Detection of Extended Spectrum β-lactamases Producing by *Klebsiella pneumonia* Isolated from Urinary tract Infection Patients by Using mPCR Technique

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الخلاصة:

الهدف: في هذه الدراسة عزلت جرثومة *Klebsiella pneumonia* من عينات البول وحُدِّدَ الطيف الواسع لإنزيمات البيتالاكتام بإستخدام تقنية mPCR.

طريقة العمل: أُخضِعت العزلات قيد الدراسة لإختبار الحساسية (بطريقة إنتشار الأقراص) تجاه بعض المضادات الجرثومية وبإتباع تقنية multiplex PCR تم تحديد الطيف الواسع للمورثات المسؤولة عن فعالية إنزيمات البيتالاكتام (*bla TEM, bla SHV, bla CTX-M* and *bla AMPC*).

النتائج: الطيف الواسع لمورثات إنزيمات البيتالاكتام بإستخدام تقنية تفاعل سلسلة إنزيم البلمرة أعطى النسب المئوية للمورثات *bla CTX-M* (93.75/30%) و *bla SHV* (78.12/25%) و *bla TEM* (56.25/18%) و *bla AMPC* (68.15/22%)، على التوالي.

الإستنتاج: عزلات *K. pneumonia* من أخماج مرضى المجاري البولية إرتبطت بقوة مع ظهور مورثة *bla CTX-M* β-Lactamase التي أثبتت فائدتها العلاجية الجيدة

الكلمات المفتاحية: *K. pneumonia*، إنزيمات البيتالاكتام، المضادات الجرثومية.

Abstract:

Objective: In this study *Klebsiella pneumonia* where isolated from urine samples and detection of extended spectrum β-Lactamases by using mPCR technique.

Method: This isolated subjected to the antimicrobial susceptibility test (Disc diffusion) for some antibiotics following by multiplex PCR techniques for detection extended spectrum β-Lactamase genes (*bla TEM, bla SHV, bla CTX-M* and *bla AMPC*).

Results: The extended spectrum β-Lactamase genes by PCR techniques were given *bla CTX-M* (30/93.75%), *bla SHV* (25/78.12%), *bla TEM* (18/56.25%) and *bla AMPC* (22/68.15%), respectively.

Conclusion: *K. pneumonia* isolates of urinary tract infection patients highly associated with the emergence of *bla CTX-M* β-Lactamase that provides useful good treatment.

Key words: *K. pneumonia*, β-Lactamase, antimicrobial.

Introduction

Extended-spectrum β-lactamases (ESBLs) at this time represent a major problem, antibiotic resistance in enterobacteria family (1). Extended-spectrum β-lactamases (ESBLs) are plasmid-mediated enzymes that give resistance to all penicillin, ampicillin and cephalosporin, including the sulbactam and clavulanic acid such as aztreonam (2). Extended spectrum β-lactamases are often plasmid mediated and derived from mutations in classic TEM, SHV, CTX-M, and AMPC genes by one or more amino acid substitution around the active site (3). ESBLs are most commonly detected in *K. pneumoniae*, which is an opportunistic pathogen associated with severe infections in hospitalized patients, including immunocompromised hosts with severe underlying diseases (4). *K. pneumoniae* is found on mucosal surfaces of mammals and the common sites of colonization in healthy humans are the gastrointestinal tract, eyes, respiratory tract and

genitourinary tract (5). The bloodstream infections associated with *K. pneumoniae* may arise as a consequence of pneumonia (community- and ventilator-acquired), the urinary tract, intra-abdominal pathologies, and central venous line-related infections (6). Extended-spectrum β-lactamases ESBLs such as SHV and TEM are the classical B-lactamase had resistance to penicillin and narrospectrum cephalosporin, the CTX-M β-lactamases are more active against cefotoxim and cefriaxon than ceftazidime, the AMPC β-lactamases has cephalosporin activity in K. pneumonia (7) .In addition, outbreak of multidrug resistant Klebsiella spp. Especially extended-spectrum B-lactamase has lead the treatment to limited option in recent year (8). This study aimed to determination of Extended-spectrum β-lactamases (ESBLs) (blaTEM, blaSHV, blaCTX-M and blaAMPC genes) found in *K. pneumonia* that isolated from urine samples by multiplex polymerase chain reaction (mPCR).

