Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

Identification of ESBL CTX-M-15 genes from isolates of urinary tract infections.

Zahid S.Aziz Abbas Sh. J. and Salman A. Addoos

Abstract:

The study aimed to evaluate the distribution of CTX-M genes, which coding for ,Extended Spectrum β- lactamases (ESBLs) which hydrolyzed the extended spectrum β- lactams .

Total of 800 urine samples taken from patients suffering from urinary tract infections were collected during the period from February to September from Hospitals in Al-Najaf province.

All samples were cultured on MacConkey agar , were the results revealed that 250 isolates were lactose fermentative versus to 50 isolates were lactose non fermentative.

The isolates succumbed to conventional tests (IMVC), Indol, Methyl red , Voges- Proskauer and Citrate utilization as well as motility test.

Lactose fermentative and non motile isolates were candidate to be confirmed by Vitek2 system. Initial screening tests by Disk Diffusion test and by MacConkey supplemented with 2 mg / liter of Ceftazidime had been conducted, the resultsof disk diffusion test revealed different resistance phenotypes as well as multi drug resistance patterns Ceftriaxone were found to be the most resistant antibiotics in our isolated bacteria with resistance of 100%. The next most resistant antibiotics were Ceftazidime (98.11%), followed by aztreonam (90.57%); and cefotaxime (90.27%). While the result of screening with Ceftazidime reported that 31/53(58.49%) of isolates were initially ESBLs producers.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

The results of genotyping tests showed that out of 53 isolates which had been screened for the prevalence of bla CTX-M genes, 47(88.6%) were positive. Nucleotide sequence of bla CTX-M of amplicons (19, 20, 22, 23, 24) showed high identity (99%) with CTX-M-15 of NCBI-BLAST.

As concerning with protein sequence, the results showed high identity (98%) with reference protein of gene bank. The results of other protein sequences revealed great degree of identity reach to (100%) of identity.

Introduction:

Urinary tract infection is the most common serious bacterial infection causing illness (NICE,2007). It is one of the most common bacterial infections encountered by clinicians in developing countries (Tessema,etal.,2007). One hundred and fifty million patients are diagnosed with UTI yearly, resulting in at least $6 billion in health care expenditures (Tanagho,etal.,2004). In the year 1997 UTI accounts approximately 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations in the United States (Ghedria,etal.,2004). Most of urinary tract infections are caused by gram-negative bacteria like *Escherichia coli, Klebsiella species, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter, and Serratia.* 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria .Gram-positive bacteria include *Enterococcus, Staphylococcus, and Streptococcus agalactiae* (Lazarevic,etal.,1998). The production of ß-lactamases is the major mechanism of bacterial resistance to ß-lactam antibiotics which considered the most widely used class of antibiotic. Curiously, the detection of the first ß-lactamase was reported before the use of penicillin in the medical field. Extended-spectrum-ß-lactams have been introduced

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

in the medical practice in the 1980s for the treatment of serious gram negative bacteria but the resistance to this class of antibiotic has emerged rapidly due to production of a new class of ß-lactamase later termed extended-spectrum ß-lactamase (ESBL) (Al-Jasser, 2006).

In this duration *Klebsiella pneumoniae* was the main ESBL-producer. Later-on, prevalence of TEM and SHV and a new ESBL family, CTX-M which is produced mainly by *E. coli*, has emerged. During the next few years, CTX-M has become the predominant ESBL family and CTX-M-producing *E. coli* has spread globally and has been involved in nosocomial outbreaks and community acquired infections (Canton and Coque, 2006; Marcade *et al*., 2009). The first *bla*CTX-M was detected in clinical *E. coli* isolate in Germany 1990 (Bauernfeind *et al*., 1990) then CTX-M-producing *Enterobacteriaceae* has globally been detected. CTX-M is named after their higher hydrolytic activity against cefotaxime than ceftazidime and the place of first isolation (Munich, Germany). *Bla*CTX-M is a 291 amino acids encoding enzyme and the change in any one of them result in a new CTX-M variant (Naseer and Sundsfjord, 2011).
CTX-M-15 was identified for the first time in 1999 from an isolate from India **(**Karim *et al*., 2001) and reported for the first time in the African continent 2005 in Tanzania (Blomberg *et al*., 2005).

