DOI Number: 10.37506/v20/i1/2020/mlu/194499

Detection of blaCTX-M gene in Klebsiella sp. Causing UTIs in Al-Yarmouk Teaching Hospital

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Abstract

Background : Klebsiella sp. causes a wide range of bacterial diseases like pneumonia, UTI and sepsis. Therefore, this study was done to assess the prevalence and molecular characteristics of Klebsiella sp. in 50 samples of men and women isolated from Iraqi patients which was suffering from urinary tract infection (UTI) compared with (10) of healthy individuals. All samples were collected from Al-Yarmouk teaching hospital/Baghdad during January – March 2019. Identification of bacterial isolated were done by using manual culturalprocedures and VITEK 2automated (bioMérieux, France)system, while antibiotic sensitivity test of Klebsiella sp. was achieved by Kirby-Bauer and MIC testing was madeby VITEK 2. The creation of ESBL was phenotypically distinguished by twofold plate cooperative energy test as assigned by to the CLSI rules. Location of bla-quality encoded CTX-M was seen by customary P.C.R system. Out of fifty separates ofK. Pneumonia,26%were planted generation of ESBL utilizing CDT, the MIC utilized withdiverse anti-infection agents in this investigation, 13(26%) of secludes by utilizing VITEK2 AST-GN30 which exhibited that, all disconnects were totally impervious to every one of ceftazidime, cefepime and ceftriaxone with MIC(≥16-≥64) µg/ml,and the outcome demonstrated that 61.53% separated of ESBL producion were delicate carbapenem with MIC (≤0.25) µg/ml. Furthermore, PCR examine uncovered that 4(30.76)%of the ESBL creating isolatesharbouredblaCTX-M gene.

Keyword : *Molecular study ; virulence genes ; Klebsiella sp.; urinary tract infections (UTIs)*

Introduction

In human body, the urinary system is a usually sterile andfar from from the colonization ofnormal by innate epithelial barrier and the antibacterial action of the bladder mucosa and the excretion of urine. However, urinary tract infections (UTIs) are represent principal infectious diseases, occurred in all individuals and all age groups(1).

Globally, more than 6 million physician clinics were visits in year due to UTI by two-thirds by women(2). Numerousrevisionssubmitted that daily sexual relationhave a role in occurrence of bacterialvaginosis. this might be sexually transmitted by sex or by therapeutic routs especially when males suffered from these infection though they applied to heavy program of treatment(3,4).

Urinary tract infections are an important health problem for millions of individuals each year (5). Notwithstanding the commonobtainability of antibiotics, it stills the second most public bacterial infection in the population, and not forget that in women more frequent infected with UTI than men(6,7).

The majority of UTI infection occurs with exposure to Klebsiella , it account about 6 to 17% of all nosocomialUTIs and reveal high occurrence in patients with risk, e.g., DM patients. Klebsiellaecausing UTIs are clinically can not be distinguished from UTIs of other origins. So, the aim of this work was to study the role of virulence gene associated with Klebsiella sp. causing UTI in Baghdad hospital(8).

Material and Method

Collection of samples:

Sample of urine were gathered as aseptically as conceivable in sterile holders (general containers or pee packs). mid-stream examples were gathered from the UTIs patients and the control person. All examples were gathered from Al-Yarmouk showing emergency clinic/ Baghdad during January – March 2019. The gathered examples were moved to the research center inside 20 minutes of accumulation.

Cultivation

A calibrated loop measures 0.01ml of urine,using one plate of each a MacConkey and blood agar, the loop touched to center of plate in such a manner and as mentioned in in standard bacterial cultivation procedures in order to produce isolation colonies. "The plates were incubated at 37oC overnight" and examined on the following day for growth. Colonies were counted on each plate. Colonies were morphologically studied on MacConkey agar and blood agar by using a magnifying lens,As well as biochemical tests.

Sensitivity Test To Antimicrobial Agents:

Disc diffusion test (Kirby-Bauer) was used as initial susceptibility test because of its ease of performance, reproducibility and proven values as a guide to antimicrobials therapy.All Klebsiella sp.secludes were societies on MacConkey agar . The antimicrobial helplessness of these detaches was finished by Kirby-Bauer technique and VITEK 2 framework as indicated by the Clinicaland Laboratory Standards Institute(CLSI) rules. CLSI guidlines(9). The MIC for phenotypically ESBL creating segregates was gotten.

