Molecular Study And Antibiotic Susceptibility Patterns Of Some Extended Spectrum Beta-Lactamase Genes (ESBL) Of Klebsiella Pneumpniae In Urinary Tract Infections

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Abstract:The present study included the collection of 121 samples from MSU for investigating the presence of K.pneumoniae in UTIs, the samples have been collected from Al-Shaheed Mohammed Baqir AL-Hakeem hospital and private laboratories in Baghdad province. The study was carried out through March 2019 to the beginning of June 2019. The samples were identified based on the morphological and microscopically characteristics of the colonies when they were culturing on number of culture media as well as biochemical tests, molecular identification were also used as a final diagnostic test for isolates that were positive as they belong to K.pneumoniae bacteria during previous tests based on the blaTEM, blaSHV and blaCTX-M genes which has specific sequences for K.pneumoniae bacteria as a detection gene and also consider as virulence factor so it have a synonyms mechanism to antibiotic resistance. The results of the final diagnosis showed that 38 isolates belong to target bacteria, The examination of the sensitivity of all bacterial isolates was done for selected 38 isolation towards the 16 antibiotics by a Vitek2 compact ASTN system and the isolates were resistant for a number of antibiotics used such as; Amikacin (5.26%), Imepenem (5.26%), Ertapenem (7.89%), Meropenem (10.52%), Gentamicin (21.05%), Ciprofloxacin (26.32%), Cefoxitin (39.47%), Trimethoprim/Sulfamethoxazole (50%), Ceftriaxone (52.63%), Fosfomycin (55.26%), Piperacillin/Tazobactam (57.89%), Nitrofurantoin (57.89%), Ceftazidime (65.79%), Cefuroxime (71.05%), Cefixime (73.68%) and Ampicillin (100%). The presence of Extended Spectrum Beta-Lactamase genes in 38 K.pneumoniae isolates were 65.8 % of the ESBL genotypes expressed blaSHV genes followed by 52.6 % blaTEM and 42.1 % for blaCTX-M.

Keywords: Klebsiella pneumoniae, urinary tract infection, antibiotic susceptibility, blaTEM, blaSHV, blaCTX-M.

1. INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, gas-producing and rod-shaped bacterium, *K. pneumoniae* is able to grow either with or without free oxygen, deeming it a facultative anaerobe. *Klebsiella pneumoniae* causes a wide range of infections, including pneumonias, urinary tract infections, bacteremias, and liver abscesses. *Klebsiella pneumoniae* strains have become increasingly resistant to antibiotics, rendering infection by these strains very challenging to treat. Four factors; capsule, lipopolysaccharide, fimbriae, and siderophores, have been well studied and

are important for virulence in at least one infection model. (Paczosa and Mecsas, 2016). Extended spectrum beta-lactamases are a group of enzymes produced by certain bacteria that are able to hydrolyze extended spectrum antibiotics belonging to the penicillin and cephalosporin groups and monobactam. ESBLs are found in Gram-negative bacteria, especially in Enterobacteriaceae and *P.aeruginosa* (Ejaz *et al.,* 2013). Extended spectrum beta-lactamases are often located on plasmids that are transferable from strain to strain and between bacterial species. Although the prevalence of ESBLs is not known, it is clearly increasing. ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues (Rupp and Fey, 2003). The SHV-1 b-lactamase is responsible for up to 20% of the plasmid-mediated ampicillin resistance in *K. pneumoniae* species (Huang *et al.,* 2016).

2. MATERIALS AND METHODS:

Clinical isolates

A total of (121) clinical specimen were collected from the beginning March 2019 to the beginning of June 2019 from Al-Shaheed Mohammed Baqir Al-Hakeem Hospital in Baghdad city and private laboratories. From patients with UTIs.

Isolation and identification of *Klebsiella pneumoniae* by Traditional methods: colony morphology and growing on different medium (CHROMagar Orientation, blood and macckongy agar), catalase test, citrate utilization, Triple-sugar iron agar, Indole test, oxidase reaction, urease test, growth at 37 C˚, and identify by VITEK 2 compact.