The recent developments in sequencing technologies have created new opportunities to compete with the traditional molecular tools. Sequencing does not require culturing and offers better sensitivity and specificity(Schmieder 2012).

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

Materials and Methods:

Plasmid DNA Extraction :

Plasmid DNA extraction by using High-Speed Plasmid Mini Kit was performed according the protocol of manufactured company (Geneaid, South Korea).

Polymerase Chain Reaction Protocol:

The extracted plasmid DNA from all isolates were subjected to bla CTX-M genes amplifications.

The primers (Bioneer , South Korea) for CTX-M amplification and PCR conditions used as suggested by Messai et.al.(2006) as following : CTX-M F CTA CCG CAG CAG AGT CTT TG and CTX-M R AAC CAG TTT TGC CTT ACC. The premix tube (1 µl Taq DNApolymerase, dNTPs each 250 µM, Tris - Hcl (pH = 9.0) 10mM, KCL30Mm, Mgcl2 1.5 Mm and trace of stabilizer and tracking dye1) completed to 20 µl volume of reaction with recommended amount of DNA template 5 µl of 5-50 ng , 2.5 µl for each primer of 5-10 pmole and 5 µl of deionized distilled water.

The Program was running by Sure cycler 8800 (Agilent ,USA), the program of thermocycling conditions for CTX-M bla genes were as follow: initial denaturation 94ºC for 3 minutes ,then 30 cycles (denaturation temperature 94 ºC for 30 seconds ,annealing temperature 60 ºC for 45 seconds ,72 ºC for 45seconds) followed by final elongation temperature 72 ºC for 10 minutes.

Gel electrophoresis and documentation :

The amplified PCR products were separated in 1% agarose gel after staining with ethidium bromide 5 µl of 0.5 µg / ml , The electric current

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

was set on 75 volt for 1.5 hr. and visualized with UV light, using gel documentation system. The positive results were distinguished when the DNA band base pairs of sample was equal to the target product size compared with molecular DNA ladder(100 bp plus DNA ladder and 100 bp DNA ladder , Geneaid , South Korea) . Finally, the gel was photographed, using Cleaver gel documentation system.

Automated DNA sequencing:

Purification of PCR products from Gel were done according to the instructions of the Manufacturer Company (Geneaid, South Korea) by using Gel / PCR DNA fragments extraction kit. The purified PCR product concentrations were measured by NanoDrop ND-1000 spectrophotometer , then the Taq-terminator amplicon were performed as concerning with directions of protocol of automated DNA sequencing .

For screenig and partial sequncing for our products. Five purified PCR products of positve genes had been candidated for DNAsequencing , the isolates were sent to Republic of Suoth Korea (BIONEER,CO.LTD) and automated sequencer (instument model/name 3730x1Bioneer had been used, then the data interpreted by picking up the data of electophorogram in NCBI-BLASTprogrammes, nucleotide blast and protein blast using blastx program.

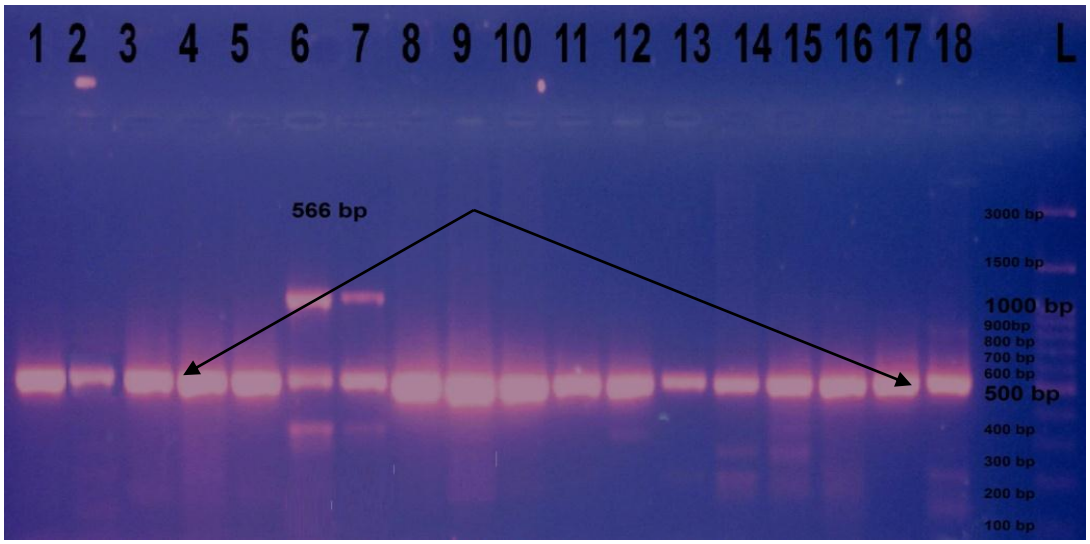
Results and discussion:

