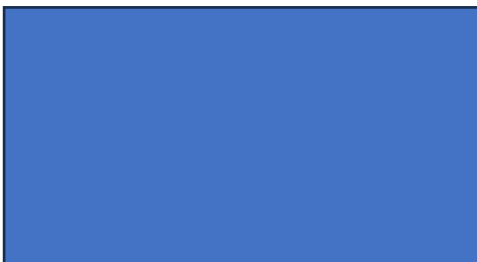




Department of Microbiology

Certificate



NAME OF COURSE	[REDACTED]
NAME OF SUBJECT	[REDACTED]
ADMISSION YEAR/ ACADEMIC YEAR	[REDACTED]

**A MICROBIOLOGICAL EVALUATION OF NON-FERMENTING
GRAM-NEGATIVE BACILLI ISOLATED FROM VARIOUS CLINICAL
SAMPLES IN A RURAL TERTIARY CARE HOSPITAL**

ABSTRACT

Background: Non-fermenting Gram-negative bacilli (NFGNB) are opportunistic pathogens associated with hospital-acquired infections, particularly in critically ill patients. Their ability to develop multidrug resistance poses a major challenge, especially in rural healthcare settings.

Aim: [REDACTED]
[REDACTED]

Methods: [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Results: [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Conclusion: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Keywords: Non-fermenting Gram-negative bacilli, antimicrobial resistance, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, rural hospital, Metallo- β -lactamase.

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INTRODUCTION

INTRODUCTION

The Non-fermentative gram- negative bacilli (NFGNB) are a broad category of aerobic, non-spore-forming bacilli that either do not use carbohydrates as a source of energy or break them down via different metabolic routes than fermentation.¹

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

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along with their antibiotic sensitivity pattern from various clinical samples attending rural tertiary care hospital.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

AIM:

[REDACTED]

OBJECTIVES:

1. To isolate and identify Non-fermenting gram- negative bacilli (NFGNB) from various clinical Samples.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

REVIEW OF LITERATURE

REVIEW OF LITERATURE

WHO Definition

Non-fermenting Gram-negative bacilli (NFGNB) are defined as a diverse group of aerobic, non-spore-forming, rod-shaped bacteria that do not ferment glucose or other carbohydrates in standard fermentation tests such as Triple Sugar Iron (TSI) agar or oxidative/fermentative (O/F) medium. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

These bacteria do not fall into one taxonomic group but are instead unified by their metabolic behaviour and clinical impact.²⁰

Thus, in summary, non-fermenting Gram-negative bacilli (NFGNB) are clinically important, glucose non-fermenting, aerobic Gram-negative rods, many of which exhibit multidrug resistance and are major contributors to nosocomial infections worldwide, especially in ICU settings.

Historical Overview

The clinical relevance of non-fermenting Gram-negative bacilli (NFGNB) has evolved significantly over the past century. [REDACTED]

[REDACTED]

[REDACTED] However, their pathogenic

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] The spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of non-fermenting Gram-negative bacilli (NFGNB) has resulted in increased morbidity, mortality, and healthcare costs, particularly in low- and middle-income countries where laboratory resources are limited. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Their persistence in the healthcare environment and evolving resistance mechanisms continue to challenge infection control and antimicrobial stewardship worldwide.⁴⁷

Epidemiology

Global

Non-fermenting Gram-negative bacilli (NFGNB) have emerged as major pathogens in healthcare settings worldwide, particularly in intensive care units (ICUs), burn wards, and among immunocompromised patients. [REDACTED]

[REDACTED] ⁴⁶

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Mortality from NFGNB infections can be high, particularly when caused by extensively drug-resistant (XDR) strains in critically ill patients, often exceeding 40–50% in ICU settings.⁴⁸

Indian and Rural region

In India, the burden of infections caused by non-fermenting Gram-negative bacilli (NFGNB) is alarmingly high and continues to grow, particularly in tertiary care centers and rural hospitals. Studies from multiple Indian institutions report that 20% to 35% of all Gram-negative isolates from ICU patients are NFGNB, with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex being the predominant species. [REDACTED]

Indian hospitals have reported carbapenem resistance rates of 40–80% in *Acinetobacter baumannii* complex and 20–50% in *Pseudomonas aeruginosa*. The widespread and often irrational use of broad-spectrum antibiotics in hospital and outpatient settings, combined with inadequate infection control, has accelerated the emergence of multidrug-resistant strains. Factors contributing to this trend include:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

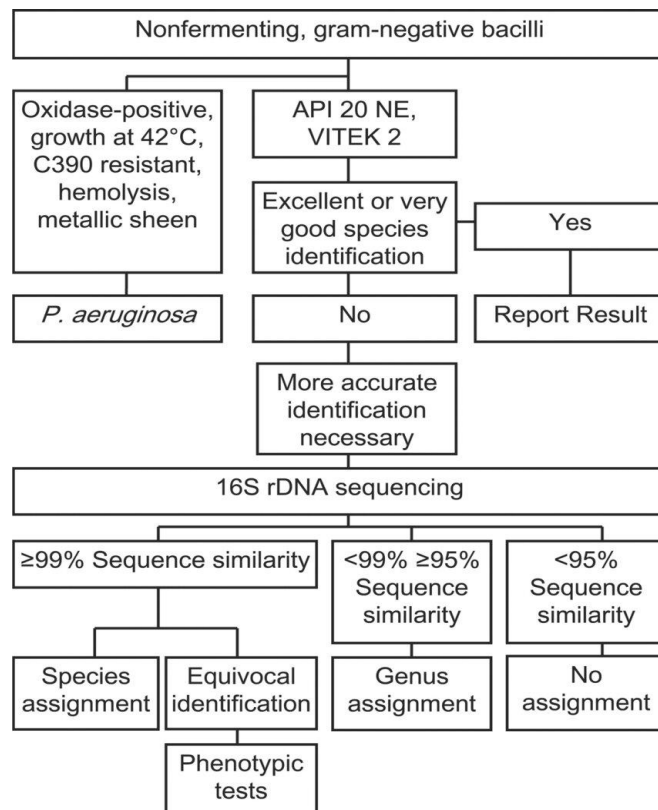


Fig. 1 'Algorithm for the identification of Non-fermenting Gram-negative bacilli (NFGNB).'

Microbial Metabolism of Non-Fermenting Gram-Negative Bacilli (NFGNB) ⁷⁵

Non-fermenting Gram-negative bacilli (NFGNB) are distinguished from enteric Gram-negative organisms by their unique metabolic pathways, particularly their inability to ferment carbohydrates like glucose.

Most NFGNB are obligate aerobes, meaning they require oxygen for their energy production.

[REDACTED]

[REDACTED] This is in contrast to fermenters like *Escherichia coli*, which produce acid in both tubes. [REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- Oxidative phosphorylation in the presence of oxygen for ATP generation

Some non-fermenting Gram-negative bacilli (NFGNB) exhibit metabolic versatility. For instance:

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- *Stenotrophomonas maltophilia* shows limited carbohydrate utilization but can metabolize amino acids and other non-glucose substrates for survival.

These organisms are also capable of surviving in nutrient-poor and high-stress environments, often due to the expression of stress-response genes and the ability to

enter metabolically dormant states, particularly within biofilms. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

In clinical microbiology, these metabolic characteristics not only aid in the identification and differentiation of NFGNB but also explain their survival advantage, environmental resilience, and pathogenic success in nosocomial settings.

[REDACTED]

[REDACTED]

Classification of Non-Fermenting Gram-Negative Bacilli (NFGNB)

Non-fermenting Gram-negative bacilli (NFGNB) are a heterogeneous group of organisms that cannot ferment glucose and rely instead on oxidative metabolism.

[REDACTED]

[REDACTED] The classification of NFGNB has evolved over time, from conventional biochemical groupings to modern molecular and proteomic-based systems.¹¹²

A. Traditional (Conventional) Classification – Phenotypic/Metabolic Approach

This approach, widely used before molecular diagnostics were available, classifies NFGNB based on:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Biochemical characteristics (TSI, catalase, urease, etc.)

1. Oxidase-Positive NFGNB

- *Pseudomonas aeruginosa*

[REDACTED]

[REDACTED]

- *Achromobacter xylosoxidans*

2. Oxidase-Negative NFGNB

- *Acinetobacter baumannii* complex

[REDACTED]

- *Alcaligenes faecalis* (oxidase variable)

3. Pigment Production

[REDACTED]

[REDACTED]

[REDACTED]

This system, while still used in resource-limited microbiology labs, has limitations due to phenotypic overlaps and inconsistent results in slow-growing or atypical strains.

B. Modern (Current) Classification – Molecular and Genomic Approach

[REDACTED]

[REDACTED]

[REDACTED]

1. 16S rRNA Gene Sequencing

[REDACTED]

[REDACTED]

[REDACTED]

2. Multilocus Sequence Typing (MLST)

Used to type strains within species, especially for epidemiological tracking of

[REDACTED]

3. MALDI-TOF Mass Spectrometry

Rapidly identifies NFGNB based on protein fingerprinting, widely used in modern

[REDACTED]

4. Whole Genome Sequencing (WGS)

Provides complete genetic information, used for research, outbreak investigations, and antibiotic resistance gene profiling.

B. Taxonomic Families and Genera of Common NFGNB (As per Bergey’s Manual and NCBI) ⁷⁵

Table 1: Motile with polar flagella

Family	Genus
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]
[REDACTED]	[REDACTED] [REDACTED] [REDACTED]
[REDACTED]	[REDACTED] [REDACTED]
[REDACTED]	[REDACTED] [REDACTED]
[REDACTED]	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]
[REDACTED]	[REDACTED]

Table 2: Motile with Peritrichous Flagella

Family	Genus/Species

Table 3: Nonmotile, Oxidase-Positive

██████████	██████████
██████████	██████████ ██████████
██████████	██ ████████████████████████████████████
██████████	████████████████████
██████████	██████████ ████████████████████
██ ██	

Table 4: Nonmotile, Oxidase-Negative

Moraxellaceae	Acinetobacter
██████████	██████████ ████████████████████████████████████
██ ████████████████████ ████████████████████	

Taxonomic Families and Genera of Common NFGNB (As per Bergey's Manual and NCBI)

Table 5: Evolution of Classification

HABITAT:¹¹³

Aerobic *Pseudomonas* species are widespread in the environment and play a role in breaking down organic matter. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] In hospitals, they are known to cause infections, often introduced through contaminated items like plants, flowers, or visitors.

Pseudomonas aeruginosa is one of the most prominent opportunistic pathogens among NFGNB, notorious for causing a wide range of healthcare-associated infections (HAIs)⁵⁶. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Otitis externa (especially in diabetic and immunocompromised individuals)

Its ability to survive in aqueous environments, colonize medical devices, and form biofilms enables it to persist in hospital settings. [REDACTED]

[REDACTED]

[REDACTED]

Resistance Mechanisms:^{75,114,113}

Pseudomonas aeruginosa exhibits both intrinsic and acquired resistance, making it a challenging organism to treat. Key mechanisms include:

■ **β-lactamase production:** [REDACTED]

[REDACTED]

■ **Efflux pumps:** [REDACTED]

[REDACTED]

■ **Porin loss or alteration:** [REDACTED]

[REDACTED]

■ **Target site mutations:** [REDACTED]

[REDACTED]

■ **Biofilm-mediated resistance:** [REDACTED]

[REDACTED]

Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) is a serious global concern, often requiring treatment with polymyxins, aminoglycosides, or novel β-lactamase inhibitor combinations.

[REDACTED]

[REDACTED] They are distinct from other *Pseudomonas* species due to their resistance to Polymyxin B and Colistin antibiotics.

***Acinetobacter baumannii* complex**

Acinetobacter baumannii complex has emerged as a major cause of nosocomial infections, particularly in ICUs. It is well known for causing:

[REDACTED]

[REDACTED]

[REDACTED]

- Meningitis (especially post-neurosurgical)

It is ubiquitous in the hospital environment, surviving on dry surfaces for prolonged periods. In India, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Resistance Mechanisms:^{75,113,38,114}

Acinetobacter baumannii complex is a model of extensive drug resistance (XDR), harbouring multiple resistance genes through plasmids, integrons, and transposons.

Key mechanisms include:

■ OXA-type carbapenemases (e.g., OXA-23, OXA-58) – ■

■

■ Metallo-β-lactamases (e.g., NDM-1) – ■

■

■ Efflux pumps (AdeABC system) – ■

■

■

■

■

■

■

***Burkholderia cepacia* Complex (BCC)**

Burkholderia cepacia complex is a group of genetically similar but phenotypically diverse species. ■

■

■

■ It is intrinsically resistant to multiple antibiotics including aminoglycosides and polymyxins, making treatment options limited.⁵⁹

Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is an opportunistic NFGNB increasingly isolated from respiratory tract infections, bacteraemia, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Most cephalosporins

It remains susceptible to trimethoprim-sulfamethoxazole (TMP-SMX), though resistance to this drug is also rising. [REDACTED]

[REDACTED]

[REDACTED]

β-lactamases

[REDACTED]

[REDACTED]

■ Class B metallo-β-lactamases (e.g., VIM, IMP, NDM) – [REDACTED]

[REDACTED]

- Class D oxacillinases (e.g., OXA-23, OXA-51) – [REDACTED]

■ ESBLs (e.g., TEM, SHV, CTX-M) – [REDACTED]

[REDACTED]

Efflux Pumps

Efflux pumps actively expel antibiotics, reducing intracellular drug concentrations.

