- If fungal MC&S is requested, add BHI broth and Calcofluor White if specimen is sufficient.
- Remove the yellow plastic top and clean the rubber plug with a gauze swab dipped in alcohol and inject the fluid into the bottle. It is worth inverting the bottle, aspirating some of the broth and then re-injecting, so as to help flush out the syringe.

- Add 2ml of fetal calf serum to the blood culture bottle. This is stored in aliquots @ 2^0-8^0C.
- Label the bottle and form as you would for a routine blood culture (using the barcode labels).

7) **Registration of specimens:**
   - Blood cultures are registered in the same way as other specimens with the exception that the manufacturer's barcode must be scanned into the 'Bottle 1' field.
   - Ensure that the specimens are labeled – unlabeled blood cultures should not be summarily discarded before consultation, as the patient may have been started on antibiotics, thus reducing the chance of culturing an organism on a repeat blood culture. unlabeled bottles should be left at room temperature in BC lab. until the clinician has been located and identity of bottle has been established or confirmed via the registrar on call.
   - Order BC for routine blood culture bottles
   - Once registered, label bottle and form with the assigned episode number.
   - Sign, scan and file forms.
   - The protocol for routine bottles is 5 days.

8) **Loading the instrument:**
   - Ensure that the labels on the bottle do not cover the manufacturer's barcode (or part thereof) nor the bottom of the bottle. Remove label covering the bottom of the bottle.
   - On the main screen press the load bottle icon. All available drawers will show a green drawer light. Load bottle into drawer labelled "IN USE". If full- load into drawer labelled "NEXT".
   - Scan the bottle (manufacturer's) barcode and then the episode number barcode.
   - Open any drawer whose indicator light is green and place the bottle into any empty cell with an illuminated light. This only applies if "IN USE" and "NEXT" drawers are full or blood culture machine has almost reached full capacity.
   - Press the check button when all bottles have been loaded.
   - Make sure all blood culture bottles which have been referred from another site have been received via the Test Sets Send/Receive icon on the LIS before loading them into the instrument.

9) **Backup procedure for the BacT/ALERT:**

Weekly backup:
- Press the next screen button
- Enter the default password for the setup screen
- Press the tick sign
- Press the reports button
- Select report #1, the load report
- On the left-hand side of the screen, select the "save" button (this is in the form of a floppy disc icon).
- An editable screen will pop up which reads REPORTS\LoadRepo.txt, replace the "Load-Repo" part of the name with the date on which the backup is performed, ensuring the rest of the format is unchanged e.g. REPORTS\01012000.txt
- Select the check button when this is complete.
- Wait 5 seconds before exiting this screen to ensure the backup is successful. Exiting the screen immediately after performing the backup will result in the backup being unsuccessful.
- Sign the tick sheet indicating that you have performed the weekly backup.

Monthly backup:

- Ensure all weekly backups for the month has been performed.
- Open the front compartment of the BacT/ALERT which contains the scanner and memory stick.
- Remove the memory stick.
- Insert the memory stick into a computer and transfer all the data into the Blood Culture Reports folder on the S Drive (Files, S Drive, Laboratories, Microbiology, Common, Read Write, Blood Culture Reports).
- Once the transfer is complete, safely remove the memory stick from the computer and insert it back into the BacT/ALERT.

NB. Erase the data on the memory stick found in the BacT/ALERT only once the memory is full.

- The monthly backup is performed in duplicate.
- Copy all the transferred data from the Blood Culture Reports folder on S drive onto a separate memory stick named BC Monthly Archive. This memory stick can be found in the Blood Culture laboratory.
- Sign the tick sheet indicating that you have performed the monthly backup.

Specimens received after hours

Routine culture:

- The blood culture samples are sorted and registered by C17 pre-analytical staff and loaded into the machine by C17 chemistry staff.

10) **Limitations:**

- Remove any label covering the bottom of the bottle, where the sensor is, as this will cause the machine to falsely flag the bottle as positive.
- Sampling volume
- Technique in collecting the sample (aseptic technique vital)

11) **Quality Control:**

- See GPL1863 for quality control.

Processing of BacT/ALERT blood culture bottles

Procedure:
(BacT/ALERT® 3D user's manual)

1. **Removal of negative bottles:**

This is performed daily on completion of the incubation period.

- From the Main Screen press the Unload Negative button.
- Open any drawer with a lit green drawer light.
- Remove negative bottles, which are indicated by illuminated cell lights, one at a time. Stored information will display on the screen.
- Press the Check button when all negative bottles have been unloaded.
- **If an incorrect bottle is removed, you will immediately receive a 911 error. Do NOT press the check button to accept the error. Reload the bottle back into the original cell with the blinking light.**
- Close the drawer

2. Removal of positive bottles:

System notifies new positive cultures by:

a) The main screen background is yellow.
b) The number of positives will be indicated on the bottle status table.
c) The Unload Positive function will be blue in colour.
d) Audible alarm sound.

- From the Main screen, press the unload positive button.
- Open any drawer with a lit green drawer light.
- Remove positive bottles, which are indicated by illuminated cell lights, one at a time. Stored information appears on the screen.
- Press the Check button when all positive bottles have been unloaded.
- Close the drawer.
- If an incorrect bottle is removed, you will immediately receive a 911 error. Do NOT press the check button to accept the error. Reload the bottle back into the original cell with the blinking light.
- If a positive bottle is removed and later needs to be reloaded due to a false positive, reload the bottle (within 1 hour) using the ` Load Bottles' function and scanning the bottle barcode. The status will automatically revert to Negative – to – Date.

3. Processing of positive blood cultures:

- Clean the septum with 70% alcohol, and insert an insulin syringe.
- Draw up some fluid and prepare a smear for Gram staining. Return the syringe to the bottle.
- Subsequent processing depends on the Gram stain.
- Repeat Gram using the Cytospin method if No bacteria observed or if the Gram stain interpretation is doubtful. The syringe is used to inoculate media.

If a bottle is flagged positive due to the bottom of the bottle completely covered by a label:

- Draw up some fluid and prepare a smear for Gram staining. Return the syringe to the bottle.
- Subsequent processing depends on the Gram stain.
- Repeat Gram using the Cytospin method if no bacteria are observed.
- If NBO on the cytospin- reload bottle to the machine as indicated above.
- If same bottle flags positive again, follow steps as for A, B and C following below.

A. If NO organisms seen on the Gram stain:

Perform a cytospin on BacT/ALERT and BCTB (≤5days) bottles.

If still no organisms are visible, the vials are sub-cultured as follows:

2% BA (AN) + Staph streak
BBA (CO_2)
Brucella agar (2dayAN)
TBA (2 day Microaerophilic)

- After subculturing, re-enter the bottle into the BacT/ALERT machine.
- If growth on subbing - go back to the vial for further processing e.g. Gram.
- If no growth after 24 hours, re-incubate for a further 24 hours.
- Remember to delete Bottle type, "POS" and "TIME" from the result screen on the LIS and enter NBO (No bacteria observed) at Gram stain.
- Enter canned text comment "NBOC" (This may be a false positive signal from the automated blood culture instrument. Culture is ongoing for 5days) at comment field.
- Record all work process in the notes field on LIS.
- If there is no growth after 5 days – the bottle will be taken out with the negatives and will usually have a date next to the bottle number. The final NG5 result has to be entered manually.

B. <u>If organisms are seen on the Gram stain: (Blood/Vitreous fluid)</u>:
Record Gram result on the blood culture worksheet, and sign and date.
Inoculate directly from blood culture as indicated below according to the microscopic characteristics.
Following inoculation of the plates the positive bottle is stored on the shelf as per dated storage container.
This facilitates one month storage after flagging positive.
All Gram stain results must be checked and countersigned.

<u>Gram-negative bacilli</u>:

- Inoculate the following plates: (Using insulin syringe)
- 2% BA (AN)
- BBA (CO_2)
- MCC (O_2).
- Uri select (O_2) may be added if 2 different morphologies of GNB's can be seen.
- Add manual sensitivity X for all ICU patients/Haematology wards/request from registrars. (4 drops)

(Method from CLSI Table 3E-1. Test for Performing disk diffusion directly from positive blood culture broth)

1. Invert blood culture bottle 5–10 times to thoroughly mix.
2. Sterilize the top of the bottle with an alcohol wipe (allow to dry) and insert 20-gauge venting needle/syringe into the blood culture bottle.
3. Dispense 4 drops of blood culture broth onto an MHA plate.
4. Spread blood culture broth across the entire surface of the MHA plate using a sterile cotton swab.
5. Repeat this procedure by streaking twice more, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum.
6. Leave the lid ajar for 3–5 minutes (ideally) but no more than 15 minutes.
7. Dispense antimicrobial disks onto the surface of the inoculated MHA plate.
8. Press each disk down to ensure complete contact with the agar surface.
9. Invert the plate and place in the incubator within 15 minutes of disks being applied.

- Discard 1ml insulin syringe in sharps container.
- Inoculate 4 ml of blood into a serum separating tube. (Using 5ml syringe aseptically)
- Spin at 4000 rpm for 10 min and remove the supernatant.
- Using a swab, emulsify the buffy layer into 3 ml saline to obtain a MacFarland standard of between 0.6 and 0.8.
- Identify and perform sensitivity on Vitek 2.

The above method has not been validated for Myco/F Lytic bottles (TB BC bottles). Do not process them as above.

If the Vitek 2 is out of order/ Myco/F Lytic bottles are received, inoculate the following:

- Saline for doing manual sensitivities.
- 2% BA (AN)
- BBA (CO_2)
- Mcc (AER)

} single colonies

- From saline, inoculate MH and perform sensitivity O1, O2, and X. Incubate at 37^0 C. Identification and sensitivities via Vitek 2/ MS Prime will be performed the following day.

<u>Curved /Seagull/Helical shaped Gram-negative bacilli</u>:

- Plates and procedure as for GNB above **INCLUDING**
- 1x TBA Camp jar (microaerophilically) **37^0C** single colonies
- 1x TBA Microaerophilically **42^0C** single colonies
- SPN with CIPR, TETRA, ERYT in Camp jar/incubator (microaerophilically) **42^0C**

Gram-negative coccobacilli:

- Plates and procedure as for GNB above **INCLUDING**
- HTM (CO_2) sens H
- BA (CO_2) single cols with Staph streak.

Gram-negative cocci: Inoculate plates in a BSC.

- 2% BA (AN)
- BBA (CO_2)
- NYC (CO_2)

  } single colonies
- SPN (CO_2) with PENG, RIFA
- SPN (CO_2) with CTAX, CIPR

Gram-positive cocci in chains/pairs:

- 2% BA (AN)
- 2% BA (CO_2)

  } single colonies
- SPN (CO_2) with sens S
- SPN (CO_2) with sens N
- BEA

Gram-positive cocci (in clusters):

- 2% BA (CO_2)
- 2% BA (AN)

  } single colonies
- DNAse
- MH (AER) Cefoxitin disc

Gram-positive cocci (or if not sure whether Gram-positive cocci are in clusters or chains/pairs):

- 2% BA (AN)
- 2% BA (CO_2)

  } single colonies
- SPN (CO_2) with sens S
- SPN (CO_2) with sens N
- BEA
- DNAse
- MH (AER) Cefoxitin disc

Gram-positive bacilli (Large):

- 2% BA (AN)
- 2% BA (CO_2)

  } single colonies

Gram-positive bacilli (small) or Gram-positive bacilli (not sure small or large):

- 2% BA (AN)
- 2% BA (CO_2)

  } single colonies
- BEA

Gram-positive bacilli (branching):

- 2% BA (AN)
- 2% BA (CO_2)
- BBA (AER)
- SABS (AER)
- NYC (CO_2)

  } Single colonies
- Modified Ziehl Neelsen (If clinically required or suspicious colonies resembling *Nocardia*, *Actinomyces* or *Streptomyces* have grown)

Yeasts:

- BA (AER)
- SABS (without amik)

Mixed Cultures:

1. GNB and GPC/GPC (mixed) **or** GNB and GPB	2% BA (AN) BBA (CO_2) McC Uriselect Col/Genta (CO_2) with optochin. O1, O2, and X	Single colonies
2. GNC and GPC **or** GNC and GPB	2% BA (AN) BBA (CO_2) McC Uriselect Col/Genta (CO_2) with optochin. NYC O1, O2, and X	Single colonies
3. GPC and GPCL **or** GPCL and GPCH/GPDC **or** GPCH/GPDC and GPB	2% BA (AN) 2% BA (CO_2) McC Col/Genta (CO_2) with optochin. SPN (CO_2) sens S & N BEA MH (AER) Cefoxitin disc DNASE	Single colonies
4. Mixed orgs and yeasts:	Add SABS+AMIK	
5. Specimens from Post Mortem patients (pure and mixed cultures).	2% BA (AN) BBA (CO_2) McC NYC (CO_2) Col/Genta with optochin (CO_2) Uriselect	Single colonies

C. If organisms are seen on the Gram stain: (Fluids received in BC/BCTB bottles):

Record Gram result on the blood culture worksheet, and sign and date.
Inoculate directly from blood culture bottle as indicated below.
Following inoculation of the plates the positive bottle is stored on the shelf as per dated storage container.
This facilitates one month storage after flagging positive.
All Gram stain results must be checked and countersigned.