Out of 53 isolates which had been screened for the prevalence of bla CTX-M genes , 47(88.6%) were positive. As shown in Figures (1, 2, 3).

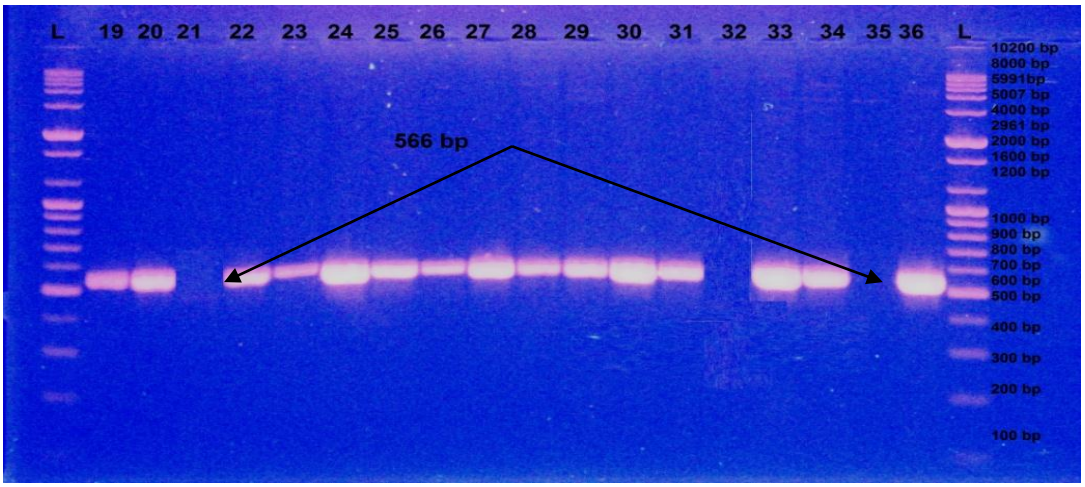
The results of PCR amplification of bla CTX-M genes were appeared in Figures (1,2,3) , the gene bands were appeared in all

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

isolates except (21, 32 , 35) in Figure (2) and (39 ,44,49) in figure (3) respectively.

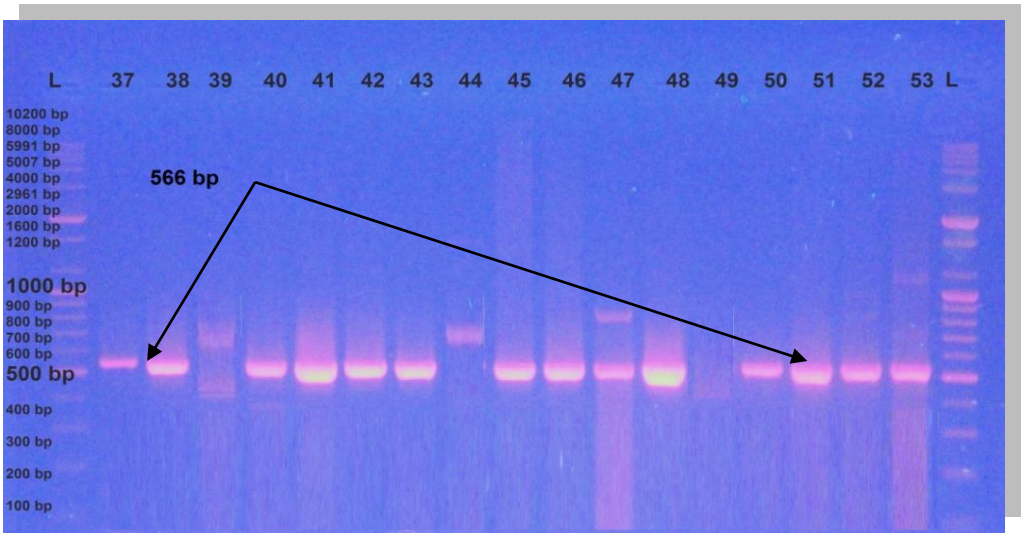


Figure(1): electrophoresis diagram of bla CTX-M PCR amplicon , Lanes (1-18), positive (566bp) and lane (L) , molecular size marker (100-3000 bp).The electrophoresis was performed at 70 volt for 2hrs, agarose gel was stained with Ethidium bromide.



