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Detection of antibiogram pattern and prevalence of blaNDM and blaVIM genes among carbapenem resistant *Eschericia coli* isolates in a tertiary care hospital

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ABSTRACT

Background: Clinical significance of *Eschericia coli* is due to its capacity to develop resistance against many antibiotics specially carbapenem by producing carbapenemase which limits treatment options. blaNDM and blaVIM genes are responsible for this multi-drug-resistance. This is an alarming condition for society as well as clinician because they are very difficult to treat due to limited options of antibiotics and its high virulence. **Aims and Objectives:** The study was conducted to detect antibiogram pattern and prevalence of blaNDM and blaVIM genes among carbapenem resistant *E. coli* isolates in a tertiary care hospital. Our study will also help to identify molecular basis of Carbapenem resistance including treatment guideline. **Materials and Methods:** Cross-sectional study was performed during January'2021 to June'2022 time period. Identification of *E. coli* was followed by standard protocol and antibiotic sensitivity was done by Kirby-Bauer disk-diffusion method following CLSI guideline, using VITEK-2D-SYSTEM. Detection of genes was done by polymerase chain reaction (PCR). **Results:** Out of 1867 samples, 161 isolates were identified as *E. coli*, among which 90 were resistant to carbapenem. Among those, 50 (31%) were resistant to all three carbapenems. Among those, 100% were resistant to ampicillin, amoxycillin-clavulanic acid, cefuroxime, cefuroxime-axetil, cefoperazone-sulbactam, ceftriaxone, cefepime, piperacillin-tazobactam, nalidixic acid, ciprofloxacin; 52% were resistant to amikacin; 54% were resistant to gentamicin; 86% were resistant to cotrimoxazole; 22% were resistant to nitrofurantoin and 96% were sensitive to tigecycline; 100% were intermediate sensitive to colistin. blaNDM gene is detected in 17 (34%) isolates and blaVIM gene is detected in 2 (4%) isolates by PCR. **Conclusion:** *E. coli* leads to common clinical presentations such as urinary tract infection, blood stream infection, pneumonia, and wound infection. High burden of carbapenem resistant *E. coli* was associated with urinary indoor samples. Rising incidence of multi-drug-resistance occurs due to lack of infection control measures, irrational use of antibiotics. Continuous surveillance will prevent development of multi-drug-resistance and help to improve treatment guideline.

Key words: *Eschericia coli*; blaNDM, blaVIM; Carbapenem resistant; Multi-drug resistant

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INTRODUCTION

Eschericia coli is a Gram-negative facultative anaerobic rod-shaped coliform bacteria commonly found in Intestines[1] as commensal. Although they are commonly harmless bacteria, they may cause uncomplicated lower urinary tract infection, rarely upper urinary tract infections by some strain.

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It may cause opportunistic infections like appendicular abscess along with anaerobic bacteria.

It is very much associated with acute gastroenteritis, traveler's diarrhea in all age groups, cholecystitis, peritonitis especially in abdominal intestinal operations, blood stream infection in immunocompromised critically ill patients and meningitis in neonates and occasionally pneumonia. They are often present in lower respiratory tract sputum in debilitated patient.[2]

Carbapenems are antibiotics which are used for the treatment of infection in multidrug resistant (MDR) bacteria. The carbapenems are structurally related to beta lactam antibiotics. Examples are meropenem, imipenem, ertapenem, and doripenem. They have a wide spectrum with good activities against many Gram-negative rods including *E. coli*. They are used frequently in hospitalized patients.

Along with other bacteria, *E. coli* also produce carbapenem resistance either by formation of Carbapenemase which is class B beta lactamase that is metallo beta lactamase or by non-carbapenemase mediated that is up regulated efflux pump and/or loss of outer membrane protein.

