

# *Genotyping of Multidrug- Resistant Escherichia Coli*

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Clinical and Chemical pathology*

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## **Abstract**

Extended-spectrum B-Lactamase (ESBL) producing organisms are among the fastest growing problems in our hospitals. The aim of this work was to study the genetic characterization of extended spectrum b-lactamase producing *E.coli* by detection of *TEM-1,SHV-1,INT-1,DELTA ,CTXM-1* genes then to analyze the relationship between these isolates using Pulsed Field Gel Electrophoresis (PFGE) followed by bionumerical analysis to reveal if there is an outbreak of a specific strain.

All strains showed high resistance pattern to ampicillin, gentamicin, tetracycline, trimethoprim, sulfonamides, quinolones, and third generation cephalosporins. All samples were positive for TEM-1 gene , SHV-1 gene ,80% of samples were positive for DELTA gene 60% of samples were positive for INT-1 gene ,All samples were negative for CTXM-1 gene by PCR .The selected isolates were subtyped by pulsed-field gel electrophoresis (PFGE) in accordance with the standardized *E. coli* protocol using (*Xba I*) restriction enzyme. No clonal relationship was found among the ESBL producing *E.coli* for most of the samples .In conclusion, ESBL producing *E.coli* isolates were found to be endemic in our hospitals with the threat of intra-hospital dissemination , control measures are Highly indicated.

### **Key Words:**

ESBLs: Extended-spectrum beta-lactamases.

PCR: Polymerase Chain Reaction.

PFGE: Pulsed-Field Gel Electrophoresis.

## **Contents**

<b>-Introduction-----</b>	<b>1</b>
<b>-Aim of work-----</b>	<b>3</b>
<b>-Review of literature-----</b>	<b>4</b>
<b>-Pathogenic <i>Escherichia coli</i></b>	
• Introduction -----	4
• Pathogenesis of <i>E. coli</i> -----	4
• Urinary Tract Infections -----	6
• Neonatal Meningitis -----	8
• Intestinal Diseases Caused by <i>E. coli</i> -----	8
• Enterotoxigenic <i>E. coli</i> (ETEC) -----	9
• Enteroinvasive <i>E. coli</i> (EIEC) -----	10
• Enteropathogenic <i>E. coli</i> (EPEC) -----	10
• Enteroaggregative <i>E. coli</i> (EAEC) -----	11
• Enterohemorrhagic <i>E. coli</i> (EHEC) -----	12
<b>- Antimicrobial resistance in bacteria</b>	
• Mode of action of antimicrobial agents -----	14
• Resistance as a medical health problem -----	14
• Mechanisms of resistance to antimicrobial agents -----	16
• Prevention of resistance -----	18
• Multidrug resistant organisms (MDROs) -----	18

• Clinical importance of MDRO -----	19
• MDRO Prevention and Control -----	19
• Extended-spectrum B-lactamases -----	21
• Diversity of ESBL types -----	26
• Integrons & ESBLs -----	32
• Epidemiology of ESBL producing organisms -----	35
• Molecular epidemiology of ESBLs -----	38
• Risk factors of ESBLs colonization and infection -----	39
• Community acquired ESBLs infections -----	40
 <b><i>-Laboratory diagnosis of ESBLs</i></b>	
• CLSI Recommended Methods for ESBL Detection -----	42
• Phenotypic Confirmatory Tests for ESBL Production -----	44
• Commercially Available Methods for ESBL Detection -----	45
• Other Methods for ESBL Detection -----	48
• Use of typing methods -----	49
• Phenotypic methods -----	50
• Genotyping of ESBL producing gram negative organisms	51
• Pulse Field Gel Electrophoresis (PFGE)-----	55
• Polymerase chain reaction (PCR)-----	56
• Multiplex PCR -----	56
• Nested PCR -----	57
• Arbitrarily primed PCR (AP-PCR) -----	57

• Amplified fragment length polymorphism (AFLP) -----	59
• Other PCR-associated typing methods -----	60
• Comparison and selection of techniques -----	60
• Safety -----	62
• Treatment and outcome of infections with ESBL-producing organisms -----	65
• Antibiotic Choice for Serious Infections -----	66
• Outcome of Serious ESBL- and Non-ESBL-Producing Infections -----	70
- <i>Materials and Methods</i> -----	72
- <i>Results</i> -----	87
- <i>Discussion</i> -----	104
- <i>Summary</i> -----	110
- <i>References</i> -----	112
- <i>Arabic Summary</i>	-

