Biochem. Cell. Arch. Vol. 21, No. 2, pp. 4705-4710, 2021 | www.connectjournals.com/bca | *ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.4705 | *eISSN 0976-1772*

DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE GENES (bla-CTX AND bla-SHV) AND SAME VIRULENT GENES IN *K. PNEUMONIAE* ISOLATED FROM URINARY TRACT INFECTION

Burooj Mohammed Razooqi Al-Aajem*[1], Ali Jaffar Saleem[2] and Hamed Majed Jasim[3]

[1]Department of Microbiology, College of Medicine, Diyala University, Diyala, Iraq.
[2]College of Education for Pure Science, University of Diyala, Iraq.
[3]College of Biotechnology, University of Al-Nahreen, Iraq.
*e-mail : m.rburooj@yahoo.com

(Received 3 April 2021, Revised 30 May 2021, Accepted 7 June 2021)

ABSTRACT : The epidemiology of ESBL genes is changing rapidly and shows marked geographic differences in distribution of genotypes of â-lactamases genes (bla-CTX and bla- SHV). *K. pneumoniae* have more virulence factors according encoding for many virulent genes. Aim of the study, determination of extended spectrum beta- lactamase genes (bla-CTX and bla-SHV) and same virulent genes in *K. pneumoniae* isolated from urinary tract infection such as (fim H, K2A). Samples were cultured on MacConky agar, blood agar, nutrient agar and Chrom agar Orientation for isolation and identification of bacterial isolates based on standard bacteriological methods, biochemical tests. And used Vitek -2- was used to identify the bacterial isolates, se. Genomic DNA was extracted using promega™ gDNA Bacteria Kit. DNA was extracted from fresh cultures of the selected bacterial isolates. After PCR amplification, for detection *K. pneumoniae*, β-lactamases genes (bla-CTX, bla-SHV), fim H and K2A. The results of PCR reaction for β-Lactamase genes showed 30 (100%) of *K. pneumoniae* contains β-Lactamase gene (blaCTX and blaSHV). This prevalent of â-Lactamase gene in all isolates explains the high resistance to β-Lactam antibiotics. And K2A genes showed 8 (26.6%) of *K. pneumoniae* contains this gene and the results of PCR reaction showed fimH genes in rates 26 (86.66%) of *K. pneumoniae* contains this genes. In conclusion, prevalent of β-Lactamase gene and adhesion genes in all most isolates explains the high resistance to β-Lactam antibiotics and highest ability to adhere to human cell. The high adhesion ability of *K. pneumonia*e isolated from UTI play important role in pathogenicity.

Key words : *K. pneumoniae*, β-lactamases genes, bla-CTX, bla-SHV, K2A, fim H.

How to cite : Burooj Mohammed Razooqi Al-Aajem, Ali Jaffar Saleem and Hamed Majed Jasim (2021) Detection of extended spectrum beta-lactamase genes (bla-CTX and bla-SHV) and same virulent genes in *K. pneumoniae* isolated from urinary tract infection. *Biochem. Cell. Arch.* **21**, 4705-4710. DocID: https://connectjournals.com/03896.2021.21.4705

INTRODUCTION

K. pneumoniae is one of the most common organisms which carry plasmids encoding extended spectrum β-lactamases (ESBLs). ESBLs producing *K. Pneumoniae* are susceptible to carbapenems such as imipenem and meropenem. Both antibiotics are administered for the treatment of ESBLs producing *K. pneumoniae* (Woodford *et al*, 2004). Acquiring the plasmid type of β-lactamases TEM-1, 2 and SHV-1 genes and chromosomal type of SHV-1 gene in *K. pneumonia* with low level resistance to penicillins may cause high level resistance to these antibiotic. Number of ESBL variants occurring through amino acid mutations has progressively increased, while demonstrating geographic variations. SHV-type ESBLs are mostly derivatives of a non-ESBL SHV-1 and quickly invaded several continents (Kiratisin *et al*, 2008; Al-Mualm *et al*, 2020).