Materials and Methods

Bacterial isolates: 32 *K. pneumonia* that isolated from urine samples provided from Microbiology laboratory of Al-Diwanyia Hospital. After that *K. pneumonia* isolates were inoculated on Mueller- Hinton agar media and incubation at 37°C overnight. Then, the antimicrobial susceptibility test was done by using of (10µg) penicillin, (10µg) ampicillin, (10µg) cephalosporin, (30µg) cefotaxime, (10µg) cloxacillin, (10µg)ceftriaxone, (10µg) ceftazidime and(10µg) cefoxitin (Hi-Media) By using by disk diffusion methods.

Bacterial genomic DNA extraction: Bacterial genomic DNA was extracted from *K. pneumonia* isolates by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid. USA). 1ml of overnight bacterial growth on Brain heart infusion broth was placed in 1.5ml microcentrifuge tubes and then transferred in centrifuge at 10000 rpm for 1 minute. After that, the supernatant discarded and the bacterial cells pellets used in genomic DNA extraction and the extraction done according to manfuctural instruction. After that, the extracted gDNA checked by Nanodrop spectrophotometer, then store in -20ºC at refrigerator until perform PCR assay.

Multiplex Polymerase chain reaction (PCR): mPCR assay was performed for detection of Extended-spectrum β-lactamases (ESBLs), (blaTEM, blaSHV, blaCTX-M and blaAMPC genes) according to method described by (Parveen *et al*. 2011) (9) by using specific ESBLs primers that designed by using NCBI-GenBank and primer 3 plus design online. As show in the following table:

Primer		Sequence	Amplicon	GenBank
BlaCTX-M	F	AGCGATAACGTGGCGATGAA	247bp	JN411912.1
	R	TCATCCATGTCACCAGCTGC		
blaSHV	F	CCGCCATTACCATGAGCGAT	410bp	FJ668798.1
	R	AATCACCACAATGCGCTCTG		
blaTEM	F	GGTGCACGAGTGGGTTACAT	531bp	JN037848.1
	R	TGCAACTTTATCCGCCTCCA		
blaAMPC	F	AAACGACGCTCTGCACCTTA	670bp	AY533245.1
	R	TGTACTGCCTTACCTTCGCG		

These primers were provided by (Bioneer Company. Korea). Then PCR master mix was prepared by using (AccuPower® multiplex PCR PreMix kit. Bioneer. Korea). The PCR premix tube

contains freeze-dried pellet of (Taq DNA polymerase 5U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction performed in a thermocycler (Mygene Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 s, annealing 58 °C for 30 s, and extension 72 °C for 1min and then final extension at 72 °C for 10 min. The PCR products examined by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

Results

The antimicrobial susceptibility test were done as phenotypic antibiotics resistance profile of K. pneumonia isolates. Where, the results show that the penicillin and ampicillin were given high resistance K. pneumonia isolates at 28 (87.75%) and the cefoxitin was given lower resistance K. pneumonia isolates at 12 (37.12%) as following table:

Antibiotic	Sensitive	Intermediate	Resistant
Penicillin	0 (0%)	4 (12.5%)	28 (87.75%)
Ampicillin	0 (0%)	8 (32%)	24 (75%)
Cephalosporin	0 (21.87)	2 (6.25)	30 (93.75)
Ceftazidime	1 (3.12%)	2 (6.25)	29 (90.62%)
Cloxacillin	3 (9.37%)	3 (9.37%)	26 (40.62%)
Ceftriaxone	8 (32%)	6 (18.75%)	18 (56.25%)
Ceftazidime	8 (32%)	10 (31.25%)	16 (50%)
Cefoxitin	10 (31.25%)	7 (21.87)	15 (46.87%)

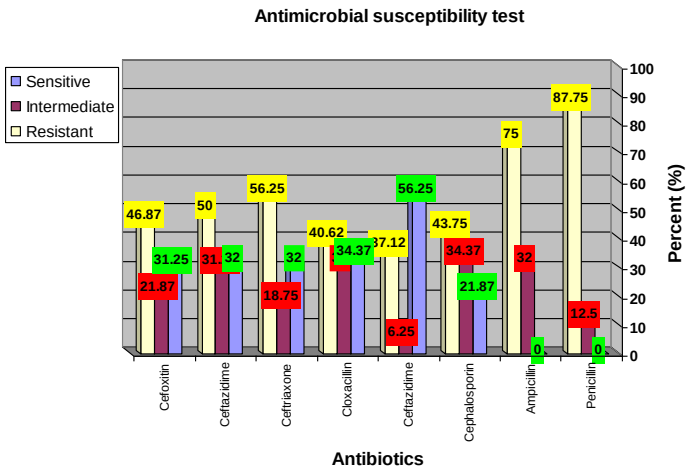

Figure (1): The antimicrobial susceptibility *K. pneumonia* isolates by using disc diffusion method.

