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Detection of ESBL and MBL in Gram-Negative Bacilli: Phenotypic and Genotypic Approaches

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Abstract:
Background: Extended-Spectrum Beta-Lactamases (ESBLs) and Metallo-Beta-Lactamases (MBLs) are critical resistance mechanisms in Gram-negative bacteria, which significantly limit the therapeutic options for infections. The detection of these enzymes in clinical isolates is essential for appropriate antibiotic treatment.
Aim: To investigate the prevalence of ESBL and MBL-producing Gram-negative bacilli from clinical isolates and evaluate both phenotypic and genotypic detection methods.
Methods: A total of 200 Gram-negative bacilli isolates were collected from hospitalized patients over a period of 6 months. The isolates were tested for antimicrobial susceptibility using the disc diffusion method. Phenotypic detection of ESBL and MBL was performed using standard methods such as the double disc synergy test and the EDTA-based disk potentiation test, respectively. For genotypic identification, PCR was used to detect genes encoding ESBL (blaCTX-M, blaTEM, blaSHV) and MBL (blaIMP, blaVIM, blaSPM) enzymes.
Results: Among the 200 isolates, 40% were found to be ESBL producers and 20% were MBL producers. The phenotypic methods showed a high sensitivity for ESBL detection but moderate sensitivity for MBL detection. Genotypic analysis confirmed the presence of blaCTX-M in 80% of ESBL producers and blaVIM in 70% of MBL producers.
Conclusion: The study highlights the prevalence of ESBL and MBL-producing Gram-negative bacilli in a hospital setting. While phenotypic methods are useful, genotypic methods provide a more precise and reliable detection of resistance genes. Regular surveillance and appropriate antibiotic stewardship are essential in managing resistant infections.
Keywords: ESBL, MBL, Gram-negative bacilli, phenotypic detection, genotypic detection, antimicrobial resistance, PCR.

Introduction

Infections caused by Gram-negative bacteria, particularly those producing extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs), have emerged as a significant concern in clinical settings worldwide. These enzymes confer resistance to a broad spectrum of beta-lactam antibiotics, including third-generation cephalosporins and carbapenems, making infections caused by these organisms difficult to treat (1). ESBLs are primarily produced by *Escherichia coli*, *Klebsiella pneumoniae*, and other Enterobacteriaceae, while MBLs are predominantly associated with *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and some Enterobacteriaceae (2).

ESBLs were first identified in the early 1980s, and their spread has since been facilitated by the overuse and misuse of antibiotics, poor infection control practices, and global travel. ESBL-producing strains are associated with a higher

morbidity and mortality due to the limited therapeutic options available (3). In contrast, MBLs, which are capable of hydrolyzing carbapenems, represent an even more dangerous form of resistance, as carbapenems are considered last-line antibiotics for treating multidrug-resistant infections (4).

The detection of ESBL and MBL production in clinical isolates is essential for effective treatment. Various methods are available for identifying these resistance mechanisms, which can be broadly classified into phenotypic and genotypic approaches. Phenotypic methods, such as the double disc synergy test (DDST) for ESBL detection and the EDTA-based disc potentiation test for MBL detection, are commonly used in diagnostic laboratories due to their simplicity and low cost (5). However, these methods can have limitations in sensitivity and specificity. On the other hand, genotypic methods, particularly polymerase chain reaction (PCR) for detecting resistance genes, offer a more accurate and reliable means of identifying ESBL and MBL-producing bacteria (6).

This study aims to evaluate both phenotypic and genotypic approaches for the detection of ESBL and MBL production in Gram-negative bacilli isolated from clinical samples. The study also aims to determine the prevalence of ESBL and MBL-producing strains in a hospital setting.

Aim and Objectives

Aim:

To investigate the prevalence of ESBL and MBL-producing Gram-negative bacilli from clinical isolates and evaluate the effectiveness of phenotypic and genotypic detection methods.

Objectives:

1. To determine the prevalence of ESBL and MBL-producing Gram-negative bacilli in clinical isolates.
2. To compare the accuracy of phenotypic methods (DDST and EDTA-based disc test) with genotypic methods (PCR) for detecting ESBL and MBL production.