Fluids: Pericardial, pleural, joint:

- 2% BA (AN)
- BBA (CO_2)
- Mcc (AER)

} single colonies

*Add SABS+AMIK (If yeasts observed on Gram stain)

- Identification and sensitivities via Vitek 2 will be performed the following day.

Fluids: (Ascitic/peritoneal/peritoneal dialysis (PD) /Bile):

- 2% BA (AN)
- BBA (CO_2)
- Mcc (AER)

} single colonies

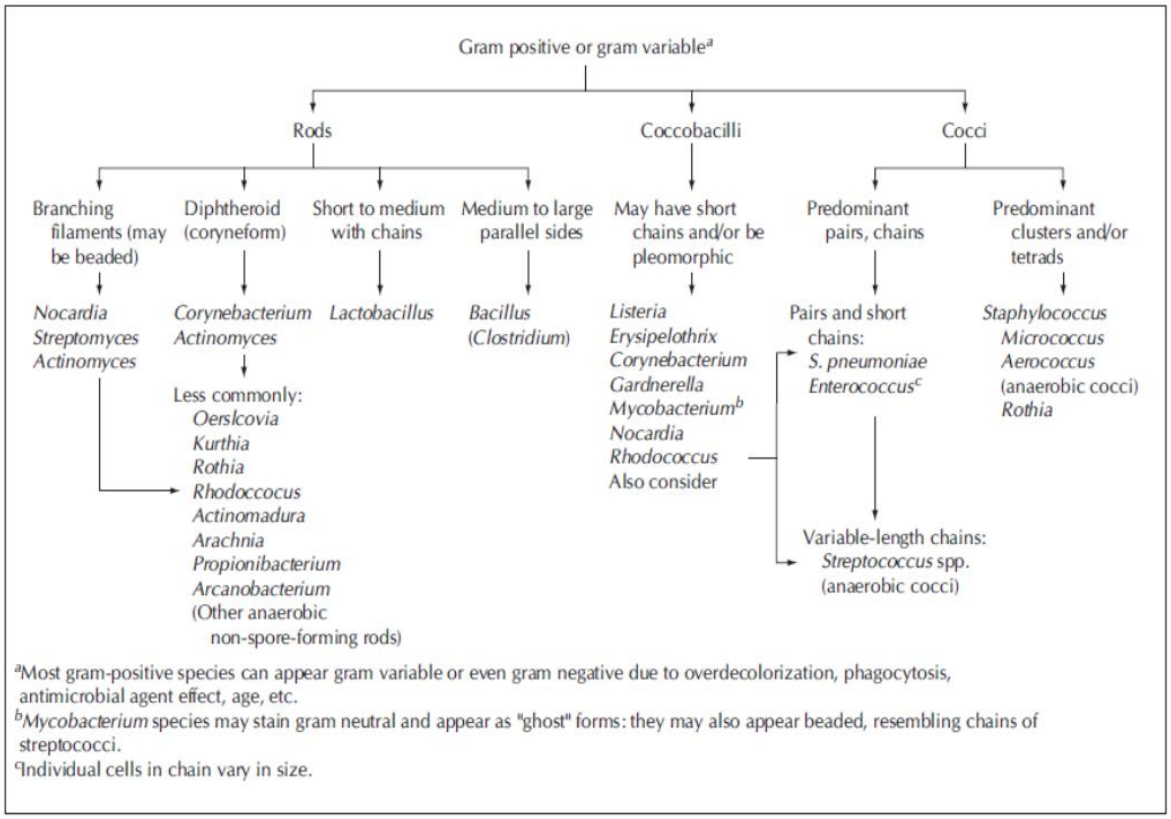
*Add McC agar with an ertapenem disc (If GNB observed on Gram Stain)
*Add SABS+AMIK (If yeasts observed on Gram stain)

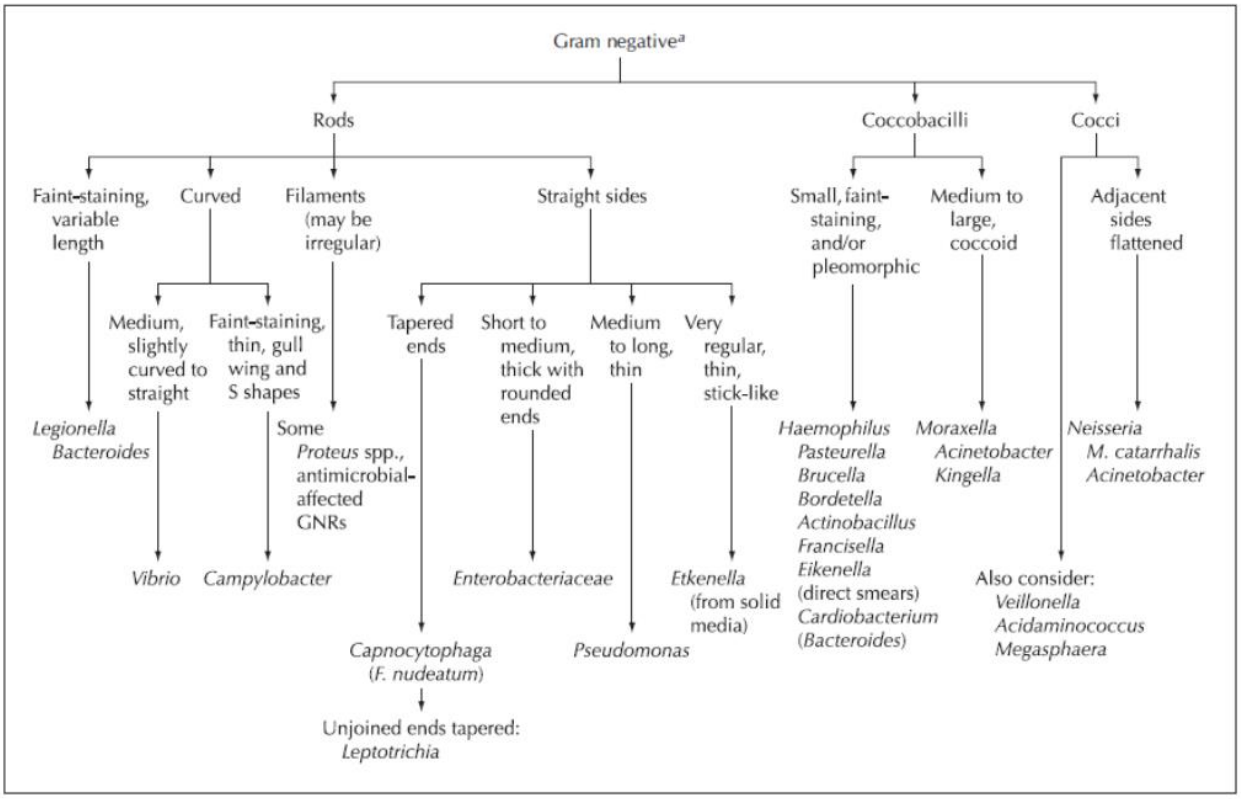
- Identification and sensitivities via Vitek 2 will be performed the following day on clinically significant isolates.

Follow up organisms as per SOP MIC0694 (Refer plates to pus bench for follow up if in doubt)

On Day 2:

Organisms should be identified as described in the relevant SOPs, and sensitivity tests read and reported as described in the relevant SOPs. See below snapshot from **Clinical Microbiology Procedures Handbook 4th Edition** of typical Gram Stain morphologies and possible organisms related to them. See following flow diagrams for guidance.





Yeast

↓

Check purity on plates

↓

Perform ID and Sens.
(Vitek 2 YST)

- **All yeasts should be fully identified and sensitivities performed using the Vitek 2.**
- **Refer yeast isolates that have low discrimination identification on Vitek 2. A supplied info form needs to be completed and submitted with the isolate.**

Gram-positive bacilli-small (GPBS)

↓

Bile aesculin

Positive

↓

Catalase

Positive

Negative
Rule out *Actinomyces*

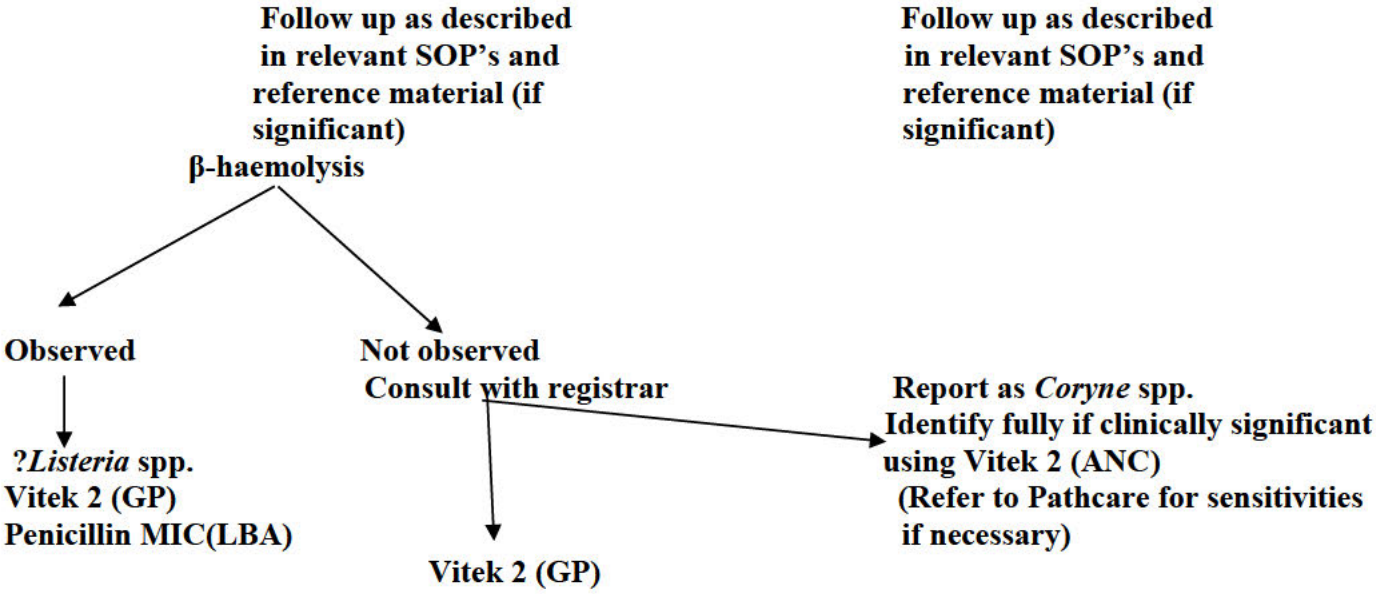
Negative

↓

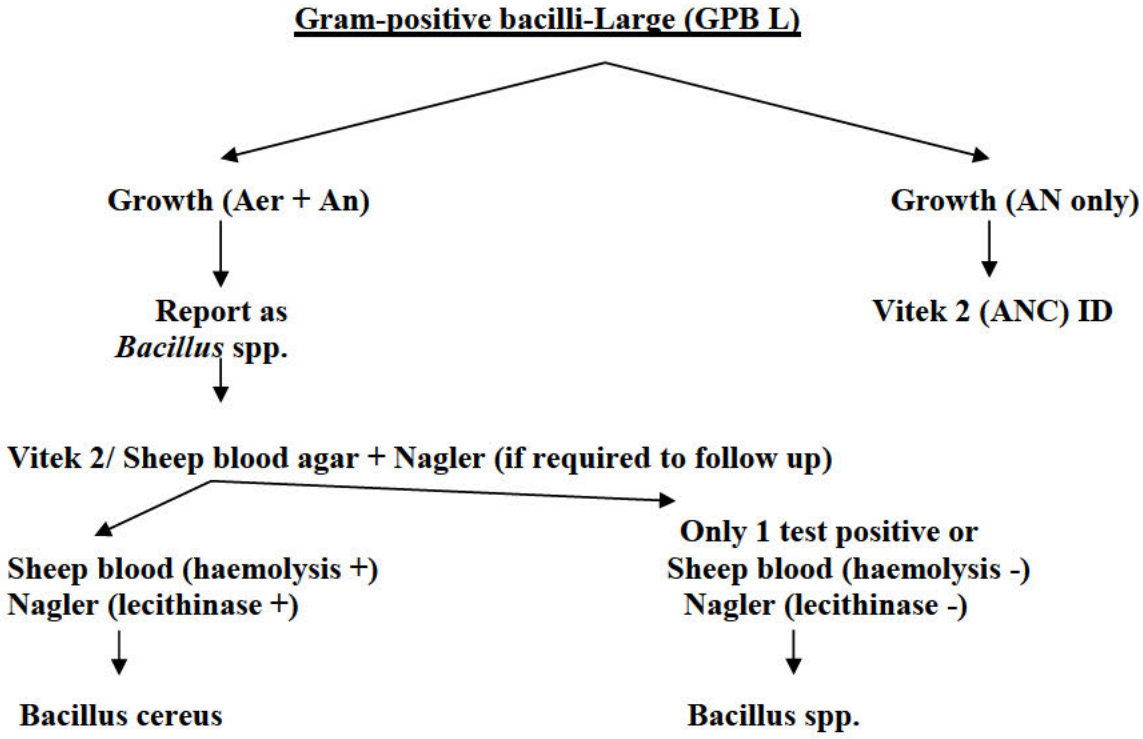
Catalase

Positive

Negative
Rule out *Erysipelothrix*
and *Arcanobacterium*



If growth only on anaerobic 2%BA, treat as a potential anaerobe and perform Vitek 2 (ANC).

