

CTX-M-15 in Gram-Negative Bacteria Isolated from Afghanian Refugees in Iran (2012-2013)

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Abstract— CTX-M beta-lactamases are enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (e.g., ceftazidime, ceftriaxone, or cefepime). The main aim of this study was to determine CTX-M-15 in gram-negative bacteria isolated from Afghanian refugee in Iran. From August 2012 to February 2013, 100 non-duplicate non-consecutive *K. pneumoniae* and *Escherichia coli* were isolated from subjects of study. DNA was extracted using PCR method. Our findings showed that 32 isolated samples had CTX-M-15 gene and other genes were not observed. Of 32 samples containing CTX-M-15 gene, 24 samples belonged to *E. coli* and 8 samples belonged to *Klebsiella pneumoniae*.

Keywords--- CTX-M-15, Gram-negative Bacteria, Afghanian Refugee, Iran

I. INTRODUCTION

THE Enterobacteriaceae are a large family of Gram-negative bacteria including pathogens, such as: *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. Members of the Enterobacteriaceae can be trivially referred to as enterobacteria or "enteric bacteria", as several members live in the intestines of animals [1].

Many members of this family are a normal part of the gut flora found in the intestines of humans and other animals, while others are found in water or soil, or are parasites on a variety of different animals and plants. *Escherichia coli* is one of the most important model organisms, and its genetics and biochemistry have been closely studied. Most members of Enterobacteriaceae have peritrichous, type I fimbriae involved in the adhesion of the bacterial cells to their hosts. Some enterobacteria produce endotoxins. Endotoxins reside in the cell cytoplasm and are released when the cell dies and the cell wall disintegrates. Some members of the Enterobacteriaceae produce endotoxins that, when released into the bloodstream following cell lysis, cause a systemic inflammatory and vasodilatory response. The most severe form of this is known as endotoxic shock, which can be rapidly fatal. Enterobacteriaceae are also the most common cause of urinary

tract infections (UTIs) in both community and healthcare settings [2].

Among gram-negative bacteria, *Escherichia coli* remains one of the most frequent causes of several common bacterial infections in humans and animals. *E. coli* is the prominent cause of enteritis, urinary tract infection, septicemia and other clinical infections, such as neonatal meningitis. *E. coli* is also prominently associated with diarrhoea in pet and farm animals [3].

Klebsiella pneumoniae is the causative agent of a variety of diseases, including pneumonia, urinary tract infections, septicemia, and the recently recognized pyogenic liver abscesses (PLA) [4].

Bacteria have also the genetic ability to transfer and acquire drug resistance factors. In recent years, inappropriate use of antibiotics has led to drug resistance among bacteria, which is the cause of untreatable "superbugs" and high death rates in clinics throughout the world, particularly among patients with suppressed immunity. The increasing prevalence of hospital and community-acquired infections caused by multidrug-resistant (MDR) bacterial pathogens is limiting the options for effective antibiotic therapy. Moreover, this alarming spread of antimicrobial resistance has not been paralleled by the development of novel antimicrobials. Resistance to the scarce new antibiotics is also emerging [5].

Beta-lactamases are enzymes produced by some bacteria that provide resistance to β -Lactam antibiotics like penicillins and cephamycins. Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a β -Lactam. Through hydrolysis, the lactamase enzyme breaks the β -Lactam ring open, deactivating the molecule's antibacterial properties.

Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria.

Beta-lactamases produced by Gram-negative organisms are usually secreted, especially when antibiotics are present in the environment [6].

CTX-M beta-lactamases are enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates (e.g., ceftazidime, ceftriaxone, or cefepime). Rather than arising by mutation, they represent examples of plasmid acquisition of beta-lactamase genes normally found on the chromosome of *Kluyvera* species, a group of rarely pathogenic commensal organisms. More than 80 CTX-M enzymes are currently known. CTX-M-14, CTX-M-3, and CTX-M-2 are the

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most widespread. CTX-M-15 is the most widespread type in *E. coli* the UK and is widely prevalent in the community [7]. The main aim of this study was to determine CTMX-15 in gram-negative bacteria isolated from Afghaninan refugee in iran.

II. MATERIAL AND METHODS

From August 2012 to February 2013,100 non-duplicate non-consecutive *K. pneumoniae* & *Escherichia coli* were isolated. Out of total samples,50% were from afghanian migration(Pishva-ye-Varamin),12% of bacterial isolated were from patienys who refered to Pishva-ye-Varamin Laboratoris.10% were related to migration who refered to health centers of Isfahan and 28% were related to Kerman.These samples were 68% of men patients and 32% of women. Out of 100 samples,50 cases were isolated of urine samples and 50 cases were stool samples. For DNA extract, a loopful of colony was suspended in 100 μ l of sterile distilled water in a 1.5-ml Eppendorf tube. The tube was vortexed for 5 to 10 s, floated in boiling water for 10 min, chilled on ice for 5 min, and then centrifuged at $12,000 \times g$ for 2 min to remove debris. The supernatant (1 μ l) was added directly to the PCR mixture. The PCR purification kit (Bioneer Co., Korea) was used to purify PCR products and sequencing was performed by the Bioneer Company (Korea). The nucleotide sequences were analyzed with FinchTV 1.45 software and BLAST in NCBI.

III. RESULTS

Our findings showed that 32 isolated samples had CTX-M15 gene and other genes were not observed. Of 32 samples containing CTX-M15 gene, 24 samples belonged to *E. coli* and 8 samples belonged to *Klebsiella pneumoniae*. Figure I shows the sequencing of CTX-M15 gene.



Fig. 1 Sequencing of CTX-M15 gene

IV. DISCUSSION

We determined CTX-M15 in our study for a considerable number of subjects suffering *E. coli* and *Klebsiella pneumoniae* isolated from Afghanian refugee in Iran. Resistance to β -lactams among Gram-negatives is a worldwide issue. Increased prevalence of extended spectrum β -lactamase (ESBL)-producers and the dissemination of carbapenem resistance genes are particularly concerning. Available data also suggest that CTX-M (CTX-M-15 in particular) is the

predominant ESBL in Enterobacteriaceae [8]. The *Klebsiella pneumoniae* producing CTX-M-15 has been determine recently in Switzerland [9]. The prevalence of extended-spectrum beta lactamases (ESBLs), the blaCTX-M genes, and aminoglycoside modifying enzymes genes in clinical isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) was also determined in Tehran- Iran. The results show that CTX-M-15 is the dominant type of CTX-M, which associates with entities that have high aminoglycoside resistance [10]. A recent study also reports the first examples of bla(CTX-M-15) in UK river sediment; the prevalence of bla(CTX-M-15) was dramatically increased downstream of the waste water treatment plant [11].

V. CONCLUSION

We determined CTX-M15 in our study for a considerable number of subjects suffering *E. coli* and *Klebsiella pneumoniae* isolated from Afghanian refugee in Iran.

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