Examples include:

- *Pseudomonas aeruginosa*: MexAB-OprM, MexXY-OprM

■ *Acinetobacter baumannii* complex: ■
■

Porin Loss

■

- *Pseudomonas aeruginosa* – loss of OprD associated with imipenem resistance

■ *Acinetobacter* species – ■
■

■

■

Pathogenic Mechanisms and Virulence Factors of Non-Fermenting Gram-Negative

Bacilli (NFGNB)⁷⁵

Non-fermenting Gram-negative bacilli (NFGNB) such as *Pseudomonas aeruginosa*,

■

■

■

■

Adherence and Colonization

A fundamental step in NFGNB infection is the ability to adhere to epithelial surfaces and medical devices. [REDACTED]

[REDACTED]

[REDACTED]

- Outer membrane proteins (OMPs) and surface adhesins facilitating colonization of mucosal surfaces.

[REDACTED]

[REDACTED]

Biofilm Formation

One of the most important virulence traits of NFGNB is their ability to form biofilms—structured communities of bacteria embedded in an extracellular polymeric substance (EPS). Biofilms form on:

[REDACTED]

[REDACTED]

[REDACTED]

- Wounds and prosthetic surfaces

Biofilm-associated cells exhibit:

- Reduced metabolic activity, making them less susceptible to antibiotics

[REDACTED]

[REDACTED]

Pseudomonas aeruginosa forms complex biofilms regulated by quorum sensing genes

[REDACTED]

[REDACTED]

Invasion and Tissue Damage

[REDACTED]

[REDACTED]

- Exotoxin A (ETA) in *Pseudomonas aeruginosa* inhibits protein synthesis by ADP-ribosylation of elongation factor-2.

[REDACTED]

[REDACTED]

- Hemolysins and cytotoxins promote lysis of red blood cells and epithelial cells.

[REDACTED]

[REDACTED]

Immune Evasion

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Additionally, *Pseudomonas aeruginosa* can alter its antigenic surface via phenotypic switching, leading to immune evasion and chronic infection.

Quorum Sensing and Regulation of Virulence

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Intrinsic and Acquired Antibiotic Resistance (as a Virulence Trait)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Efflux pumps (e.g. [REDACTED])

[REDACTED] β -lactamases (e.g. [REDACTED])

[REDACTED]

[REDACTED]

The pathogenic potential of NFGNB lies not in a single virulence factor but in the complex interplay of structural, enzymatic, metabolic, and regulatory components.

Virulence Mechanisms of Non-Fermenting Gram-Negative Bacilli

Adherence & Colonization

- Fimbriae and pili facilitate attachment to host epithelial surfaces
- Outer membrane proteins (OMPs) enable mucosal colonization
- *A. baumannii* OmpA induces host cell apoptosis

Biofilm Formation

- Structured communities embedded in extracellular polymeric substance
- Forms on medical devices and tissue surfaces
- Regulated by quorum sensing systems (e.g., las, rhl in *P. aeruginosa*)
- Confers antibiotic resistance and immune evasion

Tissue Damage & Immune Evasion

- Exotoxin A inhibits protein synthesis
- Elastases and proteases degrade host tissues
- Capsules prevent phagocytosis
- LPS modifications resist antimicrobial peptides

Antibiotic Resistance

- Efflux pumps (MexAB-OprM)
- β -lactamases (OXA, VIM, NDM)
- Porin loss reduces antibiotic entry
- Target site mutations prevent binding

These opportunistic pathogens (*P. aeruginosa*, *A. baumannii*, *S. maltophilia*, *B. cepacia*) employ multiple coordinated mechanisms to establish infection, particularly in immunocompromised hosts and healthcare settings. Their pathogenicity stems from the synergistic effect of these virulence factors rather than any single determinant.

Fig. 2 Pathogenic Mechanisms and Virulence Factors of Non-Fermenting Gram-Negative Bacilli (NFGNB)

Antibiotic resistance and Biofilm formation in non-fermenting Gram-negative bacilli (NFGNB)

Antibiotic resistance in non-fermenting Gram-negative bacilli (NFGNB) is one of the most concerning challenges in modern healthcare, especially due to their intrinsic and acquired resistance mechanisms. [REDACTED]

[REDACTED]

[REDACTED]

Mechanisms of Antibiotic Resistance

NFGNB possess a broad range of resistance mechanisms, including:

■ **β -lactamase production:** [REDACTED]

[REDACTED]

[REDACTED]

■ **Efflux pumps:** Systems like MexAB-OprM in *Pseudomonas aeruginosa* actively expel a wide range of antibiotics from the bacterial cell, [REDACTED]

[REDACTED]

■ **Porin channel alterations:** Decreased expression or structural mutations in outer membrane porins reduce permeability, [REDACTED]

[REDACTED]

- **Target site modification:** Mutations in target enzymes such as DNA gyrase or ribosomal proteins lead to resistance against fluoroquinolones and aminoglycosides.
- **Enzymatic modification:** Aminoglycoside-modifying enzymes alter the drug structure and reduce binding to its target.

[REDACTED]

[REDACTED]

Role of Biofilm Formation

Biofilms are structured communities of bacterial cells enclosed in a self-produced polymeric matrix adherent to surfaces such as endotracheal tubes, [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Biofilm bacteria display up to 1000 times greater resistance to antibiotics compared to their planktonic counterparts.

Biofilms protect bacteria through:

- Restricted penetration of antibiotics
- Slower growth rate, making bacteria less susceptible to antibiotic action
- Inactivation of immune responses

- Increased horizontal gene transfer within the biofilm

[REDACTED]

[REDACTED]

Importance in Clinical Microbiology

[REDACTED]

[REDACTED]

[REDACTED]

High-Risk Environments

NFGNB are commonly isolated from:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Burn wound sepsis

Persistence in Hospital Settings

These bacteria survive:

[REDACTED] On dry and moist hospital surfaces for extended periods (e.g. [REDACTED])

[REDACTED]

[REDACTED]

- In biofilms on medical devices, increasing the risk of cross-contamination

Diagnostic and Treatment Challenges

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Clinical Outcomes

Infections caused by resistant NFGNB are associated with:

[REDACTED]

[REDACTED]

[REDACTED]

- Elevated morbidity and mortality

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Biofilm Formation and Clinical Implications

Biofilm formation is a key virulence mechanism of non-fermenting Gram-negative bacilli (NFGNB), enabling them to persist in hostile environments and evade both host immune responses and antimicrobial therapies. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Mechanism of Biofilm Formation

Biofilm development occurs in five stages:

1. Initial attachment – [REDACTED]

[REDACTED]

2. Irreversible attachment – [REDACTED]

3. Maturation I & II – [REDACTED]

4. Dispersion – [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Clinical Relevance

Biofilm-associated infections are often:

[REDACTED]

- [REDACTED]
- Difficult to diagnose and eradicate

Common clinical scenarios include:

- Ventilator-associated pneumonia (VAP)
- [REDACTED]
- [REDACTED]
- [REDACTED]

[REDACTED] Furthermore, biofilms act as a reservoir for horizontal gene transfer, facilitating the spread of resistance genes within the microbial community.¹¹⁹

Implications

Biofilm-forming NFGNB contribute to:

- Increased hospital stay
- [REDACTED]
- [REDACTED]

- Frequent device-related infections
- [REDACTED]
- [REDACTED]
- [REDACTED]

Detection Methods in Resource-Limited Settings

Identifying NFGNB accurately is essential for initiating effective treatment and controlling infection spread. However, resource-limited rural healthcare settings often lack access to advanced diagnostics. [REDACTED]

[REDACTED]

[REDACTED]

Sample collection:^{77, 78,79,75,38}

Pus and wound swabs

[REDACTED]

[REDACTED]

Urine

[REDACTED]

[REDACTED]

[REDACTED]

Respiratory samples (Sputum, Endotreacheal aspirates, Tracheal aspirates

Bronchoalveolar lavage)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Blood Sample

Blood was collected before the start of antimicrobial treatment and onset of fever. [REDACTED]

[REDACTED] By using a sterile syringe and needle, 5-10 ml of blood from an adults and 1-5 ml from paediatric patients was collected and inoculated in Brain heart infusion broth on the patient's bed side after cleaning the site with 70% isopropyl alcohol.

Cerebrospinal fluid

The Cerebrospinal fluid was collected from the arachnoid space. [REDACTED]

Body fluids (Pleural, Pericardial, Synovial, Ascitic, Hydrocele fluid etc.)

Transport of samples: ^{77, 78,79,75,38}

After collection, all samples should be transported to the laboratory without any delay and under optimal conditions. [REDACTED]

[REDACTED] If a delay longer than 2 hours is anticipated, the sample should be placed in Cary-Blair transport medium.

Cary-Blair transport medium-

Processing of samples:

Gross examination:

All the received samples should be grossly examined

[REDACTED]

[REDACTED].

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Microscopic examination

Wet mount preparation:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Gram staining:

After smear preparation, the slide is stained and initially examined under the 40× objective to assess the quality of staining and observe the distribution of material.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Samples with a Barlett's score of 1 or higher are deemed acceptable and are processed further for microbiological analysis.

Culture and Isolation:

[REDACTED]

Blood Agar:

[REDACTED]

[REDACTED]

MacConkey Agar:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Nutrient agar:

[REDACTED]

[REDACTED]

Selective Media:

Burkholderia cepacia agar: [REDACTED]

[REDACTED]

[REDACTED]

Ashdown medium: [REDACTED]

[REDACTED]

Cetrimide Agar:

Contains cetyltrimethylammonium bromide, a selective agent that inhibits most bacteria by acting as a detergent. [REDACTED]

[REDACTED]

Preliminary Identification

Initial identification of bacterial isolates was carried out through the observation and Documentation of colony morphology on various culture media, including Nutrient agar, Blood agar, and MacConkey agar. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- **Gram stain:** to [REDACTED]
[REDACTED].

Catalase test: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Other Biochemical Tests

[REDACTED]

[REDACTED]

[REDACTED]

- **Triple Sugar Iron (TSI):** NFGNB show alkaline slant/alkaline butt (K/K) with no gas or H₂S

Oxidation-Fermentation (O/F) (Hugh-Leifson) Test: Glucose, Lactose, Sucrose,

[REDACTED]

[REDACTED]

- Indole test, Urease test, Nitrate reduction test, Gelatin hydrolysis

[REDACTED]

[REDACTED]

- Growth in 6.5% sodium chloride

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Identification *Pseudomonas aeruginosa*

- Nutrient agar- Large, circular, greyish, flat, spreading, metallic sheen with bluish-green pigment

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Poymyxin B (300 Unit) – sensitive

Idenification of *Acinetobacter baumannii* complex:

- Nutrient agar - Medium, circular, regular, raised, greyish, smooth, opaque

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Growth at 44⁰C, OF Dextrose positive

Identification of *Stenotrophomonas maltophilia*:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1. CDC Scheme – Weyant and Associates

- Uses a 3-part guide:

[REDACTED]

[REDACTED]

3. Codebook to match test results with species names.

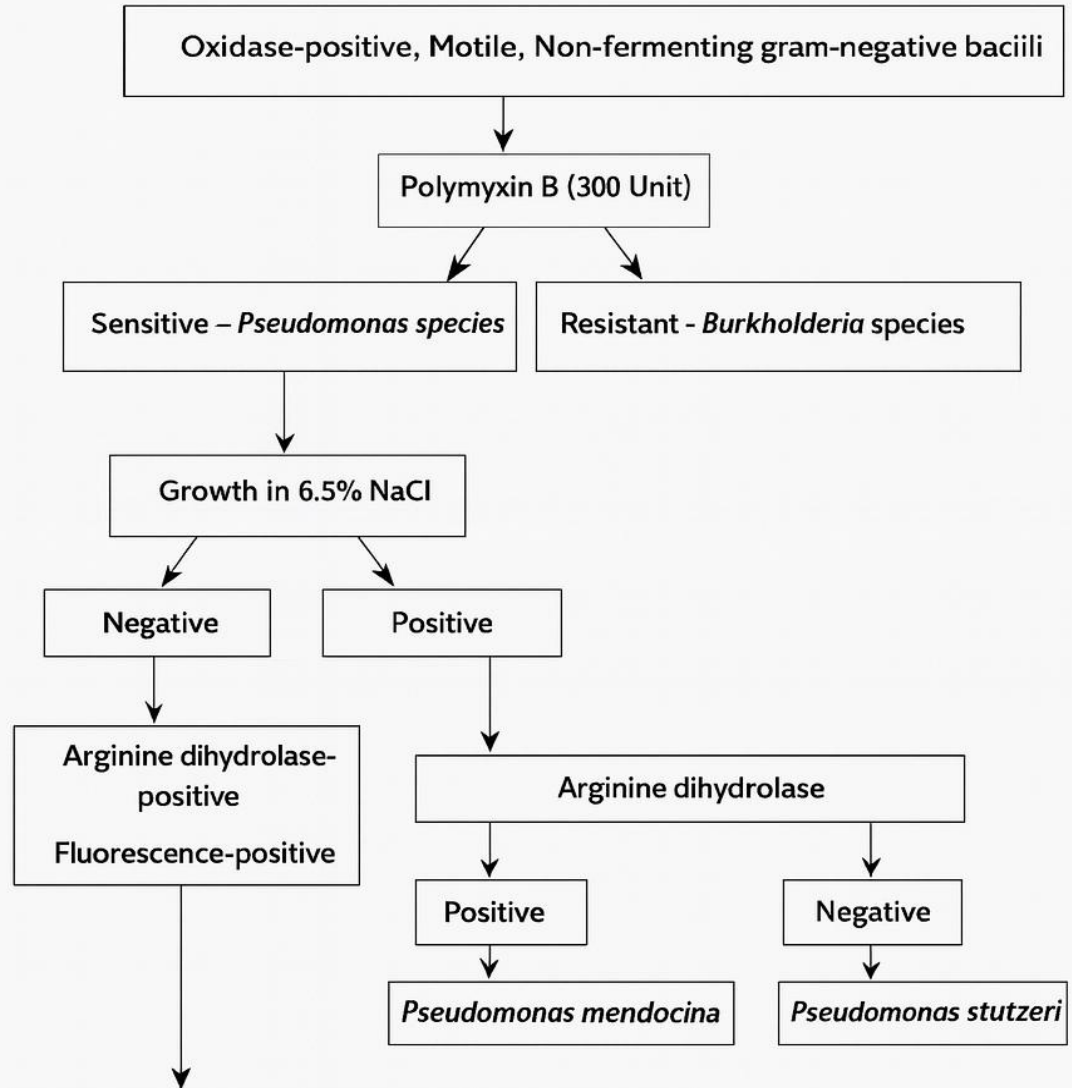
2. Schreckenberger's Scheme

[REDACTED]