According to Ambler classification beta lactamase are of four types[3]: Class A (KPC), C (AmpC), D (OXA) are active site serine beta lactamase. Their mechanism of action is totally different from class B which is metallo beta lactamase which hydrolyse beta lactamase by enzymatic process with the help of metal ion usually zinc. Various genes like Imipenemase (IMP), Verona integrin-encoded metallo beta-lactamase (VIM), Seoul Imipenemase (SIM), Sao Paulo metallo beta-lactamase, German Imipenemase (GIM), New Delhi metallo beta lactamase (NDM) etc. encodes metallo beta lactamase in bacteria. Among those gene, NDM and VIM gene are most prevalent in our setup.

The spread of carbapenemase resistant *E. coli* has been strikingly rapid worldwide, indicating that continuous monitoring systems are absolutely required. Therapeutic options for infections due to carbapenemase producing *E. coli* have also become limited. Antimicrobial susceptibility patterns detected in this study would help in the implementation of appropriate antibiotic usage and infection control measures.

This study will give a clear idea about the detection of carbapenemase producing *E. coli* by standard phenotypic methods, confirmation by VITEK and VIM and NDM gene detection by polymerase chain reaction (PCR).

Aims and objectives

- To detect carbapenem resistant Eschericia coli isolates
- To detect Anti-microbial susceptibility pattern of such isolates.
- To detect presence of blaNDM and blaVIM gene in relevant isolates

MATERIALS AND METHODS

Study design

An observational cross-sectional study is conducted on a total of 50 isolates of carbapenemase producing *E. coli* (resistant to all three carbapenem) collected from cultures of clinical specimens (urine and blood mainly) and processed by standard laboratory methods.

Isolation of *E. coli* by biochemical tests and special tests
↓
Kirby–Bauer method for sensitivity test (Screening for carbapenemase detection)
↓
VITEK 2D as confirmatory test (for minimum inhibitory concentration [MIC] detection)
↓
Conventional PCR for VIM and NDM gene detection

Place of study

Unit of Bacteriology, Department of Microbiology, CSTM Department of Biochemistry and Medical Biotechnology, CSTM.

Period of study

The study period will be for one and half year (from January 2021 to June 2022).

Study population

Patients admitted in hospital (indoor/wards and Coronary Care Unit) of the Calcutta School of Tropical Medicine.

Sample size

Fifty confirmed cases of carbapenemase resistant *E. coli*. (Justification of sample size-calculated by sample size calculation formula and also based on our record of previous 12 months; during that period of time, total of 39 carbapenemase resistant *E. coli* were isolated).

Inclusion criteria

The following criteria were included in the study:

Carbapenem resistant *E. coli* isolates (resistant to all three carbapenem) and patients willing to participate in the study with informed consent.

Exclusion criteria

The following criteria were excluded from the study:

Patients whose relevant samples

- Patient/patient's relatives not willing to take part in the study

- Carbapenem sensitive *E. coli* isolates.

Quality control at all steps will be done by *E. coli* ATCC 25922.

Work plan

- Detection of carbapenemase producing *E. coli* isolates in bacteriology laboratory from clinical materials collected as part of routine care
- Written consent from patient/patient's party will be taken and relevant clinical history was be taken (as purpose of data analysis)
- Screening of carbapenemase production by imepenem and imepenem EDTA and confirmed by VITEK 2D
- Detection of VIM and NDM gene by conventional PCR with the help of Department of Biochemistry and Medical Biotechnology
- All data were compiled for interpretation and analysis.
- Activity schedule of research work.

Activities 3 months, 6 months, 9 months, 12 months, 15 months, 18 months

- Sample collection (1st 6 months)
- Phenotyping and Genotyping (2nd 6 months)
- Data analysis and thesis writing (3rd 6 months).

RESULTS

This study was conducted in the Department of Microbiology and Department of Biotechnology of School of Tropical Medicine where *E. coli* were isolated from different specimens of patients attended the hospital.

This study was conducted on 161 isolates of *E. coli* collected from different clinical specimens. The isolates were identified by gram's stain, series of biochemical tests and VITEK 2 Compact System. Antibiotic sensitivity was performed by Kirby–Bauer disk diffusion method and VITEK 2D Compact System.