ESBL Producion

Emulsions that are potential ESBL producers were isolated by preliminary examination using a nutrient broth to adjust the density of the vaccine equal to the density of the McFarland sour criteria 0.5was performed in all isolates presumed to be an ESBL. In this test, Ceftazidime tablets alone and in combination with clavulanic acid (10)

Extraction of DNA

Beta-mercaptoethanol was added to the DNA restricting support to a last weakening of 0.5% (v/v) i.e., 500 µl/100 ml(2).

1. A measure of 100 mg(wet weight) bacterial cells that resuspended was included up to two hundred µl of water or isotonic cushion, added (750) µl of this Lysis Solution to the cylinder 2 (Figure 3.4).

2. Secure in a dot blender fitted with a 2 ml cylinder holder gathering and procedure at 10,000 x g for five minutes.

3. The ZR BashingBead™ Lysis Tube was centrifuged in a microcentrifuge at 10,000 x g for 1 minute.

4. 400 µl supernatant was moved to a Zymo-Spin™ IV turn channel (orange top) in a gathering tube and centrifuged at 7,000 x g for 1 minute.

5. A measure of 1,200 µl of bacterial DNA restricting cushion was added to the filtrate in the accumulation tube from Step(4).

6. A measure of 800 µl of the blend from Step (5)was moved to a Zymo-Spin™ IIC Column3 in an accumulation cylinder and axis at (10,000) x g for one minute.

7. Dispose of the move through from the Collection Tube and rehash Step(6).

8. 200 µl of DNA pre-wash cushion was added to the Zymo-Spin™ IIC segment in another accumulation tube ,then centrifuged at(10,000 x g) for 1 minute.

9. 500 µl DNA wash support was added to the Zymo-Spin™ IIC section and axis at 10,000 x g for 1 minute.

10. The Zymo-Spin™ IIC segment was moved to a clean 1.5 ml microcentrifuge cylinder and include 100 µl DNA elution cradle legitimately to the segment lattice, centrifuged at 10,000 x g for 30 seconds to elute the DNA.

Detection of CTX-M genes :

identified phenotypically in resistant isolates of Klebsiella sp. by CTX-M genes by used primers targeting blaCTX-M gene. amplification mixture of PCR was prepared according to manufacturer's instructions (Intron,Korea).

A- Primers:

In amplification CTX-M gene , used primers which listed in Table- 1,below:

Table 1: primersequences for detection of blaCTX-M genes(14)

CTX-M gene	Sequence(5'-3')	product Size (b.p)
F R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550

Statistical Analysis

Statistical tables including observed frequencies with their percentages and Graphical presentation by (bar - charts) as well as Chi-Square ($\chi 2$) for calculation ofP. value ($P<0.05$: significant).

Finding

In this study, 26% of Klebsiella isolates were ESBL prodcers as used by CDT methods. Number 13 of Klebsiella sp. Isolate was differed from other isolates in view of cpfalosporin resistance. 100% of Klebsiella spp were with MIC above 16 for CFT and more that 64 MIC for the both antbiotics. Some of Klebsiella sp. Isolates with MDR were resist to imipenem&meropenem with level of 16Mg of MIC/

Figure 1: Typical combination disk method with +ve result.

In this work, all Klebsiella isolates were non-susceptible to CAZ accompanied with increasing in clear zone around CZC. Additionally, majority of isolates extended antibiotic betalactames procing were recoverd from urine samples and lowest rate of them were considere MDR due to stability against 10 discs and as mentioned in Table below.

Table 2:Antibiotic susceptibility of ESBL producing Klebsiella sp. isolates

Isolate number	VITEK 2 system result of MIC									
	Imepenem	**ciproloxacin**	**gentamycin**	**Ceftaz**	**Amp.**	**ceftriaxone**	**tobramycin**	**meropenem**	**FEP**	**LEVO**
1	R*1	R*4	R*1	R*2	R*3	R*1	R*1	R*1	R*2	R*1
2	S*1	R*4	R*1	R*3	R*3	R*2	R*1	S*1	R*2	S*1
3	S*1	R*4	R*1	R*2	S*1	R*2	R*1	S*1	R*2	S*1
4	R*1	R*4	R*1	R*2	R*3	R*2	R*1	R*1	R*2	R*1