Polymerase chain reaction PCR results were show that assay was Extended-spectrum β-lactamases (ESBLs) (BlaCTX-M, blaSHV, blaTEM, and blaAMPC genes) as following table:

ESBLs gene	Percent (%)
BlaCTX-M	30 (93.75)
blaSHV	25 (78.12%)
blaTEM	18 (56.25%)
blaAMPC	22 (68.75%)

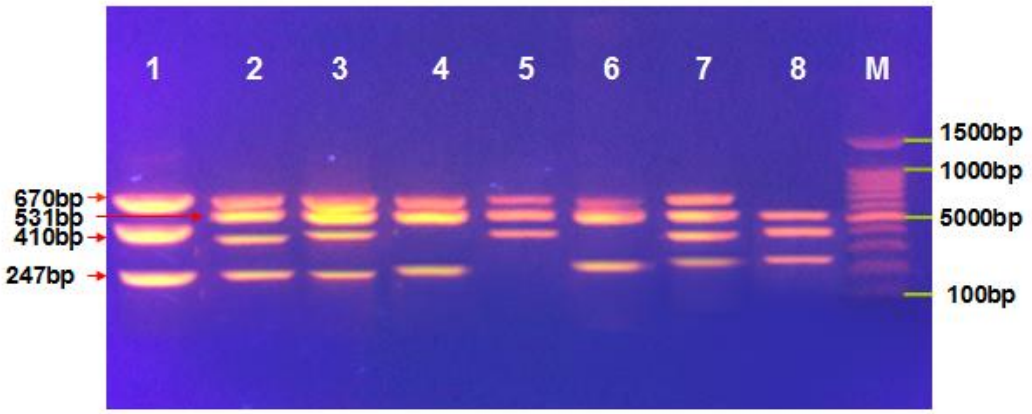

Figure (2): Agarose gel electrophoresis of PCR assay show the some positive *K. pneumonia* isolates results of Extended-spectrum β-lactamases gene. Where, Lane (M) DNA marker (1500-100bp), Lane (1-8) show positive (blaTEM, blaSHV, blaCTX-M, and blaAMPC genes) at 670bp, 531bp, 410bp, and 247bp PCR product respectively.

Discussion

K. pneumoniae consider as an important cause of hospital-acquired infections, especially among patients in the neonatal intensive care unit and can be causes mortality rates as high (70%) over the last two decades, the incidence of infections caused by multidrug-resistant Klebsiella strains has increased (10). Extended Extended-spectrum β-lactamases (ESBLs) enzymes were first described in *Serratia marcescens* and K. pneumoniae isolates in 1983 in Europe country (11). In United States in 1989 were described *K. pneumoniae* and *Escherichia coli* isolates that marked increase in the incidence of bacteria that produce ESBL enzymes and show about 20% of strains were resistant to ceftazidime in some teaching hospitals (12). Epidemiological studies proposed that the increasingly extensive use of third-generation cephalosporin is a major risk factor that has contributed to the emergence of Extended-spectrum β-lactamases -producing K. pneumoniae (13). Numerous additional risk factors for colonization and infection with ESBL-producing *K. pneumoniae* have been reported and include arterial and central venous catheterization, gastrointestinal tract colonization with ESBL- producing *K. pneumoniae*, prolonged length of stay in an intensive-care unit, low birth weight in preterm infants, prior antibiotic use, and mechanical ventilation (14). In our results of the 32 isolates were investigated, 30 (93.75%) were found to be resistant to cephalosporin and among these 32 isolates,

36 (86.5%) were found to be ESBL positive by phenotypic test. The extended spectrum β-lactamases genes by mPCR techniques were given BlaCTX-M (30/ 93.75), blaSHV (25 /78.12%), blaTEM (18 / 56.25%), and blaAMPC (22 /68.75%) respectively. These results agreement with (15, 16) which explained CTX-M-type ESBLs have become more prevalent worldwide. In conclusion, this study emphasizes the major role that Extended-spectrum β-lactamases CTX-M plays in facilitating ESBL-mediated antimicrobial resistance in *K. pneumoniae* of urinary tract infection that association with multiple antibiotic resistance determinants, include cephalosporin resistance.

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