



Detection of *CTX-M-1* gene Among *Klebsiella pneumoniae* Isolates in An Najaf Province

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Abstract: Resistance of *Klebsiella pneumoniae* strains to the broad-spectrum antibiotics may be caused by extended-spectrum β -lactamases (ESBLs). This study was aimed to determine the antimicrobial resistance patterns and prevalence of extended spectrum beta lactamase gene (*CTX-M-1*) among isolates of *K. pneumoniae*. A total of 62 *K. pneumoniae* isolates were collected from two hospitals in An Najaf - Iraq, during 6-month study (2013-2014). The antimicrobial susceptibility of isolates was determined by disc diffusion method and interpreted according to the clinical and laboratory standards institute (CLSI) recommendations. Production of ESBL was determined by the presence of *CTX-M-1* gene using PCR technique. Most of the isolates showed high level of resistance: 37 isolates were simultaneously resistant to Amoxicillin-Clavulanic acid, Cefotaxime, Ceftriaxone, Aztreonam, and Ceftazidime (37/62, 59.6%). All were susceptible to Imipenem and Ciprofloxacin. The results of PCR study revealed that 60.1% of the isolates were ESBL positive. This study highlighted the need to establish antimicrobial resistance surveillance networks for *K. pneumoniae* to determine the appropriate empirical treatment regimens.

Key words: *CTX-M-1*, *K.pneumoniae*, PCE Antibiotics, ESBL.

الكشف عن جين *CTX-M-1* في عزلات *Klebsiella pneumoniae* في محافظة النجف

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الخلاصة: يعود سبب مقاومة سلالات البكتريا *Klebsiella pneumoniae* للمضادات واسعة الطيف الى امتلاكها للانزيم extended-spectrum -lactamases (ESBLs). هدفت هذه الدراسة الى تحديد نمط المقاومة الدوائية و وجود الجين *CTX-M-1* في عزلات البكتريا. تم جمع 62 عزلة من مستشفيات في محافظة النجف للفترة من تشرين الاول 2013 لغاية نيسان 2014. تم فحص الحساسية الدوائية للعزلات بطريقة الانتشار بالاقراص وقيمت اعتمادا على توجيهات مؤسسة المقاييس المختبرية والسريية (CLSI) وتم تحديد انتاج انزيم extended-spectrum β -lactamases (ESBLs) من خلال الكشف عن وجود الجين *CTX-M-1* باستخدام تقنية سلسلة تفاعلات البلمرة (PCR). اظهرت معظم العزلات مستويات عالية من المقاومة: 37 عزلة كانت مقاومة لكل من Amoxicillin-Clavulanic acid ، Cefotaxime ، Ceftriaxone ، Aztreonam و (37/62, 59.6%) Ceftazidime. بينما كانت جميعها حساسة لـ Imipenem و Ciprofloxacin. اظهرت نتائج الـ PCR ان 60.1% من العزلات تمتلك extended-spectrum -lactamases (ESBLs). سلطت هذه الدراسة الضوء على الحاجة الى نظام مراقبة للمقاومة الدوائية لبكتريا *Klebsiella pneumoniae* و تحديد العلاج الملائم.

Introduction

Klebsiella pneumoniae is a successful opportunistic pathogen and has been associated with various ailments such as urinary tract infection, septicemia, respiratory tract infection and diarrhea (1). Epidemic and endemic nosocomial infections caused by *Klebsiella* species are leading causes of morbidity and mortality (2). Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate using available antibiotics. Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and the development of multidrug-resistant strains that produce extended-spectrum beta-lactamase (ESBL). Epidemic strains of cephalosporin resistant *K. pneumoniae* have been associated with increased morbidity and mortality in hospitalized patients (3).

Extended-spectrum β -lactamases (ESBLs) have been observed in virtually all species of the family *Enterobacteriaceae* mainly *Escherichia coli* and *Klebsiella pneumoniae* (4). Among ESBLs the most widespread and clinically relevant are class A ESBLs of TEM, SHV, and CTX-M types. CTX-M-type β -lactamases are increasingly becoming the predominant ESBLs globally in recent years, including Asia (5). These enzymes have been classified into five major groups by amino acid sequence similarities:

clusters of CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25/26. They have a preferential hydrolysis of Cefotaxime over Ceftazidime; although some CTX-M type ESBLs, including CTX-M-15, show good activity against Ceftazidime (6). Recent advances in the molecular characterization of antibiotic resistance mechanisms have resulted in the discovery of genetic elements, called integrons which can integrate antibiotic resistance genes. Several studies have reported integron distributions in multidrug resistant strains isolated from animals and humans. The role of integrons in the development of multiple resistances relies on their unique capacity to acquire gene cassettes and express cassette-associated genes (7,8). An integron, which can be located either on the bacterial chromosome or on a plasmid, includes the gene for an integrase site (*int*) and for an adjacent recombination site (*attI*) (8). So far three classes of antibiotic-resistance-encoding integrons have been identified. Each class has its own integrase. Among the antibiotic-resistance integrons, class 1 integrons are the most common integron type, class 2 integrons are embedded in Tn7-family transposons and only one example of a class 3 integron is known (9).

The aim of this study was to investigate the antimicrobial susceptibility and to determine the prevalence of CTXM-1 group enzymes genes among *K. pneumoniae* isolates.

Materials and Methods

Bacterial Isolates and Identification

A total of 62 *K.pneumoniae* isolates were collected from Al-Sader Medical City and Al-Hakim General Hospital in An Najaf, Iraq between October 2013 and April 2014. They were identified as *K. pneumonia* using biochemical tests.

Identification

The identification were carried out by using the API 20E system (bioMe'rieux, Marcy l'Etoile, France).

