Genotyping of Multidrug- Resistant Escherichia Coli

Thesis Submitted for fulfillment of Master Degree of Clinical and Chemical pathology

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2008

ACKNOWLEDGEMENT

Thanks to (ALLAH) who granted me the power to accomplish this work

I would like to express my deep gratitude to***, Professor.Dr. Amani Ali El Kholy,*** Professor of Clinical Pathology faculty of Medicine, Cairo University for her continuous support and advice throughout the course of this research.

Also, great appreciation should go to ***Professor.Dr. Howaida Ezz el Din***, Professor of Clinical Pathology, National Research Centre for her generous help and continuous support.

Many thanks to ***Dr. Mariam Adel Labib*** Lecturer of Clinical Pathology faculty of Medicine, Cairo university who provided me the valuable comments and experience needed to achieve this work.

Many thanks also to ***Dr. Iman Hamza Thabet***, Researcher of Clinical Pathology, National Research Centre, for her great support in this work.

Special thanks also to ***Dr.Mervat Gaber***, Assistant Professor of Clinical Pathology faculty of Medicine, Cairo University **& *Dr.Mona Mohieddeen***, Assistant Professor of Clinical Pathology faculty of Medicine, Cairo University for their help throughout this study.

Lastly I want to thank ***Dr. Pimentel*** and all the staff of molecular Lab.& Disease surveillance programme in **NAMRU-3** for their valuable help and the great experience they provided me , so all my respect goes to them .

Ahmed Toson Ahmed Salem Hendeya
2008

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 chephalosporins. 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.

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LIST OF ABBREVIATIONS

AK : Amikacin.

AFLP : Amplified fragment length polymorphism.

AP-PCR : Arbitrarily primed PCR.

AMP : Ampicillin.

ARI : Antibiotic Resistance Integrons.

ATCC : American Type Culture Collection.

ATM : Aztreonam.

BL : Blood.

bp : Base pair.

C : Chloramephenicol.

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 : Enteroaggregative Escherichia coli .

EAST : EnteroAggregative 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**