[REDACTED]

IDENTIFICATION OF OXIDASE POSITIVE, MOTILE, NON-FERMENTING

NEGATIVE BACILLI



	Growth at 42 °C	Gelatin hydrolysis
<i>Pseudomonas aeru-</i>	Positive	Variable
<i>Pseudomonas fluor-</i>	Negative	Positive
<i>Pseudomonas putida</i>	Negative	Negative

IDENTIFICATION OF OXIDASE NEGATIVE, NON MOTILE, NON-FERMENTING GRAM-NEGATIVE COCCOBACILLI

Oxidase negative, Non motile, Non-fermenter Gram-negative bacilli

Brown soluble pigment

Bordetella holmesii

Urease hydrolysis positive

Bordetella parapertusis

No pigment and
Urease hydrolysis negative

Growth at 44°C

Yes

No

(*Acinetobacter baumannii* complex)

OF Dextrose positive

(Other

Acinetobacter
***species*)**

Hemolysis on Blood agar

No

Yes

Acinetobacter
hemolyticus

	Gelatin hydrolysis	OF Dextrose	Arginine dihydrolase
<i>Acinetobacter junii</i>	-	-	+
<i>Acinetobacter lwoffii</i>	-	-	-
Other <i>Acinetobacter</i> species	-/+	-/+	-/+

Methods for Identifying Bacteria Using Commercial Kit Systems

Commercial kit systems are developed to help identify non-fermenting bacilli. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

3. Inappropriate test inclusion – Some kits contain tests meant for other bacteria, which are not useful for identifying non-fermenters.

Common Commercial Kits for Non-fermenter Identification

- API 20E (BioMérieux, Hazelwood, MO)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Biolog System (Biolog, Hayward, CA)

Methods for Identification Using Automated Systems ^{75,109}

The Vitek 2 System

VITEK 2 is an automated system that identifies pathogens and performs antibiotic susceptibility testing. [REDACTED]

[REDACTED]

[REDACTED]

VITEK 2 system comes in three versions (VITEK 2 compact, VITEK 2, and VITEK 2 XL) with varying levels of capacity and automation.

The Microscan Walkaway-96, Walkaway-40, and Autoscan-4

[REDACTED]

[REDACTED]

[REDACTED]

The Sensititre AP80 System – Summary

The Sensititre AP80 System (by TREK Diagnostic Systems) is used to identify gram-negative bacteria. The panels can be used manually or with automated devices like the Sensititre Autoreader or ARIS.

The Phoenix System

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Methods for Identification Using Molecular Systems

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry⁷⁶

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

16S rRNA Gene Sequencing

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The Epsilometer test is a quantitative approach for confirming resistance profiles. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Following incubation, the minimum inhibitory concentration (MIC) is determined at the point where the elliptical inhibition zone intersects the scale on the strip.

Macro Broth Dilution

In this procedure, a series of antimicrobial dilutions are prepared in Mueller–Hinton broth. [REDACTED]

[REDACTED]

[REDACTED] To establish the minimum bactericidal concentration (MBC), aliquots from tubes with no visible growth are sub-cultured onto nutrient agar to confirm bacterial killing.

Micro Broth Dilution

This method involves performing the test in microtiter plates and is frequently employed in automated platforms for MIC determination. Systems such as the VITEK 2 provide both identification and susceptibility profiles of bacterial isolates.

Challenges in Resource-Limited Labs

■ Lack of automated systems (e.g. ■)

■

■

- Limited availability of specialized reagents or diagnostic kits

Strategies to Overcome

- Regular training in manual biochemical identification

■

- ■

■

■ Their integration with antibiotic sensitivity testing is critical for managing infections effectively and curbing resistance spread in rural tertiary care centers.¹²⁰

Outbreaks and Nosocomial Infections Caused by NFGNB

Non-fermenting Gram-negative bacilli (NFGNB) are major contributors to nosocomial infections globally, often implicated in hospital outbreaks. ■

■

■

■

Outbreak Settings and Sources

Outbreaks involving NFGNB have been reported from:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Dialysis units

Common sources of outbreaks include:

- Contaminated ventilator circuits and endotracheal tubes

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Notably, *Acinetobacter baumannii* complex has caused prolonged ICU outbreaks due to its ability to survive on dry surfaces for weeks.⁹⁰

Contributing Factors to Outbreaks

[REDACTED] Lapses in infection control practices (e.g. [REDACTED])

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Delayed microbiological identification and reporting

Clinical and Epidemiological Impact

- Prolonged hospitalization

[REDACTED]

[REDACTED]

[REDACTED]

- Spread to other units and between patients

[REDACTED]

[REDACTED]

[REDACTED]

Rural Healthcare Burden and Diagnostic Challenges

Burden of Disease in Rural Settings

- High incidence of device-associated infections in patients admitted to rural ICUs and post-operative wards.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Diagnostic Limitations

■ Absence of automated systems (e.g. [REDACTED])

[REDACTED]

[REDACTED]

[REDACTED]

■ Inadequate laboratory infrastructure (e.g. [REDACTED])

[REDACTED]

[REDACTED]

[REDACTED]

- No surveillance systems or antibiogram tracking

Impact on Clinical Management

- Empirical therapy often chosen without microbiological guidance, leading to:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- Recurrent hospital admissions and out-of-pocket expenditure

[illegible]

Vinutha D.R. (2008) focused on the microbiological characterization of non-fermenting gram-negative bacilli (NFGNB) isolated from various clinical specimens in a tertiary care setup.

[REDACTED]

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[REDACTED]

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[REDACTED]

Malini A. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] and

Acinetobacter species. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Bhatnagar R.

[REDACTED]

[REDACTED]

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Jayaprakash C. [REDACTED]

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Agarwal S. [REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] It offers an insightful analysis into the epidemiology of CR-NFGNB and the need for a robust microbiological and clinical response. [REDACTED]

Solanki S.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] This article is highly pertinent

for microbiologists working in smaller institutions, as it bridges the knowledge gap between metropolitan and rural antimicrobial resistance data. ■

Berwal A. ■

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Banu A. ■

■
■

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

Sharma S. [REDACTED]

[REDACTED]

[REDACTED]

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Chawla K. [REDACTED]

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[REDACTED]



Fig 3. Research studies Literature Gaps

[REDACTED]

[REDACTED]

Rural Data Deficiency: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

MATERIALS AND METHODS

MATERIALS AND METHODS

[REDACTED]

[REDACTED]

Study Type – [REDACTED]

Study Area [REDACTED]

[REDACTED]

Study Duration - [REDACTED]

[REDACTED]

Ethical Consideration - [REDACTED]

[REDACTED]

Consent - [REDACTED]

[REDACTED]

Study Group – [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Study Sample Size - [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

SELECTION OF CASES

Inclusion Criteria:

1. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Exclusion Criteria:

1. [REDACTED]

[REDACTED]

METHODOLOGY

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

COLLECTION OF SAMPLES:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

TRANSPORT OF SAMPLES:-

[REDACTED]

[REDACTED]

PROCESSING OF SAMPLES: ^{75,78,79,38}

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

GROSS EXAMINATION

[REDACTED]

[REDACTED]

MICROSCOPIC EXAMINATION

Gram staining was done for detection of pus cells, RBCs, epithelial cells, gram reaction of organisms.

[REDACTED]

[REDACTED] Samples with a Bartlett score of ≥ 1 were accepted for further processing. ⁷⁵

CULTURE INOCULATION:

- All the samples were inoculated on Nutrient agar, Blood agar, MacConkey Agar.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

OBSERVATION OF COLONY CHARACTERISTICS:

- All inoculated plates were observed for colony characteristics.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

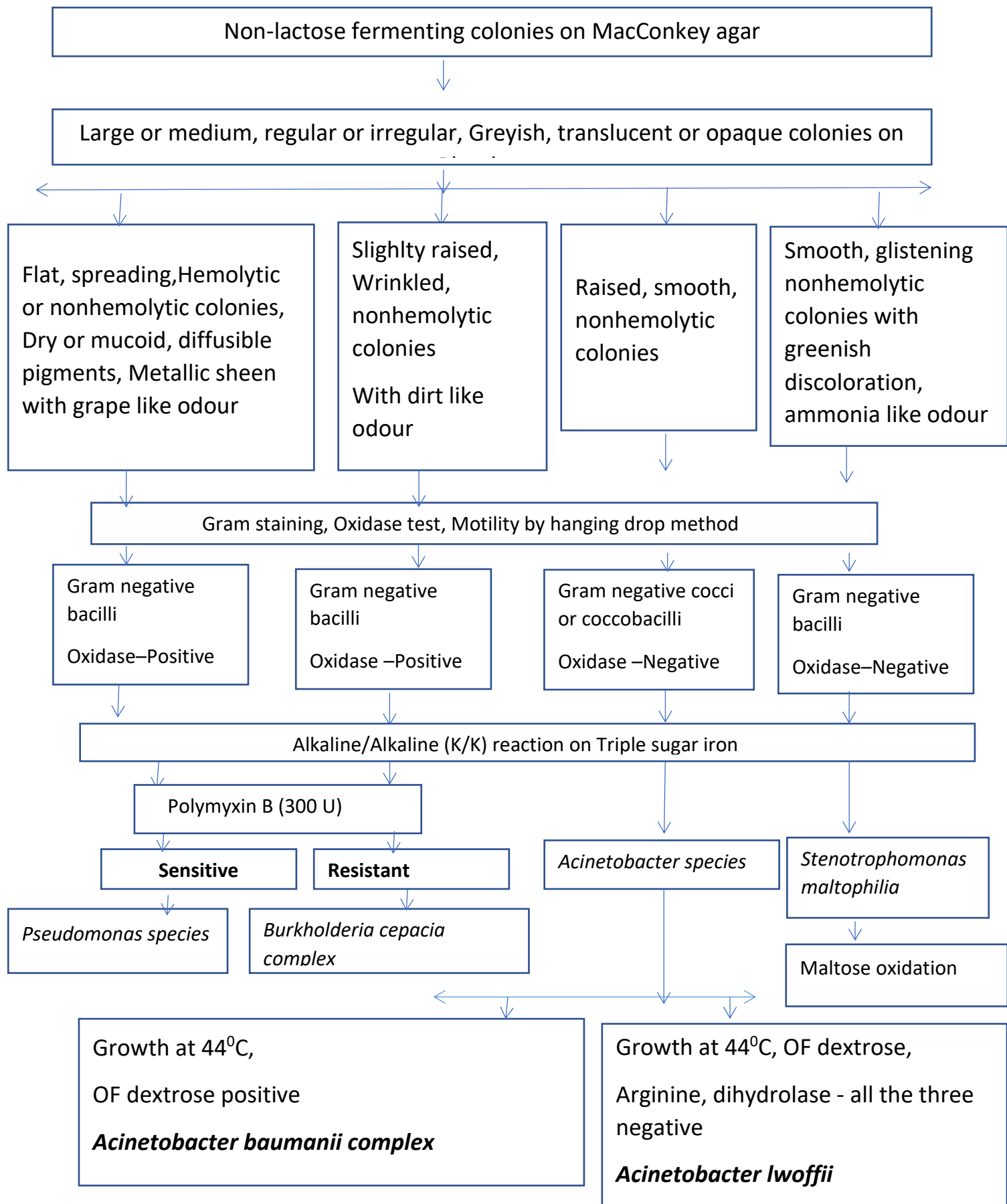
[REDACTED]

[REDACTED]