Antimicrobial susceptibility:

The drug susceptibility test was carried out for all the isolates on VITEK 2 compact system to; Gentamicin, Nitrofurantoin, Trimethoprim /Sulfamethoxazole, Piperacillin /Tazobactam, Ceftazidime, Imipenem, Meropenem, Amikacin, Ciprofloxacin, Ampicillin, Ertapenem, Ceftriaxone, Cefoxitin, Cefuroxime, Cefixime and Fosfomycin.

Molecular detection:

DNA was extracted from activated pure culture of *Klebsiella pneumoniae* bacteria using DNA Bacteria Kit (geneaid). Detection of DNA bands using Agarose gelelectrophoresis (1%). The primers used in this study are shown in Table (1). PCR amplification of for the detection of *K.pneumoniae* genus was performed on all phenotypically tested strains of *K.pneumoniae.*

Table (1): Sequences of primers used for conventional PCR to detect *K.pneumonia ESBL* genes.

Target gene	**Oligonucleotide primer sequence 5' to 3'**	**Amplicon size (bp)**	**Reference**
***bla*SHV-F** ***bla*SHV-R**	TCCCATGATGAGCACCTTTAAA TCCTGCTGGCGATAGTGGAT	104	Roschanski *et al.*, (2014)
***bla*CTX-M-F** ***bla*CTX-M-R**	TCTTCCAGAATAAGGAATCCC CCGTTTCCGCTATTACAAAC	909	Chouchani *et al*., 2011
***bla*TEM-F** ***bla*TEM-R**	TCCGCTCATGAGACAATAACC TTGGTCTGACAGTTACCAATGC	931	Eghbalpoor *et al.*, 2019

3. PCR AMPLIFICATION:

5x FIREPol® Master Mix is a premixed ready-to-use solution containing all reagents required for PCR (except template, primers and water). Using 20 µL of PCR reaction, 5 µl DNA template was amplified using 4 µl of 5x FIREPol® master mix (Solis BioDyne/ Europe) and 1 µl were added from each of forwarded and reversed primers, up to the final volume 20 µl with nucleases free water (9 µl). Negative control contained all material except DNA, that D.W. was added instead of template DNA, the PCR programs were set on Thermal-cycler (Applied BioSystem, Singapore). Amplification reactions was started according to the program described in the table (2).

Table (2): Conditions of uniplex PCR reaction for ESBL genes detection.

Target gene	**PCR conditions**	**No. of cycles**
*bla*TEM and *bla*CTX-M	Initial denaturation at 95ºC for 5 min Denaturation 95 ºC for 30 sec Annealing 60 ºC for 30 sec Extension 72 ºC for 1 min Final extension at 72 ºC for 5 min	36 cycle
*bla*SHV	Initial denaturation for 5 min at 94ºC Denaturation 95 ºC for 30 sec Annealing 50 ºC for 30 sec Extension 72 ºC for I min Final extension at 72 ºC for 5 min	36 cycle

4. RESULTS AND DISCUSSION:

The first or initial diagnosis that based on microscopic examination and characteristic of the bacterial colonies, a total of 121 clinical specimens of urine were cultured on selective medium CHROMagar Orientation the 38 isolates of *K.pneumoniae* appeared as mucoid metallic blue colonies as in figure (1).

Figure (4.3): *K.pneumoniae* on CHROMagar orientation.

The results of biochemical tests for *K.pneumoniae* were negative results for indole and oxidase tests and gave positive result for citrate utilization, catalase and urease tests. *K.pneumoniae* were non-motile and On the TSI test strains produced acids both in butt and slant along with gas production and no H2S production.

Antimicrobial susceptibility was performed on all 38 *K. pneumoniae* isolates to 16 different antibiotics by Vitek2 compact ASTN system that showed in chart (1).

