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Original Research Article

EVALUATION OF PHENOTYPIC AND GENOTYPIC METHODS FOR DETECTION OF EXTENDED-SPECTRUM BETA-LACTAMASE IN ENTEROBACTERIACEAE: A CROSS-SECTIONAL STUDY

Shivam Kumar[1], Divakar Srivastava[2], Samridhi Tewari[3]

[1]PG Resident, Department of Microbiology, Hind Institute of Medical Sciences, Sitapur, India.
[2]Professor, Department of Microbiology, Hind Institute of Medical Sciences, Sitapur, India.
[3]Assistant Professor, Department of Anaesthesia, Hind Institute of Medical Sciences, Sitapur, India.

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Corresponding Author:
Dr. Divakar Srivastava,
Email: drdivakarsrivastava@gmail.com

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ABSTRACT

Background: Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae pose a significant global public health threat. Optimal detection methods are crucial for guiding antimicrobial therapy. This study evaluated phenotypic and genotypic methods for ESBL detection in Enterobacteriaceae clinical isolates. **Materials and Methods:** A cross-sectional observational study was conducted at a tertiary care hospital in North India over 18 months. Seventy ESBL screen-positive Enterobacteriaceae isolates from various clinical specimens were included. Phenotypic detection employed combined disc tests using ceftazidime-clavulanic acid (CAZ-CAC) and cefotaxime-clavulanic acid (CTX-CEC). Genotypic detection utilized PCR targeting blaCTX-M, OXA10/11, and blaSHV genes. Antibiotic susceptibility testing was performed per CLSI guidelines. **Results:** Phenotypic detection rate (92.9%) was significantly higher than genotypic rate (78.6%) (p=0.016). CAZ-CAC showed superior detection (88.6%) compared to CTX-CEC (74.3%) (p=0.030). Among genotypes, CTX-M was most prevalent (75.7%), followed by SHV (40.0%) and OXA10/11 (14.3%). Phenotypic methods demonstrated 100% sensitivity but only 33.3% specificity (κ=0.440). CAZ-CAC showed 96.4% sensitivity and 40.0% specificity; CTX-CEC showed 83.6% sensitivity and 60.0% specificity. ESBL-positive isolates exhibited significantly lower sensitivity to cefepime, imipenem, and tetracycline. **Conclusion:** Phenotypic methods are highly sensitive for ESBL detection and suitable for resource-limited settings. CAZ-CAC combined disc test is recommended as the preferred phenotypic method. Genotypic confirmation remains valuable for epidemiological surveillance.

INTRODUCTION

Enterobacteriaceae constitute a large family of Gram-negative facultative anaerobic bacilli that commonly inhabit the gastrointestinal tract of humans and animals. Clinically important genera include Escherichia, Klebsiella, Enterobacter, Proteus, Citrobacter, and Serratia, which are responsible for a wide range of community-acquired and nosocomial infections including urinary tract infections, bloodstream infections, pneumonia, and intra-abdominal infections.[1,2]

The emergence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae has become a significant global health concern. ESBLs are enzymes that hydrolyzeoxyimino-cephalosporins (ceftriaxone, cefotaxime, ceftazidime) and aztreonam but are usually inhibited by beta-lactamase inhibitors such as clavulanic acid.[3] The global burden of ESBL-producing Enterobacteriaceae is rising, particularly in Asia, Africa, and Latin America, with up to 50% of E. coli and K. pneumoniae isolates in some regions producing ESBLs.[4]

Detection of ESBLs is crucial for appropriate antimicrobial therapy and infection control. Phenotypic methods including combined disc test and double-disk synergy test remain the mainstay in clinical laboratories due to their cost-effectiveness and feasibility.[5] Genotypic methods such as PCR provide definitive identification of ESBL genes but are resource-intensive.[6] The profile and antimicrobial resistance pattern of Enterobacteriaceae members are highly dependent on ESBL status; therefore, identification of ESBL producers is essential for planning empirical therapy in environment-specific settings. This study aimed to

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evaluate different phenotypic and genotypic methods for ESBL detection in Enterobacteriaceae from clinical specimens in a tertiary care hospital.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional observational study was conducted in the Bacteriology & Molecular Laboratory, Department of Microbiology, Hind Institute of Medical Sciences, Sitapur, Uttar Pradesh, India, over 18 months. The study protocol was approved by the Institutional Ethics Committee (IEC). Written informed consent was obtained from all participants. The study followed STROBE guidelines for cross-sectional studies.

