Prevalence of BLA CTX-M, Gene in Escherichia Coli Isolated From Urinary Tract Infection at a Tertiary Care Centre in Kanpur

R. Sujatha[1], Deepak Sameer[2], Suneet Kr Yadav[3], Nashra A[4], SarahK5

Abstract:

Back ground: Escherichia coli is the most common organism that causes UTI. However, the incidence of community acquired UTI caused by Extended spectrum beta-lactamase (ESBL)-producing strains of E. coli, in particular CTX-M genes, is on the rise worldwide.

Aim: To determine the prevalence of ESBLs (bla CTX M) produced by E. coli from urinary isolates.

Material & Methods: The present study was carried out in the Department of Microbiology, Rama Medical College, and Kanpur over a period from Oct 2021-Dec 2021. It was approved by the Institutional Ethical Committee. Escherichia coli isolated from urinary isolates from various clinical departments including OPD and IPD of all age groups and both genders were included. Identification of isolates was carried out using conventional biochemical Methods and All the E. coli isolates was phenotypic ally tested for ESBL production by double disk diffusion test, According to CLSI guidelines, 2021. After screening for ESBL, the CTX-M, genes were detected among ESBL- producing isolates using PCR.

Results: Out of 110 urine samples , females were (50.90%) and males were 49.09%, growth was seen in 41/110(37.27%) and E.coli was seen in 20/41(48.78%), ESBL was detected in 13/20(65%) of E. coli that carried BLA (CTX-M) genes in 46.15%(6/13).

Conclusion: Due to the high resistance of E. coli to beta-lactam drugs in this region, these drugs have limited effects for treatment of UTI in outpatient. The frequency of CTX beta-lactamases is high which indicates the spread of drug resistance. Proper infection control policy and antibiotic stewardship should be implicated to combat this resistance

Keywords: Extended spectrum beta lactamase (ESBL), BLA (CTX-M)

Introduction

E. coli is the main and prevalent cause of UTIs in all ages, it is important to recognize its regional susceptibility pattern to antibiotics [1]. The sensitivity of bacteria to diverse antibiotics varies in different regions, which can be the consequence for the usage of various types and quantity of antibiotics in each region [2] The main resistance mechanism used in gram-negative bacteria against beta-lactam antibiotics is the production of beta-lactamase enzymes to hydrolyze the beta-lactam ring of antibiotics [3]. .Beta –lactamases are enzymes that are major cause of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins, Cephamycins and Carbapenems. [4, 5, 6]. The ESBL genes are mostly plasmid encoded. Most ESBL genotypes are TEM, SHV, CTX-M.On the basis of primary structure, beta –lactamase are grouped into four classes A, B, C and D enzymes. Enzymes of classes A, C and D have serine at the active site whereas the class B enzymes are Zine- metal enzyme. Classical ESBLs have been evolved from the wide spread plasmid encoded enzyme families Temoniera (TEM), Sulfhydryl variable (SHV) and Oxacillin (OXA), have an extended substrate profile which allows hydrolysis of all cephalosporin's, penicillins, and aztreonam[7]. The ESBL enzyme is mostly produced by Escherichia coli and Klebsiella. During the last three decades, ESBLs among urinary E. coli have been reported worldwide, and their occurrence has increased in both outpatients and inpatients diagnosed with UTIs. The present study aims to determine the prevalence of ESBL-producing E. coli of both community and noso comial origin isolated from urine samples taken from patients diagnosed with UTIs, to detect their drug resistance pattern to commonly used antibiotics in medical practice and to detect bla CTX-M genes in these multi-drug resistant isolates.

Material and Methods

The present study was carried out in the Department of Microbiology, Rama Medical College, and Kanpur over a period from Oct 2021-Dec 2021. It was approved by the Institutional Ethical Committee. Escherichia coli isolated from urinary isolates from various clinical departments including OPD and IPD of all age groups and both genders were included. Identification of isolates were carried out using conventional biochemical methods and All the E. coli isolates were

[1]*Prof. & Head, Dept of Microbiology Rama Medical College Hospital and Research Centre, Mandhana Kanpur*

[2]*Tutor, Dept of Microbiology Rama Medical College Hospital and Research Centre, Mandhana Kanpur.*

[3,5] *Assistant Professor, Dept of Microbiology Rama Medical College Hospital and Research Centre, Mandhana Kanpur*

[4] *Research Assistant, Central Laboratory Rama Medical College Hospital and Research Centre, Mandhana Kanpur*

phenotypic ally tested for ESBL production by double disk diffusion test, According to CLSI guidelines, 2021[8]. After screening for ESBL,the CTX-M, genes were detected among ESBL- producing isolates using PCR Ceftazidime plus clavulanic acid(30/10 mcg) and cefotaxime plus clavulanic acid(30/10 mcg) discs were also included along with ceftazidime (30 mcg) and cefotaxime (30 mcg)discs on Muller Hinton agar. An organism was considered as ESBL producer if there was a ≥5 mm increase in the zone diameter of ceftazidime/clavulanic acid disc and that of ceftazidime disc alone and/or ≥5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc and that of cefotaxime disc alone. E. coli ATCC25922 (Hi-Media) were used as negative and K. pneumonia ATCC 700603(Hi-Media) used as positive control. Genotypic characterization of ESBL genes by PCR. The Deoxyribonucleic acid (DNA) was extracted from all phenotypic ESBL confirmatory test positive Escherichia coli isolates. Polymerase chain reaction (PCR) amplification was done with specific gene primers for CTX-M types. The genomic DNA from E.coli strains was extracted by using bacterial DNA isolation kit (CHROMOUS BIOTECH).PCR cycling temperature for CTX-M.5 min at 94°C and 32 cycles of amplification consisting of 30 s at 95°C, 1 min at54°C, and 2 min 72°C, with 5 min at 72°C for the final extension. Analysis of PCR products (amp icons) after amplification, the amp icons were visualized on 1.5% agar's gels for the presence of band. The agarose gel were scanned under UV illumination, visualized and digitized with the gel documentation system.