The encoded enzyme SHV–1 proved its activity against penicillins and first generation cephalosporins and was confirmed part of the conjugative plasmid p453 (Liakopoulos *et al*, 2016). The CTX-M family, first described in 1992, is known to be the most dominant non-TEM, non-SHV ESBL among *Enterobacteriaceae* and is recognized as a rapidly growing family of ESBLs that selectively prefer to hydrolyze cefotaxime rather than ceftazidime. However, variants of CTX-M with increased hydrolyzing activity against ceftazidime have emerged (Izadi *et al*, 2016). The insertion sequence (IS) element IS*Ecp1* is commonly present in the upstream region of

the CTX-M gene and is likely responsible for the transposition process of the gene (Overdevest *et al*, 2011). Extended Spectrum β-Lactamases Enzymes (ESBLs) genes are located on plasmids that can be easily transferred between and within bacterial species. Some ESBL genes are mutant derivatives of established plasmid-mediated â-lactamases (*blaCTX- bla*-SHV) and others are mobilized from environmental bacteria (Izadi *et al*, 2016). The epidemiology of ESBL genes is changing rapidly and shows marked geographic differences in distribution of genotypes of *bla*CTX-M β-lactamases (Overdevest *et al*, 2011). Extended Spectrum β-Lactamases Enzymes (ESBLs) are derived from genes for the narrow-spectrum β-lactamases (TEM-1, TEM-2, or SHV-1) by mutations that alter the amino acid configuration around the enzyme active site. They are encoded by plasmids. More than 350 different natural ESBL variants are known that have been classified into nine distinct structural and evolutionary families based upon their amino acid sequence comparisons such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA and OXA (Bajpai *et al*, 2017). *K. pneumoniae* is a well-known opportunistic pathogen and responsible for rate of nosocomial bacterial infections. This bacterium can infect almost every part of the human body, the urinary and respiratory tracts are most commonly affected (Curiel *et al*, 2013). *K. pneumoniae* has several virulence factors, which facilitate the microbe's ability to spread and evade body immune system, and cause disease of human hosts. Include capsular polysaccharide, lipopolysaccharide, fimbriae and siderosphores (Fange *et al*, 2012). *K. pneumoniae* employs many strategies to grow and protect itself from the host immunity. There are four major classes of virulence factors that have been well characterized in *K. pneumoniae*, including the production of hypercapsule in HV strains; lipopolysaccharide (LPS), siderophores, and fimbriae, also known as pili (Pan *et al*, 2015). Several other factors were recently identified as factors for *K. pneumoniae* virulence. These virulence factors include outer membrane proteins (OMPs), porins, efflux pumps and iron transport systems. These virulence factors play various roles in infection and in different strains of *K. pneumoniae* (Follador *et al*, 2016).

Aim of the study : Determination of extended spectrum beta- lactamase genes (bla-CTX and bla-SHV) and same virulent genes in *K. pneumoniae* isolated from urinary tract infection.

MATERIALS AND METHODS

A total of 302 urine samples were collected from patients women suffering from UTI attends Baquba teaching hospital and Al- Batool teaching hospital during the period from November 2019 to June 2020. Ages of patients women were ranged between 5-65 years old. A volume of 10 ml aliquots of mid-stream urine samples were taken from each subjects and kept in sterile screw-capped tube and transferred immediately to the laboratory and analyzed within two hours. Samples were cultured on MacConky agar, blood agar, nutrient agar and Chrom agar Orientation for isolation and identification of bacterial isolates based on standard bacteriological methods, in addition to many of biochemical tests (Baron *et al*, 1994; Collee *et al*, 1996). And used Vitek-2 was used to identify the bacterial isolates, sensitivity test and colonies counting. Genomic DNA was extracted using promega™ gDNA Bacteria Kit. According to the protocol stated by the kit manufacturer, DNA was extracted from fresh cultures of the selected bacterial isolates. Quantus Fluorometer was used to detect the purity of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 ìl of DNA, 199 ìl of diluted Quantifluor Dye was mixed. After 5min of incubation at room temperature, DNA purity was measured and the concentration was calculated throughout the following formula: DNA concentration (Mg/ml) 50Xod (260)x dilution factor. After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplified products. PCR was completely dependable on the extracted DNA criteria.