Sample Size and Selection: Sample size was calculated using the formula $n = (z)^2 p (1-p)/d^2$, with 95% confidence interval, 8% margin of error, and estimated ESBL proportion of 22.76% from a previous study,[7] yielding a minimum of 68 samples; 70 samples were enrolled. Simple random sampling with consecutive patient enrollment was employed.

Inclusion criteria

Bacterial isolates identified as Enterobacteriaceae from clinical samples; complete data availability; ESBL screening positive by phenotypic methods.

Exclusion criteria

Non-Enterobacteriaceae isolates; ESBL screening negative; incomplete data; contaminated samples.

Specimen Collection: Clinical specimens (urine, pus, blood, sputum, body fluids, stool, throat swabs) were collected from outpatient and inpatient departments. Patient demographics, specimen type, and ward source were recorded.

Phenotypic Methods: Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method per CLSI 2024 guidelines. ESBL screening used cefotaxime, ceftriaxone, ceftazidime, aztreonam, and cefpodoxime. Phenotypic confirmatory disc diffusion test (PCDDT) used ceftazidime-clavulanic acid (CAZ-CAC) and cefotaxime-clavulanic acid (CTX-CEC). An increase in zone of inhibition ≥5 mm around combination disc compared to antibiotic disc alone indicated ESBL production.

Genotypic Methods: DNA templates were prepared from ESBL-positive isolates. PCR amplification was performed using primers specific for blaCTX-M, OXA10/11, and blaSHV genes (Hi-Media) on Bio-Rad CFX96 real-time PCR system.

Quality Control: Quality control strains included Klebsiellapneumoniae ATCC 700603 (positive control) and Escherichia coli ATCC 25922 (negative control).

Statistical Analysis

Data were analyzed using IBM SPSS 25.0. Categorical data were presented as numbers and percentages. Chi-square test compared detection rates. Diagnostic efficacy (sensitivity, specificity, positive predictive value, negative predictive value, accuracy) of phenotypic methods was calculated against genotypic methods as reference. Cohen's kappa coefficient assessed agreement. Fisher's exact test compared antibiotic sensitivity between ESBL-positive and -negative genotypes. P-value <0.05 indicated statistical significance.

RESULTS

Patient and Specimen Characteristics: Seventy patients (34 males, 36 females; mean age 42.21±19.83 years, range 2-78 years) were enrolled. Majority (55.7%) were aged >40 years. Urine (32.9%), sputum (24.3%), and pus (24.3%) were the most common specimen types. General medicine (35.7%) and respiratory medicine (22.9%) were the primary sources. E. coli (70.0%) was the predominant isolate, followed by K. pneumoniae (28.8%) and Klebsiella spp. (1.4%).