R*1*: MIC equal or more 16 Mg/ml

R*2: MIC equal or more 64 Mg/ml

R*3 : MIC equal or more 32Mg/ml

R*4: MIC equal or more 4 Mg/ml

S*1 : MIC equal or lese that 0.25 Mg/ml

Additionally, CEF resistant Klebsiella isolates were further underwent molecular evaluation for ESBL pheonotype using specific procedure for detection of blaCTX-M and according to manufacture instructions. It was observed by above PCR technique that 30.76% of Klebsiella isolates (the 4 MDR isolates) were virtually harboured blaCTX-M genes.

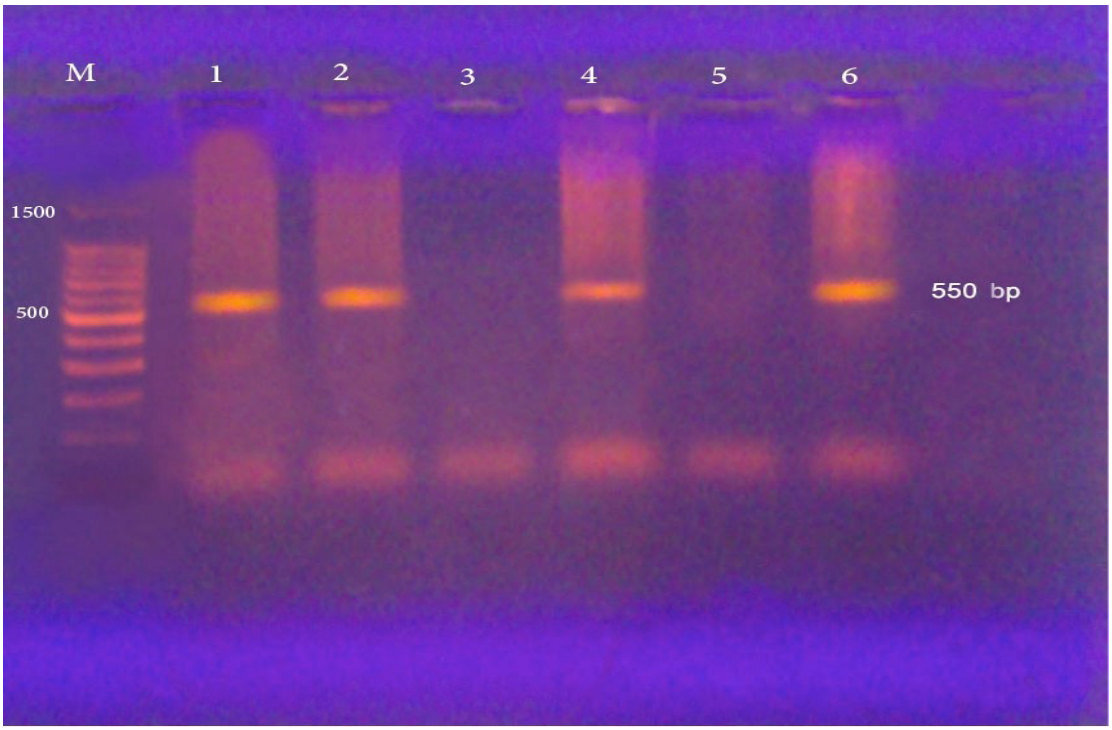

Figure 2: Agarose gel was stained by Ethidium bromide to show amplification of PCR out product with*bla*CTX-M gene (550 bp) primers for *Klebsiella sp.* extracted DNA.M: 100 bp average size reference marker. Lane 1: *K1* shows positive result with *bla*CTX-M gene Lane 2: *K2* shows positive result with *bla*CTX-M gene. Lane 3: *K3* shows negative result with *bla*CTX-M gene. Lane 4: *K4* shows positive result with *bla*CTX-M gene. Lane 5: *K5* shows negative result with *bla*CTX-M gene. Lane 6: *K6* shows positive result with *bla*CTX-M gene.