Primers preparation : Lyophilized primers indicated in Table 2 were dissolved in a nuclease free water to give a final concentration of 100pmol/ìl as a stock solution. A working solution of these primers was prepared by adding 10ìl of primer stock solution (stored at freezer -20ºC) to 90ìl of nuclease free water to obtain a working primer concentration of 10pmol/ìl as indicated in Table 1.

Optimization of PCR program

Optimum conditions for implication of each transposable element was described in Tables 3 and 4.

Table 1 : Primers preparation.

Primer name	Vol. of nuclease free water (ìl)	Concentration (pmol/ìl)
K2A-F	300	100
K2A-R	300	100
fimH-F	300	100
fimH-R	300	100
blaCTX-F	320	100
blaCTX-R	320	100
blaSHV-F	300	100
blaSHV-R	300	100

Table 2 : Oligonucleotide primers.

Primer name	Sequence (5 → 3)	Annealing temp. (°C)	Amplicon size (bp)
K2A	F: CAACCATGGTGGTCGATTAG	55	532
	R: TGGTAGCCATATCCCTTTGG		
fimH	F: TGTTCACCACCCTGCTGCTG	55	512
	R: CACCACGTCGTTCTTGGCGT		
blaCTX	F: CGCTTTGCGATGTGCAG	60	551
	R: ACCGCGATATCGTTGGT		
blaSHV	F: AGCCGCTTGAGCAAATTAAAC	60	714
	R: ATCCCGCAGATAAATCACCAC		

Table 3 : PCR program for amplification of 12, APHA1, ERMB1, int, xis transposable elements.

Step	Temperature (°C)	Time m:s	No. of cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

Table 4 : PCR program for amplification of TETM2 transposable elements.

Step	Temperature (°C)	Time (m:s)	No. of cycles
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:45	
Extension	72	00:45	
Final extension	72	07:00	1

RESULTS

All urine samples have been cultured on blood agar, MacConky agar, for 24 hrs. at 37°C for primary isolation and identification. Identification of bacterial isolates based on standard bacteriological methods. Bacterial isolates diagnosed based on culture characteristics and morphological examination and more accurate test were done for *K. pneumoniae*. The positive result was identified by depending on the colony morphology such as (color, shape, texture and size). Colonies of *K. pneumoniae* appeared on MacConkey agar after 24 hrs at 37°C as large, mucoid, convex, circular colonies, pink color because it ferments the lactose sugar as shown in Fig. 1. Non-hemolytic grey-white, mucoid colonies on blood agar. On EMB agar appear as dark pink colony. Isolates of *K. pneumoniae* appeared on Chrom agar Orientation as metallic blue colonies at 37°C for 24 hours as. Other bacterial isolates diagnosed according to culture and morphological characteristics.

All collected bacterial cultures diagnosed according to Biochemical tests has been approved were according to Baron *et al* (1994) and Collee *et al* (1996) and identified by Vitek2 compact system.

Molecular detection of â-Lactamase genes (bla CTX-M, blaSHV) in *K. pneumoniae*

The results of PCR reaction for *â-Lactamase* genes showed 30 (100%) of *K. pneumoniae* contains â-Lactamase gene(blaCTX and blaSHV) as appear in Fig. 2, 3. This prevalent of â-Lactamase gene in all isolates explains the high resistance to â-Lactam antibiotics. The genotypic detection was almost similar with genotyping in rate (100%). All isolates of *K. pneumoniae* were high resistant to Ampicillin, Cefazolin and Ceftriaxone and showed a highest resistance to Cefipime (86.11%), Ceftazidime (80.56%), Piperacillin (75.0%) and Aztreonam (69.44%). All *K. pneumoniae* (100%) of the genotypes expressed1 bla CTX-M, bla SHV genes. The results were in agreement with local study of Aljanaby and Alhasnawi (2017) in Al-Kufa hospital and Salman (2019) in Baghdad Teaching Hospital, were found *K. pneumoniae* isolated from UTI most have resistance gene blaSHV (86.04%) (100%), respectively. In the present study, majority of the isolates expressed *bla*SHV and bla CTX-M genes. Similar findings were reported in a study conducted in Turkey among patients with ESBL-producing carbapenem-resistant *K. pneumoniae* strains, where the most prevalent gene was blaSHV (97%), followed and *bla*CTX-M-1 (62%) (Iraz *et al*, 2015). The report from other parts of the world, was found the blaCTX-M most dominant gene in Saudi Arabia (74.1%) (Elhassan *et al*, 2016), Isfahan, Iran (92%) (Maleki *et al*, 2018) and also, CTX-M-producing *K. pneumoniae* isolates demonstrated rapid emergence and spread at the 2000s in the United States (Wang *et al*, 2013). In *K. pneumoniae*, A multidrug-resistant transferable plasmid encoding the SHV-5 beta-lactamase, causing unusually high resistance to Ceftazidime and Aztreonam (69%) of

Fig. 1 : Colonies of *K. pneumoniae* on MacConkey agar.