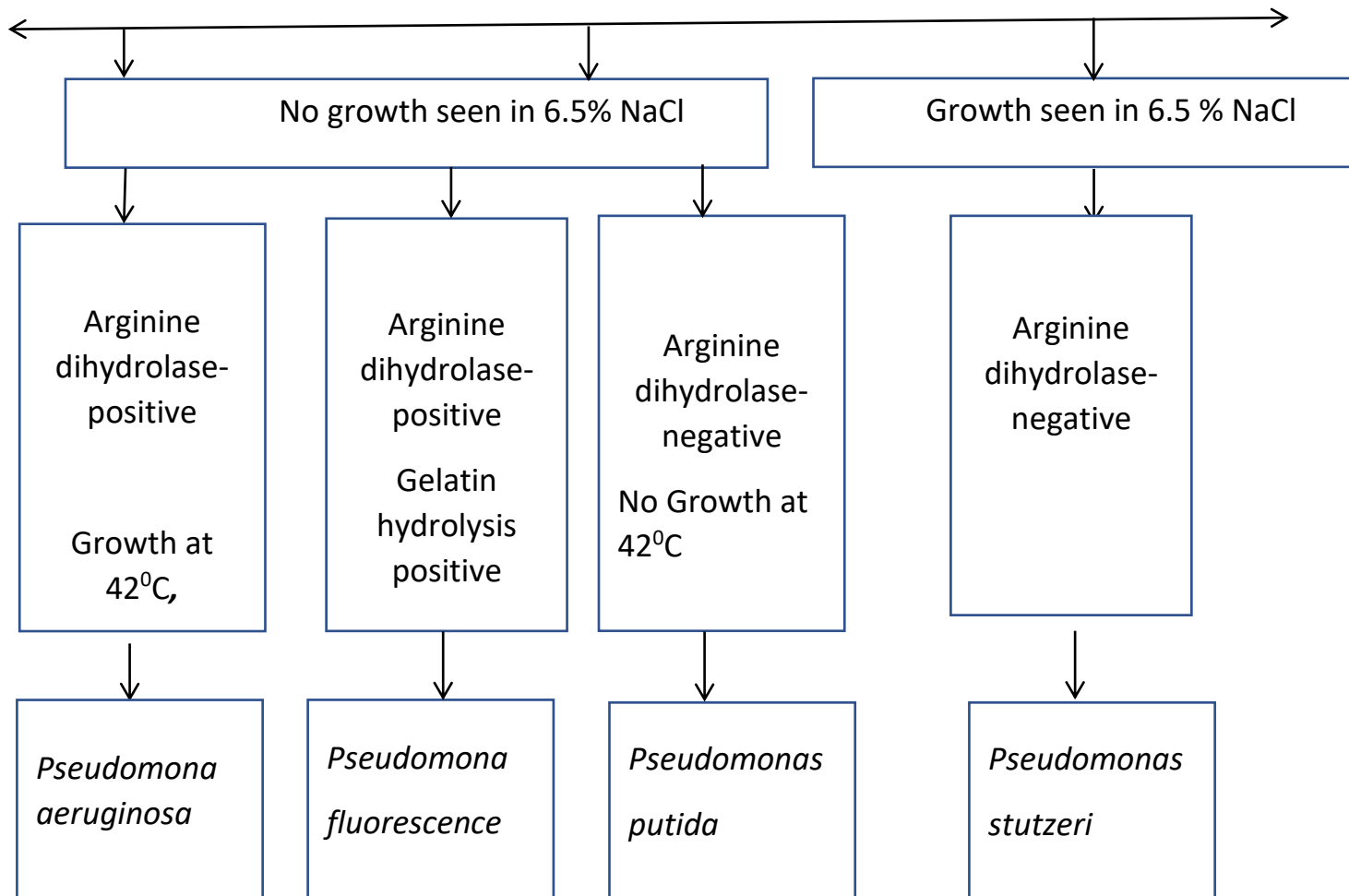
[REDACTED]

- [REDACTED]

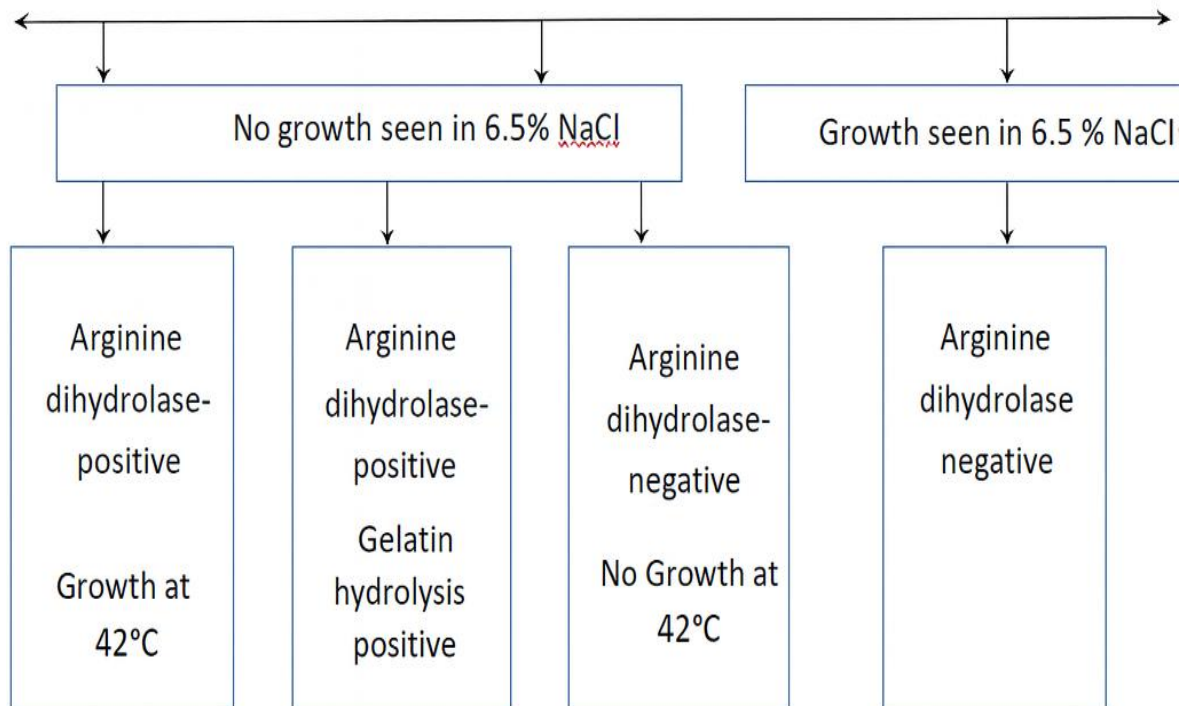
The organisms that produced an alkaline/ alkaline (K/K) reaction when grown on Triple Sugar Iron Agar were temporarily regarded as gram-negative, non-fermentative bacilli and were included in this study. ⁷⁵



Further speciation of Pseudomonas species



Further speciation of Pseudomonas species



**ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST) BY MODIFIED KIRBY - BAUER DISC
DIFFUSION METHOD ⁷⁷**

Well-isolated 3–5 morphologically similar colonies from 18–24-hour-old agar plate was taken by using a sterile loop and suspended in 4–5 mL of 0.9% saline solution. ³⁸

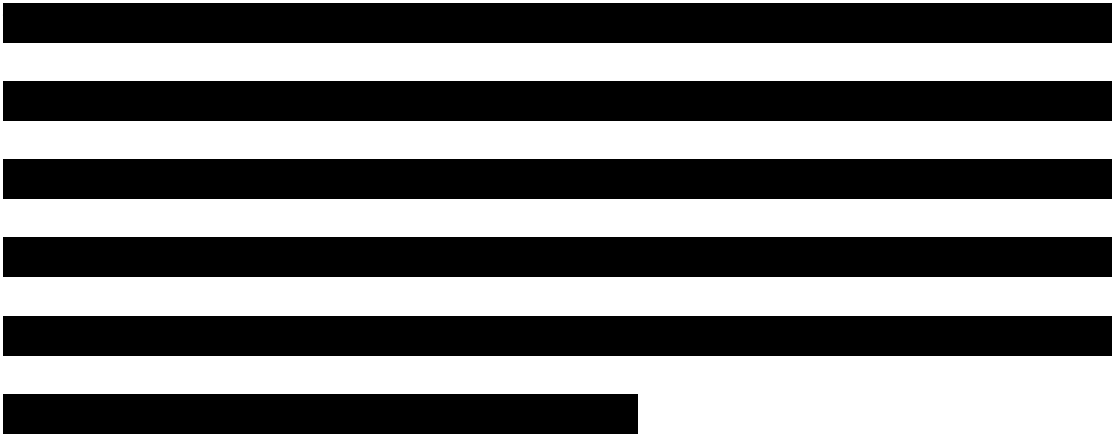


Table 6: Antibiotic disc and their interpretation of Zone diameter for *Pseudomonas aeruginosa*⁷⁷

Antibiotic discs	Concentrations	Interpretive categories and zone diameter breakpoints, nearest whole mm		
		S	I	R
				
				
				
				

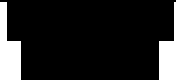
				
				
				
				
				
				
				

Table 7: Antibiotic disc and their interpretation of Zone diameter for *Acinetobacter* species⁷⁷

Antibiotic discs	Concentrations	Interpretive categories and zone diameter breakpoints, nearest whole mm		
		S	I	R
 				
				
				
				
				
				
				
				

Table 8: Antibiotic disc and their interpretation of Zone diameter for *Burkholderia cepacia complex*.⁷⁷

Antibiotic discs	Concentrations	Interpretive categories and zone diameter breakpoints, nearest whole mm		
		S	I	R

Table 9: Antibiotic disc and their interpretation of Zone diameter for *Stenotrophomonas maltophilia*.⁷⁷

Antibiotics	Concentration	Interpretive categories and zone diameter breakpoints, nearest whole mm
-------------	---------------	---

		S	I	R
				
				
 				

Detection of Metallo- β -lactamase (MBL)⁷⁷

Method for detection of Metallo- β -lactamase (MBL) Production in Non-fermenting Gram-negative bacilli (NFGNB)

Initial screening was done by identifying isolates are 



Confirmatory Detection of Metallo- β -lactamase (MBL) by Modified Carbapenem Inactivation Method (mCIM)

Isolates that screened positive for potential carbapenem resistance were subjected to







[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

DATA ANALYSIS

Data was analysed using Microsoft Excel 2010, open EPI - Info Version 3.01 updated on 2013/04/06. [REDACTED]

[REDACTED]

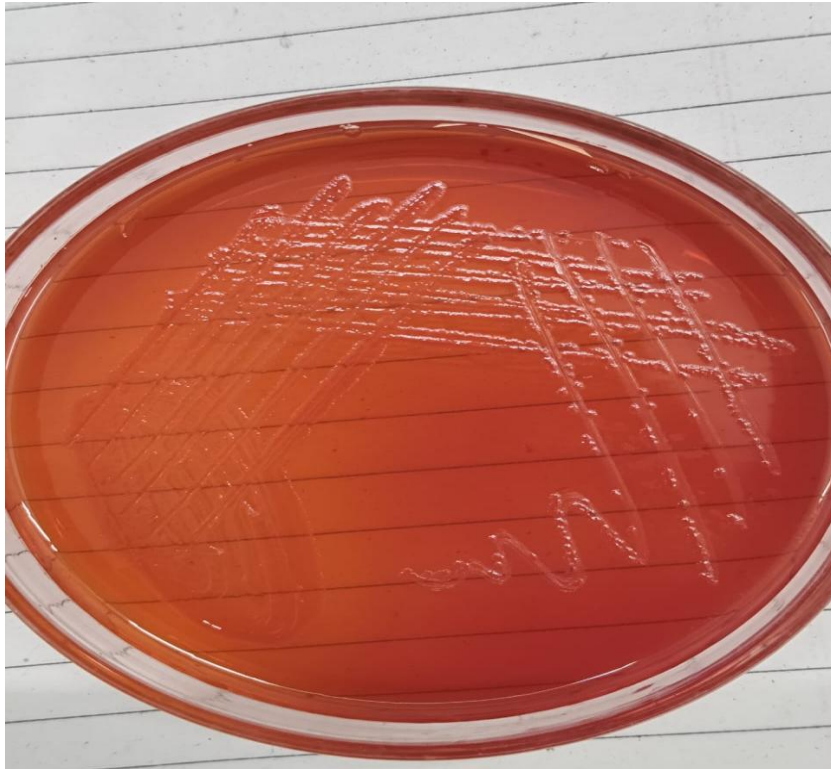


Photo No.1-Non-lactose fermenting colonies on MacConkey agar.



**Photo No.2- Dry, wrinkled, brown to buff colored colonies of *Pseudomonas stutzeri*
on Blood agar**

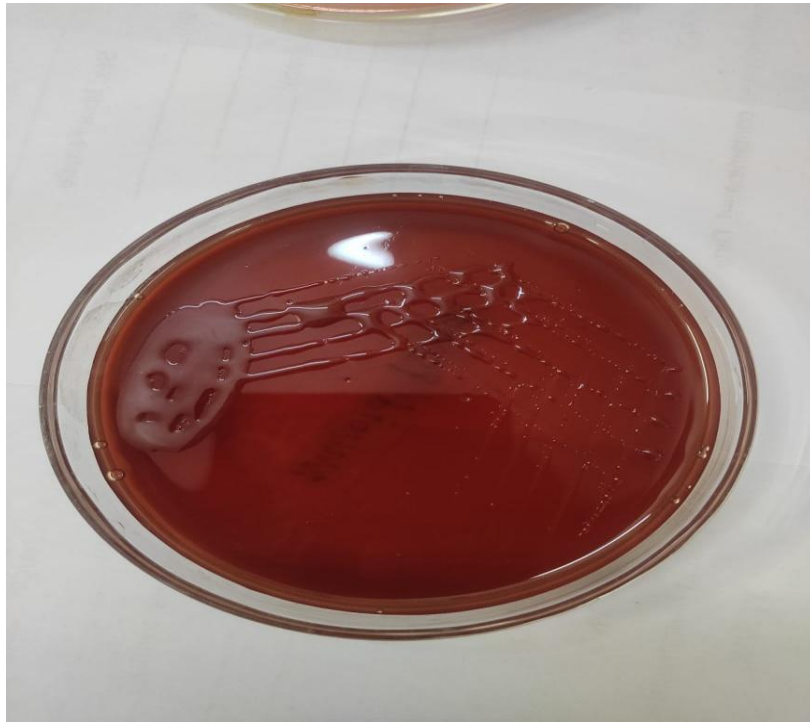


Photo No.3- Greyish, Translucent, Mucoid colonies of *Pseudomonas aeruginosa*.