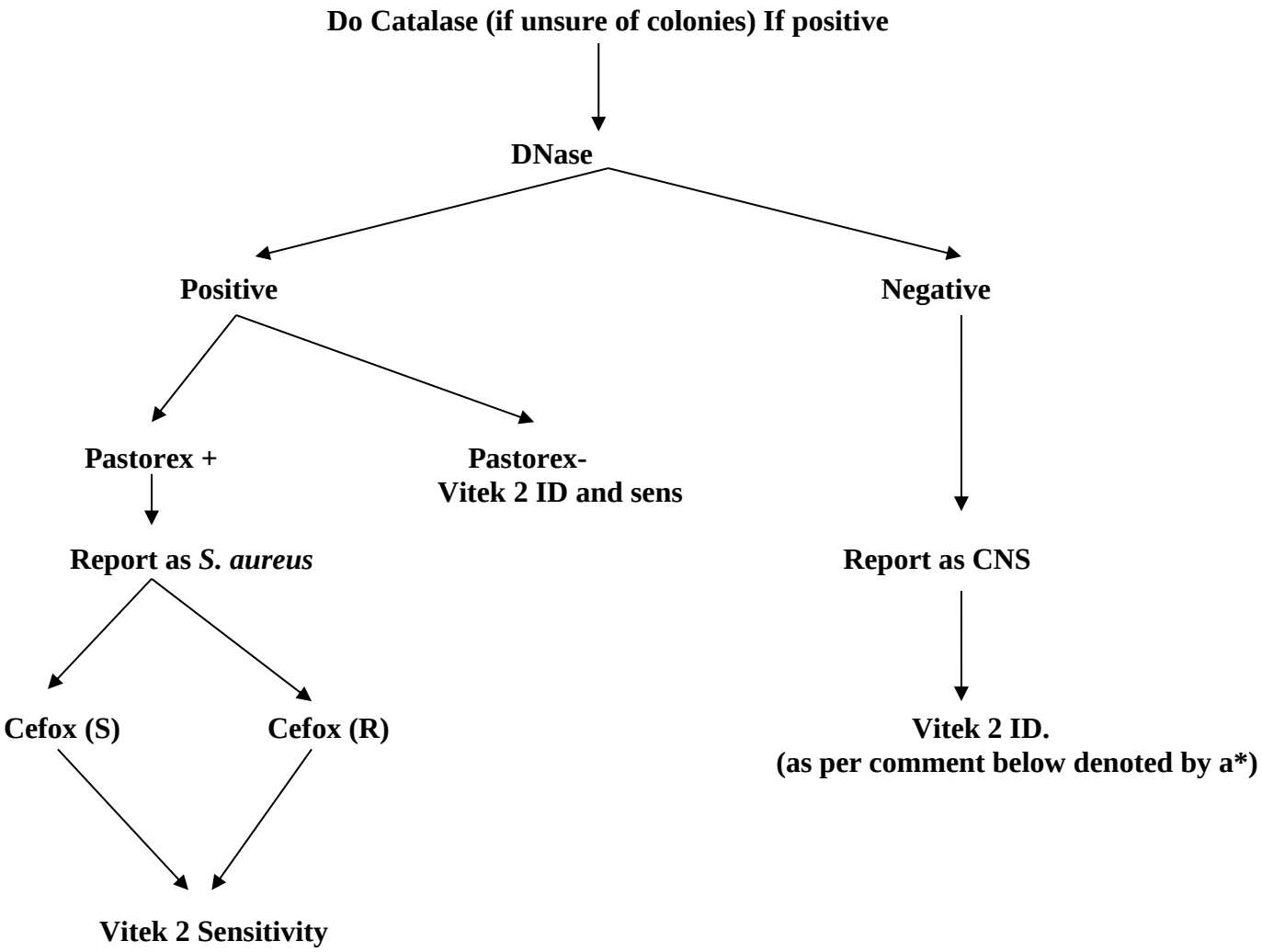


If growth only on anaerobic 2%BA, treat as a potential anaerobe and perform VITEK 2 (ANC).

Gram-positive bacilli (branching): See MIC0688 and MIC0720 for ID procedures.
Some isolates may require 16s PCR for identification/confirmation.

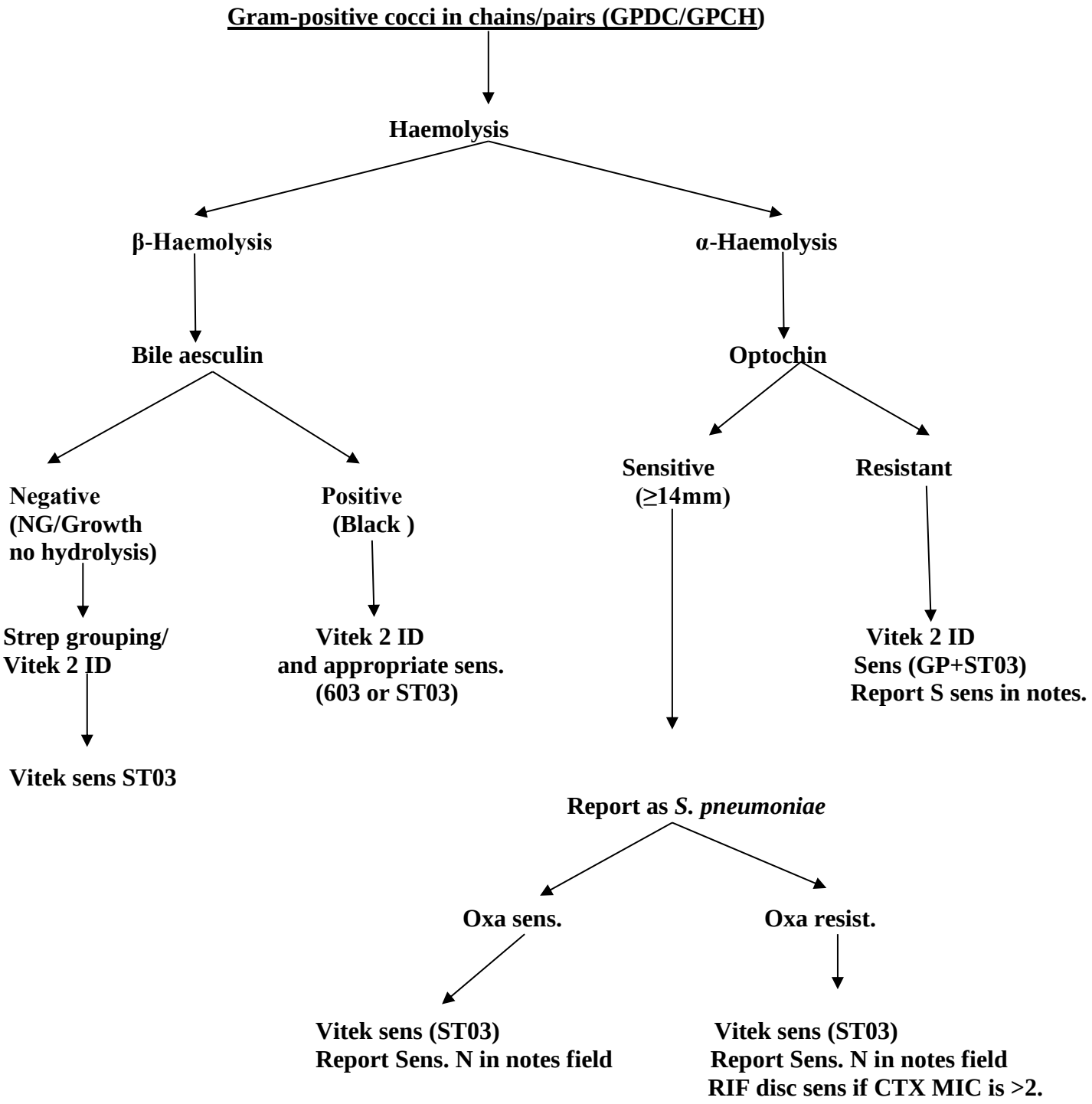
Consult reference material for additional information if above criteria is not fulfilled
Identify fully ONLY if the isolate is felt to be clinically significant. Discuss all these isolates with the registrar / consultant on call.

Gram-positive cocci in clusters (GPCL)



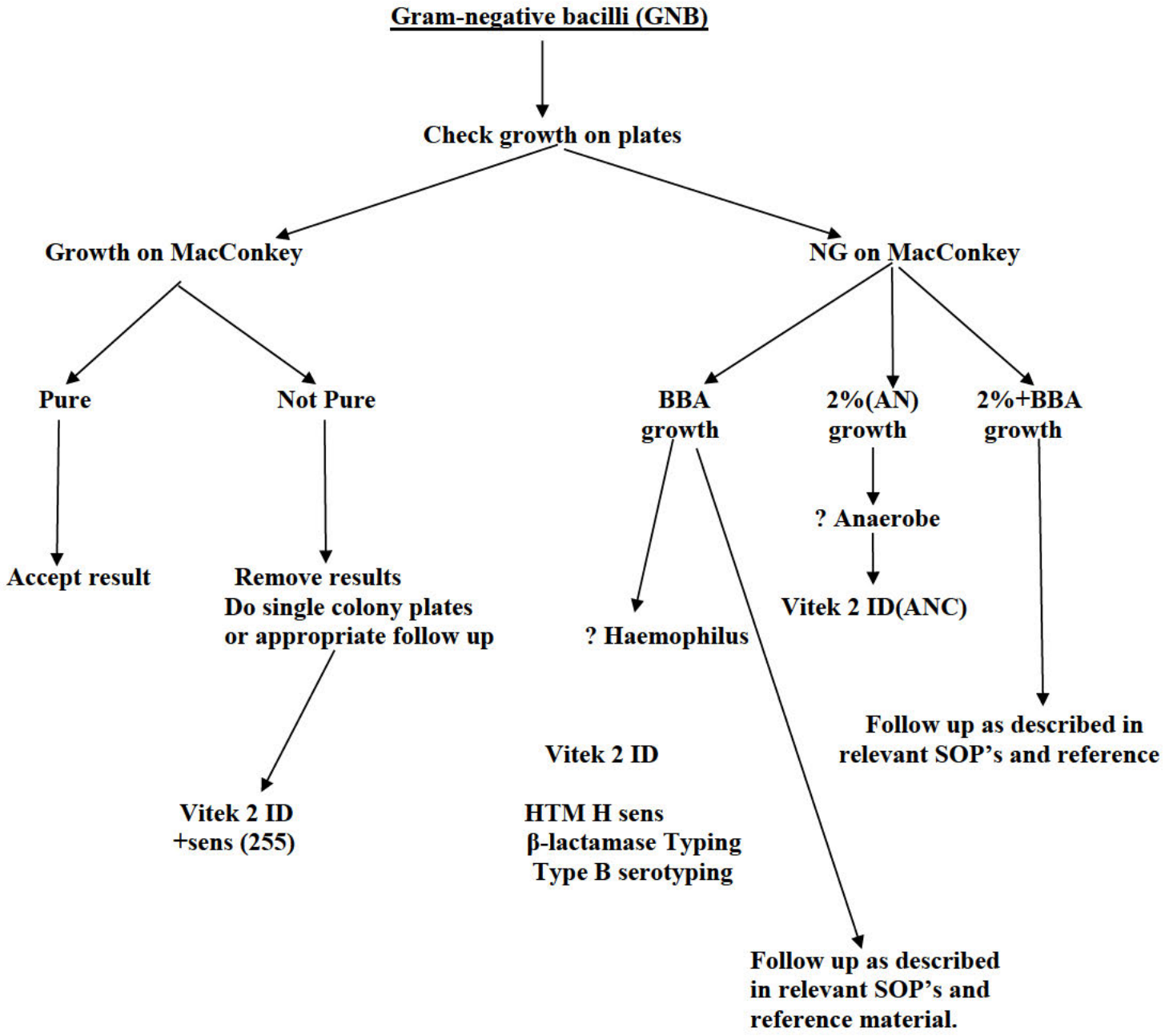
If growth only on anaerobic 2%BA, treat as a potential anaerobe and perform Vitek 2 (ANC).