Molecular detection of fimbrial adhesion genes (fimH) in *K. pneumoniae*

In order to detect the presence of fimbrial adhesins gene (fimH) and determination the prevalence of gene among 30 isolates of *K. pneumoniae*, polymerase chain reaction (PCR) for each DNA extracted sample have been used. The PCR products have been confirmed by analysis of the bands on gel electrophoresis and by comparing their molecular weight with 512 bp DNA Ladder. The results of PCR reaction for fimH gene showed 26 (86.66%) of *K. pneumoniae* contains this genes as showed in Fig. 5. Mahmood and Abdullah (2015)

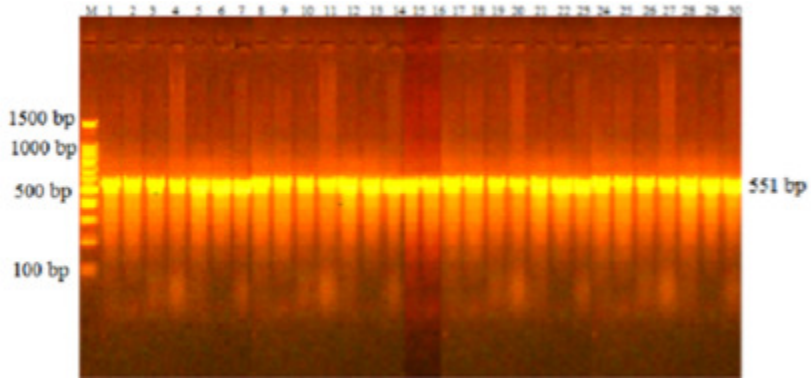

Fig. 2 : Amplification of bla-CTX gene of *K. pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-7 resemble 551bp PCR products.

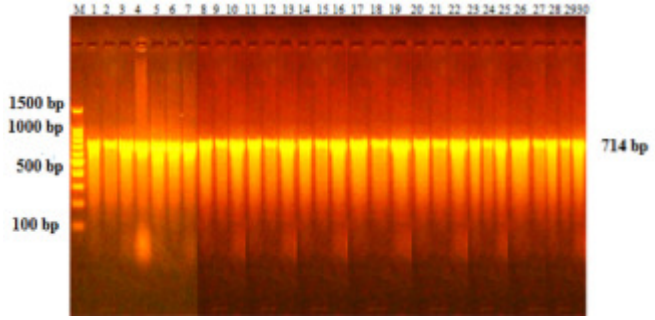

Fig. 3 : Amplification of blaSHV gene of *K. pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-7 resemble 714bp PCR products.

K. pneumoniae were resistance to Aztreonam. While salman (2019) found bla CTX-M in rate of (4.5%) in *K. pneumoniae* isolated from UTI (Wang *et al*, 2013).

Molecular detection of capsular genes(K2A) of *K. pneumoniae*

In order to detect the presence of capsule genes (K2A) and prevalence of gene among 30 isolates of *K. pneumoniae*, polymerase chain reaction (PCR) for each DNA extracted sample have been used. The results of PCR reaction for K2A genes showed 8 (26.6%) of *K. pneumoniae* contains this gene as appear in Fig. 4.