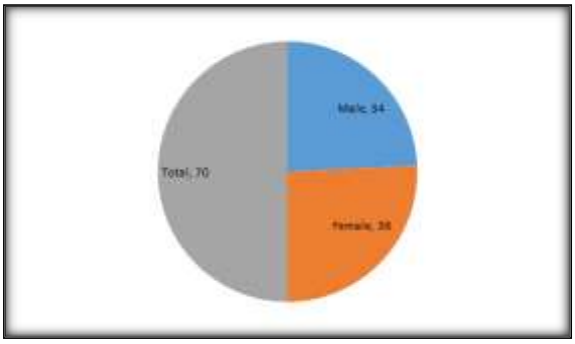

Chart:1 Gender Distribution

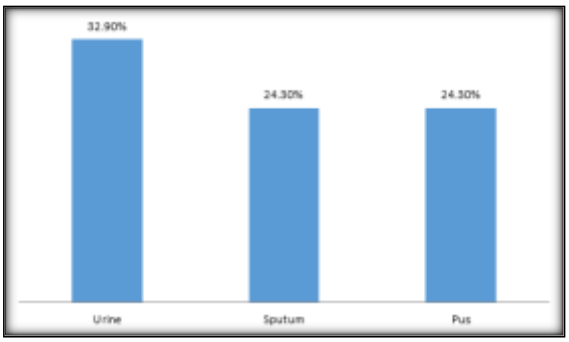

Chart 2: Sample distribution

Detection Rates: Phenotypic detection rate (92.9%, 65/70) was significantly higher than genotypic detection rate (78.6%, 55/70) (p=0.016). Among phenotypes, CAZ-CAC showed significantly higher detection (88.6%, 62/70) compared to CTX-CEC (74.3%, 52/70) (p=0.030). Among genotypes, CTX-M was most prevalent (75.7%, 53/70), followed by SHV (40.0%, 28/70) and OXA10/11 (14.3%, 10/70) (p<0.001 for inter-genotype comparison).

Diagnostic Efficacy: Compared to genotypic methods, overall phenotypic methods demonstrated 100% sensitivity, 33.3% specificity, 84.6% positive predictive value, 100% negative predictive value, and 85.7% accuracy, with moderate agreement (κ=0.440, p<0.05). CAZ-CAC showed 96.4% sensitivity,

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40.0% specificity, 85.5% PPV, 75.0% NPV, and 84.3% accuracy (κ=0.438). CTX-CEC showed 83.6% sensitivity, 60.0% specificity, 88.5% PPV, 50.0% NPV, and 78.6% accuracy (κ=0.407).

Antibiotic Susceptibility: Maximum sensitivity was observed for nitrofurantoin (69.6%), ertapenem (62.9%), and tobramycin (60.0%). Maximum resistance was observed for ampicillin (94.3%) and cefoxitin (94.3%). ESBL-positive genotype isolates showed significantly lower sensitivity to cefepime (29.1% vs 60.0%, p=0.011), imipenem (49.1% vs 80.0%, p=0.042), and tetracycline (45.5% vs 80.0%, p=0.021) compared to ESBL-negative isolates.

Table 1: Detection rates for phenotypic and genotypic methods

Method	No. of ESBL positive cases (N=70)	Percentage	χ^2	p-value
Phenotypic (overall)	65	92.9		
CAZ-CAC	62	88.6	4.723	0.030*
CTX-CEC	52	74.3		
Genotypic (overall)	55	78.6	61.59	<0.001*
CTX-M	53	75.7		
SHV	28	40.0	55.82	<0.001†
OXA10/11	10	14.3		

*Comparison between phenotypic and genotypic overall rates; †Comparison among genotypes.

Table 2: Efficacy of phenotypic methods against genotypic detection

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	κ	p-value
Overall Phenotypic	100	33.3	84.6	100	85.7	0.440	<0.05
CAZ-CAC	96.4	40.0	85.5	75.0	84.3	0.438	<0.05
CTX-CEC	83.6	60.0	88.5	50.0	78.6	0.407	<0.05

Table 3: Comparison of antibiotic sensitivity between ESBL genotype positive and negative isolates

Antibiotic	Total sensitive N (%)	ESBL Genotype Positive (n=55)	ESBL Genotype Negative (n=15)	p-value*
Cefepime	25 (35.7)	16 (29.1)	9 (60.0)	0.011
Imipenem	39 (55.7)	27 (49.1)	12 (80.0)	0.042
Tetracycline	37 (52.9)	25 (45.5)	12 (80.0)	0.021
Ampicillin	4 (5.7)	2 (3.6)	2 (13.3)	0.199
Gentamicin	35 (50.0)	27 (49.1)	8 (53.3)	1.000