- **If manual sensitivities are performed on *Staphylococcus* spp., and the organism is D test positive- Report clindamycin as resistant.**
- **If there is a small DNAse zone (smaller than positive control) and Pastorex is negative-Report as CNS.**
- **If there is a small DNAse zone (smaller than positive control) and Pastorex is positive-Perform a Vitek 2 identification and sensitivity testing.**
- **If colonies are yellow and NG on 2% BA (AN)- Report as *Micrococcus* spp.**
- **Record cefoxitin zone diameter**
  *** Coagulase negative *Staphylococcus* spp. from Haematology/Oncology, Dialysis patients, or where a coagulase negative *Staphylococcus* spp. has been isolated from more than one blood culture, may need to be identified fully using the Vitek 2.**
  *** For ICU patients only follow up after consultation with consultant/registrar as this may be unnecessary.**
- **All DNase positive *Staphylococcus* spp. must be confirmed with a pastorex. Perform Vitek 2 identification if there are any discrepancies.**



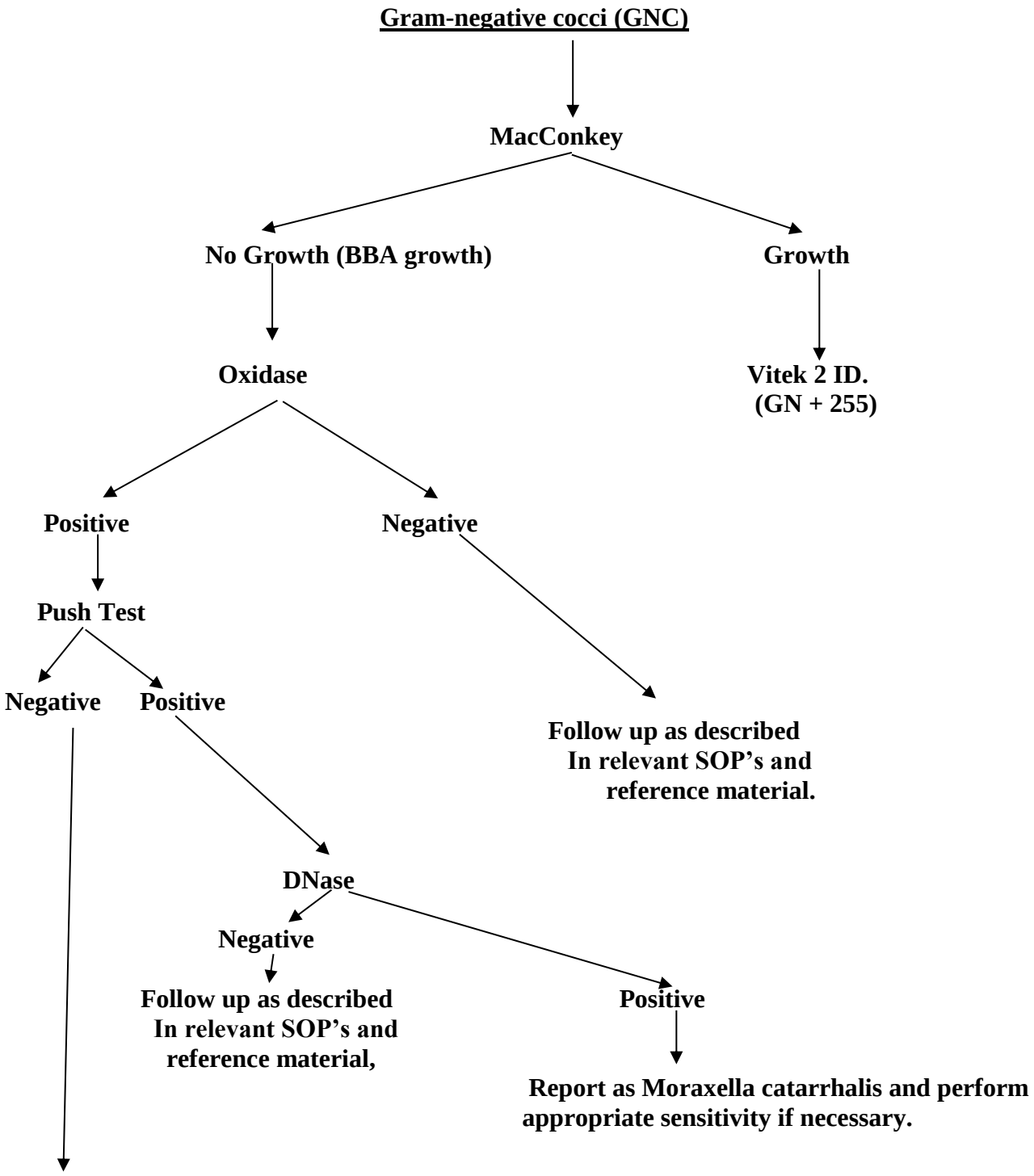
If growth only on anaerobic 2%BA, treat as a potential anaerobe and perform Vitek 2 (ANC).

- **If Gram-positive cocci in chains observed on Gram stain but NG on culture plates-sub on 2% BA (CO_2) with *S. aureus* streak over inoculated plates to exclude *Nutritionally variant streptococci* (NVS). It is a type of Gram-positive coccus that exhibits satellitism around colonies of other bacteria.**
- **If Gram positive cocci in pairs observed on Gram stain but NG on culture plates-send an aliquot of blood culture sample to NICD for Bacterial PCR.**



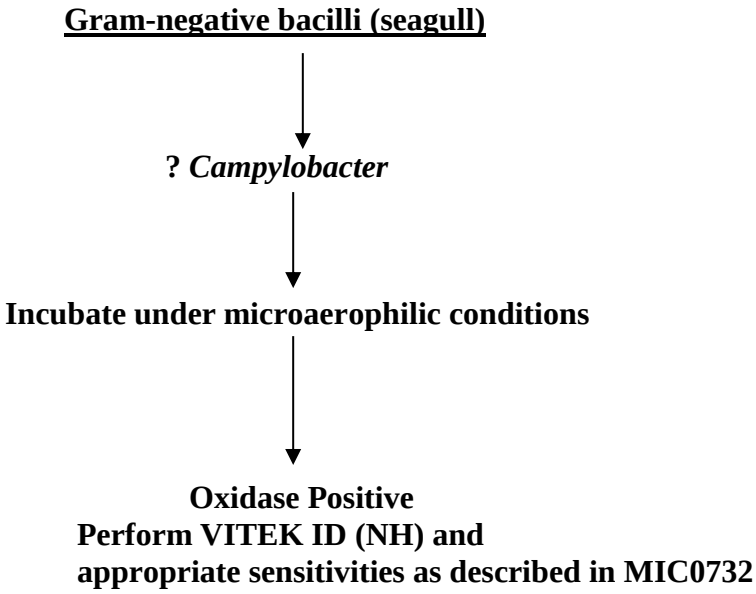
If growth only on anaerobic 2%BA, treat as a potential anaerobe and perform Vitek 2 (ANC).

- **Repeat Vitek 2 ID and sensitivities, if Vitek 2 ID results (using inoculum directly from BC bottle) is identified as an unusual non fermenter or colonies do not match identification.**
- **Add Sens X if an *Acinetobacter* spp., and/or *Serratia marcescens* has been identified.**



Vitek 2 ID (NH) and appropriate sensitivity. Follow up as described in relevant SOP's and reference material.

Suspected Gonococci:
Susceptibility testing must be repeated using appropriate media as per the sensitivity testing SOP.



ALL isolates that are considered to be clinically significant should have appropriate susceptibility tests performed and reported. In the case of unusual or fastidious organisms, please discuss with the registrar / consultant on call to decide the most appropriate agents to test.

Storage of isolates:

- All significant isolates for NICD surveillance must be inoculated onto cryotubes and stored at -70°C.Cryotubes must be labeled with episode number and patient's name. Record on LIS under test method "MSTOR" the storage box number and slot number.
  Inoculate a surveillance dorset egg medium and send specific isolates as requested by NICD surveillance unit (See updated NICD list on BC notice board).
- All isolates referred for additional/confirmatory identification and sensitivities should follow the same procedure as above. An appropriate test code has to be added with all the required tests populated and required info forms should accompany the isolates.
- The cryotubes of samples referred for BDMIC and CRE isolates is stored using the created TRAK storage.

5. Reporting of results:

Microscopy:

- As the blood culture laboratory is paperless, results of all blood culture bottles that have flagged positive must be recorded on the blood culture worksheet. It consists of the date, patient episode number, name, ward, Gram result, signature, counter signature, processor's signature, and amendment. All columns must be completed. A follow-up form (only ***one*** follow-up form per patient - obtain previous form from filing box for repeat isolates) is completed by a registrar or consultant. The result should then be phoned through to the ward by the registrar and as much relevant clinical information as possible recorded.
- Gram stain results are called out by a technologist during after-hours when the registrar on call has left the laboratory. See MIC0721. On occasion the technologist will require the assistance of the on-call registrar if they are experiencing difficulties in locating the clinician.
- The result of the Gram stain must be entered onto the LIS in the same manner as other microscopy results. The name of the person informed as well as the date and time should be entered under "PHONM"

Culture:

- It is the aim of this laboratory to provide relevant information to the clinicians, as it becomes available. When a sensitivity report is known it should be made available to the clinicians even if the identification of the organism is not complete - for example, sensitivities with a non-specific identification such as "Gram negative bacillus" can be entered onto the computer. The identity can then be added once it is known.
- All work processes must be recorded in the notes field on LIS. Final results must be recorded by the registrar on white follow up forms.
- The follow-up forms will be reviewed by the registrar who will then decide whether further communication with the clinicians is necessary e.g. if the patient is on inappropriate antibiotics.
- All negative results which have not gone through on the LIS for some reason must be entered manually. This will be evident on the outstanding BC test list on LIS system

Retention and disposal of samples:

- Negative bottles are discarded after removal and checking of OTL.
- Positive routine bottles are kept for a month at room temperature.
- Certain significant isolates are stored in -70 freezer indefinitely.
- All bottles are discarded into plastic biohazard waste buckets.
- Culture plates are kept on the bench until the report has been authorized and then discarded into biohazard waste containers.

Quality control:

All incubator temperatures are monitored and recorded each working day.
All media are validated before being put into routine use.
Positive and negative controls are used to check biochemical reactions.
A Gram stain control is performed daily.

Rejection criteria:

Reject blood culture bottles in the following instances:

- I**nformation on the form and specimen does not match (mislabeled).**
  - Bottles are kept in the daily processing containers for a month to give sufficient time for clinicians or responsible parties, responsible for specimen collection, to correctly label bottles/forms.
- **Specimens are unlabeled.**
  - Bottles are stored in the blood culture incubator for 7 days to give sufficient time for clinicians or staff (responsible for specimen collection), to correctly label BC bottles.
- **Expired blood culture bottles.**
  - Bottles are kept in the daily processing containers for a month to give sufficient time for clinicians or staff (responsible for specimen collection) to request incubation due to special cases/circumstance.
  - In these cases, the comment "**bcexpire**" is inserted on TrakCare under the MCOM test set.

    BCEXPIRE: An expired blood culture bottle was received. Please treat the result with reserve and repeat the culture if clinically indicated.

References:

BacT/ALERT®3D User Manual Version B.25
Clinical Microbiology Procedures Manual – Isenberg 2nd Edition
The BacT/ALERT®MB Operator's Manual
Clinical Microbiology Procedures Manual 4th Edition

Appendix 2: Antimicrobial susceptibility – Kirby-Bauer disk diffusion method standard operating procedure (SOP).

This method was adopted from the NHLS Q-pulse website (Q-Pulse7/docs/active/MIC1462v6).

Introduction:

Disk diffusion tests, based solely on the presence or absence of a zone of inhibition without regard to the size of the zone or of the inoculum are not acceptable. Reliable results can only be obtained with disk diffusion tests that use the principle of standardized methodology and zone diameter measurements correlated with minimum inhibitory concentrations (MIC's) with strains known to be susceptible and resistant to various antibiotics. The methods described below must be followed explicitly to obtain reproducible results.

Objective:

To provide a uniform and accurate procedure for antimicrobial susceptibility testing to obtain reproducible results.

Responsibility:

All medical technologists and technicians trained to perform these test procedures are responsible.

Safety precautions:

All procedures are performed using universal safety precautions.