Figure(2): electrophoresis diagram of bla CTX-M PCR amplicon , Lanes (19 , 20 , 22 , 23 , 24 , 25 , 26, 27, 28 , 29 , 31 , 33, 34 , 36)= positive (566bp) , Lane (21,32,35) , Negative and lane (L) , molecular size marker(100-10200bp).The electrophoresis was performed at 70 volt for 2hrs, agarose gel was stained with Ethidium bromide.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/



Figure(3) : electrophoresis diagram of bla CTX-M PCR amplicon , Lanes (37, 38 , 40 , 41 , 42 , 43 ,45, 46 ,47, 48, 50, 51, 52, 53) , positive (566 bp) , Lanes (39, 44 ,49) , Negative and lane (L) , molecular size marker (100-10200 bp) The electrophoresis was performed at 70 volt for 2hrs , agarose gel was stained with Ethidium bromide.

As shown in Table (1) and Appendices (1,2) , the results revealed high degree of similarity between query (sequenced genes) and subject gene (reference genes) of NCBI- BLAST, that could be demonstrated by the high percentage of identity, which range between 98- 99%, with decrease gaps , and the most important category ,E-value , which referred to expected value which in turn indicated the matching between query and subject, the lesser value , the greater matching , this value range between (0- 1).

Table (1) nucleotide and protein sequence of query and subject genes.

Gene	Nucleotide sequence of query and subject					Protein sequence of query and subject				
	Score	Expect value	Identities	Gaps	Accession NO.	Score	Expect value	Identities	Gaps	Accession NO.
CTX-M-15	933 bits 505	0.0	513/516 (99%)	3/516 (0%)	KF055402.1	337 bits 864	1e-117	166/170 (98%)	0/170 (0%)	ACG58889.1
CTX-M-15	929 bits 503	0.0	510/513 (99%)	2l513 (0%)	KF055400.1	337 bits 863	2e-117	164/164 (100%)	0/164 (0%)	AGB07538.1

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

Nucleotide sequence of CTX-M amplicons (19,20,22,23,24) showed high rate of identity (99%) with CTX-M-15 of NCBI-BLAST ,with no gaps (0.0), and E.value (0.0) ,this agreed with results obtained by Liu *etal*.(2013) , Mshana *etal*.(2007) and Huang and Zhu (2013) successively with Accession NO. (KF055402.1) and Accession NO.(KF055400.1). As concerning with protein sequence , the results showed high identity (98%) ,there was no gaps(0.0) and E.value(1e-117) ,these were belong Accession NO. (ACG58889.1),which agreed with that confirmed by Kingsley and Verghese (2008) . And by Pachkunov *etal.* (2013) belong Accession NO.(AGH15625.1) and E.value (2e-117).

The results of other protein sequences revealed great degree of identity with reference of genbank. (100%) of identity, (0.0) gaps and E.value (2e-117), these results were compared with that of Jayahar Bharathi (2012) with Accession NO.(AGB07538.1) and Pachkunov etal.(2013) with Accession NO.(AGH15625.1) with high agreement .