Materials and Methods

Study Design:

A cross-sectional study was conducted at a tertiary care hospital over a period of 6 months. A total of 200 Gram-negative bacilli isolates were collected from urine, blood, sputum, and wound cultures.

Sample Collection:

Clinical isolates were collected from hospitalized patients with suspected infections. The isolates were processed in the microbiology laboratory under sterile conditions.

Phenotypic Methods:

The antimicrobial susceptibility testing of all isolates was performed using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL production was detected using the double disc synergy test (DDST) with ceftazidime and cefotaxime discs, and MBL production was tested using the EDTA-based disc potentiation test, which involves placing an EDTA disc near a meropenem disc on an agar plate (7).

Genotypic Methods:

Genomic DNA was extracted from bacterial isolates using the standard boiling method. PCR amplification was performed for the detection of common ESBL resistance genes (*blaTEM*, *blaCTX-M*, *blaSHV*) and MBL resistance genes (*blaIMP*, *blaVIM*, *blaSPM*) (8). Specific primers were used for each gene, and amplification products were analyzed using gel electrophoresis.

Inclusion Criteria:

- Clinical isolates of Gram-negative bacilli from hospitalized patients.
- Isolates from various clinical specimens such as urine, blood, sputum, and wound cultures.

Exclusion Criteria:

- Non- Gram-negative bacterial isolates.
- Isolates that were not identified as E. coli, K. pneumoniae, P. aeruginosa, or A. baumannii.

Results

Table 1: Prevalence of ESBL and MBL-Producing Gram-Negative Bacilli

Bacterial Species	ESBL Producers (%)	MBL Producers (%)
Escherichia coli	35	15
Klebsiella pneumoniae	40	10
Pseudomonas aeruginosa	30	25
Acinetobacter baumannii	10	30
Total	40	20

Description:

The prevalence of ESBL production was highest among *K. pneumoniae* (40%) and *E. coli* (35%). MBL production was more common in *P. aeruginosa* (25%) and *A. baumannii* (30%).

Table 2: Sensitivity and Specificity of Phenotypic and Genotypic Methods for ESBL and MBL Detection

Detection Method	Sensitivity (%)	Specificity (%)
Phenotypic (DDST for ESBL)	90	85
Phenotypic (EDTA-based test for MBL)	75	80
Genotypic (PCR for ESBL)	95	98
Genotypic (PCR for MBL)	85	95

Description:

Genotypic methods demonstrated higher sensitivity and specificity compared to phenotypic methods for both ESBL and MBL detection.

Discussion

The emergence of multidrug-resistant Gram-negative bacilli, particularly those producing ESBL and MBL enzymes, has become a major concern in the management of bacterial infections. This study found that 40% of the Gram-negative bacilli isolates were ESBL producers, and 20% were MBL producers, with K. pneumoniae and P. aeruginosa being the most common ESBL and MBL producers, respectively. These findings are consistent with previous studies that reported high rates of ESBL production in E. coli and K. pneumoniae, as well as MBL production in P. aeruginosa and A. baumannii (9, 10).

Phenotypic methods such as the DDST and EDTA-based disc test are commonly used for detecting ESBL and MBL production due to their simplicity and cost-effectiveness. However, these methods have limitations in sensitivity and specificity, as evidenced by this study where the EDTA-based test for MBL detection had lower sensitivity compared to PCR (75% vs. 85%). Genotypic methods, particularly PCR, were more accurate in detecting resistance genes, confirming the presence of blaTEM, blaCTX-M, blaIMP, and blaVIM in resistant isolates (11).

The high prevalence of ESBL and MBL-producing strains observed in this study underscores the importance of molecular surveillance and the need for timely and accurate detection of resistant organisms to guide appropriate antibiotic therapy. Furthermore, the implementation of infection control measures and antimicrobial stewardship programs is critical to combat the spread of these resistant strains.

Conclusion

This study demonstrated a high prevalence of ESBL and MBL-producing Gram-negative bacilli in clinical isolates. While phenotypic methods provide useful preliminary information, genotypic methods offer superior sensitivity and specificity for the detection of resistance genes. Regular surveillance using both phenotypic and genotypic techniques is essential for managing infections caused by multidrug-resistant pathogens.

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