Abbreviations

ATCC - American Type Culture Collection
CLSI - Clinical and Laboratory Standards Institute
CoNS – Coagulase negative *Staphylococcus*
CSF – Cerebrospinal fluid
ESBL - Extended-spectrum β-lactamase
E-test – Epsilometer test
EQA - External Quality Assessment
FGNB1/FGNB1 – Facultative Gram-negative bacilli 1/ Facultative Gram-negative bacilli 2
GSH - Groote Schuur Hospital
IBL – Inducible β-lactamase
LIS - Laboratory Information System
MIC – Minimum Inhibitory Concentration
MRSA – Methicillin-resistant *S. aureus*
MRO - Multidrug-resistant organisms
NCCLS - National Committee for Clinical Laboratory Standards
NFGNB – Non-fermentative Gram-negative bacilli
NHLS - National Health Laboratory Services
NICD - National Institute for Communicable Diseases
PCR - Polymerase chain reaction
QAD - Quality assurance division
QC - Quality control
SOP - Standard operating procedure

Principle:

A standardised inoculum of the organism to be tested is swabbed over the entire surface of a Mueller Hinton agar plate. Antibiotic impregnated disks are placed onto the surface of the agar. As soon as the disk comes into contact with the moist agar surface, water is absorbed into the filter paper and the antibiotic diffuses into the medium. After overnight incubation at 35°C, the diameter of the zone of inhibition around each disk is measured and recorded. The size of the zone is inversely proportional to the MIC of the organism.

Equipment and Materials:
Mueller Hinton agar plates (with and without blood)
Haemophilus test medium
Appropriate antimicrobial disks/rings. Disc dispensers if available
Nutrient broth or saline
Sterile forceps
Sterile cotton wool swabs
Measuring device to measure inhibition zones.
Calibrated 0.5 McFarland turbidity standard
Incubators 35 ± 2ºC

Procedure:
Bring plates and antimicrobial disks to room temperature (maximum 2hrs at 22ºC). Ensure that all plates are dry before use.

Inoculum Preparation:
Transfer single colonies from a fresh (18-24 hour) agar plate to a Nutrient broth or saline, using a loop. Ensure that a homogenous suspension is obtained. Adjust the inoculum so that a 0.5 McFarland turbidity is reached, either by adding more colonies or more nutrient broth or saline. Either check visually against the standard or use the DENSICHECK densitometer to obtain 0.5 McFarland turbidity. To inoculate plates, dip a swab into the broth and rotate against the side of the tube above the broth to remove excess liquid. Swab the entire surface of the agar plate three times, rotating approximately 60º between swabbing, ensuring that the swab is rotated continually.

Allow plate to stand for at least 3 minutes, but no longer than 15minutes before applying disks. Disks are applied to the agar surface either by dispenser or by sterile forceps. Apply gentle pressure to ensure complete contact of disk with agar surface. Do not apply disks closer than 24mm from center to center i.e. no more than 7 disks on a 90mm plate. Do not relocate disk once it has made contact with the plate surface as diffusion begins instantly. Incubate plates within 15minutes of disk application. Plates are incubated overnight at 35±2°C aerobically or at 35±2°C in CO_2 overnight depending on organism type. (CO_2 incubation altars the surface pH, affecting the activity of some antimicrobial agents so should be avoided).

Reading of the plates:
Read according to zone sizes (in mm), using a table for that particular organism and provided in the most recent and updated Clinical and Laboratory Standards Institute (CLSI) guideline, and report as sensitive or resistant. If an organism is a very resistant strain, then report those antibiotics that are intermediate.

Read plates only if the lawn of growth is confluent (kissing colonies) or nearly confluent and measure diameter of inhibition zone, (from edge of growth across the centre of disk to opposite edge) to the nearest whole millimetre, using a ruler or callipers.

1. **Translucent media (e.g., Mueller Hinton agar)**
   a. Rest plate (lid down) on a dark non-reflecting surface.
   b. Illuminate plate with reflected light directed from above at a 45º angle
   c. Measure diameter of inhibition zone. For staphylococci examine closely. consider any growth within the zone of inhibition around the cefoxitin disk as evidence of resistance.

2. **Opaque media (e.g., blood-supplemented Mueller Hinton agar)**
   a. Remove cover of agar plate.
   b. Illuminate plate with reflected light directed from above at a 45º angle.
   c. Measure diameter of inhibition zone.
   d. When testing haemolytic organisms, be careful to measure the diameter of the zone

of inhibition of growth and not the zone of inhibition of haemolysis.

3. **Additional considerations:**
   a. Always disregard minute colonies, except for colonies of oxacillin-resistant staphylococci (any growth within zone of inhibition is evidence of resistance) or vancomycin-resistant enterococci.
   b. Disregard swarming of *Proteus* spp., and measure obvious edge of inhibition.
   c. When measuring zones for sulphonamides, trimethoprim, or cotrimoxazole, disregard light growth and measure the edges of heavy growth.

Interpretation of results:

Providing quality control is acceptable, interpret diameters of zones of inhibition according to accepted Clinical and Laboratory Standards Institute/National Committee for Clinical Laboratory Standards (CLSI/NCCLS) criteria.

Report results as:

- Susceptible **S**
- Intermediate **I (only if organism is very resistant to most antimicrobials)**
- Resistant **R**

Quality control:

The goals of a quality control program for antimicrobial discs are to assist in monitoring the following:

- Precision and accuracy of the susceptibility test procedure
- The performance of reagents, media and antibiotics used in the test.
- The performances of persons who carry out the tests and interpret the result.

Quality control (QC) is performed once a week using American Type Culture Collection (ATCC) stock control strains received from National Institute for Communicable Diseases (NICD) External Quality Assessment (EQA) department annually if available or from another supplier. The stock control strains are received as a lyophilised culture in a vial containing >12 glass beads labelled as P2. These strains are sub-cultured once a week, the first culture is P3. The strains should not be sub-cultured beyond 6-7 passages. Some stock controls are received on a swab. Culture according to instructions supplied. For the preparation of working stock reference cultures refer to the instructions from the Quality Assurance Division (QAD) department issuing the organisms. For the reference ranges (mm) for Kirby-Bauer disc susceptibility testing refer to the validation certificate received with each organism or CLSI guidelines. All results are to be recorded on the QC templates for each organism and signed and dated.

Quality assurance:

Corrective action:

When control results are out of the ranges for QC strains, inform the supervisor and proceed with corrective action as follows:

- Review clerical procedures.
- Hold back patients results
- Check materials (including reference strains)
- Check equipment (refrigerators, freezers, incubators etc)
- Review technical aspects of test performance with individuals performing testing.
- If repeat testing is required, consider testing old and new lots of materials with ATCC strains.
- Repeat testing of patient's sample if necessary.

Interpretation of results:
Numerous factors can affect results:

- Inoculum size
- Rate of growth
- Medium formulation and pH
- Incubation environment and length
- Disk content and drug diffusion rate
- Measurement of endpoints.

Storage:
ATCC control strains (beads) and antimicrobial discs are stored between 2-8ºC.
Plate cultures from beads should be stored on the bench for one week away from direct sunlight.
Fastidious organisms should be stored in the incubator.

Antimicrobial selection panels (organism specific)

Enterobacterales and non-Enterobacterales
Medium: Mueller Hinton agar
Inoculum: 0.5 McFarland
Incubation: 35±2^{O}C aerobically
Antimicrobials:
For Enterobacterales: NHLS FGNB1 and NHLS FGNB2 and extra discs–tazobactam and meropenem. Add ertapenem for all Enterobacterales, as decreased ertapenem susceptibility suggests a risk of carbapenemase production.

For non-Enterobacterales/*Pseudomonas*/*Acinetobacter*: NHLS NFGNB and extra discs: meropenem, tobramycin, cefepime, imipenem and colistin (or any other antimicrobial requested by physician. If colistin is required for *Acinetobacter* spp. an MIC must be performed.

For *Stenotrophomonas maltophilia*: CLSI disk criteria exist for cotrimoxazole and levofloxacin. If resistant to cotrimoxazole, perform ceftazidime Epsilometer test (E-test).

Refer to mastering profiles-templates for zone sizes

Antimicrobial agents for Enterobacterales and non-fermenters	**Disk content**	**Resistant**	**Intermediate**	**Susceptible**
β-lactams penicillins				
ampicillin /amoxycillin	10µg	≤ 13	14-16	≥ 17
β-lactam/β-lactamase inhibitor combinations				
amoxicillin/clavulanic acid	20/10µg	≤ 13	14-17	≥ 18
piperacillin-tazobactam*				
Enterobacterales (including *Acinetobacter baumannii*)	100/10µg	≤ 17	18-20	≥ 21
Pseudomonas aeruginosa	100/10µg	≤ 14	15-20	≥ 21
Lipopeptides				
colistin				
Pseudomonas aeruginosa	10 µg	≤ 10	N/A	≥ 11
Cephalosporins and other				

Cephems				
cefoxitin (Enterobacterales)	30µg	≤ 14	15-17	≥ 18
ceftazidime (*Pseudomonas*)	30µg	≤ 14	15-17	≥ 18
ceftazidime (Enterobacterales)	30µg	≤ 17	18-20	≥ 21
ceftriaxone (Enterobacterales)	30µg	≤ 19	20-22	≥ 23
cefuroxime sodium-parenteral (Enterobacterales)	30µg	≤ 14	15-17	≥ 18
cefuroxime sodium - Oral	30µg	≤ 14	15-22	≥ 23
cefepime*	30µg	≤ 14	19-24	≥ 25
Carbapenems (*Acinetobacter*)				
imipenem*	10µg	≤ 18	19-21	≥ 22
meropenem*	10µg	≤ 14	15-17	≤ 18
Carbapenems (*Pseudomonas*)				
imipenem	10µg	≤ 15	16-18	≥ 19
meropenem	10µg	≤ 15	16-18	≥ 19
Carbapenems (Enterobacterales)				
imipenem*	10µg	≥ 19	20-22	≥ 23
meropenem*	10µg	≥ 19	20-22	≥ 23
ertapenem*	10µg	≥ 18	19-21	≥ 22
Aminoglycosides				
amikacin	30µg	≤ 14	15-16	≥ 17
gentamicin	10µg	≤ 12	13-14	≥ 15
tobramycin*	10µg	≤ 12	13-14	≥ 15
Quinolones				
ciprofloxacin (Enterobacterales other than *S. typhi* and extraintestinal *Salmonella* spp.)	5µg	≤ 15	16-20	≥ 21
ciprofloxacin (*S. typhi* and extraintestinal *Salmonella* spp.)	5µg	≤ 20	21-30	≥ 31
nalidixic acid	30µg	≤ 13	14-18	≥ 19
ofloxacin	5µg	≤ 12	13-15	≥ 16
levofloxacin (*Stenotrophomonas*)	5µg	≤ 13	14-16	≥ 17
Others				
chloramphenicol	30µg	≤ 12	13-17	≥ 18
tetracycline	30µg	≤ 11	12-14	≥ 15
nitrofurantoin	300µg	≤ 14	15-16	≥ 17
Cotrimoxazole (including *S. maltophilia*)	1.25/23.75µg	≤ 10	11-15	≥ 16

NB: The table is modified in accordance to the font, current naming (e.g., Enterobacterales) and antimicrobial writing (small letters) on the thesis. *

Reported on ICU patients and resistant organisms for non-Enterobacterales. N/A: Not applicable.

If Enterobacterales show resistance to the carbapenems: meropenem, ertapenem or imipenem, repeat sensitivities using E-test for the resistant carbapenem, confirm purity, inform infection control services

and clinician (explain any uncertainty about initial findings if relevant), and make arrangements for the isolate to be sent to the NICD Molecular Epidemiology Unit at Groote Schuur Hospital (GSH) (or other laboratory) for a carbapenemase Polymerase Chain Reaction (PCR) if required for that organism.

Other non-Enterobacterales include *Pseudomonas* spp. (not *P. aeruginosa*) and other non-fastidious, glucose-non-fermenting, Gram-negative bacilli, but exclude *P. aeruginosa*, *Acinetobacter* spp. *Burkholderia cepacia*, *B. mallei*, *B. pseudomallei*, *Stenotrophomonas maltophilia*. For this organism group, disk diffusion is not currently recommended. Refer to CLSI document.

For non-fermenters: report tazobactam, ceftazidime, meropenem and ciprofloxacin.

Staphylococcus
Medium: Mueller Hinton agar
Inoculum: 0.5 McFarland
Incubation: 35±2^{o}C aerobically
Antimicrobials: NHLS Staph-ring (or loose discs if not available)
cefoxitin is used as a surrogate to report oxacillin. Based on the cefoxitin result, report oxacillin as susceptible or resistant. Refer to the NHLS mastering profiles-templates for zone sizes.

Antimicrobial agents for *Staphylococcus*	**Disk content**	**Resistant**	**Intermediate**	**Susceptible**
β-lactams/penicillins				
cefoxitin for *S. aureus* and *S. lugdunensis*	30µg	≤ 21	N/A	≥ 22
cefoxitin for CoNS except *S. lugdunensis*	30µg	≤ 24	N/A	≥ 25
penicillin	10 units	≤ 28	N/A	≥ 29
Glycopeptides				
vancomycin	30µg	N/A	N/A	N/A
teicoplanin	30µg	≤ 10	11-13	≥ 14
Macrolides				
erythromycin	15µg	≤ 13	14-22	≥ 23
Others				
clindamycin	2µg	≤ 14	15-20	≥ 21
cotrimoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16
chloramphenicol (for CSF's and eyes)	30µg	≤ 19	13-17	≥ 16
ciprofloxacin (eyes and urines)	5µg	≤ 15	16-20	≥ 21

NB: The table is modified in accordance to the font, current naming (e.g., Enterobacterales) and antimicrobial writing (small letters) on the thesis.