Al-Agamy,etal.,(2009) ,stated that the PCR assays revealed that the prevalence of SHV, TEM and CTX-M genes was 97.3% ,84.1% , and 34.1% respectively, in ESBL-producing isolates. In Pakistan, the prevalence of ESBL-producing *K pneumoniae* was very high (70%). (Shah, etal., 2004) In the Arabian Peninsula , 23.5% of ESBL-producing *K pneumoniae* were identified as having the ESBL phenotype in Kuwait.(Jamal,etal., 2005) However, in the United Arab Emirates, 36% of *K pneumoniae* was found to harbor the ESBL phenotype. (Al-Zarouni etal.,2008) In Jordan, 70% of *K pneumoniae* isolates express ESBL phenotypes and this prevalence is alarming.(Shehabi.2000) Resistance to β-lactams, especially third-generation cephalosporins and non-β-lactams, among clinical isolates of gram-negative bacteria is increasing worldwide (Andrews,2001) .In Iran, the prevalence of *bla*$_{SHV}$ and *bla*$_{TEM}$ among ESBL-producing *K*

pneumoniae was 69.6% and 32.1%, respectively(Shahcheraghi,etal.,2007) The higher prevalence of CTX-M in Kuwait is due to most of the CTX-M being detected in non-Kuwaiti immigrants, mainly from South Asia, where CTX-M is endemic (Ensor, etal.,2008) . In Arab countries, the first description of CTX-M-15 was in Egypt and then in the United Arab Emirates and in Kuwait (Ensor, etal.,2008) CTX-M-15 is the predominant ESBL in Egypt, United Arab Emirates and in Kuwait (Sonnevend,etal.,2006) .

References:

- Al-Agamy MH, Shibl AM and Tawfik AF (2009) Prevalence and molecular characterization of extended spectrum beta lactamase producing Klebsiella pneumoniae in Riyadh, Saudi Arabia. Annals of Saudi Medicine 29 (4) 253-7.
- Al-Jasser. (2006). Extended -Spectrum Beta-Lactamases (ESBLs): Aglobal problem. *Kuwait Medical Journal, 38*(3), 171-185.
- Al-Zarouni M, Senok A, Rashid F, Al-Jesmi SM, Panigrahi D. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med Princ Pract. 2008; 17(1):32–36.
- Andrews JM. BSAC working party on susceptibility testing: BSAC standardized susceptibility testing method. J Antimicrob Chemother. 2001; 48:43–57.
- Bauernfeind, A., Grimm, H. & Schweighart, S. (1990). A new plasmidic cefotaximase in a clinical isolate of *E. coli*. Infection 18: 294-298.
- Blomberg, B., Jureen, R., Manji, K. P., Tamim, B. S ., Mwakagile, D. S., Urassa, W. K., Fataki, M., Msangi, V., Tellevik, M. G., Maselle, S. Y. and N. Langeland (2005) High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum ß-lactamases in Dar es Salaam, Tanzania. *J. Clin. Microbiol.* 43 (2): 745-749.
- Cantón, R., and T. M. Coque. 2006. The CTX-M - lactamase pandemic. Curr. Opin. Microbiol. 9:466–475.

- Ensor VM, Jamal W, Rotimi VO, Evans JT, Hawkey PM. Predominance of CTX-M-15 extended spectrum beta-lactamases in diverse Escherichia coli and Klebsiella pneumoniae from hospital and community patients in Kuwait. Int J Antimicrob Agents. 2008 Dec; 33(5):487–489.
- Ghedira, L., Messaoudi, A., Ben Meriem, C., Guediche, MN. (2004) Profile of antimicrobial resistance of agents causing urinary tract infections in children. Tunis Med. 82(3):299-305.
- Huang,J.and Zhu,N.(2013) pKo6, a novel multidrug-resistant IncN plasmid conferring resistance to carbapenem ,cephalosporins, quinolones,aminoglycosides , trimethoprim , and rifapicin from Klebsiella ozeanae. submitted to the GenBank databases.
- Jamal W, Rotimi VO, Khodakhast F, Saleem R, Pazhoor A, Al Hashim G. Prevalence of extended-spectrum beta-lactamases in Enterobacteriaceae, Pseudomonas and Stenotrophomonas as determined by the VITEK 2 and E test systems in a Kuwait teaching hospital. Med Princ Pract.2005; 14(5):325–31.
- Jayahar Bharathi M., Rameshkumar G., Ramakrishnan R.(2012) **CTX-M type ESBL among ocular isolates.** Submitted to the EMBL/GenBank/DDBJdatabases.
- Karim, A., Poirel, L., Nagarajan, S. & Nordmann, P. (2001). Plasmid-mediated extended-spectrum β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence IS*Ecp1*. FEMS Microbiol Lett 201: 237-241.
- Kingsley ,J.D.and Verghese,S.(2008) Report of CTX-M-15 from Klebsiella pneumonia for the first time in South India , submitted to the GenBank databases.
- Lazarevic, G., Petreska, D., Pavlovic, S. (1998) Antibiotic sensitivity of bacteria isolated from the urine of children with urinary tract infections from 1986 to 1995. Srp Arh Celok Lek. 126(11-12):423-9.