Among those, 90 isolates were carbapenem resistant. Carbapenem resistant *E. coli* was main subject of interest of this study which was identified by MIC value of different antibiotics (meropenem, imepenem, and ertapenem), as shown in Table 1 below.

Among those, 50 isolates were resistant to all three Carbapenem. PCR was done to detect blaNDM and blaVIM genes among them.

As per Tables 2 and 3, study shows incidence of blaNDM and blaVIM gene are more among carbapenem resistant isolates with high MIC value.

DISCUSSION

This study conducted in the Department of Microbiology and Department of Biotechnology of School of Tropical Medicine.

This study was conducted on 161 isolates of *E. coli* collected in this study period. Among those, 90 isolates were carbapenem resistant and among those 90 isolates, 50 isolates were resistant to all three carbapenem (meropenem, imepenem and ertapenem) and prevalance of blaNDM and blaVIM gene was detected among those 50 isolates.

Reports before 2006 indicated that most *E. coli* isolates were sensitive to Carbapenems. Studies carried out by Akram et al.,[4] (2002–2006) and Babypadmini and Appalaraju[5] (2002–2003) in Northern India reported 100% susceptibility to Imipenem for urinary isolates of *E. coli*.

Menon et al.,[6] in their study from Southern India in 2003 reported similar pattern of susceptibility for Imipenem. However, subsequent reports in a study by Nagaraj et al., indicated emergence of Carbapenem resistance among *E. coli*.[7]

In a study by Sarma et al., three of 30 (10%) *E. coli* isolates were resistant to both meropenem and imipenem. This was in accordance to studies from elsewhere in India, namely, Delhi, Guwahati, and Mumbai reporting resistance from 5.1% to 14% for both these antimicrobials.[8-10]

In another study from Southern India by Shenoy et al., of the 4976 samples tested, 74 (1.48%) yielded MDR isolates that included 10 *E. coli* isolates resistant to both Meropenem and Imipenem.[11]

In a study from Kashmir by Fomda et al., of the 1625 Gram-negative isolates, 6.0% were resistant to both Meropenem and Imipenem.[12]

In a study by Guh et al., 95% of carbapenem resistant *E. coli*, were susceptible to tigecycline with variation of MIC value and mostly resistant to other group of antibiotics like β-lactam/β-lactamase inhibitor (BL-BLI), fluroquinolone, cephalosporin aminoglycoside. Which concordant with this study showed 100% isolates were resistant to BL-BLI, cephalosporin, fluroquinolone, 48% were resistant to amikacin; 44% were resistant to gentamicin; 96.66% were sensitive to tigecycline with variation of MIC value.[13]

In our study, 34% isolates were found to be blaNDM positive and 4% isolates were found to be blaVIM positive. 2% isolates were found to be both blaNDM and blaVIM positive. Deshpande et al.,[14] reported blaNDM-1 in nine *E. coli* isolates among 24 carbapenem resistant Enterobacteriaceae in a tertiary care set up.[15]