found that (100%) of *K. pneumoniae* isolated from UTI in Mosul were contain *mrkD* genes. Infection caused by pathogenic *K. pneumoniae* are often initiated by binding of the bacteria to the host cell surface via specific bacterial adhesins with fimbriae (Heydari *et al*, 2013). Most uropathogenic isolates of *K. pneumoniae* express type 1 fimbriae fimH, (mannose-sensitive) and type 3 fimbriae mrkD (mannose-resistant) fimbrial adhesins (Hojati *et al*, 2015). Which have a relation with biofilm formation, the main cause of chronic UTI infections (Jarjees, 2014). Biofilms have a major medical significance as they decrease susceptibility to antimicrobial agents. by multiple factors, including physical impairment of diffusion of antimicrobial agents, reduced bacterial growth rates and local alterations of the microorganisms that may impair activity of the antimicrobial agent (Pramodhini *et al*, 2012; Al-Aajem, 2020). Fimbria type1 which is encoded by the fimH genes and type 3 fimbria are encoded by the mrkDV genes cluster. *K. pneumoniae* type 3 fimbriae contribute to UTIs (Struve *et al*, 2009). Most *K. pneumoniae* isolates express type 3 fimbrial adhesin Which contribute to the invasion of bladder cells by and to biofilm formation in the bladder during UTI (Bandeira *et al*, 2014). Many studies have revealed that type 3 fimbriae (mrkDV gene)

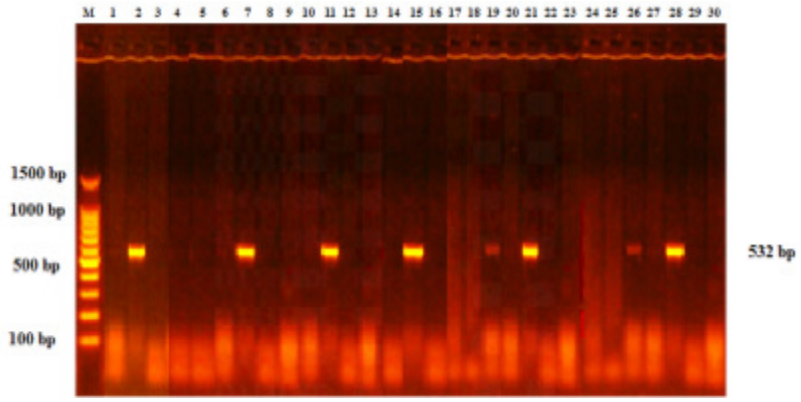

Fig. 4 : Amplification of K2A gene of *K. pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-7 resemble 532bp PCR products.

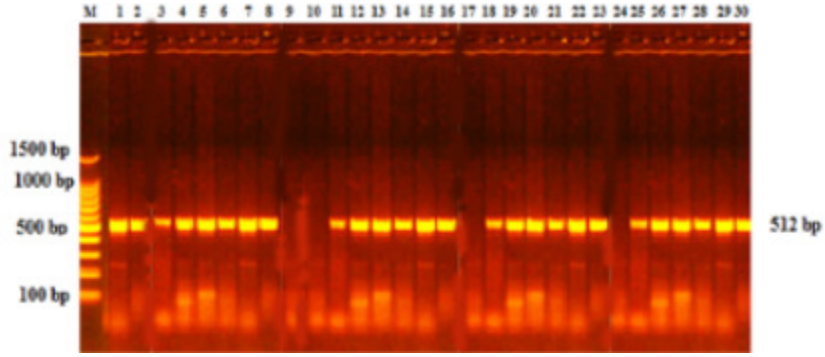

Fig. 5 : Amplification of FimH gene of *K. pneumoniae* samples fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 2-7 resemble 512bp PCR products.

are important in *K. pneumoniae* biofilm formation (Ong *et al*, 2010; Bellifa *et al*, 2013). Mahmood and Abdullah (2015) It were found that all biofilm producing *K. pneumoniae* 9(100%) were positive for mrkDV gene.

CONCLUSION

This prevalent of β-Lactamase gene in all isolates explains the high resistance to β-Lactam antibiotics and In current study 26 (86.66%) of *K. pneumoniae* isolates showed the highest ability to adhere to human epithelial cells. As result to found genes encoding for adhesion fimbrial factors. The high adhesion ability of *K. pneumonia*e isolated from UTI play important role in pathogenicity. The ability of bacterial isolates to adherence to human epithelial surfaces, which is the first step in inducing inflammation.

ACKNOWLEDGMENTS

I would like to thank the cadre of the educational labs at Baquba Teaching Hospital and the specialized doctors to help complete this useful scientific study.

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