Discussion

The vast majority of the diseases are procured in clinic settingtherefore, it is accounted for to be the among the 10 most normal nosocomial pathogen in different examinations(9). Nowadays,Klebsiella pneumoniae diseases are entangled by increment in Extended Spectrum Beta Lactamase (ESBL)producing segregates(10,11). Subsequently, this examination is being led with the target to fi nd out the commonness ofESBL delivering Klebsiella pneumoniaein different clinical examples and to find out there affectability design(12).Flare-ups of ESBL-delivering living beings have been depicted. Asymptomatic patients colonized with ESBL-delivering K. pneumoniae can fill in as stores for this pathogen with resulting persistent to-tolerant spread through the hands of medicinal services laborers. What's more, sullied persistent consideration things and fake fingernails worn by human services laborers have been involved in transmission(9).Most examinations have exhibited a poor adherence to contamination control strategies as a significant factor. Flare-ups of ESBL-delivering K. pneumoniae in NICUs have been outstanding for high assault rates and huge quantities of colonized infants(1,2). The neonates at most serious hazard for colonization are those with a more extended length of remain, a lower evaluated gestational age and additionally a lower birth weight.(11,13).Studies directed in Ghana have announced K. pneumoniae as a noteworthy pathogen in charge of UTI (14). A research center based across the nation reconnaissance of antimicrobial obstruction in Ghana by Opintan and collaborators announced that K. pneumoniae spoke to 1.06% of every bacterial disease and 1.4% of Gram-negative bacilli (11).Another study showed an expanded K. pneumoniae opposition of 118.9% ofGram-negative microscopic organisms in their investigation on MDR bacterial diseases in a showing medical clinic(15). Notwithstanding the risk presented by multidrug safe Gram-negative microscopic organisms in social insurance, there is lack of sub-atomic the study of disease transmission ponders. This examination, which structures some portion of a more extensive investigation on the atomic profile of Gram-negative ESKAPE pathogens in a patientsof private clinic, portrays the clonal genealogies, anti-infection resistome and plasmid replicons of a sub-set of K. pneumoniae with protection from the second and third-age cephalosporins utilizing entire genome sequencing (14).

Ceftazidime is one of type of3rd generation cephalosporin used commonly for the treatment K. pneumoniae related infections.Nevertheless, high rate of resistance towad ceftazidime is occurred continuously in clinical managing of infection with these isolates. In the current study, anelevated rate of ceftazidime resistance was detectedin patents infected with Klebsiella sp. , This result similar to results obtained by Aheret al(4) showed ceftazidime resistant Klebsiella. additionally, isolates producing ESBL were with high rate of resistance to all antibiotics testedas compared with non- ESBL producer, otherrevealed a resistance to aminoglycosides fluoroquinoloes, and methoprim in organismsproducingESBL(5). The development and polyclonal spread of CTX-M–delivering K. pneumoniae as happened among detaches with various hereditary foundations. This speculation appears differently in relation to discoveries with respect to KPC-delivering K. pneumoniae: clonal spread of KPC-delivering(K. Pneumoniae) segregates having a place with the ST258 heredity was seen by ZacharCzuket al(7).

In clinical strains,theCTX-M–encoding qualities has normally been situated on plasmids that differ in size from(7 -160)kb(2). Plasmid-intervened transmission of CTX-M qualities in Enterobacteriaceae that includes a few motile.hereditary components has portrayed (7,9).givenstrength of CTX-M-15 genotype,among hereditarily heterogeneous(K.Pneumoniae) secludes, theexamination additionally suggests the same level exchange of a hereditary component conveying blaCTX-M amongK. pneumoniae disconnects. Second, the CTX-M–delivering K. pneumoniae study disconnects displayed high paces of protection from gentamicin and trimethoprim-sulfamethoxazole as percentage(68 and960%respectively , so antibiotic medication as80%, notwithstanding protection from ciprofloxacin (88%) and pipercillin-tazobactam (64%) as portrayed beforehand (16).

Conclusion

In this study, the high rate of beta lactamases production of Klebsiella sp. is necessary to avoid treatment failure condition and need to adopt appropriate control measures to reduce the ESBL. Also, usefulness of PCR technique for detection ESBLs and their effect on antimicrobial resistance. Remarkably, 41.67% of bacterial isolates have three of cephalosporine β-lactamase genes and this could be due to the common used of cephalosporine for treatment.

Conflict of Interest: Non

Source of Findings: Self

Ethical Clearance: Nil

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