Table 1: Antibiogram pattern of carbapenem resistant *Eschericia coli*

Drug tested with MIC value			Number of carbapenem resistant *Eschericia coli* (n=50)	Percentage
β Lactum-β Lactum inhibitor	R	Amoxicillin/clavulanic acid (MIC≥32)	50	100
		Ampicillin (MIC≥32)	50	100
		Piperacillin/tazobactam (MIC≥128)	50	100
Cephalosporin	R	Cefepime (MIC≥64)	50	100
		Ceftriaxone (MIC≥64)	50	100
		Cefuroxime (MIC≥64)	50	100
		Cefuroxime axetil (MIC≥64)	50	100
		Cefoperazone/Sulbactam (MIC≥64)	50	100
Ciprofloxacin	R	Ciprofloxacin (MIC≥4)	50	100
Trimethoprim/ sulfamethoxazole	S	Trimethoprim/sulfamethoxazole (MIC≤20)	7	14
	R	Trimethoprim/sulfamethoxazole (MIC≥320)	43	86
Amikacin	S	Amikacin (MIC≤2)	21	42
		Amikacin (MIC=4)	1	2
		Amikacin (MIC=8)	2	4
	R	Amikacin (MIC≥64)	26	52
Gentamicin	S	Gentamicin (MIC≤1)	21	42
		Gentamicin (MIC=2)	1	2
	I	Gentamicin (MIC=8)	1	2
	R	Gentamicin (MIC≥16)	27	54
Nalidixic acid	R	Nalidixic acid (MIC≥32)	50	100
Nitrofurantoin	S	Nitrofurantoin (MIC≤16)	10	20
		Nitrofurantoin (MIC=32)	8	16
	I	Nitrofurantoin (MIC=64)	21	42
	R	Nitrofurantoin (MIC=128)	6	12
		Nitrofurantoin (MIC=256)	4	8
		Nitrofurantoin (MIC>512)	1	2
Tigecycline	S	Tigecycline (MIC≤0.5)	41	82
		Tigecycline (MIC=1)	7	14
	R	Tigecycline (MIC=2)	1	2
		Tigecycline (MIC≥8)	1	2
Ertapenem	R	Ertapenem (≥8)	50	100
Imipenem	R	Imipenem (8)	3	6
		Imipenem (≥16)	47	94
Meropenem	R	Meropenem (4)	1	2
		Meropenem (8)	2	4
		Meropenem (≥16)	47	94

MIC: Minimum inhibitory concentration

Table 2: Association between MIC value of carbapenem and presence of blaNDM gene

Drug tested with MIC value			Number of blaNDM positive carbapenem resistant *Eschericia coli* (n=17)	Percentage
Imipenem				
R		Imipenem (8)	2	12
		Imipenem (≥16)	15	88
Meropenem				
R		Meropenem (4)	0	0
		Meropenem (8)	1	6
		Meropenem (≥16)	16	94

MIC: Minimum inhibitory concentration

Table 3: Association between MIC value of carbapenem and presence of blaVIM gene

Drug tested with MIC value			Number of blaVIM positive carbapenem resistant *Eschericia coli* (n=2)	Percentage
Imipenem				
R		Imipenem (8)	0	0
		Imipenem (≥16)	2	100
Meropenem				
R		Meropenem (4)	0	0
		Meropenem (8)	0	0
		Meropenem (≥16)	2	100

A study by Shenoy et al., of the 74 *E. coli* isolates showing resistance to carbapenems, 34 were positive for blaNDM gene by PCR.[11]

Study by Albiger et al., showed blaVIM-producing carbapenem resistant *E. coli* are even less common; per the CDC, only 17 (4%) isolates from seven states; as of April 2016.[16]

As per CDC, in Spain, Italy, and Hungary, blaVIM is the predominant MBL; in these countries, "inter- regional

spread" (epidemiological stage 4) of blaVIM producers has been documented.

Limitations of the study

- Due to financial constraints of PCR to detect other genes encoding multi-drug resistance, could not be done.
- Genetic sequencing to determine blaNDM AND blaVIM could not be done.
- Amplified rRNA gene restriction analysis to understand the predictability and accuracy of blaNDM AND blaVIM detection.

CONCLUSION

The present study highlighted the continued threat by carbapenem resistant *E. coli* in the hospital setting. The detection of prevalence of blaNDM and blaVIM gene was producing strains in the clinical setting and to review future treatment strategies for hospitalized patients. The present study also indicates the importance of regular surveillance of drug resistance in the hospital for an urgent action to be taken for antibiotics stewardship in the country.

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I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or processional qualification except as specified.

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SK- Definition of intellectual content, literature survey, prepared first draft of manuscript, implementation of study protocol, data collection, data analysis, manuscript preparation, and submission of article; **BC**- Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **AT**- Design of study, statistical analysis, and interpretation; **SM**- Literature survey and preparation of figures.

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