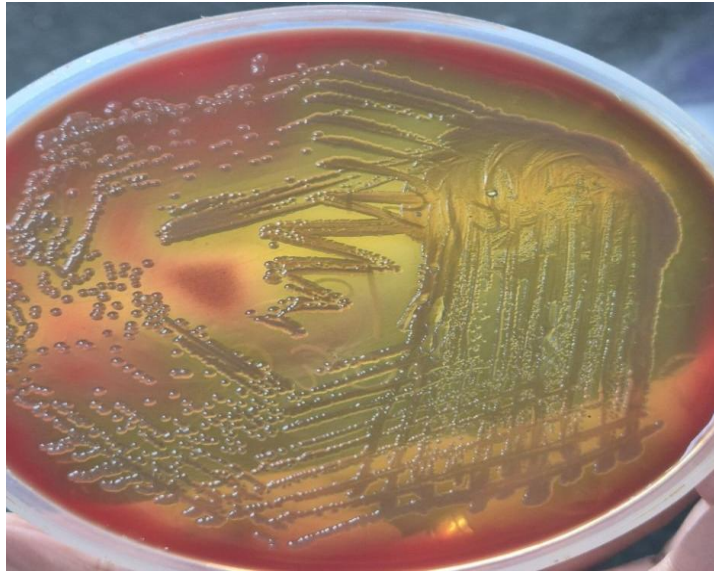


Photo No.4- *Stenotrophomonas maltophilia* producing Smooth, glistening colonies with greenish discoloration beneath the growth

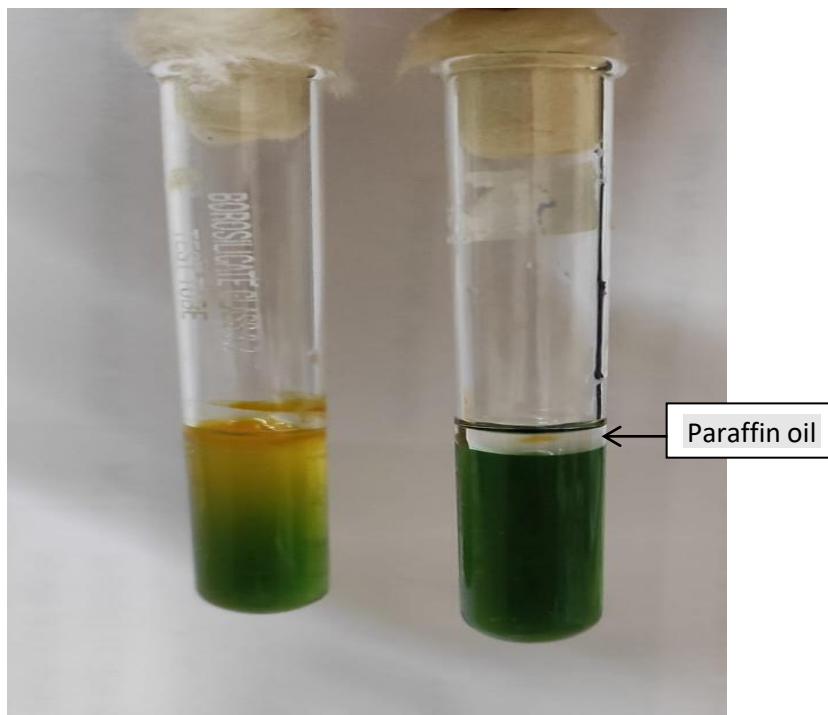


Photo No.5- Oxidation-Fermentation test of *Pseudomonas aeruginosa* shows acid production (yellow colour) in the open tube, indicating its oxidative nature.

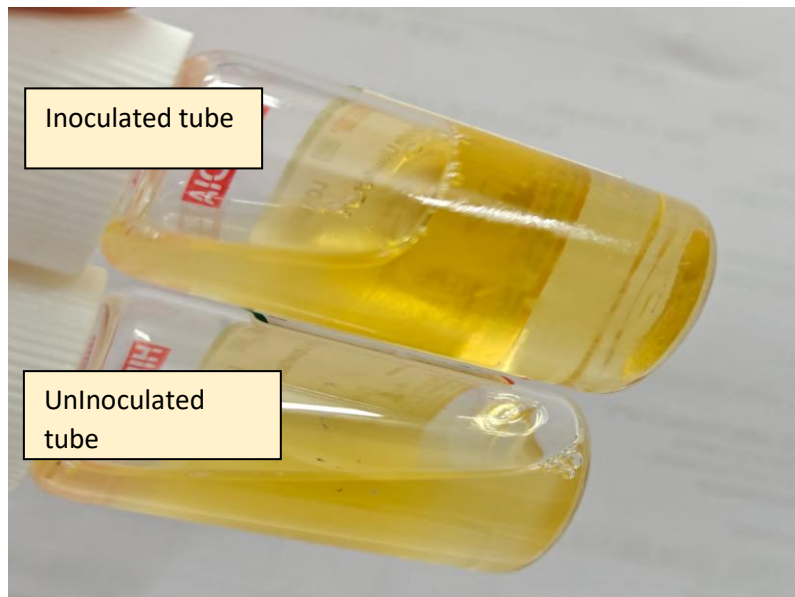


Photo No.6- Partial Gelatin hydrolysis was observed in the tube inoculated with *Pseudomonas fluorescens*.

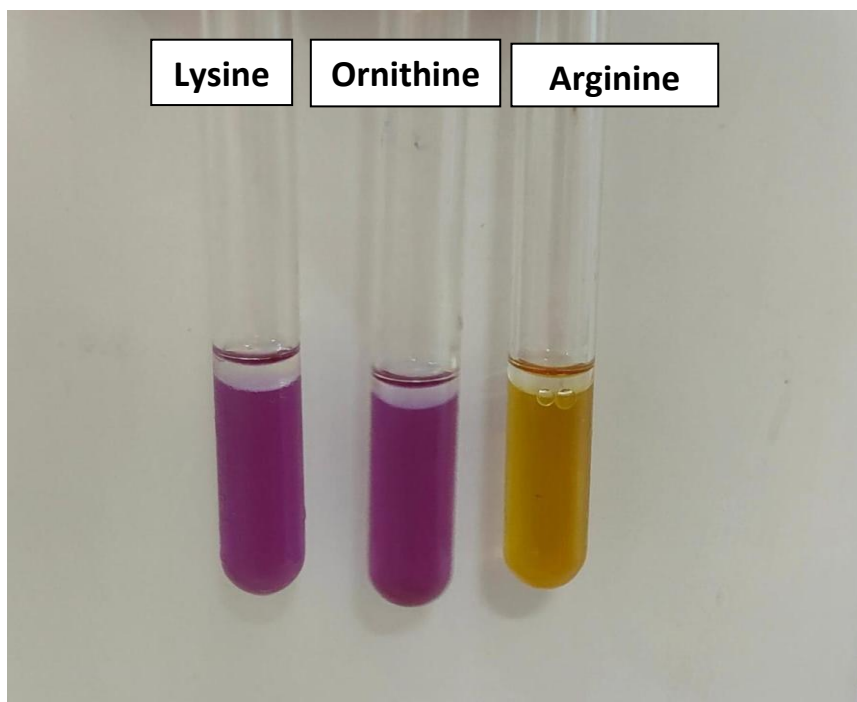


Photo No.7- Fluorescent group of *Pseudomonas* showing negative- Lysine and Ornithine decarboxylation, but positive- Arginine dihydrolase



Photo No.8- Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa* producing pigment (pyocyanin + pyoverdine)



Photo No.9- Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa* producing blue-green pigment (pyocyanin)



Photo No.10- Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa* producing yellow - green pigment (Pyoverdine)

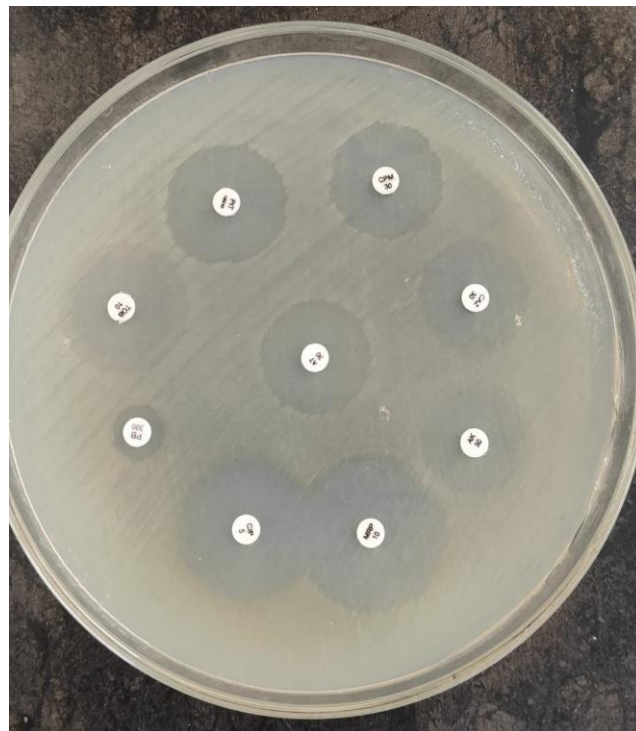


Photo No.12- Antimicrobial Susceptibility Testing of *Pseudomonas species* without any pigment production

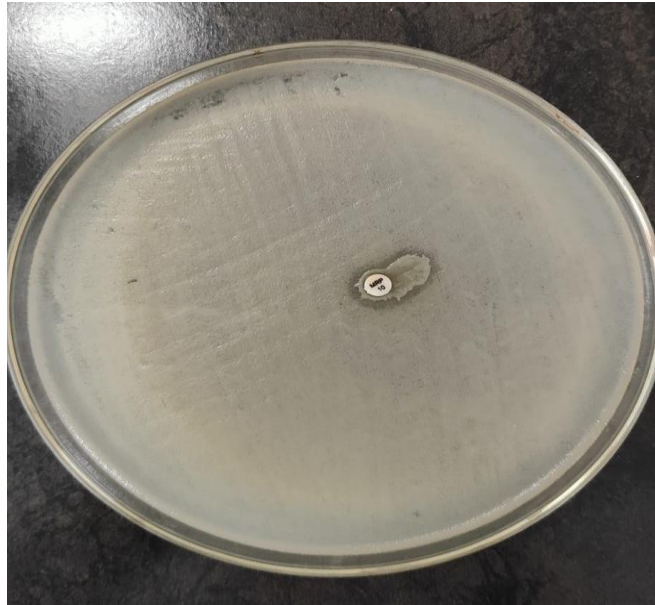


Photo No.13- Metallo- β -lactamase-producing *Pseudomonas species*

RESULTS

RESULTS

The present study was carried out in the Department of microbiology of a rural tertiary care hospital during the study period duration from July 2023 to June 2025. [REDACTED]

[REDACTED]

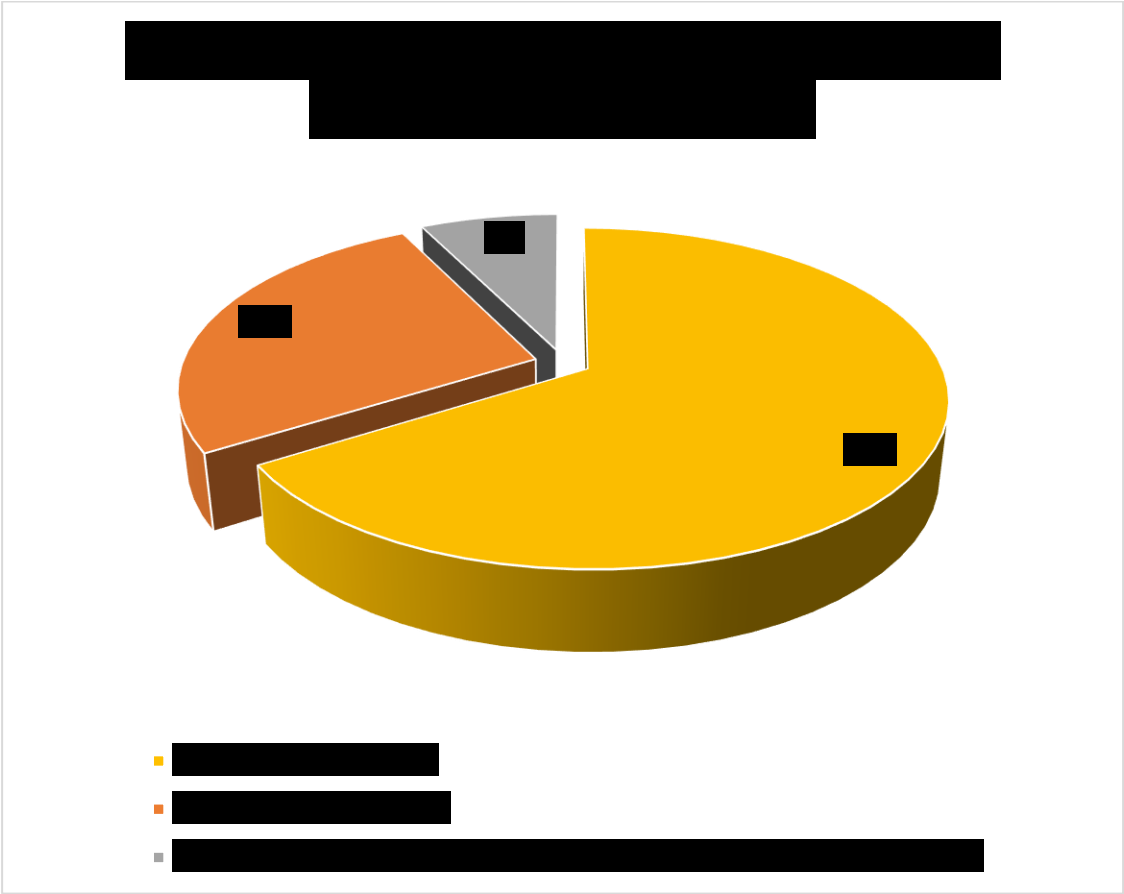
[REDACTED]

[REDACTED]

TABLE 10: Percentage Distribution of Culture-Positive, Culture-Negative Clinical Samples and Non-fermenting gram-negative bacilli

Total samples received	Culture-Positive samples	%	Culture-Negative samples	%	Non-fermenting gram-negative bacilli (NFGNB) in Culture-Positive samples
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

Graph 1: Distribution of Culture-Positive and Culture-Negative Clinical Samples



[Redacted text block]

TABLE 11: Age and Gender-wise distribution of Non-fermenting Gram -negative bacilli (NFGNB) Isolates. (n=322)

Age groups					Total isolates
	Male	%	Female	%	
0-20 yr	■	■	■	■	■
21-40 yr	■	■	■	■	■
41-60 yr	■	■	■	■	■
61-80 yr	■	■	■	■	■
≥81 yr	■	■	■	■	■
Total	■	■	■	■	■

Graph 2: Age and Gender-wise distribution of Non-fermenting Gram -negative bacilli (NFGNB) Isolates.