- Liu,L.Wang,X,An.S. and Gao,Z.(2013)Klebsiella pneumonia e insertion sequence IS26,partial sequence;and beta-lactamase TEM-1(blaTEM-1) gene, complete cds. submitted to the GenBank databases.
- Marcade´, G., C. Deschamps, A. Boyd, V. Gautier, B. Picard, C. Branger, E. Denamur, and G. Arlet. 2009. Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum -lactamases. J. Antimicrob. Chemother.63:67–71.
- Messai Y, Benhassine T, Naim M, Paul G, Bakour R.(2006) Prevalence of beta-lactams resistance among Escherichia coli clinical isolates from a hospital in Algiers. Rev ESP Quimioter. 2006 Jun; 19(2):144-51.
- **Mshana,**S.E**., Imirzalioglu**,C.; **Hossain**,H. **Hain**,T.; **Domann**,E.. and **Chakraborty**,T. (2009). Conjugative IncFI plasmids carrying CTX-M-15 among*Escherichia coli* ESBL producing isolates at a University hospital in Germany .*BMC Infectious Diseases* 2009, **9:**97.
- Naseer, U and A. Sundsfjord (2011) The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microb. Drug Resist.* 17: 83-97.
- NICE :National Institute for Health and Clinical Excellence. (2007) Urinary tract infection in children: diagnosis, treatment and long-term management. . NICE clinical guideline 54. Available: http:// www.nice.org.uk/cg054.
- Pachkunov, D. M , Kartsev, N. N, Ageeva, E.N. , Pryamchuk , S.D. and Fursova, N. K. (2013) Beta-lactamase CTX-M-15, partial(Klebsiella pneumonia). Submitted to the GenBank databases.
- Schmieder,R.A.(2012) A framework for identifing antibiotic resistance in the Human microbiome. A Dissertation submitted to the Faculty of Claremont Graduate University and San Diego State University in partial

fulfillment of the requirements for the degree of Doctor of Philosophy in Computational Science.

- Shah AA, Hasan F, Ahmed S, Hameed A. Prevalence of extended spectrum B-lactamases in nosocomial and outpatients (ambulatory) Pak J Med Sci. 2004;19:187–191.
- Shahcheraghi F, Moezi H, Feizabadi MM. Distribution of TEM and SHV beta-lactamase genes among Klebsiella pneumoniae strains isolated from patients in Tehran. Med Sci Monit.2007; 13(11):BR247–250.
- Shehabi AA, Mahafzah A, Baadran I, Qadar FA, Dajani N. High incidence of Klebsiella pneumoniae clinical isolates to extended-spectrum β-lactam drugs in intensive care units. Diagn Microbiol Infect Dis. 2000; 36(1):53–56.
- Sonnevend A, Al Dhaheri K, Mag T, Herpay M, Kolodziejek J, Nowotny N, Usmani A, Sheikh FA, Pál T. CTX-M-15-producing multidrug-resistant enteroaggregative Escherichia coli in the United Arab Emirates. Clin Microbiol Infect. 2006; 12(6):582–585.
- Tanagho, Emil A., Mcaninch, Jack W., editors.(2004) Smith's General Urology.United States of America: McGraw-Hill companies Inc; .Bacterial Infections of the genitourinary tract p 203-227.
- Tessema, B., Kassu, A., Mulu, A.,Yismaw, G.(2007) Predominant isolates of urinary tract pathogens and their antimicrobial susceptibility patterns in Gondar University Teaching Hospital, northwest Ethiopia. Ethiop Med J. 1(61-7).

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

الكشف عن جينات الانزيمات المحللة للمضادات الحياتية بيتالاكتام الواسعة الطيف نوع CTX-M-15 من عزلات التهابات المجاري البولية.

زاهد سعدون عزيز عباس شاكر المحنه سلمان عزيز عدوس

الخلاصة

الدراسة كانت تهدف لتقييم انتشار جينات انزيمات السيفوتاكسيم المقاومة للمضادات الحياتية بيتا لاكتام واسعة الطيف ودراسة تتابع النيوكليوتيدات والبروتين لبعض منها.