### **List of Tables**

Table 1. Summary of the Virulence Determinants of Pathogenic <i>E. coli</i> .	5
Table 2. Summary of diarrheagenic <i>E. coli</i> (virulence determinants and characteristics of disease)	13
Table 3. Type of Plasmid-Mediated Extended-Spectrum $\beta$ -Lactamase Enzymes	25
Table 4. Criteria for interpreting PFGE patterns	55
Table 5. Recommended treatment for infections with ESBL-producing organisms	69
Table 6. Zone diameters Interpretive of antimicrobial agents for Enterobacteriaceae according to CLSI guidelines, 2005	75
Table 7. Primers used in PCR reaction	78
Table 8. Master mix reagents and amount for SHV-1 PCR reaction	80
Table 9. Master mix reagents and amount for TEM-1, INT-1, DELTA-1, CTXM-1 PCR reaction	80
Table 10. Amplicon Expected molecular weight for PCR reaction for each gene	82
Table 11. Types and percentage of isolates	87
Table 12. Results for API 20E system reactions for <i>E. coli</i> representative isolates and biotype of each isolate	88
Table 13. The percentages of positive reactions of the API 20E system for <i>E. coli</i> isolates	89
Table 14. Results of antimicrobial susceptibility for the isolates	90
Table 15. Results of antimicrobial resistant ESBL positive isolates	91
Table 16. Ten isolates chosen for genotyping using PCR	92
Table 17. Phenotypic & Genotypic analysis for the ten samples	101
Table 18. Master table for isolates	102

## **List of Figures**

<u>Figure 1:</u> Amino acid substitutions in TEM ESBL derivatives	28
<u>Figure 2:</u> Amino acid substitutions in SHV ESBL derivatives	29
<u>Figure 3:</u> Schematic Diagramme of Genetic units Encoding Various B-lactamses	34
<u>Figure 4:</u> Double-disk diffusion(A) and Etest (B), (C) ESBL detection tests	44
<u>Figure 5:</u> PFGE patterns of chromosomal DNA Restriction fragments of E.coli MG1655 Digested by <i>XbaI</i>	55
<u>Figure 6:</u> PCR test for TEM-1 gene	93
<u>Figure 7:</u> PCR test for SHV-1 gene	94
<u>Figure 8:</u> PCR test for Delta -1 gene	95
<u>Figure 9:</u> PCR test for INT -1 gene	96
<u>Figure 10:</u> PCR test for CTXM -1 gene	97
<u>Figure 11:</u> PFGE analysis using <i>XbaI</i> restriction enzyme	98
<u>Figure 12:</u> Dendrogram for samples showing percentage relation in between	99
<u>Figure 13:</u> DICE coefficient correlation analysis for the selected samples	100



## **LIST OF ABBREVIATIONS**

AK	: Amikacin.
AFLP	: Amplified fragment length polymorphism.
AP-PCR	: Arbitrarily primed PCR.
AMP	: Ampicillin.
ARI	: Antibiotic Resistance Integrans.
ATCC	: American Type Culture Collection.
ATM	: Aztreonam.
BL	: Blood.
bp	: Base pair.
C	: Chloramphenicol.
cAMP	: Cyclic adenosine mono- phosphate.
CAZ	: Ceftazidime.
CDC	: Centers for disease control and prevention.
CF	: Cephalothine.
CFA I	: Colonizing Fimbrial Adhesin one.
CFA II	: Colonizing Fimbrial Adhesin two.
cGMP	: Cyclic guanosine mono- phosphate.
CHEF	: Contour-clamped homogenous electric field .
CIP	: Ciprofloxacin.
CLSI	:Clinical Standard laboratory Institute.
CN	: Gentamycin.
CRO	: Ceftriaxone.
CSB	: Cell suspension buffer.
Ctx	: Cholera toxin.
CTX	: Cefotaxime.

CVL-BSIs	: Central-venous-line associated bloodstream infections.
DAEC	: Diffusely adherent E.coli.
DNA	: Deoxyribonucleic acid.
E. coli	: Escherichia coli.
EAEC	: Enteraggregative Escherichia coli .
EAST	: Enteraggregative heat stable toxin.
EHEC	: Enterohemorrhagic Escherichia coli.
EIEC	: Enteroinvasive Escherichia coli.
EPAF	: Enteropathogenic adherence factor.
EPEC	: Enteropathogenic Escherichia coli.
ESBLs	: Extended spectrum beta lactamases.
ETEC	: Enterotoxigenic Escherichia coli.
FEP	: Cefepime.
GI	: Gastrointestinal.
GNB	: Gram negative bacilli.
HC	: Hemorrhagic colitis.
HUS	: Hemolytic uremic syndrome.
ICU	: Intensive care units .
IPM	: Imipenem.
K- ag	: Capsular antigen.
KIA	: Kligler's iron agar.
LIA	: Lysine iron agar.
LPS	: Lipopolysaccharide endotoxin .
LT	: Heat-labile enterotoxin.
LTCF	: Long-term care facilities.
MDROs	: Multidrug-resistant organisms.
MHA	: Mueller Hinton agar .