Antibiotic Susceptibility Testing

The antibiotic susceptibilities were determined by disk diffusion method on Mueller-Hinton agar plates (Himedia, India) as recommended by the clinical and laboratory standards institute (CLSI) (10). The disks containing the following antibiotics (μ g) were used (Mast, UK): Cefotaxime (CTX, 30), Ceftriaxone (CRO, 30), Ceftazidime (CAZ, 30), Imipenem (IPM, 10), Amoxicillin-Clavulanic acid (AUG, 30), Aztreonam (ATM, 30), Ciprofloxacin (CIP, 5), Tobramycin (TN, 10), Tetracycline (T, 30), Trimethoprim-sulfamethoxazole (TS, 25), Gentamicin (GM, 10), Cefepime (CPM, 30), Cefoxitin (FOX, 30), and Amikacin (AK, 30).

CTX-M-1 gene Identification

The *K. pneumonia* genomic DNA was performed according to Promega (the clinical manual for isolation genomic DNA from gram negative bacteria (wizard genomic DNA purification kit, Promega, USA).

The amplification of extended spectram β -lactamase gene (CTX-M-1) was carried according to AccuPower®TLA PCR Pre Mix tube (Bioneer Corporation, USA), using the primers pair CTX-M-1-F 5'-GTAAAAAATCACTGCGTC-3' and CTX-M-1-R 5'-TTGGTGACGATTTTAGCCGC-3' (amplicon size: 864 bp) (11). The DNA amplification program consisted of: Initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min followed by a final extension at 72°C for 7 min.

PCR products were analyzed by electrophoresis with 1% agarose gels in 1XTBE (Tris-Borate-EDTA) buffer. The gels were stained with ethidiumbromide, products were visualized under the UV light.

Results

All *K. pneumonia* isolates were susceptible to Imipenem and Ciprofloxacin. Of 62 isolates, 37 (59.6%) were simultaneously resistant to Amoxicillin-Clavulanic acid, Cefotaxime, Ceftriaxone, Aztreonam, and Ceftazidime, 33 isolates were resistant to Tobramycin, Cefepime, and Amikacin (53.2%), 29 isolates were

resistant to gentamicin(46.7%), 5 isolates were resistant to Trimethoprim-Sulfamethoxazole, and Tetracycline (8.06%). (P value < 0.05).

The CTX-M-1 gene was detected in all cefotaxime-resistant isolates (37/62, 59.6%) (Figure 1).

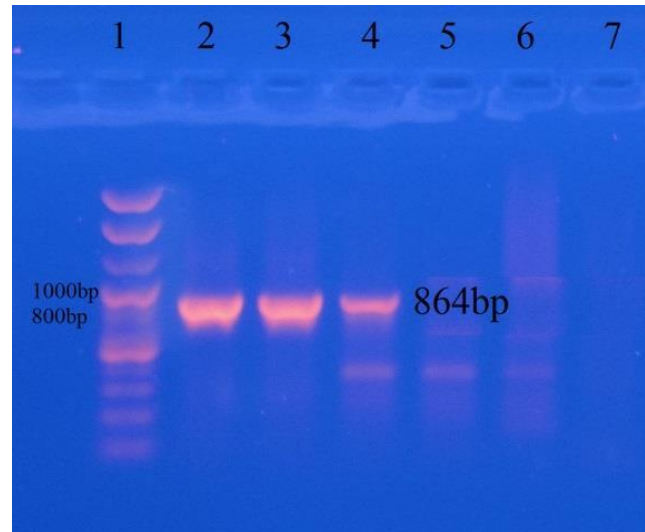


Figure1: PCR products for detection of CTX-M-1 Gene, Lane 1: 100 bp ladder; Lane 2-4: CTX-M-1 positive isolates; Lane 5-7: CTX-M-1 negative isolates.

Discussion

Infections caused by ESBL-producing bacteria are increasing in many countries (12, 13). During the last decades, the emergence of antimicrobial drug resistant strains has been reported in *K. pneumonia* isolated from community and hospital acquired infections(14-16). Resistance to antimicrobial agents is often associated with the spread of transmissible plasmids and integrons which can be located on the chromosome or plasmids. The ability of integrons to integrate resistance gene cassettes makes them prime pools for the further dissemination of antibiotic resistance among clinical isolates of gram-

negative bacteria, including *K. pneumonia* (17).

In this study, the frequency of ESBL-producing *K. pneumonia* isolates was 59.6% (37/62). The CTX-M-1-gene was detected in 60.1% of isolates. In a study performed by Karimi *et al.*, 19 isolates from 50 *K. pneumoniae* isolated from children with urinary tract infections (UTI) (38%), had positive results for ESBLs production(18). In another study performed by Seyed Javadi *et al.*, Of 30 *K. pneumoniae* isolated from children with UTI, 21(70%) were multidrug resistant (18). In a study performed by Feizabadi *et al.*, the prevalence of CTX-M-1 gene among the *K. pneumonia* isolates was 45.2% (14). Several reports have

confirmed the emergence of CTX-M-1-producing *K. pneumonia* isolates in Sweden, France, Madagascar, and Croatia. Also in Asian countries, CTX-M-1-producing *K. pneumonia* isolates have been reported from India, Kuwait, Saudi Arabia, Malaysia, The Philippines, Singapore, and Thailand (20, 21). Various factors involved in these differences including strains isolated from hospitalized patient or from outpatient, the geographic differences across the world.

In conclusion, the results of our study revealed that CTXM-1- producing *K. pneumonia* is already present in An Najaf province. *K. pneumonia* is a very efficient hospital pathogen. High rates of antimicrobial resistance suggest to monitor mechanisms of antimicrobial resistance and to emphasize on the rational use of antimicrobials to decrease the spread of ESBL producing bacteria.

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