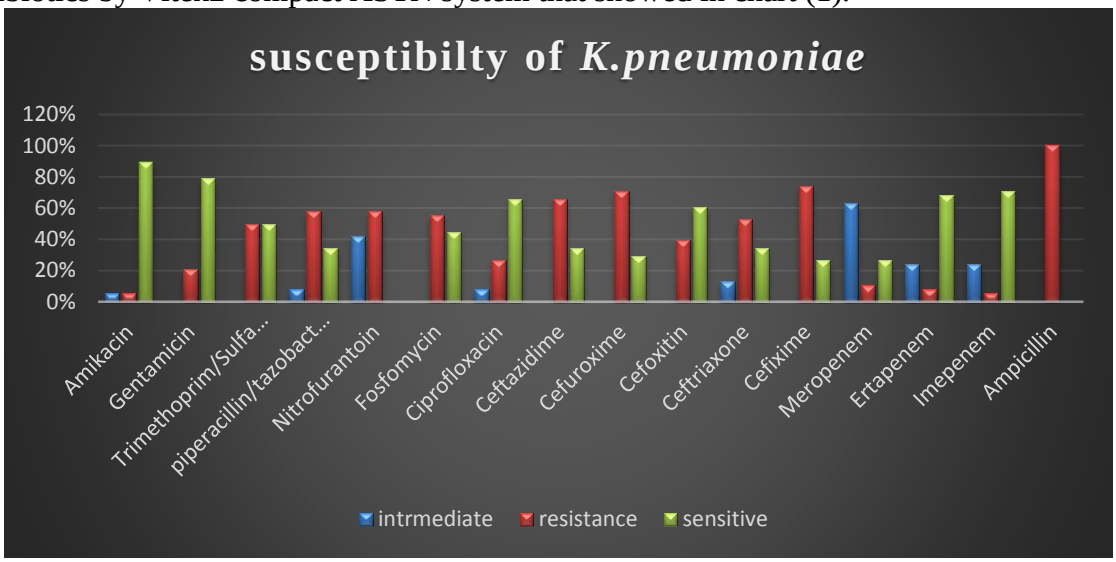

Chart (1): susceptibility of *K. pneumonia* to 16 different antibiotics by Vitek2 compact.

It was found that all isolates were (100 %) resistant to Ampicillin the results was matched with study of *(*Salih *et al*., 2016; Ghotaslou *et al*., 2018) in Iraq and Iran respectively whom isolates gram negative bacteria from UTIs patients. Depending on the results showed in chart (1) The bacterial isolates showed resistance to second and third generation cephalosporin ranged between (50% to 73%) our results with the previous reported by Ghaima *et al*., (2017) in Baghdad province from patients with UTIs and Mansury *et al*., (2016) in Iran, The second cephalosporin generation (Cefoxitin, Cefuroxime) and third generation (Ceftriaxone, Cefixime and Ceftazidime) were concordant with the results of Mishra *et al.* (2013) in India who referred that the resistance of uropathogens to cephalosporin reached to 75%. The resistant against Nitrofurantoin in current study were (57.89%) to *K.pneumoniae* back to the study of Tawgozy and Amin, (2018) in Erbil city

which were convergent to our results, nearly results were found in United State America (Kiley *et al.*, 2018) whom found that the service members who Sustained injured and infected with MDR *K.pneumoniae* in Iraq and Afghanistan were (62 %) resistant against Nitrofurantoin. Results referred also to convergent resistant against Piperacillin /Tazobactam (57.89 %) that similar with Al-Jubori, (2012) study in Baghdad and Al Wutayd *et al.,* (2018) study in Kingdom Saudi Arabia, while in Duhok city was sensitive (100%) (Assafi *et al.,* 2015). Trimethoprim/ Sulfamethoxazole and Gentamicin in this study show low resistant like Khalid and Yassin, (2017) in Duhok city from the clinical specimens including (urine, wound and the middle ear) get convergent result 33% for both of them in ESBL isolates. The current study demonstrated that *K. pneumoniae* possessed a very low-level resistance against Imipenem (10.52%), Ertapenem (7.89%), Meropenem (5.26%) and Amikacin (5.26%) that convergent many studies with UTIs infection in Iraq and worldwide include (Hussein *et al.,* 2018; Majlesi *et al.,* 2018; Veeraraghavan *et al*., 2018) in Iraq, iran and India respectively.

Total DNA was extracted using genomic DNA mini Kits then detected by gel electrophoresis as show in figure (2).