Table 1: Primers used in a master cycler

Primers	Primer sequence (5'- 3')	Product size (bp)
CTX M	CTX-M. - F 5' ACCGCCGATAATTCGCAGAT	588
CTX-M.	R- 5' GATATCGTTGGTGGTGCCATAA	

Results

Out of 110 urine samples , females were (50.90%) and males were 49.09%, growth was seen in 41/110 (37.27%) and E. coli was seen in 20/41(48.78%), ESBL was detected in 13/20 (65%) of E. coli that carried bla (CTX-M) genes in 46.15%(6/13).

Discussion

In our results CTX-M(46.15%) is more than a study by El Filewy et al [9] 2007, in which CTX M (28.8%) exceeded SHV(13.7%),[6] and less than in a study by Ahmed et al.,[10] 2013, in which CTX M (71.4%in E. coli and 68.4%in Klebsiella). Several other studies performed throughout the world showed variable results. In a Chinese study, the TEM gene predominated followed by SHV. A report from Canada showed SHV as the main group of ESBLs. However, reports from South America, Israel, Spain, New York, the United Kingdom, and several parts of Indian subcontinent revealed CTX M as the predominant gene. Until the year 2000, TEM was the most prevalent ESBL gene in the Indian bacterial population but was replaced by CTX M in the following decade. In urine isolates in our setting, CTX-M is again predominant. The differences between our study results and those of other authors indicated that the prevalence and type of ESBL genes may vary from one geographical region to another. The present study clearly demonstrates the dramatic change in the gene pool in Indian Enterobacteriaceae.

Conclusion

Due to the high resistance of E. coli to beta-lactam drugs in this region, these drugs have limited effects for treatment of UTI in outpatient. The frequency of CTX beta-lactamases is high which indicates the spread of drug resistance. Proper infection control policy and antibiotic stewardship should be implicated to combat this resistance Inappropriate identification of antibiotic resistance may lead to wrong antibiotic prescription, which may in turn choose for new resistance genes. Phenotypic tests for ESBL detection only confirm whether an ESBL is produced but cannot detect the ESBL subtype and cannot detect those genes whose expression is hidden or masked. Therefore, the genotypic method is suggested as the method of choice for detection of ESBL producing strains of Enterobacteriaceae. Molecular methods are sensitive, but they are expensive and require specialized equipment and expertise. Furthermore, genotypic methods can only detect those genes with known sequences. Phenotypic tests need to be evaluated periodically: Their performance may change with the introduction of a new enzyme, and they may detect new enzymes not included within the laboratory's test algorithm. For best results, phenotypic methods of ESBL detection should be improved.

References

1. Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-Acquired urinary tract infections. Ann Intern Med. 2001; 135: 41-50.
2. Akya A, Gheisari H, Mohammadi G, Khodadoost M. Study of the pattern of plasmid and antibiotic resistance of E. coli isolated from outpatients with urinary tract infection. Sci J Kurdistan Uni Med Science. 2015; 20: 89-96.
3. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control. 2006; 34:S20-28; discussion S64-73
4. Naseer U, Sundsfjord A., 2011."The CTX M conundrum: Dissemination of plasmids and Escherichia coli clones". Microb DrugResist 17:83 97.
5. Shahid M, Singh A, Sobia F, Rashid M, Malik A,Shukla I, et al.2011."Bla (CTX M), bla (TEM), and bla (SHV) in Enterobacteriaceae from North Indian tertiary hospital".High occurrence of combination genes. Asian Pac J Trop Med 4:101 5
6. Lal, P., Kapil, A., Das, B.K., Sood, S., 2010. "Occurrence of TEM and SHV gene in extended

spectrum beta-lactamases (ESBLs) producing Klebsiellasp. Isolated from a tertiary care hospital."Indian J. Med. Res., 125:173-8

7. El-Fiky, S., Elgandour, A., Harfoush, R., 2007."Phenotypic and Molecular Detection of TEM and SHV beta-lactamases producedby organisms of the family Enterobactericeae; Study on blood cultureclinical isolates from intensive care unit".Egyptian J. Med. Microbial., 16:2; 211-
8. Clinical Laboratory Standards Institute. Performance Standard for Antimicrobial Susceptibility Testing. 24thInformation Supplement. NCCLS Document M100 S23 24. Wayne, Pennsylvania, USA: Clinical Laboratory Standards Institute.
9. Eftekhar F, Rastegar M, Golalipoor M, Mansour Samaei N. ,2012."Detection of extended spectrum beta lactamases in urinary isolates of Klebsiella pneumonia inrelation to BlaSHV, BlaTEM, BlaCTX M gene carriage". Iran J Public Health41:127 32.
10. Ahmed AB, Omar AO, Asghar AH, ElhassanMM. 2013."Prevalence of TEM, SHV andCTX M genes in Escherichia coli and Klebsiella spp. urinary isolates from Sudan with confirmed ESBL phenotype". Life Sci J 10:191 5.