Isolation and Molecular Characterization of Multidrug Resistant ESBL producing Pathogenic *Escherichia coli*

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ABSTRACT

Escherichia coli are an opportunistic microorganism known to cause nosocomial and community borne infections. Infections like urinary tract infection (UTI), traveler's diarrhea, bacteremia, sepsis are mostly caused by multidrug resistant *E.coli.* β-lactam antibiotics are widely used drugs to treat such infections. But day by day microorganisms are evolving and modifying themselves in order to bypass the cytocidal effects of antimicrobials. These bacteria produce extended spectrum B-lactam enzymes (ESBLs) to neutralize these drugs. Severity of gram negative infections is frequently because of ESBLs. In this study we isolated multidrug resistant ESBL producing *E.coli* from various clinical specimens like urine, pus, blood etc and performed molecular characterization of resistant genes.

Keywords: ESBL, CTX-M, antibiotic resistance, *E.coli.*

INTRODUCTION

Infectious diseases are the major reason of morbidity. Irrational use of antibiotics enhances the pattern of growing resistance in pathogenic bacteria. This acquired resistance then further disseminate in environmental flora and other non resistant bacteria by horizontal gene transfer. Concisely mobile genetic elements like Plasmids and integrons are responsible for evolution of microorganism into multidrug resistance and extensively drug resistant strains. Resistance developed in these microbes making it difficult to cure from infections by conventional antibiotics. One of the well-known mechanism by which bacteria employee resistance against antibiotics is to produce drug hydrolyzing enzymes such as extended spectrum beta lactamases (ESBLs) which are responsible to breakdown β-lactam antibiotics such as pencillins, cephalosporins, carbapenams, ceftazidime etc. ESBLs are a group of plasmid mediated, very complex and rapidly evolving enzymes. They are divided into groups like SHV, TEM, OXA and CTX-M. They are cause of major therapeutic challenge in the treatment of nosocomial and community-based infections. The purpose of this study is to isolate resistant causing plasmid from pathogenic *E.coli* and understands the mechanism of growing resistance.

OBJECTIVE

- To isolate CTX-M type ESBL producers from local indigenous pathogens.
- To identify CTX-Mase producing *E. coli* from indigenous pathogens.
- To study the epidemiology of CTXmase producer in Karachi.
- To perform molecular characterization of CTX-M.

METHOODOLOGY

Multidrug resistant ESBL producing *E.coli* strains were isolated from various clinical sources like blood, urine, stool, wound, pus. E. coli strains were confirmed by using microbiological and biochemical tests. Multidrug resistance was confirmed by Kirby bauer disk diffusion test (Hudzicki, 2009).

Plasmid DNA was isolated by alkaline lysis method. Later amplification of ESBL $_{CTX\text{-}M}$ gene was performed and visualized on 2% gel Electrophoresis. Amplified green product was sequenced on AB137303 XL DNA analyzer. Various bioinformatics tools were used to analyses the obtained sequences.

RESULTS

The present study discovered that majority of the pathogenic isolates from health care setting were multidrug resistant. 60% of the isolates were CTX-M gene positive showing their ability to catabolize third and fourth generation cephalosporins like ceftazidime, cefepime, cefotaxime. Ceftazidime was found to be the most effiecnt drug against gram negative pathogens such as *E.coli*. Developing resistance basically depends on the point mutations i.e single nucleotide polymorphism in the drug binding motif, loops and active site regions of drug hydrolysine enzyme i.e CTX-Mases.

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