Table 12: Distribution of Indoor and Outdoor Patients in the Study on Non-Fermenting Gram-Negative Bacilli

Patient Type	n	%
Indoor patients	█	█
Outdoor patients	█	█
Total	█	█

Graph 3: Distribution of Indoor and Outdoor Patients in the Study on Non-Fermenting Gram-Negative Bacilli

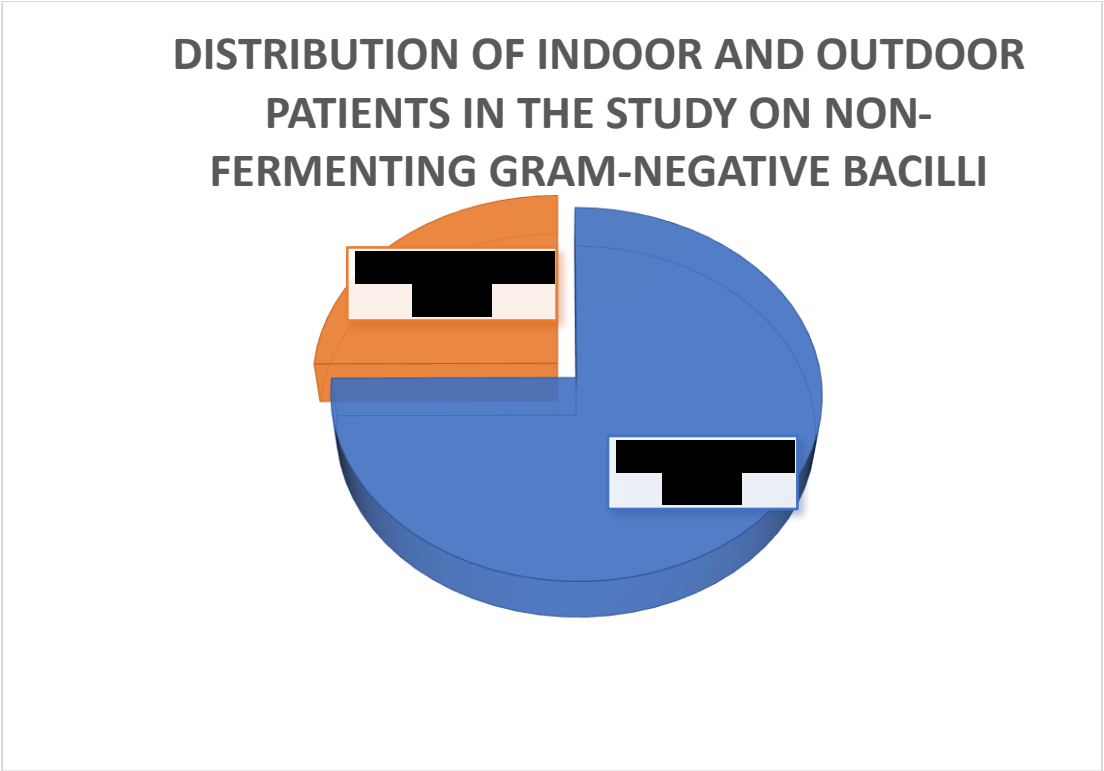
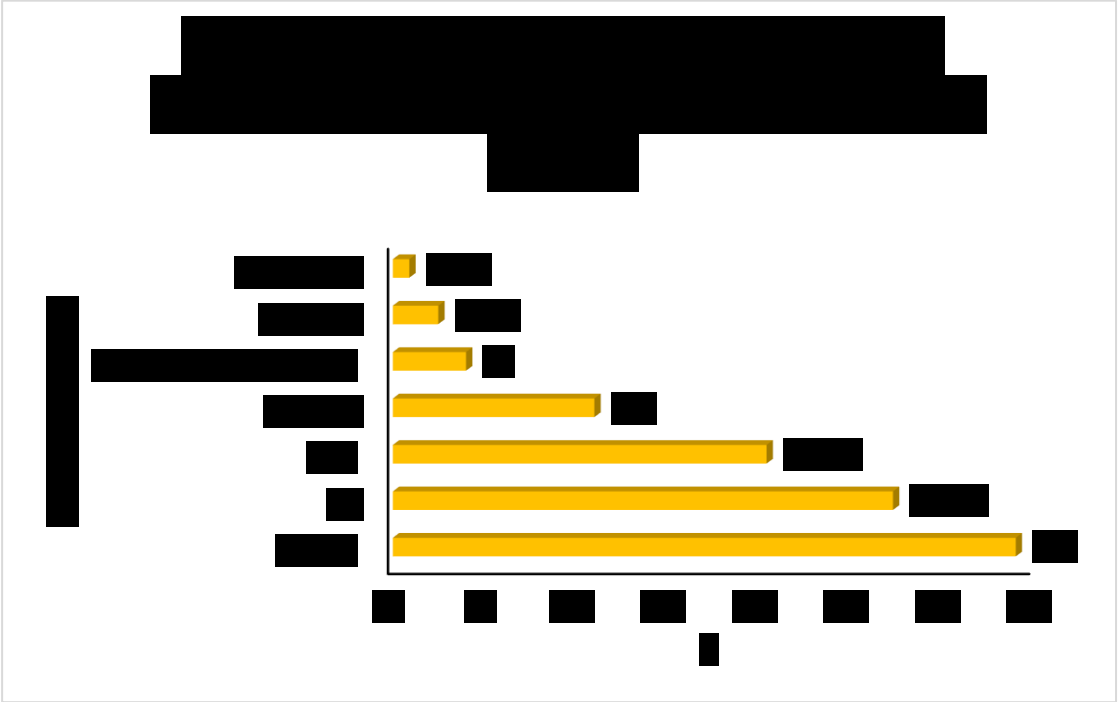




TABLE 13: Department- wise distribution of Non-fermenting gram negative bacilli (NFGNB) isolates (n=322)

Clinical Departments	n	%
Surgery	█	█
ICU	█	█
Burn	█	█
Medicine	█	█
Obstetrics & Gynaecology	█	█
Paediatrics	█	█
orthopaedics	█	█
Total	█	█

Graph 4: Department- wise distribution of Non-fermenting gram negative bacilli (NFGNB) isolates

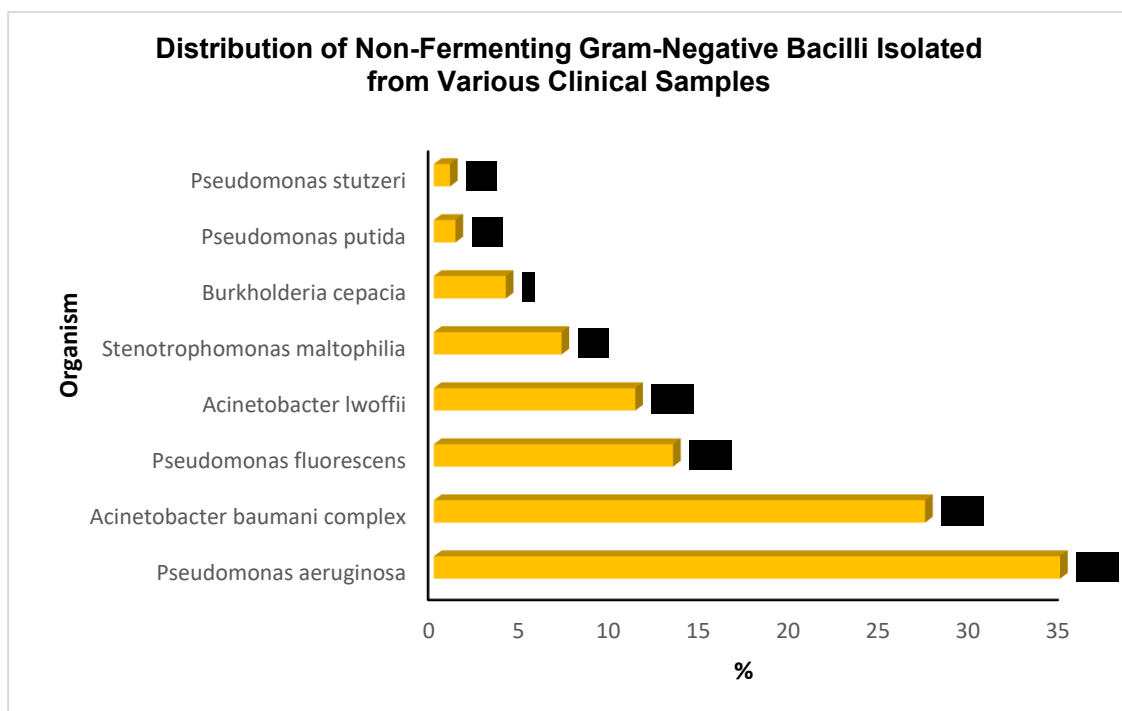


[Redacted text block]

TABLE 14: Distribution of Non-Fermenting Gram-Negative Bacilli Isolated from Various Clinical Samples

Organism	n	%
<i>Pseudomonas aeruginosa</i>	16	35
<i>Acinetobacter baumannii</i> complex	16	35
<i>Pseudomonas fluorescens</i>	16	35
<i>Acinetobacter lwoffii</i>	16	35
<i>Stenotrophomonas maltophilia</i>	16	35
<i>Burkholderia cepacia</i> complex	16	3
<i>Pseudomonas putida</i>	1	35
<i>Pseudomonas stutzeri</i>	1	35
Total	16	35

Graph 5: Distribution of Non-Fermenting Gram-Negative Bacilli Isolated from Various Clinical Samples



[REDACTED]

[REDACTED]

[REDACTED] The least commonly isolated species was *Pseudomonas stutzeri*.

TABLE 15: Sample – wise distribution of Non-fermenting Gram-negative_bacilli (NFGNB) species

Sample	Pus & Wound swab	Respiratory samples		Urine	Blood	Body fluids	Total
Organisms		Sputum	Endotracheal fluid				
<i>Pseudomonas aeruginosa</i>	■	■	■	■	■	■	■
<i>Acinetobacter baumannii</i> complex	■	■	■	■	■	■	■
<i>Pseudomonas fluorescens</i>	■	■	■	■	■	■	■
<i>Acinetobacter lwoffii</i>	■	■	■	■	■	■	■
<i>Stenotrophomonas maltophilia</i>	■	■	■	■	■	■	■
<i>Burkholderia cepacia</i> complex	■	■	■	■	■	■	■
<i>Pseudomonas putida</i>	■	■	■	■	■	■	■
<i>Pseudomonas stutzeri</i>	■	■	■	■	■	■	■

Total							
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 *Acinetobacter baumannii* complex was most commonly isolated from pus & wound samples followed by urine samples. Other Non-fermenting Gram-negative bacilli (NFGNB), such as *Pseudomonas fluorescens*, *Acinetobacter Iwoffii*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia* complex were identified in smaller proportions across various samples.

TABLE 16: Antimicrobial susceptibility pattern of *Pseudomonas* species.

Antimicrobial agent	Concentrations	<i>Pseudomonas aeruginosa</i> (n= 112)	
		Sensitive	%
Ceftazidime			
Cefepime			
Piperacillin-tazobactam			
Tobramycin			
Ciprofloxacin			
Meropenam			
Ceftazidime-avibactam			

Norfloxacin (U)				
Amikacin(U)				
Aztreonam				
Polymyxin B				

Graph 6: Antimicrobial susceptibility pattern of *Pseudomonas species*.



