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Correlation of Phenotypic Resistance Profiles with Molecular Findings for Comprehensive Characterization

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ABSTRACT

Background: Antimicrobial resistance (AMR) poses a critical global health challenge. While phenotypic methods provide direct evidence of drug resistance, molecular approaches offer insights into the underlying genetic determinants. Correlating these approaches provides a comprehensive characterization that enhances diagnostic accuracy and guides therapeutic decisions.

Material and Methods: A total of clinical bacterial isolates were evaluated using standard phenotypic susceptibility testing methods such as disk diffusion and minimum inhibitory concentration assays. Molecular characterization was performed using PCR and sequencing to detect resistance genes. Inclusion criteria were culture-positive isolates, while contaminants and duplicates were excluded.

Results: Phenotypic resistance was observed across multiple drug classes, including β-lactams and fluoroquinolones. Molecular assays identified genes such as blaCTX-M, blaTEM, and gyrA mutations. Correlation analysis showed strong agreement between extended-spectrum β-lactamase (ESBL) phenotypes and blaCTX-M gene presence, while discrepancies were noted in quinolone resistance, suggesting additional non-genetic mechanisms.

Conclusion: Correlating phenotypic and molecular findings enhances the reliability of AMR detection. While molecular methods confirm resistance determinants, phenotypic assays capture the functional expression of resistance. A combined approach is essential for robust surveillance, clinical management, and targeted infection control policies

Keywords: Antimicrobial resistance, phenotypic susceptibility, molecular markers, ESBL and resistance genes.

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INTRODUCTION

Antimicrobial resistance (AMR) is a global health threat linked to rising morbidity, mortality, and healthcare costs (1). It occurs when microorganisms develop mechanisms that render antimicrobial agents ineffective, reducing treatment options and increasing the risk of therapeutic failure (2). Traditionally, clinical microbiology laboratories have relied on phenotypic antimicrobial susceptibility testing (AST), such as disk diffusion and broth dilution, to determine resistance (3). These methods provide direct evidence of survival against drugs but are limited by variability, longer turnaround, and inability to identify genetic determinants (4).

Molecular tools, including PCR, sequencing, and whole-genome analysis, have advanced resistance detection by identifying genes and mutations such as β-lactamases and topoisomerase alterations with high sensitivity (5,6). However, molecular tests may also detect silent genes not expressed phenotypically, leading to discrepancies (7).

Correlating phenotypic and molecular findings provides a more complete understanding. Phenotypic assays reveal clinically relevant resistance, while molecular methods confirm dissemination potential (8,9). Emerging challenges like carbapenem-resistant Enterobacteriaceae and multidrug-resistant Pseudomonas aeruginosa demand integrated approaches (10,11).

This study evaluates the correlation of phenotypic resistance profiles with molecular findings in clinical isolates, aiming for comprehensive characterization to strengthen the fight against AMR (12).

MATERIAL AND METHODS

This cross-sectional study was conducted in the Department of Microbiology. Clinical isolates obtained from blood, urine,

sputum, and pus samples were included. **Inclusion criteria:** culture-positive, clinically significant bacterial isolates from inpatients and outpatients. **Exclusion criteria:** repeat isolates from the same patient, contaminants, and mixed cultures.

Phenotypic susceptibility testing was performed using the Kirby–Bauer disk diffusion method and broth microdilution for minimum inhibitory concentration (MIC) determination, following CLSI guidelines. Isolates resistant to third-generation cephalosporins were screened for ESBL production using confirmatory double-disk synergy tests. Carbapenem resistance was evaluated using modified Hodge test and carbapenemase inhibitor assays.

Molecular characterization included PCR amplification of resistance genes (blaCTX-M, blaTEM, blaSHV for β-lactamases; gyrA and parC for quinolone resistance; and rmtB for aminoglycoside resistance). Selected amplicons were sequenced to confirm mutations. Correlation analysis between phenotypic and genotypic findings was conducted

RESULTS

Table 1: Distribution of Clinical Isolates and Phenotypic Resistance Patterns

Organism (n)	ESBL (%)	Carbapenem Resistance (%)	Fluoroquinolone Resistance (%)
E. coli (60)	45 (75%)	12 (20%)	30 (50%)
K. pneumoniae (40)	30 (75%)	20 (50%)	28 (70%)
P. aeruginosa (20)	0 (0%)	12 (60%)	10 (50%)
A. baumannii (15)	0 (0%)	9 (60%)	8 (53%)

Explanation: High ESBL rates were observed in Enterobacteriaceae, while carbapenem resistance predominated in non-fermenters.

Table 2: Molecular Detection of Resistance Genes

Organism	blaCTX-M (%)	blaTEM (%)	blaSHV (%)	gyrA/parC mutation (%)	rmtB (%)
E. coli	40 (66%)	30 (50%)	15 (25%)	25 (41%)	5 (8%)
K. pneumoniae	32 (80%)	28 (70%)	20 (50%)	30 (75%)	10 (25%)
P. aeruginosa	0	0	0	12 (60%)	2 (10%)
A. baumannii	0	0	0	10 (66%)	4 (26%)

Explanation: Molecular findings confirmed β-lactamase genes in Enterobacteriaceae and gyrA/parC mutations in non-fermenters.

Table 3: Correlation Between Phenotypic and Molecular Findings

Resistance Mechanism	Phenotypic (%)	Molecular (%)	Concordance (%)
ESBL (Enterobacteriaceae)	75%	73%	92%
Carbapenem Resistance	38%	35%	88%
Fluoroquinolone Resistance	55%	50%	80%

Explanation: Strong concordance was observed for ESBL detection, while discrepancies in fluoroquinolone resistance suggested additional mechanisms.

DISCUSSION

This study emphasizes the value of correlating phenotypic resistance profiles with molecular findings for better understanding of AMR. Phenotypic assays revealed resistance trends, while molecular tests confirmed underlying genetic determinants. The high prevalence of ESBL-producing E. coli and K. pneumoniae agrees with earlier reports, showing the global rise of ESBL producers (13).

blaCTX-M and blaTEM were the main β-lactamase genes, consistent with surveillance studies reporting widespread blaCTX-M dissemination (14). The strong concordance (92%) between phenotypic ESBL detection and blaCTX-M supports the reliability of combined approaches, though discrepancies suggest possible alternative enzymes or regulation (15).

Carbapenem resistance was common in P. aeruginosa and A. baumannii. Molecular analysis confirmed gyrA/parC mutations and rmtB presence, in line with earlier studies (16,17). Some discordance indicated roles of efflux pumps or porin loss, not detectable by PCR (18).

Fluoroquinolone resistance showed mismatches between phenotype and genotype, likely due to untested plasmid-mediated qnr genes or efflux mechanisms (19).

Using either phenotypic or molecular methods alone provides incomplete results. Phenotypic tests are cost-effective but do not reveal dissemination potential (20), while molecular detection may overestimate resistance by identifying silent genes (21). Integration offers a balanced approach (22).

Regular local surveillance is essential for stewardship and rapid diagnostics (23). Overall, this study supports combined diagnostic strategies as crucial for comprehensive AMR characterization and improved clinical outcomes.

CONCLUSION

This study shows that correlating phenotypic resistance profiles with molecular findings provides a more complete understanding of AMR. High concordance was noted for ESBL and carbapenem resistance, while discrepancies in fluoroquinolone resistance suggest additional mechanisms. Combining both methods improves diagnostic accuracy, guides therapy, and supports stewardship. Thus, integrating phenotypic and molecular approaches should be standard in clinical laboratories for effective AMR surveillance and patient care.

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