***Fisher's exact test**

Table 4: Distribution of isolates by specimen type and organism

Specimen Type	N (%)	E. coli	K. pneumoniae	Klebsiella spp.
Urine	23 (32.9)	18	5	0
Sputum	17 (24.3)	11	5	1
Pus	17 (24.3)	11	6	0
HVS	5 (7.1)	3	2	0
BAL fluid	4 (5.7)	2	2	0
Blood	2 (2.9)	2	0	0
Endotracheal tube	2 (2.9)	2	0	0
Total	70 (100)	49 (70.0)	20 (28.8)	1 (1.4)

DISCUSSION

The present study evaluated phenotypic and genotypic methods for ESBL detection in 70 Enterobacteriaceae isolates from a tertiary care hospital in North India. The high prevalence of ESBL producers (92.9% by phenotypic methods, 78.6% by genotypic methods) reflects the significant burden of antimicrobial resistance in this region, consistent with previous Indian studies reporting ESBL prevalence of 74-88%.[7,8]

The significantly higher phenotypic detection rate compared to genotypic detection (p=0.016) is noteworthy. This discrepancy likely arises because phenotypic methods detect functional ESBL production regardless of specific gene type, whereas our PCR targeted only three gene families (CTX-M, OXA10/11, SHV), potentially missing other ESBL genes such as TEM variants. Muhwezi et al. similarly reported that 9.5% of phenotypically positive isolates could not be genotypically confirmed using limited primer sets.[9] This highlights that genotypic methods, while specific, require comprehensive primer panels to avoid false negatives.

Among phenotypic methods, CAZ-CAC demonstrated significantly higher detection (88.6%) than CTX-CEC (74.3%) (p=0.030). Singh and Basak reported similar findings with CAZ-CAC detection of 94.5% versus CTX-CEC at 87.3%.[10] The superior performance of CAZ-CAC may be attributed to the higher stability of ceftazidime-clavulanate interaction and better detection of certain ESBL variants, particularly CTX-M-15, which is predominant in India.[11]

The genotypic analysis revealed CTX-M as the dominant ESBL genotype (75.7%), consistent with global reports indicating CTX-M enzymes, particularly CTX-M-15, as the most common ESBL type worldwide.[3] SHV prevalence (40.0%) aligns

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with previous Indian studies.[12] Notably, OXA10/11 was detected in 14.3% of isolates, representing emerging OXA-type ESBLs in the region that have been previously underreported.

The diagnostic efficacy analysis showed that phenotypic methods had excellent sensitivity (100% overall, 96.4% for CAZ-CAC) but limited specificity (33.3% overall). This indicates that phenotypic methods are excellent for screening but may overcall ESBL production in isolates with other resistance mechanisms such as AmpC beta-lactamases or porin mutations. The moderate agreement between methods (κ=0.407-0.440) suggests that neither method is perfect alone, and a combined approach is optimal.

The antibiotic susceptibility findings are alarming, particularly the significantly reduced imipenem sensitivity in ESBL-positive isolates (49.1% vs 80.0% in negatives, p=0.042). This suggests emerging carbapenem resistance, possibly due to co-production of carbapenemases like NDM or OXA-48-like enzymes, which has been increasingly reported from India.[13] The high resistance to ampicillin and cefoxitin (>94%) reflects the extensive beta-lactam resistance in these isolates.

Study limitations include single-center design, relatively small sample size, limited primer set (missing TEM and other OXA variants), and lack of sequencing for variant confirmation.

CONCLUSION

Phenotypic methods, particularly the CAZ-CAC combined disc test, demonstrate high sensitivity (96.4%) for ESBL detection and remain valuable for routine laboratory use in resource-limited settings. CTX-M is the predominant ESBL genotype in North India. The significant agreement between CAZ-CAC and genotypic methods (κ=0.438) supports its use as a reliable screening tool. The emergence of OXA-type ESBLs and reduced carbapenem sensitivity among ESBL producers warrants continued surveillance. A pragmatic algorithm combining routine AST screening, CAZ-CAC confirmatory testing, and targeted genotypic methods for epidemiological purposes is recommended.

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