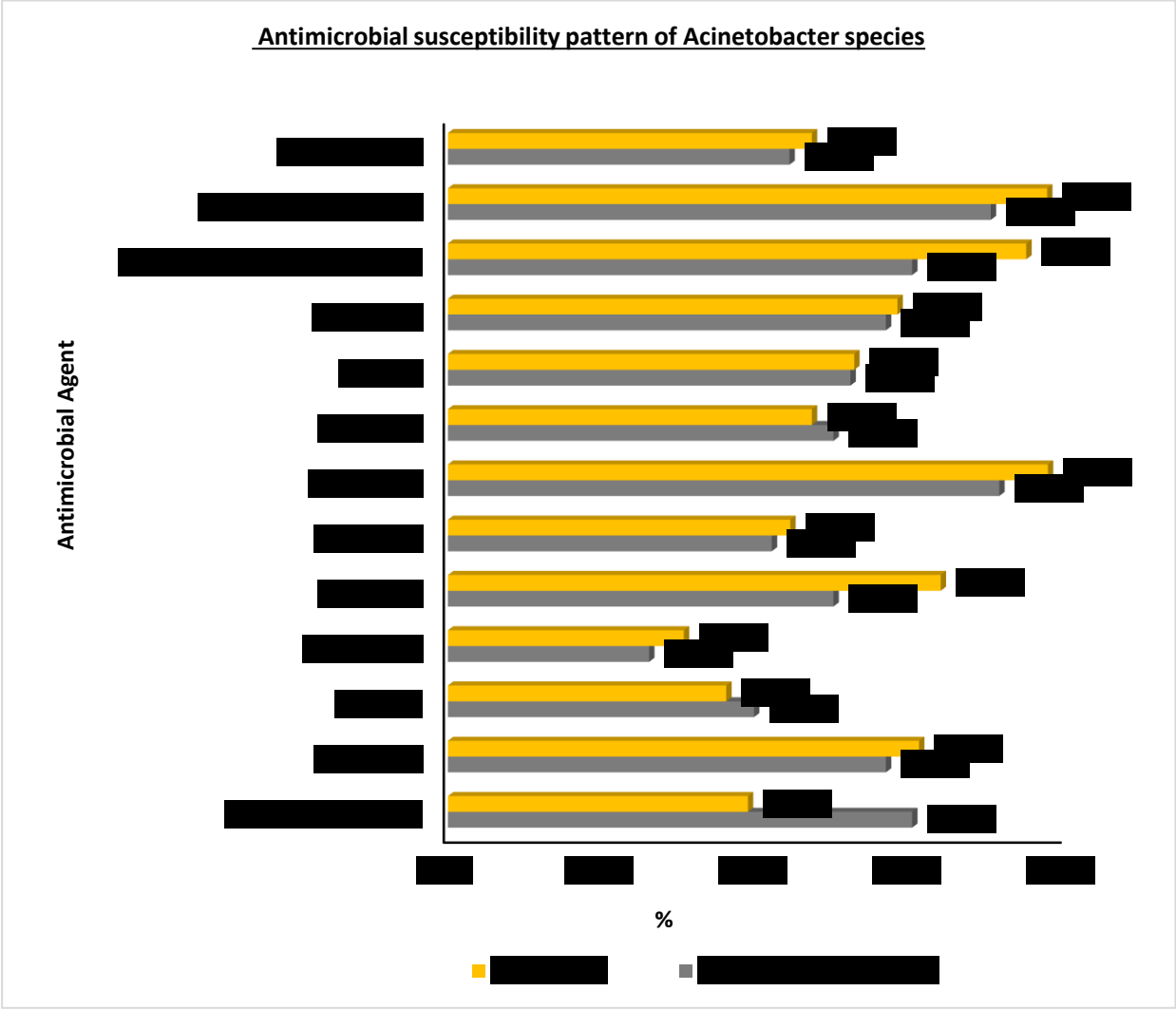
[Redacted text block]

All the *Pseudomonas* isolates were sensitive to Polymyxin B which was used to differentiate between *Pseudomonas species* and *Burkholderia species*

Table 17: Antimicrobial susceptibility pattern of *Acinetobacter species*

Antimicrobial Agent	Concentration	<i>A. baumannii</i> complex (n=88) Count	<i>A. baumannii</i> complex (%)	<i>A. lwoffii</i> (n=36) Count	<i>A. lwoffii</i> (%)
Ampicillin–Sulbactam	██████	█	██████	█	██████
Ceftazidime	████	█	██████	█	██████
Cefepime	████	█	██████	█	██████
Ciprofloxacin	████	█	██████	█	██████
Gentamicin	████	█	██████	█	██████
Tobramycin	████	█	██████	█	██████
Meropenem	████	█	██████	█	██████
Cefotaxime	████	█	██████	█	██████
Amikacin	████	█	██████	█	██████
Minocycline	████	█	██████	█	██████
Trimethoprim–Sulfamethoxazole	██████████	█	██████	█	██████
Piperacillin–Tazobactam	████████	█	██████	█	██████
Tetracycline (U)	████	█	██████	█	██████

Graph 7: Antimicrobial susceptibility pattern of *Acinetobacter* species

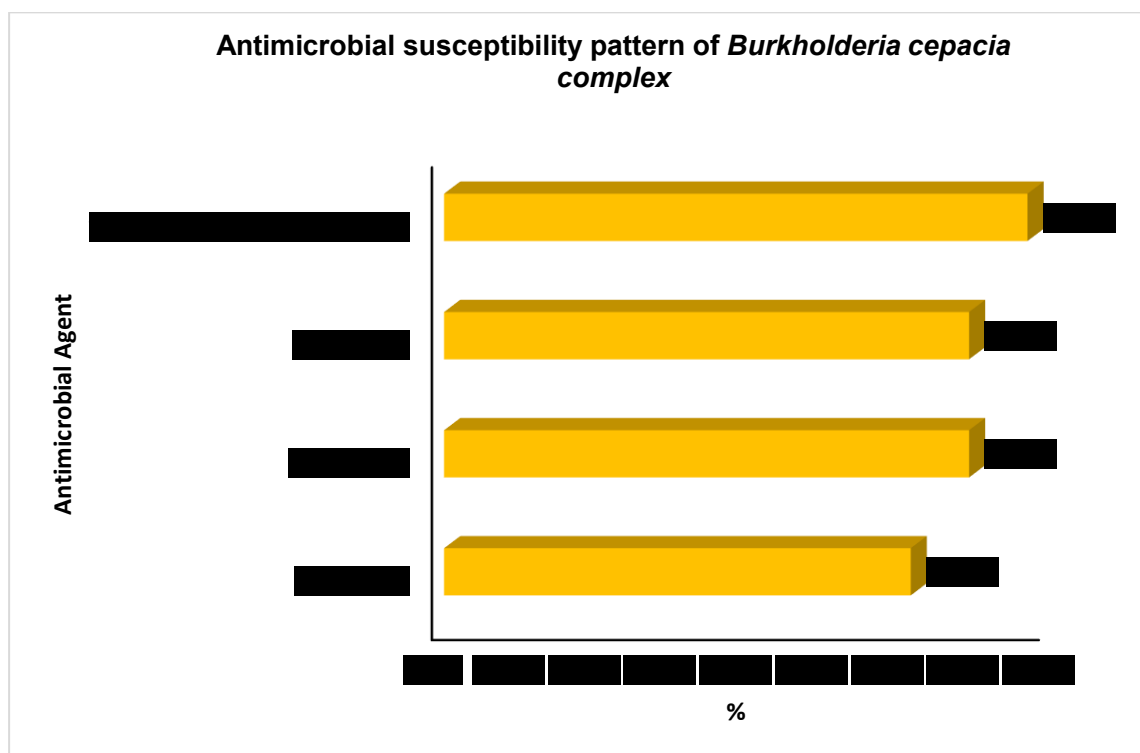


Meropenem, Piperacillin-Tazobactam, and Trimethoprim Sulfamethoxazole were found to be the most effective antibiotics against both *Acinetobacter baumannii* complex and *Acinetobacter lwoffii*. In contrast, Ciprofloxacin exhibited the lowest sensitivity in both species. *Acinetobacter baumannii* complex showed slightly higher resistance overall compared to *Acinetobacter lwoffii*.

TABLE 18: Antimicrobial susceptibility pattern of *Burkholderia cepacia* complex (n= 13)

Antimicrobial Agent	Concentration	Count	Percentage
Ceftazidime	████	█	████
Meropenem	████	█	████
Minocycline	████	█	████
Trimethoprim– Sulfamethoxazole	████████	█	████

Graph 8: Antimicrobial susceptibility pattern of *Burkholderia cepacia* complex



[REDACTED]

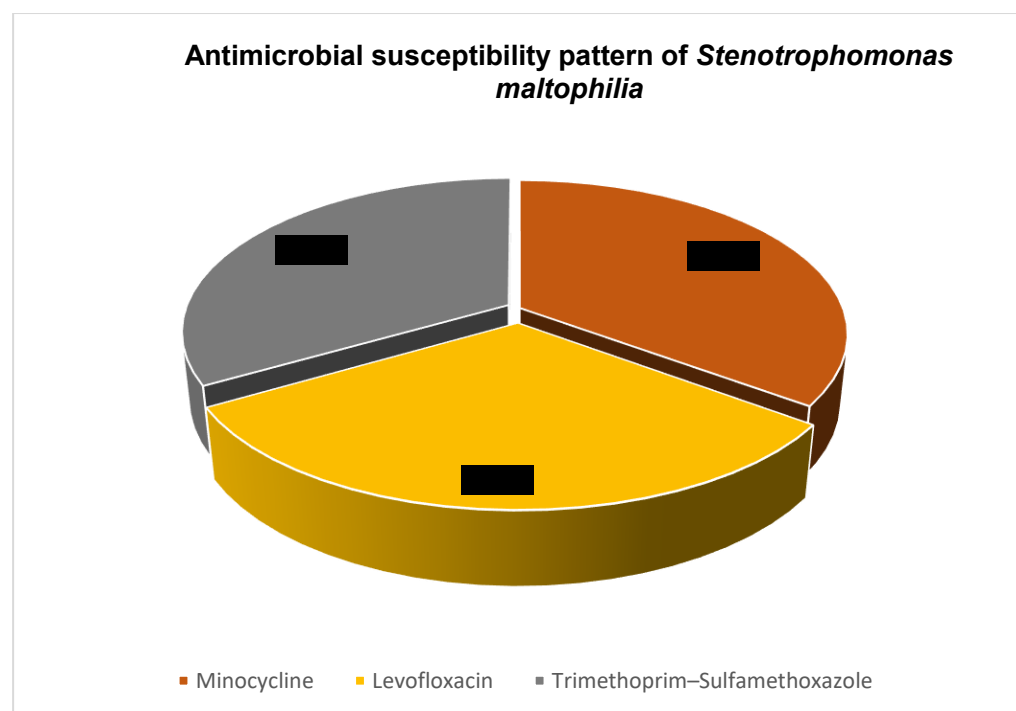
[REDACTED]

[REDACTED] All four antibiotics demonstrated acceptable sensitivity, indicating moderate susceptibility patterns in *Burkholderia cepacia complex*.

TABLE 19: Antimicrobial susceptibility pattern of *Stenotrophomonas maltophilia* (n= 23)

Antimicrobial Agent	Concentration	Count	Percentage
Minocycline	[REDACTED]	[REDACTED]	[REDACTED]
Levofloxacin	[REDACTED]	[REDACTED]	[REDACTED]
Trimethoprim– Sulfamethoxazole	[REDACTED]	[REDACTED]	[REDACTED]

Graph 9: Antimicrobial susceptibility pattern of *Stenotrophomonas maltophilia* (n= 23)



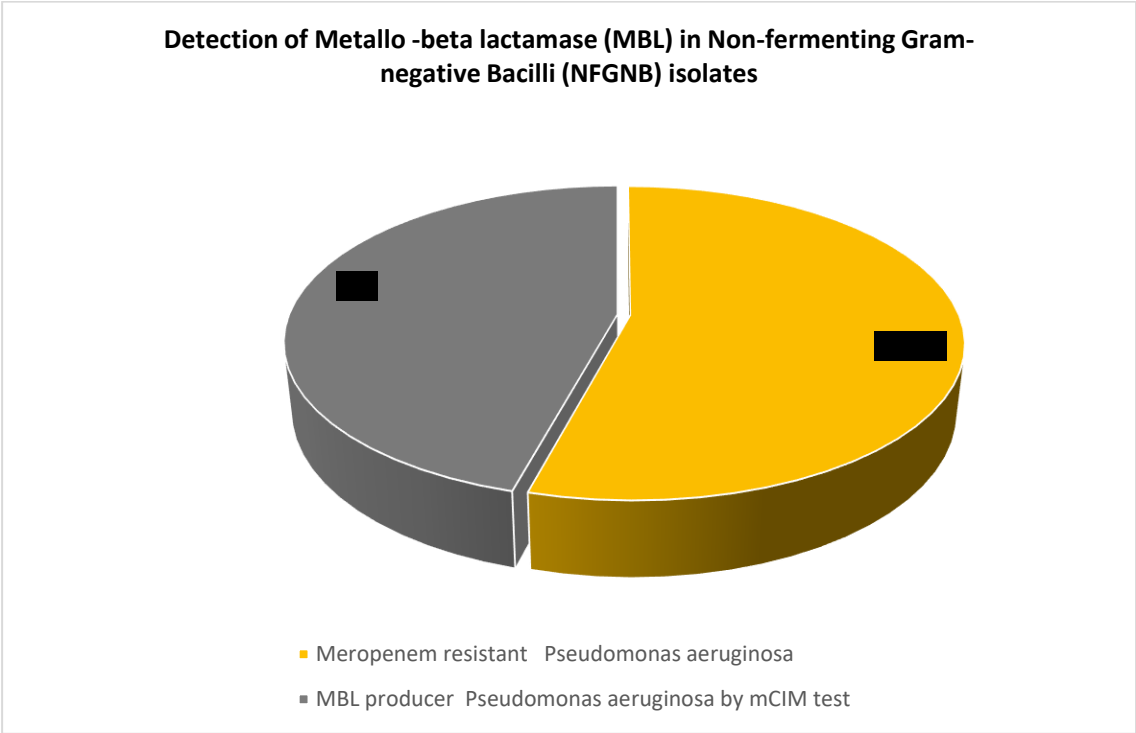
[REDACTED]

[REDACTED]

TABLE 20: Detection of Metallo -beta lactamase (MBL) in Non-fermenting Gram-negative Bacilli (NFGNB) isolates

Total <i>Pseudomonas aeruginosa</i> tested for MBL production (n=112)	Meropenem resistant <i>Pseudomonas aeruginosa</i>	Meropenem resistant <i>Pseudomonas aeruginosa</i>	MBL producer <i>Pseudomonas aeruginosa</i> by mCIM test	MBL producer <i>Pseudomonas aeruginosa</i> by mCIM test
<i>Pseudomonas aeruginosa</i> (n=112)	■	■	■	■

Graph 10: Detection of Metallo -beta lactamase (MBL) in Non-fermenting Gram-negative Bacilli (NFGNB) isolates



[Redacted text block]

DISCUSSION

DISCUSSION

Non-fermenting organisms are aerobic, Gram-negative, non-spore-forming bacilli that either lack the ability to utilize carbohydrates for energy or break them down via oxidative pathways instead of fermentation.²¹

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In our study, total of [REDACTED] culture-positive clinical samples, [REDACTED] isolates were identified as Non-fermenting Gram-negative bacilli (NFGNB), resulting in an isolation rate of [REDACTED] [REDACTED]

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In contrast, significantly lower isolation rates were reported by (4.24%) **Prudhave sumana et al. (2017)**¹¹¹, (3.5%) **Kirti Lakshmi K et al. (2014)**²⁸ and (3.5%) **Malini et al.**

[REDACTED]

[REDACTED]

infection control practices, and environmental exposure to resistant strains in the community setting. [REDACTED]

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[REDACTED] This shows that middle-aged

people were more commonly affected by NFGNB infections. Similar findings were

reported by **Praveetha C et al. (2024)**⁸², **Rajeev Kumar et al. (2020)**²², **Nand Kishor**

et al. (2021)⁸⁶ However, some studies have shown different results. For example, **R.**

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conditions such as COPD. [REDACTED]

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The predominance of *Pseudomonas aeruginosa* among the non-fermenting Gram-negative bacilli (NFGNB) isolates in this study can be attributed to its ubiquitous presence in moist environments, [REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED] Similarly, studies by **Udhayvir Singh et al. (2017)**,⁹⁸ **Maniyan G et al. (2016)** ⁵, and **Mahajan R et al. (2016)** ⁹⁹ also reported a higher prevalence of *Pseudomonas aeruginosa* in pus and wound samples, but in these study, the urine was the next most common source of isolation.