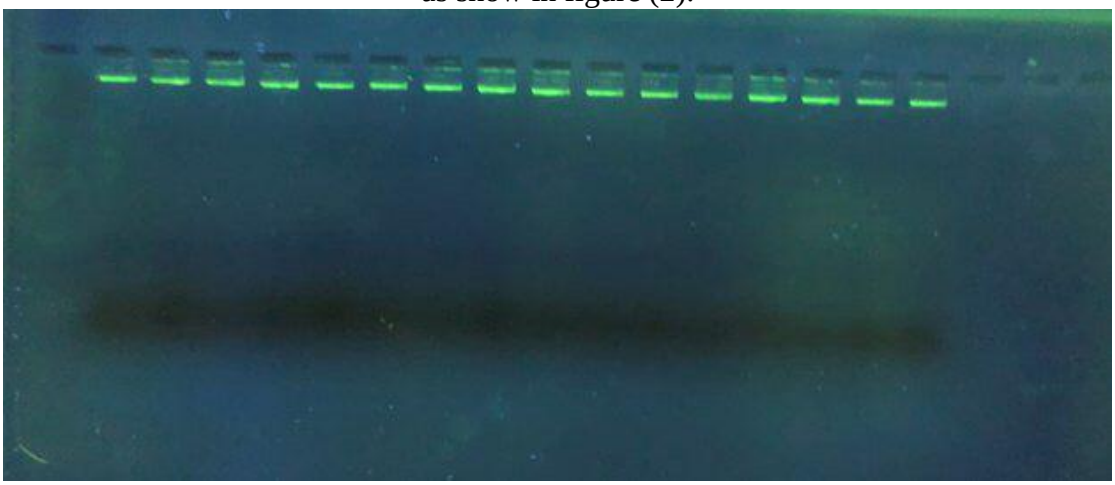

Figure (2): Agarose gel electrophoresis of extracted DNA to check purity and integrity. (70 V/ 30 min.).

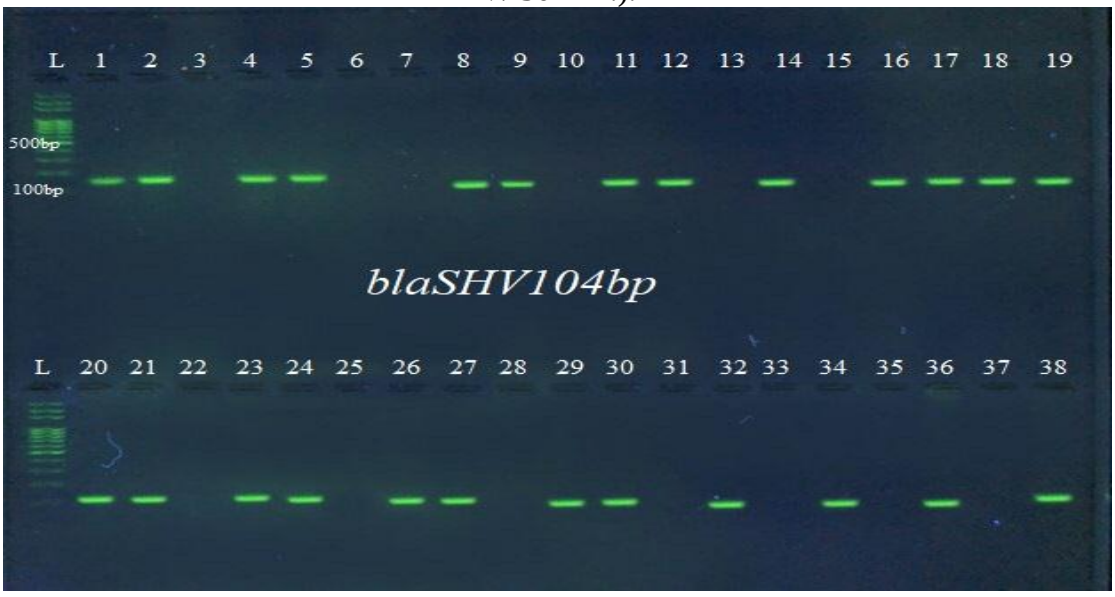

Figure (3): Agarose gel electrophoresis of PCR products for the resistance genes *bla*SHV. Lane L: 100bp DNA ladder; lanes 1-38: *K. pneumoniae* isolates. Positive result in 1,2,4,5,8,9,11,12,14,16,17,18,19,20,21,23,34,26,27,29,30,32,34,36 and 38.