N/A: Not applicable.

Check for D-zone between erythromycin and clindamycin. Perform vancomycin E-test (MIC) for MRSA from blood cultures and CSF specimens and other sterile sites if requested. An alternative is to perform a Vitek (automated) susceptibility test and to record the derived vancomycin MIC in the result. There are no CLSI zone diameter interpretive criteria for vancomycin for *Staphylococcus* spp.

For CoNS: Do not report sensitivities from conjunctival/eye swabs as no criteria exists for the accurate sensitivity reporting for topical use. Only report CoNS from sterile sites – this includes tissues, bone and pus swabs from patients with clinical diagnosis of osteomyelitis.

For *Staphylococcus saprophyticus*: Do not report sensitivities, just enter code STASA which decodes to *S. saprophyticus* susceptibility tests are not standardized.

***Streptococcus* and *Enterococcus* spp.**
Medium: Mueller Hinton agar with 5% sheep blood agar (SPN)
Inoculum: 0.5 McFarland
Incubation: 35±2^{o}C CO_2
Antimicrobials: Individual discs are placed onto the media.
Vancomycin must be read after 24hours.

Antimicrobial agents for *Streptococcus* and *Enterococcus*	**Disk content**	**Resistant**	**Intermediate**	**Susceptible**
β-lactams/penicillins				
ampicillin (*Enterococcus*)	10µg	≤ 16	N/A	≥ 17
ampicillin (*Streptococcus*, excl. *S. pneumoniae*)	10µg	N/A	N/A	≥ 24
penicillin (*Enterococcus*)	10 units	≤ 14	N/A	≥ 15
penicillin (*Streptococcus*, excl. *S. pneumoniae*)	10 units	N/A	N/A	≥ 24
Glycopeptides				
vancomycin (*Enterococcus*)	30µg	≤ 14	15-16	≤ 17
vancomycin (*Streptococcus*)	30µg	N/A	N/A	≤ 17
Macrolides				
erythromycin (*Enterococcus*)	15µg	≤ 13	14-22	≤ 23
erythromycin (*Streptococcus*)	15µg	≤ 15	16-20	≤ 21
Lincosamides				
clindamycin	N/A	≤ 15	16-18	≤ 19
Cephems				
cefotaxime/ceftriaxone (*Streptococcus*)	30µg	N/A	N/A	≥ 24
Extra sensitivities for *Enterococcus* isolates from CSF and blood cultures (sterile sites)				
High level gentamicin	120µg	N/A	N/A	≥ 10

NB: The table is modified in accordance to the font, current naming (e.g., Enterobacterales) and antimicrobial writing (small letters) on the thesis.

N/A: Not applicable.

Enterococcal endocarditis requires combined therapy with high-dose penicillin or high-dose ampicillin, or vancomycin plus gentamicin or streptomycin for bactericidal action. Because of limited alternatives, chloramphenicol, erythromycin, tetracycline and rifampicin may be used for VRE. All VRE strains are to be sent to NICD. Rifampicin must not be used alone for chemotherapy. For CSF, blood cultures, and other sterile sites, on *Enterococcus* spp., an automated ID must be performed. A vancomycin MIC must be done if ampicillin is resistant.

Streptococcus pneumoniae
Medium: Mueller Hinton agar with 5% sheep blood agar (SPN)
Inoculum: 0.5 McFarland
Incubation: 35±2^{o}C in CO_2
Antimicrobials: NHLS pneumo-ring or individual discs are placed onto the media.

Antimicrobial agents for *Streptococcus pneumoniae*	**Disk content**	**Resistant**	**Intermediate**	**Susceptible**
β-lactams/penicillins				
oxacillin (report as penicillin)	1µg	N/A	N/A	≥ 20
Macrolides				
erythromycin	15µg	≤ 15	16-20	≥ 21
Others				
chloramphenicol	30µg	≤ 20	N/A	≥ 21
cotrimoxazole	1.25/23.75µg	≤ 15	16-18	≥ 19
clindamycin	2µg	≤ 15	16-18	≥ 19
tetracycline	30µg	≤ 18	19-22	≥ 23

NB: The table is modified in accordance to the font, current naming (e.g., Enterobacterales) and antimicrobial writing (small letters) on the thesis.

N/A: Not applicable.

Extra testing for *pneumococcus*:

For non-invasive isolates (sputum, ear, eye, etc.) that are penicillin resistant, test *pneumococcus* against penicillin using a penicillin and ceftriaxone E-test strip.

For invasive isolates (CSF, and blood cultures) perform E-test for penicillin and ceftriaxone regardless of sensitivity result (refer to E-test SOP). On Laboratory Information System (LIS) for CSF's only release the penicillin result. If the E-test screen is intermediate or fully resistant to ceftriaxone, vancomycin or rifampicin may be added. Rifampicin should not be used alone for chemotherapy.

Any *viridans streptococcus* - this includes the following five groups: *mutans*, *salivarius*, *bovis*, *anginosus* (previously *S. milleri*) and *mitis* - isolated from sterile body sites, perform penicillin and ceftriaxone MIC using E-test strips.
There are no disc criteria for penicillin or ampicillin for *Streptococcus viridans* group.

Anaerobic organisms:
No CLSI/NCCLS criteria, therefore no susceptibility testing done, only screen for metronidazole, not reported. MIC's can be measured if clinically indicated.

Any other organism not covered in this document:
Refer to SOP MIC0018 and SOP NJHM0037.
Either consult with a pathologist or select antimicrobials to be reported once referred to CLSI reference document or contact NICD.

The following antimicrobial principles must be applied:

1. *Staphylococcus* that are resistant to oxacillin/cefoxitin (reported as cloxacillin resistant): Report all penicillins and cephalosporins as resistant. Add MRSA comment for oxacillin resistant *S. aureus* at comment field on LIS.
2. Use oxacillin disc to determine penicillin susceptibility on *pneumococcus*.
3. *Proteus spp*. are always resistant to nitrofurantoin.
4. *Klebsiella* are always resistant to amoxicillin/ampicillin

5. For all ESBL-producing organisms: report all cephalosporins as resistant, including cefepime. Add ESBL comment ESBLC at comment field on LIS.
6. Comments: Paediatric patients and pregnant women: Although organism is sensitive to tetracyclines, nitrofurantoin and quinolones, these agents are not recommended for the use for children and pregnant women.
7. *Enterobacter* spp. have the potential to develop resistance to cephalosporins. Consequently, these agents should be avoided for treatment of *Enterobacter* infections. If the cefoxitin is resistant – add Inducible β-lactamase (IBL) comment on the LIS. Do not release the cephalosporins, except for cefepime when ceftriaxone is sensitive.
8. If an organism is identified as *Staphylococcus epidermidis*, resistant to cloxacillin but susceptible to ampicillin, perform a Gram stain and re-identify.
9. Gram- positive organisms susceptible to penicillin will always be susceptible to ampicillin/amoxycillin. If disk susceptibility testing suggests otherwise, the disks have probably become ineffective and corrective action should be carried out.
10. Do not report aminoglycosides (GM and AK) for *Streptococcus*.
11. Enterococci are always resistant to cephalosporins, clindamycin and cotrimoxazole.
12. Do not treat blood and CSF isolates with first-generation cephalosporins.
13. For organisms isolated from pus swabs that can be skin contaminants such as CoNS, *Corynebacterium* spp., *viridans Streptococcus* and *Bacillus* spp. Antimicrobials should not be reported except for heart tissue.
14. When reading *Proteus* spp. ignore the thin veil of swarming growth in an otherwise obvious zone of growth inhibition.
15. For all resistant organisms e.g., *Acinetobacter* or *Pseudomonas*, add multidrug resistant organism (MRO) comment at
    Comment field on LIS. Add colistin comment when necessary.

For further information on resistance mechanisms and reporting, refer to the NHLS proficiency testing commentary and antimicrobial susceptibility SOPs.

References:

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: Twenty-Third Informational Supplement. 2013; M100: S23.

Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Second Edition. M45-A2; Vol 30 No. 18.

Performance standards for Antimicrobial Disk Susceptibility Tests Eleventh edition.
2012; M02-11: Vol3.

Appendix 3: Vitek 2 System Standard operating system (SOP).

This SOP was adopted from the NHLS Q-pulse (Q-Pulse5/docs/active/MIC1587v9)

Purpose:
This SOP details the procedure to be followed in the sample processing methodology of Vitek 2/XL system as well as procedure to be followed for reviewing daily results.

Scope:
All competent technical staff may perform this test. Students may perform this test under supervision of a qualified technologist.

Responsibility:
All medical technologists, medical technicians, students (under supervision) who are trained to perform these test procedures.

Abbreviations:
AES – Advanced expert system
API – Analytical profile index
AST – Antimicrobial susceptibility testing
CD - Compact disc
CO_2 – Carbon dioxide
CPU - Central processing unit
Nacl – Sodium chloride
N/H ID – *Neisseria* and *Haemophilus* identification
QC – Quality control

Principle:
The Vitek 2 is an automated instrument, capable of rapid identification and antimicrobial sensitivity testing of microorganisms. The identification is done up to species level, and includes:

- Most Gram-negative bacilli
- Gram-positive cocci
- Yeast
- *Neisseria* and *Haemophilus* group organisms
- Anaerobes
- Corynebacteria

The Gold standard analytical profile index (API) technology forms the basis of the Vitek 2 systems identification capacity.

Antibiotic susceptibility results are reported in terms of MIC's (minimum inhibitory concentration), which are more clinically and epidemiologically relevant.

The heart of the Vitek 2/XL is the advanced expert system (AES), which is responsible for analysing the data, correlating identification with sensitivity, and determining the presence of resistance mechanisms and making appropriate recommendations for antimicrobial therapy.

Equipment and reagents:

Vitek 2 system

- Clear plastic tubes.
- Saline dispenser – dispensing 3.5- 4.0ml of saline into sterile plastic tubes.
- Sterile swabs.
- Densichek.
- Blood purity plates.

- MacConkey purity plate.
- CO_2 incubator at 35-37°C.
- O_2 incubator at 35-37°C.
- Bunsen burner.
- Wire loops.
- Vitek 2 identification card.
- Vitek 2 AST (antimicrobial sensitivity) card.

Environmental and safety controls:
Universal safety precautions need to be followed at all times.

Procedure steps:
Frequency of testing as required.

Sample required:
An 18-24 hr culture of the organism to be tested

Storage of materials:

- Vitek 2 identification & AST cards at 2°C – 8°C
- Sterile saline dispenser at 18°C – 25°C

Daily check:

- Remove cards and saline from fridge and leave at room temperature.
- If Pneumo to be picked off, pre-heat saline tube at 35-37°C.
- Empty waste box of previous days cards.
- Change back up compact disc (CD) to appropriate day.
- Manually record the QC/temp check.
- Check that the saline purity plate is free of growth.
- Calibrate the Densichek with the calibration standards provided. The surface of the Densichek may be cleaned by using a damp cloth and mild detergent.

NB: Do not use alcohol to clean the instrument. Use of alcohol may damage the reading lens.

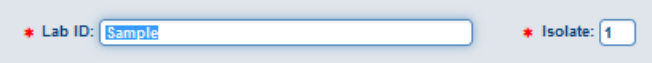
Setting up of new isolate testing:

- Aseptically dispense 3.5ml of sterile saline 0.45 – 0.5%: Sodium chloride (NaCI) pH 5-7.2 into appropriate number of clear plastic tubes.
- Mark all new purity plates with specimen numbers.
- Select well isolate colonies from a primary plate if culture requirements have been met.
  NB: If mixed cultures are present a sub-culture step should be done instead.
- Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies to a pre-dispensed saline tube.
- Prepare a homogenous organism ID suspension with a density equivalent to the recommended McFarland standard required, using a calibrated Vitek 2 Densicheck.

Gram-negative bacilli	**0.5 – 0.63 (target 0.6)**
Gram-positive bacilli	**0.5 – 0.63 (target 0.6)**
Gram-positive cocci	**0.5 – 0.63 (target 0.6)**
Yeast	**1.8 – 2.20 (target 2.0)**
NH ID	**2.7 – 3.30 (target 3.0)**
Anaerobes/Coryne	**2.7 – 3.30 (target 3.0)**

Starting at the **Cassette Identification** screen, click the **Lab ID** field and enter the Lab ID.

