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Phenotypic and Molecular Characterization of Multidrug Resistant Gram-Negative Organisms from Lower Respiratory Tract Infections: A Review

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ABSTRACT

Lower respiratory tract infections (LRTIs) remain among the leading causes of morbidity and mortality worldwide, particularly in immunocompromised and hospitalized patients. The emergence of multidrug resistant (MDR) Gram-negative bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and Escherichia coli has further complicated clinical management. Conventional phenotypic antimicrobial susceptibility testing (AST) provides essential guidance but fails to capture the complete resistance landscape. Molecular approaches, including PCR-based detection of resistance genes, offer greater precision in identifying extended-spectrum β-lactamases (ESBLs) and carbapenemases. This review synthesizes current evidence on phenotypic and molecular characterization of MDR Gram-negative bacteria in LRTIs, emphasizing their epidemiology, mechanisms of resistance, diagnostic approaches, and clinical implications.

Keywords: Multidrug resistance; Gram-negative bacteria; Lower respiratory tract infections; phenotypic characterization; Molecular characterization; Antimicrobial resistance genes and ESBL.

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INTRODUCTION

Lower respiratory tract infections (LRTIs) remain a leading cause of morbidity and mortality worldwide, particularly among hospitalized and critically ill patients (1). Gram-negative organisms — notably Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii — are frequent causes of community-acquired and hospital-acquired LRTIs (2). The rising prevalence of multidrug resistance (MDR) among these pathogens has seriously narrowed therapeutic options, increased lengths of stay, raised healthcare costs, and worsened patient outcomes (3). Understanding both the phenotypic behaviour and the underlying molecular mechanisms of resistance is therefore essential for effective treatment, infection control, and surveillance (4).

Phenotypic characterization provides the first, clinically actionable layer of information. Conventional culture and identification followed by antimicrobial susceptibility testing (AST) — using disc diffusion, broth microdilution, or automated systems — determine which agents remain active against an isolate and guide immediate therapy. Additional phenotypic assays (for example, combined disk tests, inhibitor-based methods, Modified Hodge/Carba NP tests, and screening for extended-spectrum β-lactamase [ESBL], AmpC, metallo-β-lactamase [MBL] or carbapenemase activity) help classify resistance phenotypes and detect important enzymatic mechanisms that may not be obvious from routine AST alone (5).

Molecular characterization complements phenotype by identifying the genetic determinants that drive resistance, their genetic context (plasmids, transposons, integrons), and the potential for horizontal transfer. Polymerase chain reaction (PCR) assays and targeted gene sequencing detect key resistance genes such as blaCTX-M, blaSHV, blaTEM (ESBLs), blaAmpC, and carbapenemase genes (blaNDM, blaKPC, blaVIM, blaIMP, blaOXA-48-like). More comprehensive approaches — multilocus sequence typing (MLST), plasmid replicon typing, and whole-genome sequencing (WGS) — enable high-resolution epidemiology, uncover clonal spread, trace transmission pathways, and reveal novel or emerging resistance mechanisms (6).

This review synthesizes current knowledge on the phenotypic and molecular characterization of MDR Gram-negative organisms isolated from LRTIs. We summarize common phenotypic methods and their limitations, outline the principal genetic determinants and mobile elements responsible for resistance, discuss the clinical and epidemiological implications, and highlight gaps in diagnostics and surveillance that should guide future research and policy.

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MATERIAL AND METHOD

This review was conducted using a systematic approach to identify relevant studies on phenotypic and molecular characterization of multidrug-resistant (MDR) Gram-negative organisms isolated from lower respiratory tract infections (LRTIs). A comprehensive search was performed in major electronic databases including PubMed, Scopus, Web of Science, Google Scholar, and Science Direct. The search covered studies published between 2017 to till date.

OBSERVATION AND DISCUSSION

Phenotypic Characterization of MDR Gram-Negative Organisms

Across the reviewed studies, Enterobacterales (particularly Klebsiella pneumoniae and Escherichia coli), along with Pseudomonas aeruginosa and Acinetobacter baumannii, emerged as the predominant Gram-negative pathogens isolated from lower respiratory tract infections (LRTIs). Routine antimicrobial susceptibility testing consistently demonstrated high levels of resistance to β-lactams, fluoroquinolones, and aminoglycosides, while carbapenem resistance was increasingly reported, especially among K. pneumoniae and A. baumannii (7).

Phenotypic assays such as combined disc tests, inhibitor-based methods, Modified Hodge Test, Carba NP test, and EDTA-based assays were frequently employed for the detection of extended-spectrum β-lactamase (ESBLs), AmpC β-lactamases, and carbapenemases. While these methods are relatively inexpensive and widely available, their sensitivity and specificity vary, and in some cases, overlapping resistance mechanisms complicated interpretation. For example, co-production of ESBLs and AmpC enzymes may mask phenotypic detection, leading to underestimation of resistance burden (8).

Molecular Characterization and Resistance Genes

Molecular approaches provided greater precision in identifying the genetic basis of resistance. PCR and sequencing revealed widespread distribution of bla genes, notably blaCTX-M, blaSHV, and blaTEM in Enterobacterales, while blaNDM, blaKPC, blaVIM, blaIMP, and blaOXA-48-like genes were commonly reported in carbapenem-resistant isolates (9).

In P. aeruginosa and A. baumannii, carbapenem resistance was often associated with blaOXA-type enzymes and metallo-β-lactamases, alongside efflux pump overexpression and porin loss, which were not always evident in phenotypic assays. Advanced molecular techniques such as multilocus sequence typing (MLST) and whole-genome sequencing (WGS) revealed the clonal spread of high-risk lineages (e.g., K. pneumoniae ST258, A. baumannii international clones), emphasizing the role of genetic epidemiology in outbreak investigations (10).

Correlation Between Phenotypic and Molecular Methods

A recurring observation was the discordance between phenotypic and molecular findings. Some isolates carried resistance genes without expressing them phenotypically, while others exhibited resistance due to mechanisms not easily detected by standard PCR panels (e.g., efflux pump upregulation). This highlights the need for an integrated approach, where phenotypic susceptibility guides immediate therapy, and molecular diagnostics provide confirmatory evidence and insights into transmission dynamics (11).

Clinical and Epidemiological Implications

The high prevalence of MDR Gram-negative pathogens in LRTIs significantly limits treatment options, often leaving colistin and a few novel β-lactam/β-lactamase inhibitor combinations as last-resort therapies. Molecular evidence of plasmid-mediated gene transfer indicates a high potential for rapid dissemination of resistance determinants across species and healthcare settings. These findings underscore the importance of antimicrobial stewardship, strict infection control measures, and continuous molecular surveillance to prevent outbreaks (12).

CONCLUSION

Multidrug-resistant Gram-negative organisms represent a major challenge in the management of lower respiratory tract infections, contributing to prolonged illness, limited therapeutic options, and increased mortality. An integrated approach that combines phenotypic susceptibility testing with molecular diagnostics offers the most comprehensive understanding of resistance, enabling better clinical decision-making, outbreak detection, and infection control. Strengthening laboratory capacity, promoting antimicrobial stewardship, and implementing molecular surveillance programs are crucial steps toward curbing the spread of multidrug resistance.

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