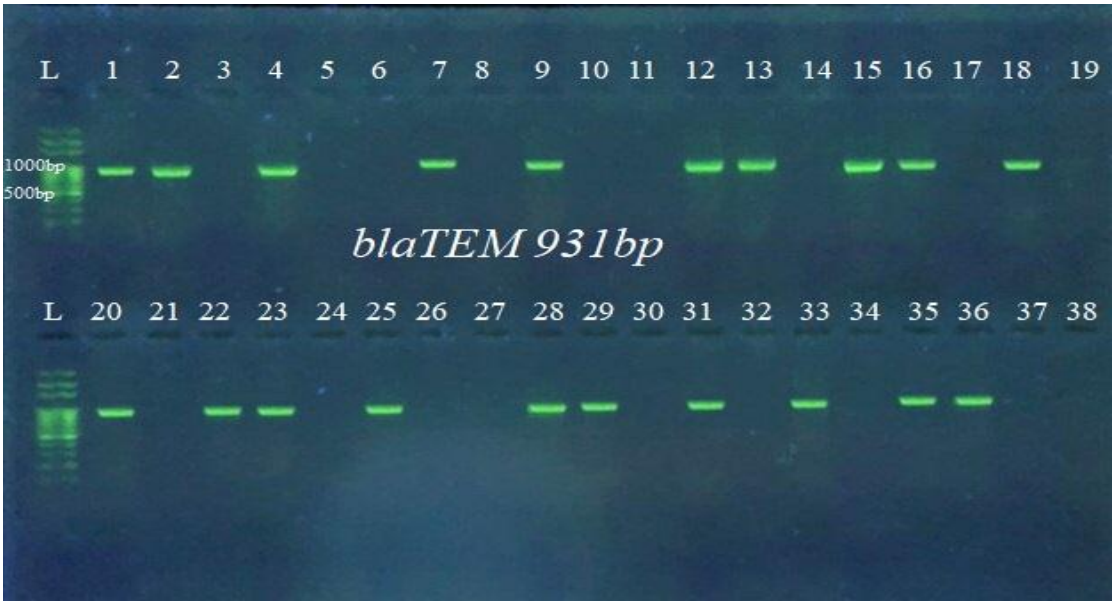

Figure (4): Agarose gel electrophoresis of PCR products for the resistance genes *bla*TEM. Lane L: 100bp DNA ladder; 1-38 *K. pneumoniae* isolate. Positive result in 1,2,4,7,9,12,13,15,16,18,20,22,23,25,28,29,31,33,35 and 36.