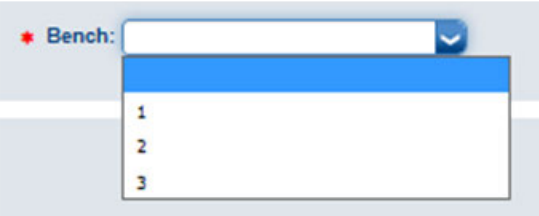
Figure 1: Lab ID and Isolate Number Fields



This can be done either by scanning the barcode or manually entering the barcode number. The isolate number will automatically populate.

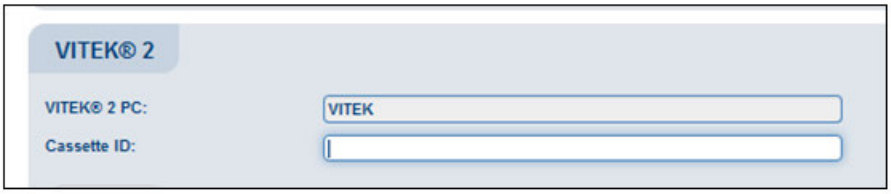
2. Select the bench name in the **Bench** drop-down field. (Only available if Bench is enabled in General Configuration and set to display in FLEXPREP™.)

Figure 2: Bench Drop-Down Field



3. Enter the desired cassette ID for the cassette in use (for VITEK® 2 60 and XL cassettes) or select a cassette number from the drop-down list (for VITEK® 2 Compact instruments). Entering Cassette Information Preparing a VITEK® 2 Cassette in VITEK® FLEXPREP™

Figure 3: Cassette ID Field



4. Press **Enter**.

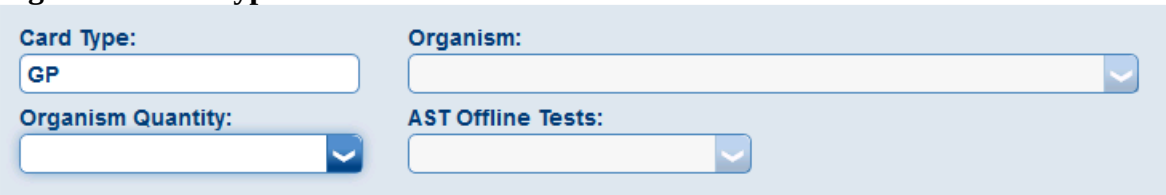
Defining Card Types:
There are 3 possible scenarios for defining card types in a cassette:
1. Single ID Card
2. ID Card with AST Card(s)
3. Single AST Card
Each scenario involves a slightly different method of data entry. All scenarios require the user to validate the isolate before defining the next isolate or sending the cassette.

Single ID Card:
1. The **Lab ID**, **Isolate** number, and **Bench** (if enabled) are entered in the Cassette Identification screen.

If the information does not auto-populate, it may be entered or edited by clicking the field and keying the information directly into the field.

2. Click the **Card Type** field and enter the barcode number. This can be done either by scanning the barcode or manually entering the barcode number.

Figure 4: Card Type Field

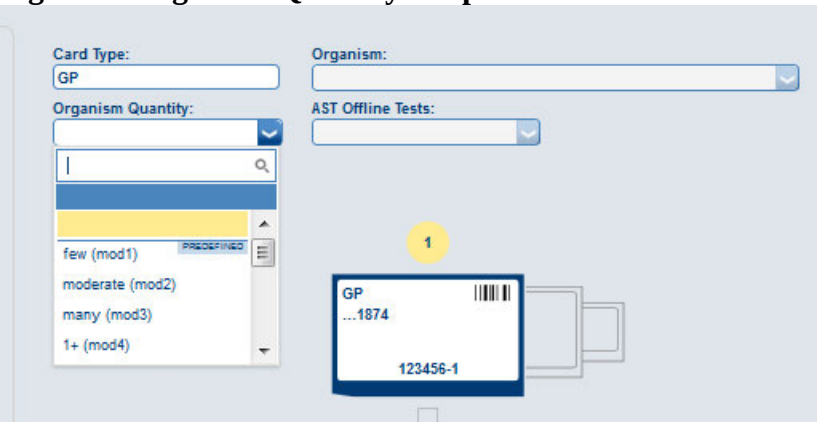


The card type should auto-populate in the **Card Type** field when the barcode is recognized. Once the card type is entered, a graphic of the isolate will be displayed. The graphic includes both a card and test tube.

Figure 5: Card Graphic

The **Organism Quantity** field is not required, but users can make a selection from the drop-down field if desired.

Figure 6: Organism Quantity Drop-Down Field



The **Organism** and **AST Offline Tests** fields will be unavailable for entry on an ID card. If enabled, the McFarland values can be entered or will auto-populate, if previously defined in Quick McFarland Entry.

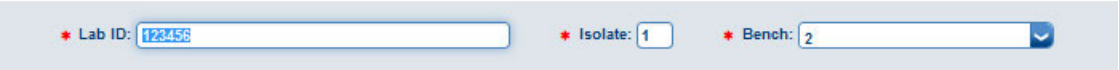
3. Click **Validate** to save the information and advance to defining the next isolate.

Note: *Once an isolate is validated, no changes are allowed. If changes need to be made, you will need to delete the isolate and re-enter the information.*

<u>ID Card with AST Card(s)</u>:

1. For the first care in the isolate, the **Lab ID**, **Isolate** number, and **Bench** (if enabled) selection is entered in the Cassette Identification screen. This information may be edited prior to validation by clicking the field and manually entering the correction. If the information does not auto-populate, it may be entered or edited by clicking the field and keying the information directly into the field.

Figure 7: Lab ID, Isolate, and Bench Fields



2. Click the **Card Type** field and enter the barcode number for the ID card. This can be done by scanning the barcode or entering it manually.

Figure 8: Card Type Field with Barcode

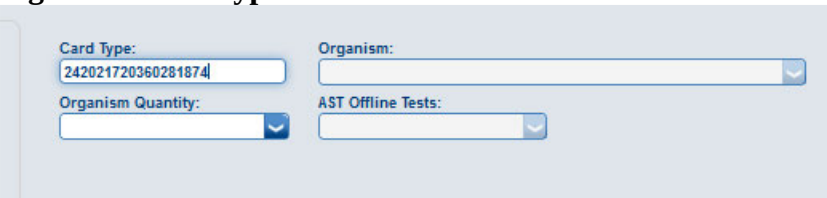
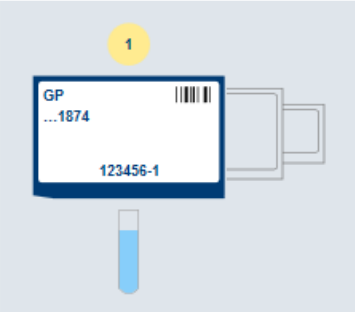


Figure 9: Card Graphic



The **Organism Quantity** field is not required, but users can make a selection from the drop-down menu if desired.

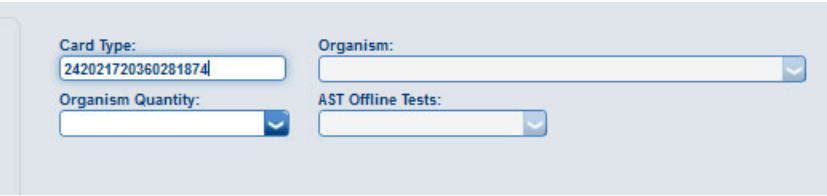
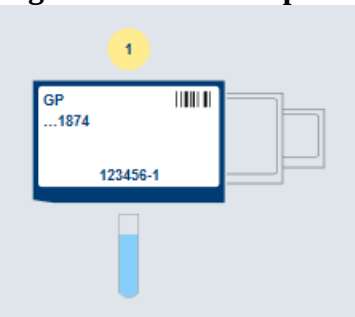
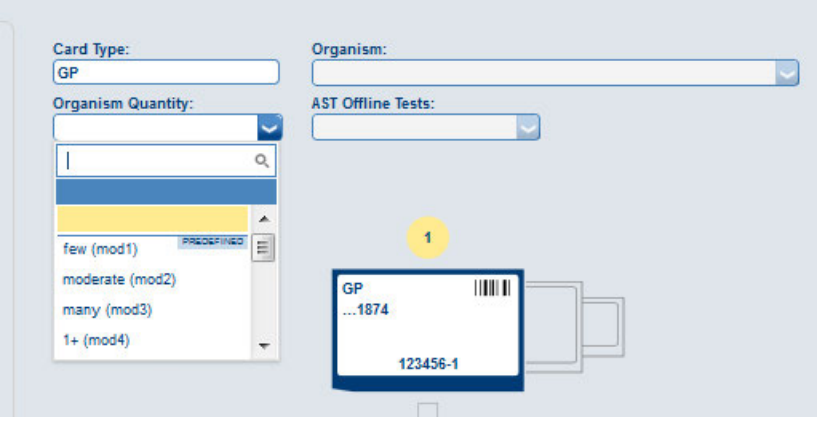


Figure 10: Card Graphic



The **Organism Quantity** field is not required, but users can make a selection from the drop-down menu if desired.

Figure 11: Organism Quantity Drop-down Menu



The **Organism** and **AST Offline Tests** will be unavailable for entry on an ID card. If enabled, McFarland can be entered. If the McFarland value was already entered using the Quick Entry McFarland screen, the appropriate McFarland will be auto populated.

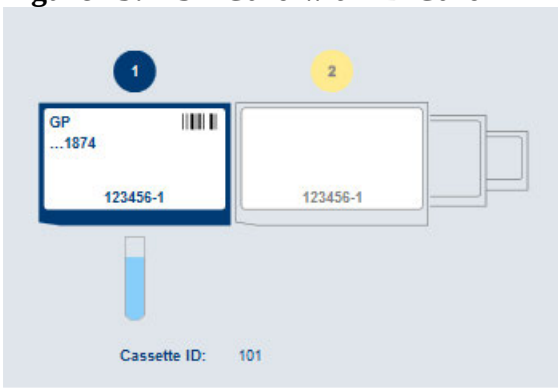
IMPORTANT: Do not click Validate. If Validate is clicked, the ID card will validate as a single card and cannot be paired with an AST card.

3. Click **Add Card** (**F8**) to add an AST card to the ID card.

Figure 12: Add Card Button

After the AST card is added, a second empty card graphic will appear beside the ID card graphic.

Figure 13: AST Card with ID Card



4. Click the **Card Type** field and enter the barcode number. This can be done either by scanning the barcode or manually entering the barcode number.

Figure 14: Card Type with Barcode

The **Card Type** field will auto-populate with the card type when the barcode is recognized.

Note: *If the card type is not recognized, it must be defined in the VITEK® 2 Systems software before the card can be added to the cassette. Refer to the VITEK® 2 Systems Software User Manual.*

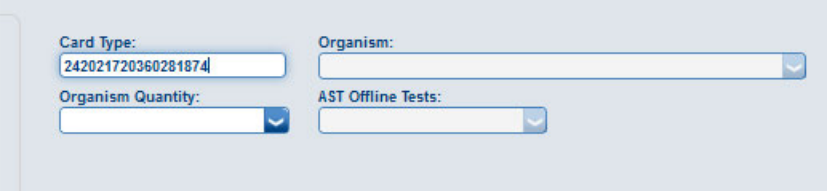
The **Organism Quantity** and **AST Offline Tests** fields are not required, but users can make a selection from the drop-down menu if desired.

Single AST Card:

1. The **Lab ID**, **Isolate** number, and **Bench** selection are entered in the Cassette Identification screen. This information may be edited by clicking the field and manually entering the correction.

2. Click the **Card Type** field and enter the barcode number. This can be done by scanning the barcode or entering it manually.

Figure 15: Card Type Field with Barcode

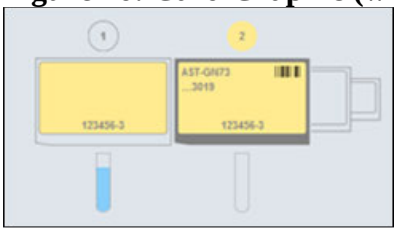


The **Card Type** field will auto-populate with the card type when the barcode is recognized.

Note: *If the card type is not recognized, it must be defined in the VITEK® 2 Systems software before the card can be added to the cassette. Refer to the VITEK® 2 Systems Software User Manual.*

Once the card type is entered, a graphic of the isolate will be displayed. If auto dilute is enabled, the first slot shows a test tube filled with saline (no card), and the second slot shows the AST card and an empty test tube. If predilute is enabled, the single AST card will be shown in the first card slot.

Figure 16: Card Graphic (with Auto Dilute Enabled)



Sending a Cassette to VITEK® 2:
Once each isolate has been defined in the cassette in VITEK® FLEXPREP™ and the user has confirmed that the software matches the physical cassette, the cassette is ready to be sent to the VITEK® 2 Systems instrument.

1. Click the **Summary** button to access the Cassette Summary screen.

2. Review the details of each card slot and ensure the software matches the physical cassette.

3. Click **Back** to return to the Cassette Definition screen.

IMPORTANT: If any cards need to be edited, delete the isolate and re-enter the correct card information.