[REDACTED]

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In the present study, *Acinetobacter baumannii complex* was most frequently isolated from pus and wound samples, followed by urine samples. [REDACTED]

[REDACTED]

[REDACTED]

Additionally, studies conducted by **Sharma D. et al. (2014)**³² and **Kaur A. et al. (2018)**¹ reported that *Acinetobacter baumannii complex* was most commonly isolated from respiratory samples, followed by pus and wound swabs. [REDACTED]

[REDACTED]

[REDACTED]

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In this study, *Pseudomonas aeruginosa* reported highest susceptibility to Meropenem followed by Piperacillin-tazobactam, Similar findings were reported by **Sindhu Cugati et al. (2018)**¹⁰⁰ However **Karthik M et al. (2018)**¹⁰¹ found Meropenem to be the most effective, followed by Amikacin, Gentamicin, and Ciprofloxacin. In contrast, **Soumya S et al (2018)**¹⁰², reported highest susceptibility to Piperacillin-tazobactam followed by Meropenem. [REDACTED]

[REDACTED]

[REDACTED]

In Contrast to this, Ciprofloxacin was the least effective antibiotic in the present study, with a sensitivity rate of [REDACTED]

[REDACTED]

In the present study, [REDACTED]

In this study, the *Acinetobacter baumannii* complex was sensitive to Meropenem, Piperacillin-Tazobactam, and Trimethoprim-Sulfamethoxazole, which was consistent with the findings of **Praveetha C et al. (2024)** ⁸² Specifically, Meropenem showed [REDACTED] sensitivity in this study, which was comparable to the findings of Narayan

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overall compared to *Acinetobacter lwoffii*, Similar to **Kaur A et al. (2018)** ¹,study.

In this study, the *Burkholderia cepacia* complex reported highest sensitivity to Trimethoprim-sulfamethoxazole, with [REDACTED] of isolates being susceptible. These

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CONCLUSION

CONCLUSION

The present study successfully isolated, identified, and characterized non-fermenting Gram-negative bacilli (NFGNB) from a variety of clinical specimens in a rural tertiary care hospital, with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex being the most frequently encountered pathogens. [REDACTED]

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Stringent infection control measures, antimicrobial stewardship, and targeted public health interventions are essential to curb the spread of these pathogens.

SUMMARY

SUMMARY

1. The study focused on the isolation, identification, and antibiotic susceptibility profiling of non-fermenting Gram-negative bacilli (NFGNB) from various clinical samples in a rural tertiary care hospital.

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

122. Raja NS, Singh NN. Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital. *Journal of Microbiology, Immunology and Infection*. 2007 Feb;40(1):45–9.

ANNEXURES

ANNEXURE-A

LIST OF ABBREVIATIONS

- **NFGNB:** Non-lactose fermenting Gram-negative bacilli
- **AST:** Antimicrobial Susceptibility Test
- **MHA:** Mueller-Hinton Agar
- **CFU:** Colony Forming Unit
- **ATCC:** American type culture collection
- **CLSI:** Clinical and Laboratory Standards Institute
- **EUCAST:** European Committee on Antimicrobial Susceptibility Testing
- **ESBL:** Extended-Spectrum Beta-Lactamases
- **MBL:** Meta Beta Lactamases
- **CTR:** Ceftriaxone
- **CAZ:** Ceftazidime
- **CAZ-CAC:** Ceftazidime- Ceftazidime/Clavulanate
- **CPM:** Cefepime
- **GEN:** Gentamicin
- **AK:** Amikacin
- **IPM:** Imipenem
- **PIT:** Piperacillin -Tazobactem
- **COT:** Cotrimoxazole
- **EDTA:** Ethylene diamine tetraacetic acid
- **AMP:** Ampicillin

- **CIP:** Ciprofloxacin
- **µg:** Microgram
- **U:** Urine
- **MIC:** Minimum inhibitory concentration
- **OPD:** Outpatient Department
- **IPD:** Inpatient Department
- **ICU:** Intensive Care Unit

ANNEXURE-B

CASE RECORD FORM

Name:

Age:

Sex:

MRD:

Address:

Occupation of patient:

Chief complaints:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Present history

Positive history:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Negative history:

Social history:

Past history:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Birth history:

Family history:

Examination of the patient:

GENERAL EXAMINATION:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

SYSTEMIC EXAMINATION:

Per abdomen: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Cardio vascular system: [REDACTED]

[REDACTED]

Respiratory system: [REDACTED]

Central nervous system: [REDACTED]

Clinical diagnosis:

MICROBIOLOGICAL STUDY: -

LAB ID NUMBER

SAMPLE

EXAMINATION OF SAMPLE:

Ø **Macroscopic examination:**

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

LABORATORY EXAMINATION:

Microscopic findings

Wet mount: [REDACTED]

Gram Stain: [REDACTED]

[REDACTED]

Culture findings:

Colony characteristics:

[REDACTED]

[REDACTED]

[REDACTED]

Gram stain

Catalase test

Oxidase test

Motility by hanging drop method

Various biochemical reactions-

Isolated organism:

AST results:

ANNEXURE- C

PROCEDURE FOR IDENTIFICATION OF NON-FERMENTATIVE GRAM-

NEGATIVE BACILLI (NFGNB):

Microscopic examination

Wet mount preparation

Materials required-

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Gram

Staining

Materials required:

[REDACTED]

Procedure:

A series of horizontal black bars of varying lengths, representing redacted text. The bars are arranged in a list-like fashion, with some bars spanning the full width of the page and others being shorter and indented. There are 11 bars in total, with lengths ranging from approximately 10% to 100% of the page width.

Quality control:

Hanging drop preparation

Materials required: [REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

MEDIA REQUIRED FOR NON-FERMENTATIVE GRAM-NEGATIVE BACILLI

(NFGNB):

Media prepared

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1) Nutrient agar:

Composition:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

2) Blood agar:

Composition:

[REDACTED]

[REDACTED]

3) Chocolate agar:

Composition:

[REDACTED]

[REDACTED]

4) MacConkey agar-

Composition:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

5) Mueller Hinton Agar (MHA) medium:

Composition:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

**BIOCHEMICAL TESTS FOR IDENTIFICATION OF NON-FERMENTATIVE GRAM-
NEGATIVE BACILLI (NFGNB):**

CATALASE TEST

Material required:

[REDACTED]

Procedure-

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

[REDACTED]

[REDACTED]

OXIDASE TEST

Material required:

[REDACTED]

Composition:

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

2) [REDACTED]

[REDACTED].

Quality Control:

[REDACTED]

[REDACTED]

INDOLE PRODUCTION (KOVAC'S METHOD)

Material required: [REDACTED]

Composition: [REDACTED]

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

[REDACTED]

[REDACTED]

UREASE TEST (CHRISTENSEN'S METHOD),

Material required: [REDACTED]

Composition: [REDACTED]

[REDACTED]

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

[REDACTED]

B [REDACTED]

TRIPLE SUGAR IRON TEST

Material required:

Composition:

Procedure:

- 1) A well-isolated colony of the test organism was picked using a sterile straight wire and inoculated into Triple Sugar Iron (TSI) agar by stabbing the butt of the medium to its base, followed by streaking along the surface of the slant.

Quality Control:

OXIDATION AND FERMENTATIVE OF MEDIUM: (LATOSE, GLUCOSE, SUCROSE, MANNITOL, MALTOSE, XYLOSE)

[REDACTED]

Composition: [REDACTED]

[REDACTED]

Procedure:

- 1) Two tubes containing Hugh and Leifson's oxidative-fermentative (OF) medium with desired sugar were inoculated by stabbing the medium with a sterile straight needle three to four times, approximately halfway to the bottom.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

[REDACTED]

[REDACTED]

GELATIN HYDROLYSIS TEST

Material required: [REDACTED]

Composition: [REDACTED]
[REDACTED]

Procedure:

- 1) Approximately [REDACTED] broth culture of the test organism were inoculated into gelatin stab tubes by making [REDACTED] to a depth of approximately [REDACTED] inch using a sterile inoculating needle.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control

[REDACTED]

[REDACTED]

GROWTH AT 42°C

Material required: [REDACTED]

Composition: [REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control

[REDACTED]

[REDACTED]

GROWTH AT 44°C

Material required: [REDACTED]

Composition: [REDACTED]

Procedure:

- 1) The test organism was inoculated by streaking the slant surface of Nutrient agar using a sterile loop.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

SALT-TOLERANCE TEST (6.5% NaCl)

Material required: [REDACTED]

Composition: [REDACTED]

[REDACTED]

Procedure:

- 1) Two or three colonies were inoculated into [REDACTED] broth.

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control

[REDACTED]

[REDACTED]

MOELLER'S DECARBOXYLASE TEST

Material required: [REDACTED]

Composition:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

	[REDACTED]	[REDACTED]
Lysine	[REDACTED]	[REDACTED]
Ornithine	[REDACTED]	[REDACTED]
Arginine	[REDACTED]	[REDACTED]
Base	[REDACTED]	[REDACTED]

KIRBY-AUER DISC DIFFUSION METHOD FOR ANTIMICROBIAL SUSCEPTIBILITY

TESTING

Material required: [REDACTED]

[REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Quality Control:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Material required: [REDACTED]

Procedure:

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

ANNEXURE- D

Timeline/ GNATT chart

FEBRUARY -MARCH 2023: DESSERTATION TOPIC SELECTION



APRIL – MAY 2023: REVIEW OF LITERATURE AND SYNOPSIS PREPRATION



JUNE 2023: APPROVAL OF DESSERTATION BY INSTITUTIONAL ETHICS COMMITTEE
AND SUSMISSION OF SYNOPSIS TO MUHS



JULY 2023 TO JUNE 2025: DATA COLLECTION, ANALYSIS OF DATA, WRITING OF
DESSERTATION AND SUBMISSION OF DESSERTATION TO MUHS, NASHIK.

ANNEXURE- E

संमतीपत्र (ADULT CONSENT FORM)

संमतीपत्र देणाऱ्याचे नाव:

मी खालील सही करणार ,संमतीपत्र लिहून देतो की “

.....

.....” या वैद्यकीय शोधनिबंधात मी स्वतःच्या मर्जीने कोणत्याही दबावाखाली न येता सहभागी होत आहे.

सदरील वैद्यकीय परीक्षणाचे उद्देश स्वरूप तसेच त्यामुळे माझ्या आरोग्यावर होणाऱ्या सर्व परिणामांची मला कल्पना देण्यात आली आहे.

मला सदरील वैद्यकीय परीक्षणातून कोणत्याही क्षणी बाहेर पडण्याची मुभा देण्यात आलेली आहे.

परीक्षकाची सही:

संमतीपत्र देणाऱ्याची सही:

ठिकाण:

दिनांक:

संमतीपत्र (PEADIATRIC CONSENT FORM)

संमतीपत्र लिहून देण्याऱ्याचे नांव:

.....आम्ही मुलाचे आई-

वडील या नात्याने आमच्या मुलाच्या औषधोपचाराकरीता संमतीपत्र लिहून

देतो की, आमचा

मुलगा.....

.....

कोणत्याही दबावाखाली न येता सहभागी होत आहे.

सदरील वैद्यकीय परीक्षणाचे उद्देश स्वरूप तसेच त्यामुळेमाझ्या आरोग्यावर होणाऱ्या सर्व परिणामांची मला कल्पना देण्यात आली आहे.

मला सदरील वैद्यकीय परीक्षणातून कोणत्याही क्षणी बाहेर पडण्याची मुभा देण्यात आलेली आहे.

परीक्षकाची स्वाक्षरी:

संमतीपत्र देण्याऱ्याची स्वाक्षरी:

ठिकाण:

दिनांक:

ANNEXURE - F

[illegible]