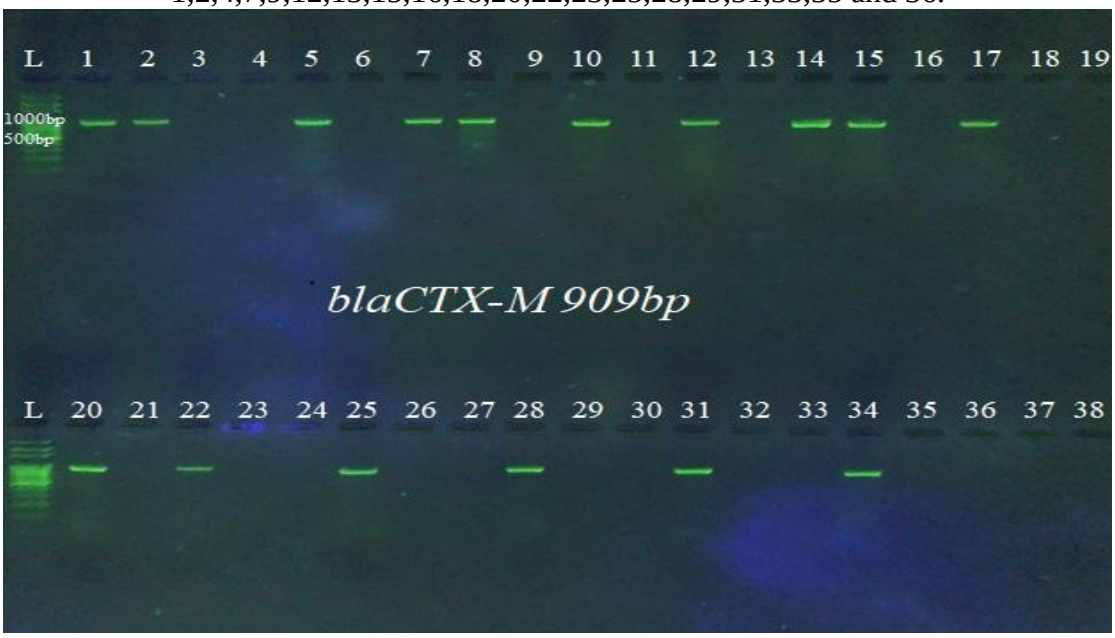

Figure (5): Agarose gel electrophoresis of PCR products for the resistance genes *bla*CTX-M. Lane L: 100bp DNA ladder; 1-38 *K. pneumoniae* isolate. Positive result in 1,2,5,7,8,10,12,14,15,17,20,22,25,28,31 and 34.

Table (3): Genotypes of ESBL Genes Detected in Extended Spectrum β-lactamases-producing *K.pnumoniae* Isolates.

Genotypes	***K.pnumoniae* (n=38)**
One genotype	
*bla*SHV	25 (65.8 %)
*bla*TEM	20 (52.6 %)
*bla*CTX-M	16 (42.1 %)

Number of selective genes in isolates	
All Three genes	4(10.5%)
Two genes	18(47.4%)
One gene	13(34.2%)
No one	3(7.9%)

The results of PCR reaction (Figures, 2 to 5) and Table (3) revealed the results of detection and genotypes of ESBL genes among *K.pneumoniae* isolates were characterized for *bla*TEM, *bla*SHV and *bla*CTX-M genes, 65.8 % of the ESBL genotypes expressed *bla*SHV genes followed by 52.6 % *bla*TEM and 42.1 % for *bla*CTX-M. The results were in agreement with local study by Aljanaby and Alhasnawi, (2017) whom demonstrated that majority of *K.pneumoniae* isolates from patients with urinary tract infection in Al-Najaf city were expressed prevalence with *bla*SHV gene followed by *bla*TEM (55.8%) then (51.2%) for *bla*CTX-M. the majority of the isolates expressed *bla*SHV genes that agreement with Salman, (2019) study in Baghdad also with UTIs patients. Similar findings reported in a study conducted in Turkey among patients with ESBL-producing carbapenem-resistant *K. pneumoniae* strains the most prevalent ESBL gene were *bla*SHV (97%), followed by *bla*TEM (92%), and *bla*CTX-M-1 (62%) (Iraz *et al.*, 2015). While the local study in Baghdad revealed to very low prevalence of *bla*CTX-M (4.5 %) (Salman, 2019). The current study found the percentage of isolates that contain two genes were (47.4%) that relative with Aljanaby and Alhasnawi, (2017) study who demonstrated that *K. pneumoniae* isolates from inpatients with urinary tract infection in Al-Kufa hospital in Al-Najaf province of Iraq, and gave high resistance rate toward ampicillin, 3rd generation cephalosporins and Piperacillin /Tazobactam. Our study show 3 isolates (10.5%) carry all three genes similar with local study for Aljanaby and Alhasnawi, (2017) in Al-Najaf province. Also convergent with Ali and Zahab, (2011) in Egypt whom found 16.7% of isolates contain (*bla*TEM, *bla*SHV and *bla*CTX-M) genes. Our research show 3 isolates (7.9%) although of absence (*bla*TEM, *bla*SHV and *bla*CTX-M) genes there are resistance to antibiotics because of other ESBL genes that the same found with (Aljanaby and Alhasnawi, 2017; Ali and Zahab, 2011) in Iraq and Egypt respectively.

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