4. Click the **Send Cassette** button.

The **Please confirm** dialog will appear. This dialog shows the available instruments attached to the VITEK® 2 PC.

Figure 17: Please Confirm Dialog:

IMPORTANT: The cassette must be loaded on one of the instruments shown in the Please confirm dialog. Loading it on an instrument not shown in the dialog will result in errors.

5. Click **OK** to send the cassette.

<u>Procedure for reviewing daily results</u>:

- From the main menu, click the view and maintain isolate results icon.
- Change the "view by" filter to date tested and "filter by" filter to show all.
- Click on each sample number, at isolate level, to be able to review.
- **Check the purity plate first. This must be done before any review or choice is made**.
- Results with a **V** require review only.
    - If purity plate is fine and you are in agreement with the results visible, you may click on the review button, then indicate print report and click ok for the correct printer.
- Results with  require technologist involvement.
    - If the purity plate is fine, then one of the following may be required:
    - Slash line – multiple organisms from the same genus are options, and 1
        - must be chosen, or the organism may belong to a group which requires
        - serological confirmation before choosing the Final Identification.
    - Low discrimination – multiple organisms from different genus are options, and
        - a choice must be made by user.
        - If uncertain about choice – repeat testing.
    - Missing patient information – enter info.
    - Missing identification (i.e. only AST card put up) – enter ID.
    - Missing offline tests – enter test info.

2. Mixed or unidentified results must be repeated, but must still be reviewed and printed. The printout must have "MIXED" written on the form, and must be filed as evidence for audit trails.

3. Results with ■ are reviewed, can be printed and reported.

   NB: *the patient's episode number on the Vitek result sheet must be checked against the episode number on the request form and then highlighted when attaching the result sheet to corresponding request form.*

4. Ultimately, all results on a daily basis must have this icon.

Maintenance:

Daily Maintenance

Removal of ejected (completed) cards

- Open the waste collection door.
- Place the index finger on one hand on the sliding retainer bar to prevent it from snapping back.
- Remove the test card collection tray from the station by slightly lifting the front edge of the tray and then pulling it outward. When the tray is clear of the station, allow the sliding retainer bar to slowly slide back into place.
- Dispose of waste cards in the tray. If the slide bar does not move all the way to the left, the system assumes no cards were removed.
- Firmly slide the tray onto its shelf, lifting the front edge to clear the retaining brackets.
- Close the waste collection door.

The Vitek 2 instrument contains 2 consumables, namely saline and pipette tips, which must be monitored daily using the status screen of the Vitek 2.

*They must be changed together, and the user must run the 9dispenser/pipettor diagnostic tests after the change.

How to change saline:

- Select utilities > Maintenance > Change Saline.
- Open the front access door, lift the saline access door.
- Pull out the filter disk to remove it from its connector.
- While pushing the dispenser release button, pull the saline dispenser tube out of its housing.
- Discard the saline dispenser tube, its tubing, and the saline bag.
- Place the new bag of saline on its shelf at the top of the instrument. Using aseptic technique, removes a new tubing assembly from its bag, take the new bag and insert the cannula into the port of a new saline bag.
- While pushing the dispenser release button, insert the saline dispenser tube into its housing.
- Push the filter disk onto the connector above the saline dispenser tube.
- Close the front panel and saline access door. Go to the UIF screen and press DONE.

How to change pipette tips:

- Select utilities > Maintenance > Change pipette tips.
- Open the front access door, rotate the pipette tip container 90°C in a counter clockwise direction and remove the pipette tip container from its base by pulling upward.
- Discard any pipette tips that remain in the container to maintain an accurate count and minimize contamination.
- Open a pipette tip replacement pack and place it on the bench top. Using aseptic technique hold the pipette tip container with its open end down and lower it over the exposed ends of the pipette tips. Invert the pipette tip container and pipette tip replacement pack so that the pipette tips fall into the pipette tip container. Shake the container gently to ensure that tips fit in properly.
- Replace the pipette tip container on its base with the alignment pin towards you. Rotate the pipette tip container 90 degrees in a clockwise direction.
- Close the front panel door and the pipette tip container lid. Go to the UIF screen and press done.

Dispenser/pipettor diagnostic test:

- Prepare a cassette containing test tubes filled with 3. 3.5 ml of saline in slots 1 & 3 and empty test tubes in slots 2, 4 & 5.
  This does not require the use of a smart carrier station.
- Select utilities > Diagnostics > Dispenser/Pipettor, open the cassette load door, load the cassette, close the door.
- Press the YES option. This test will proceed and a PASS/FAIL status will be reported.
- If the test fails, contact a Biomerieux representative for assistance.

Trouble shooting:

- In case of software / hardware failure:
- Troubleshoot first by referring to Vitek manual before calling service engineer.
- Shut down the computer and monitor
- Shut down central processing unit (CPU) (black box).
- Shut down Vitek machine by switching it off. Switch located on the side of the machine.
- Wait a few minutes and switch all components back **ON.**

If all else fails – call service centre – number displayed on the Vitek instrument.

For further information and monthly maintenance procedure Refer to Vitek 2 System Training manual or online software user manual (S10773 – 4EN – 1).

Quality control procedure:
Vitek identification and antimicrobials is tested once a month with reference strains recommended by the manufacturer.

Potential sources of variation, limitations and interferences:
Each card type has limitations, so it is important to read the package insert and take note of this before its use.

- *S. maltophilia*, only trimethoprim/sulfamethoxazole is claimed for susceptibility testing.
- Gram positive: only *Staphylococcus*, *Streptococcus Group B*, Enterococci and *Strep. Pneumoniae* are claimed for susceptibility testing.
- Pneumococci antimicrobial susceptibility testing (AST) can be difficult due to the fastidious nature of the organism, and requires some nurturing:
  - ❖ Saline tubes must be pre- heated at 35-37°C for 30 minutes before picking *Pneumococcus*.
  - ❖ McFarland should be as close as possible; organism must be picked off immediately or plate must remain in CO_2 incubator until pick – off.

References:
1. Vitek 1 System Training manual.
2. Online software user manual (S10773 – 4EN – 1)
3. Software user manual for Vitek 2 and Vitek 2 Compact

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

11 October 2023

Miss Sindiswa Charlotte Selane (215026909)
School of Laboratory Medicine & Medical Science
Medical School

Dear Miss Selane,

Protocol reference number: BREC/00004902/2022
Project title: Causes of bloodstream infections in adult Intensive Care patients at eThekwini KwaZulu-Natal Hospitals.
Degree: Masters

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 11 October 2023. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 11 October 2023. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 14 November 2023.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

ACADEMIC COMPLEX BUSINESS UNIT
DEPARTMENT OF MEDICAL MICROBIOLOGY
SCHOOL OF LMMS, CHS, UKZN & NHLS
Level 4, Pathology Laboratory Building
Inkosi Albert Luthuli Central Hospital
800 Vusi Mzimela (Bellair) Road, Mayville, 4091
Tel: +27 (0)31 240 2787
Reference:

Practice No: 5200296

Permission letter from Medical Microbiology Department

NHLS/ KZN

Date: 21 August 2023

RE: Causes of bloodstream infections in adult Intensive Care patients at eThekwini KwaZulu-Natal Hospitals

Student name: Sindiswa C. Selane

Student number: 215026909

Degree: Master of Medical Science – Medical Microbiology (MMDSC)(UKZN)

Provisional reference number: BREC/00004902/2022; **AARMS project number:** PR2232923

Supervisor: Prof. S. Moodley; **Co-supervisor:** Dr. Y. Mahabeer (Pathologist, Microbiology-NHLS)

In my capacity as the Academic Head of Department, the Discipline of Medical Microbiology at the National Health Laboratory Services (NHLS)/ University of KwaZulu -Natal (UKZN), KZN, it gives me great pleasure to write this letter in support of your MMDSC Degree Research Project as per your protocol, objectives, and methodology of the project. You as a UKZN – MMDSC student has permitted to analyze the micro- data on the ''*Causes of bloodstream infections in adult Intensive Care patients from KZN hospitals* (*King Edward VIII Tertiary Hospital, Inkosi Albert Luthuli Central Hospital and Prince Mshiyeni Memorial Regional Hospital).''* during study period under supervisor Dr Yesho Mahabeer (Micro-Pathologist-NHLS) that will be started after BREC final approval. That letter would support to get final approval of BREC.

Yours sincerely,

Prof. Khine Swe Swe/Han (MB,BS; DTMH; PDIC; FC Path; MMed; PhD(Med Micro)
Head of Academic Department (HOD)
Medical Microbiology Department- KZN
Inkosi Albert Luthuli Central Hospital Academic Complex
Univ of KwaZulu-Natal & National Health Laboratory Services
School of Laboratory Medicine & Medical Sciences
Phone: 031 2402784/2787/2793 ; Fax: 0312402786 ; Email: Sweswe-han@ukzn.ac.za;
www.nhls.ac.za; Practice Number: 5200296

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
OFFICE OF THE MEDICAL MANAGER
INKOSI ALBERT LUTHULI CENTRAL HOSPITAL

Physical Address 800 Vusi Mzimela Road, Mayville - 4058
Postal Address: Private bag X03 Mayville – 4058
Tel: 031 240 1554 Fax: 031 240 1005 Email: [redacted]
www.kznhealth.gov.za

Reference: BREC/00004902/2022
Enquiries: Dr. S. Singh

09th September 2024

Miss Sindiswa Charlotte Selane
School of Laboratory Medicine & Medical Science
UKZN Medical School

Dear Miss Selane

RE: PERMISSION TO CONDUCT RESEARCH AT IALCH

I have pleasure in informing you that permission has been granted to you by the Medical Manager to conduct research on: **Causes of bloodstream infections in adult intensive care patients at eThekwini KwaZulu Natal**.

Kindly take note of the following information before you continue:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN Department of Health.
3. Kindly ensure that this office is informed before you commence your research.
4. The hospital will not provide any resources for this research.
5. You will be expected to provide feedback once your research is complete to the Medical Manager.

Yours faithfully

[redacted]
.....
Dr. S. Singh
Medical Manager
IALCH

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:

Physical Address 800 Vusi Mzimela Road, Mayville - 4058
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www.kznhealth.gov.za

OFFICE OF THE MEDICAL MANAGER
INKOSI ALBERT LUTHULI CENTRAL HOSPITAL

Reference: BREC/00004902/2022
Enquiries: Dr. S. Singh

09th September 2024

Miss Sindiswa Charlotte Selane
School of Laboratory Medicine & Medical Science
UKZN Medical School

Dear Miss Selane

Re: Approved Research: Ref No: BREC/00004902/2022 – Causes of bloodstream infections in adult Intensive Care patients at eThekwini KwaZulu Natal

As per the policy of the Provincial Health Research Committee (PHRC), you are hereby granted permission to conduct the above-mentioned research once all relevant documentation has been submitted to PHRC inclusive of Full Ethical Approval.

Kindly note the following.

1. The research should adhere to all policies, procedures, protocols and guidelines of the KwaZulu-Natal Department of Health.
2. Research will only commence once the PHRC has granted approval to the researcher.
3. The researcher must ensure that the Medical Manager is informed before the commencement of the research by means of the approval letter by the chairperson of the PHRC.
4. The Medical Manager expects to be provided feedback on the findings of the research.
5. Kindly submit your research to:

- The application is an online process by logging on to: HTTP://NHRD.HEALTH.GOV.ZA and follow the steps as indicated on the Provincial Health Research page.

Yours faithfully

[redacted]

Dr. S. Singh
Medical Manager
IALCH

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

10 November 2023

Applicant: Sindiswa Selane
Institution: Mangosuthu University of Technology
E-mail Address: sindiswa.charlotte@gmail.com
Tel: 031 907 7518 **Cell:** 073 661 2334

Project Title: CAUSES OF BLOODSTREAM INFECTIONS IN ADULT INTENSIVE CARE PATIENTS AT ETHEKWINI KWAZULU-NATAL HOSPITALS.
Reference Number: PR2232923

Research Application Type(s):
1. Request for Data

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 NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) 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.

[redacted]

D[redacted]-Kgokong
National Manager: Academic Affairs and Research

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email: hrkm@kznhealth.gov.za

Health Research & Knowledge Management

NHRD Ref: KZ_202409_016

Dear Ms SC Selane
(UKZN)

Approval of research

1. The research proposal titled **'Causes of bloodstream infections in adult Intensive Care patients at eThekwini KwaZulu-Natal Hospitals'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at Prince Mshiyeni Memorial, Victoria Mxenge and Inkosi Albert Luthuli Central hospital.

2. You are requested to take note of the following:
   a. ***Kindly liaise with the facility manager BEFORE your research begins.***
      This is to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.
   b. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to* ***HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200*** *and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Mr. X Xaba on 033-395 2805.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Provincial Health Research Committee
Date: 19 September 2024