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Study Protocol Phenotypic and Molecular Characterization of Multidrug Resistant Gram-Negative Organisms from Lower Respiratory Tract Infections

Puvvada Sai Swaroop [1], Dhruba Hari Chandi [2], Ranjit Ambad[3]

[1]Assistant Professor and PhD Scholar Dept. of Microbiology Jawaharlal Nehru Medical College, DMIHER Wardha
[2]Associate Professor Dept. of Microbiology Jawaharlal Nehru Medical College, DMIHER Wardha
[3]Professor, Department of Biochemistry, Jawaharlal Nehru Medical College, DMIHER, Sawangi (Meghe), Wardha

Corresponding author:
Puvvada Sai Swaroop
Email.id. swaroop8288@gmail.com

ABSTRACT

Lower respiratory tract infections (LRTIs) remain a leading cause of morbidity and mortality globally, particularly among vulnerable populations. Gram-negative bacteria are increasingly implicated in these infections, posing significant therapeutic challenges due to multidrug resistance (MDR). The irrational and excessive use of antibiotics has accelerated the emergence of MDR Gram-negative organisms, thereby limiting treatment options and increasing healthcare burdens. This study aims to perform comprehensive phenotypic and molecular characterization of MDR Gram-negative organisms isolated from patients with LRTIs in a tertiary care setting. A cross-sectional observational design will be employed, with respiratory specimens such as sputum, tracheal aspirates, bronchoalveolar lavage, and pleural fluid analyzed using both phenotypic antimicrobial susceptibility testing and molecular assays for resistance gene detection. The anticipated outcomes include improved diagnostic accuracy, informed treatment strategies, and reduced reliance on broad-spectrum antibiotics, ultimately contributing to better clinical outcomes and reduced antimicrobial resistance

Keywords: LRTIs, MDR, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa.

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INTRODUCTION

Lower respiratory tract infections (LRTIs), encompassing conditions such as bronchitis and pneumonia, remain a pervasive cause of illness and death worldwide, particularly among vulnerable populations like the elderly, children, and immunocompromised individuals (1). The respiratory tract serves as a prime site for infection by various microorganisms, but Gram-negative bacteria have become significant pathogens due to their ability to cause severe infections that are difficult to treat (2). Historically, these infections were manageable with conventional antibiotics. However, relentless and often inappropriate use of these drugs has accelerated the emergence of multidrug resistant (MDR) Gram-negative bacteria, which hamper treatment success with limited therapeutic alternatives (3).

Diagnosis and management of infections caused by MDR organisms require accurate and timely detection. Routine phenotypic antimicrobial susceptibility testing (AST) guides initial therapy; yet, phenotypic methods alone often cannot unravel the complexity of resistance mechanisms involved (4).

While numerous studies have explored MDR Gram-negative bacteria in bloodstream and urinary infections, data specifically addressing their phenotypic and molecular profiles in lower respiratory tract infections—particularly in the context of regional epidemiology—are relatively limited. Understanding local pathogen profiles is critical for tailoring effective antimicrobial stewardship programs and optimizing therapeutic approaches (5). This study thus aims to fill this gap by providing an integrated phenotypic and molecular analysis of MDR Gram-negative organisms isolated from LRTI patients in a tertiary care setting.

AIM AND OBJECTIVES

Aim

To perform comprehensive phenotypic and molecular characterization of multidrug resistant Gram-negative organisms isolated from lower respiratory tract infection specimens.

Objectives

To isolate and identify Gram-negative bacteria from specimens of patients with LRTI.

To determine antimicrobial susceptibility patterns by phenotypic methods..

To detect and characterize key antimicrobial resistance genes through molecular assays.

To correlate phenotypic resistance profiles with molecular findings for comprehensive characterization.

REVIEW OF LITERATURE

Lower respiratory tract infections (LRTIs) continue to be a major cause of morbidity and mortality across the globe, particularly in developing countries where healthcare infrastructure and antimicrobial stewardship are often limited (6). The etiological agents include a diverse array of bacteria, with Gram-negative organisms emerging as leading pathogens due to their increasing resistance to multiple antibiotics (7).

Prevalence and Impact of MDR Gram-Negative Organisms

Several studies have documented a rising burden of multidrug resistant (MDR) Gram-negative bacteria in LRTIs. Gupta et al. reported that over 30% of Gram-negative respiratory isolates exhibited multidrug resistance in their tertiary care hospital setting, a figure echoed in subsequent studies worldwide (8).

Phenotypic Resistance Patterns:

Phenotypic antimicrobial susceptibility testing remains the primary method for detecting resistance in clinical microbiology laboratories. Studies frequently observe high resistance rates to cephalosporins, fluoroquinolones, and carbapenems among Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa isolates from respiratory samples (9).

Molecular Mechanisms of Resistance:

Molecular studies have elucidated various genetic determinants responsible for multidrug resistance in Gram-negative bacteria. Research has identified the widespread distribution of bla_CTX-M type ESBLs, especially in Enterobacterales isolated from respiratory infections (10).

Correlation of Phenotypic and Molecular Findings:

Du et al., studying isolates from respiratory specimens, found a significant correlation between phenotypic resistance patterns and the presence of resistance genes detected by PCR, advocating for integrated diagnostics in clinical laboratories (11).

Regional and Socioeconomic Considerations:

Resistance patterns and genetic profiles vary across regions. While some resistance genes are globally prevalent, others show geographic clustering influenced by antibiotic usage policies and healthcare practices (12). Local epidemiological data are essential to inform empirical treatment guidelines and infection control measures. Moreover, resource constraints in many low- and middle-income countries limit access to molecular diagnostics, emphasizing the need for affordable, rapid, and accurate methods that combine phenotypic and genotypic approaches.

Emerging Technologies and Future Directions:

Advancements including whole-genome sequencing and rapid molecular assays present promising tools to overcome limitations of traditional methods and enable real-time resistance monitoring (13). Integration of such technologies into routine diagnostics, supported by robust epidemiological data, is critical for combating the MDR threat.

MATERIAL AND METHODOLOGY

Methodology: This will be a Cross-sectional observational study conducted in the Department of Microbiology, at Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research Sawangi Meghe Wardha from 2022 to 2025

Study Population:

The study will include all patients diagnosed with lower respiratory tract infections at Acharya Vinoba Bhave Rural Hospital and Lower respiratory tract specimens (sputum, tracheal aspirate, BAL, pleural fluid) will be collected. Samples will be processed immediately for culture, AST and find multi drug resistant gram negative bacilli and PCR will be used for DNA Extraction and PCR for finding the resistant genes

Inclusion Criteria

Confirmed Patients of all ages with clinical and radiological diagnosis of LRTI will be enrolled.

Will give written Consent.

Exclusion Criteria

Patients on antimicrobials exceeding 48 hours prior to sample collection.

Know Diagnosed cases of carcinoma, DM, HT and any type of illness.

Expected Outcomes:

The findings from this research have direct and significant clinical applications. The data on local pathogen prevalence and their resistance profiles will serve as a foundational guide for developing effective, evidence-based empirical treatment protocols for patients with LRTIs. This will help clinicians make more informed therapeutic decisions, reducing the reliance on broad-spectrum antibiotics and potentially improving patient outcomes by shortening hospital stays and lowering mortality rates..

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