MIC	: Minimal inhibitory concentration.
MIO	: Motility-Indole-Ornithine.
MRSA	: Methicillin Resistant Staphylococcus aureus.
NA	: Nalidixic acid.
NCCLS	: National Committee for Clinical Laboratory Standards
NICU	: Neonatal intensive care unit .
PAP	: Pyelonephritis-associated pili .
PCR	: Polymerase Chain Reaction.
PFGE	: Pulsed-Field Gel Electrophoresis .
S.aureus	: Staphylococcus aureus.
SAM	: Ampicillin/Sulbactam.
SMX	: Sulfamethoxazole.
SP	: Sputum.
ST	: Heat stable toxin .
Stx	: Shiga toxins.
SXT	: Trimethoprim/Sulphamethoxazole.
TE	: Tetracycline.
TIM	: Ticracillin/Clavulanic acid.
TMP	: Trimethoprim.
UR	: Urine.
UTI	: Urinary tract infections.
VAP	: Ventilator-associated pneumonia.
VRE	: Vancomycin resistant Enterococci.

## **INTRODUCTION**

Extended-spectrum B-Lactamase- (ESBL-) producing organisms are among the fastest growing problems in our hospitals. These B-Lactamases can be produced by a variety of Enterobacteriaceae; however, the most common ESBL-producing organisms are *Klebsiella pneumoniae*, other *Klebsiella* sp., and *Escherichia coli*. With the ability to produce highly effective B-Lactamase enzymes, these organisms are resistant to all B-Lactamase antibiotics except cephamycins (cefoxitin, cefotetan) and carbapenems. In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics, including aminoglycosides and fluoroquinolones. Hence, a more appropriate name would be "multidrug resistant organisms." Other problems associated with these organisms include difficulty in correctly identifying them in the clinical microbiology laboratory, limited treatment options, and deleterious impact on clinical Outcomes (**Nathisuwan et al., 2001**).

Typically, they derive from genes for TEM-1, SHV-1 by mutations that alter the amino acid configuration around the active site of this B-Lactamase. This extends the spectrum of B-Lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have recently been described. The presence of ESBLs carries tremendous clinical significance. The ESBLs are frequently plasmid encoded. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes (**Paterson and Bonomo, 2005**).

In recent years a new family of plasmid-mediated ESBLs, called CTX-M, that referentially hydrolyze cefotaxime has arisen. They have mainly been found in strains of *Salmonella enterica* and *E. coli*, but have also been described in other species of *Enterobacteriaceae*. They include the CTX-M-type enzymes CTX-M-1 (formerly called MEN-1), CTX-M-2 through CTX-M-10 (**Gazouli et al., 1998; Bonnet et al., 2000**). Strains expressing CTX-M-type beta-lactamases have been isolated from many parts of the world, but have most often been associated with focal outbreaks in eastern Europe, South America and Japan (**Bradford et al., 1998 ; Gniadkowski et al., 1998**).

Molecular typing of multi-drug resistant isolates is useful for surveillance purposes, to monitor outbreaks and track nosocomial spread. Pulsed-field gel electrophoresis (PFGE) is the current "gold standard" for bacterial molecular typing (**Nemoy et al., 2005**).

Pulsed-field gel electrophoresis (PFGE) has been used extensively in epidemiological investigations of bacteria, especially during outbreaks of nosocomial infections. PFGE allows DNA fingerprinting of bacterial strains, and is widely used to compare bacterial isolates collected over variable times and from different places. By analyzing PFGE data, relatedness of bacterial isolates can be determined. Use of software package allows analysis of gel image and normalization of the lanes in the gel and can be used to assess the similarity among the isolates and to construct dendrograms (**Singer et al., 2004**).

### Aim of work

The aim of this work was to study the genetic characterization of extended spectrum b-lactamase producing E.coli by detection of *TEM-1*, *SHV-1-INT-1*, *DELTA*, *CTXM-1* genes using polymerase chain reaction (PCR), then to analyze the relationship between these isolates using Pulsed Field Gel Electrophoresis (PFGE) followed by bionumerical analysis to reveal if there is an outbreak of a specific strain.

## **Pathogenic Escherichia coli**

### **INTRODUCTION:**

*E. coli* is one of the dominating species of bacteria living in the lower intestines of mammals, known as gut flora (Akil et al., 2006). In the large intestine, it actually assists with waste processing, vitamin K production and food absorption (Rendon et al., 2007). *E. coli* is one of hundreds strains of bacteria that cause illness in humans. *E. coli* can generally cause several intestinal (diarrhea) and extra-intestinal infections such as urinary tract infections, pneumonia, septicemia, and meningitis. If *E. coli* bacteria escape the intestinal tract through a perforation and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment (Anatoliotaki et al., 2007).

### **Pathogenesis of *E. coli*:**

Over 700 antigenic types (**serotypes**) of *E. coli* are recognized based on **O, H, and K antigens**. Serotyping used to be important in distinguishing the small number of strains that actually cause disease. For example, the serotype O157:H7 (O refers to somatic antigen; H refers to flagellar antigen) is uniquely responsible for causing HUS (hemolytic uremic syndrome) (Nataro and Kaper, 1998).

Nowadays, particularly for diarrheagenic strains (those that cause diarrhea), pathogenic *E. coli* are classified based on their unique **virulence factors** and can only be identified by these traits. Hence, analysis for pathogenic *E. coli* usually requires that the isolates first be identified as *E. coli* before testing for virulence markers. Pathogenic strains of *E. coli* are responsible for three types of infections in humans: **urinary tract**