جمعت 800 عينة ادرار للفترة من شباط الى ايلول من العام 2012 لمرضى مصابين بالتهابات المجاري البولية من عدة مستشفيات في محافظة النجف الاشرف. جميع العينات تمزراعتها على وسط الماكونكي الصلب , 300 منه نمت على وسط الماكونكي و اذ كانت 250 منها مخمرة للاكتوز بينما كانت 50 منها غير مخمرة للاكتوز. العزلات خضعت لفحوصات الاندول و احمر المثيل وفحص فوكس بروسكاور واسهلاك السترات بالاضافه الى فحص الحركة .رشحت العزلات الخمرة للاكتوز وغير المتحركة لفحص الفايتك 2.

اجريت فحوصات انتشار الاقراص واستخدام الماكونكي الحاوي على 2 ملغم / لتر من السيفتازيديم . نتائج فحص انتشار الاقراص بين انماط مقاومة مختلفة بالاضافه الى انماط تعدد المقاومة , كانت 100% من العزلات مقاومة للمضاد الحياتي سيفترياكزون , بينما كانت المقاومة 98.11% , 90,57% و 90.27% للسيفتازيديم والازترونام والسيفوتاكسيم على التوالي.

بينت نتائج الماكونكي الحاوي على السفتازيدبم 2 ملغم / لتر ان 31 / 53 (58.49%) من العزلات ابتداءاً منتجة لانزيمات المقاومة للبيتا لاكتام الواسعة الطيف.بينت نتائج الفحوصات الوراثية لدراسة انتشار جينات انزيمات المقاومة لمضادات البيتا لاكتام واسعة الطيف ان 47(88.6%) من العزلات كانت موجبة .

دراسة تتابع نيوكليونتيدات لخمسه من العزلات (19 , 20, 22, 23, 24) بينت درجة عالية من التشابه وصلت الى (99%) مع CTX-M-15 لل NCBI - BLAST .

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

فيما يتعلق بدراسة تتابع البروتينات فان الدراسة بينت ان التشابه كان (98%) مع البروتينات القياسية لبنك الجينات كما ان بعض البروتينات اظهرت (100%) من التشابه مع البروتينات القياسية.

Appendix(1) : Alignments between query and subject.

Klebsiella pneumoniae strain F25 plasmid insertion sequence ISEcp1 insertion sequence IS1, complete sequence; and CTX-M-15 (CTX-M-15) gene, complete cds

Sequence ID: gb|KF055402.1|

Score	Expect	Identities	Gaps
933 bits(505)	0.0	513/516(99%)	3/516(0%)

```
Query  13    CGCGG-CGCGGTGCTG-AG-AAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGA  69
             ||||| |||||||||| || ||||||||||||||||||||||||||||||||||||||||
Sbjct  2048  CGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGA  2107

Query  70    GATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC  129
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2108  GATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC  2167

Query  130   GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAA  189
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2168  GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAA  2227

Query  190   TAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGG  249
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2228  TAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGG  2287

Query  250   AGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGA  309
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2288  AGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGA  2347

Query  310   TCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGG  369
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2348  TCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGG  2407

Query  370   TAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCAC  429
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2408  TAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCAC  2467

Query  430   CGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGG  489
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

```
Sbjct  2468  CGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGG  2527

Query  490   CAGCGGTGGCTATGGCACCACCAACGATATCGCGGT  525
              ||||||||||||||||||||||||||||||||||||
Sbjct  2528  CAGCGGTGGCTATGGCACCACCAACGATATCGCGGT  2563
```

extended spectrum b

Sequence ID: gb|ACG58889.1|

Score	Expect	Identities	Positives	Gaps
337 bits(864)	1e-117	166/170(98%)	167/170(98%)	0/170(0%)

```
Query  14   AARC*ESESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQYSDNVAMNK  193
            AA   +SESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQYSDNVAMNK
Sbjct  11   AAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQYSDNVAMNK  70

Query  194  LIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQTLRNLTLGK  373
            LIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQTLRNLTLGK
Sbjct  71   LIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQTLRNLTLGK  130

Query  374  ALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGSGGYGTTNDIA  523
            ALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGSGGYGTTNDIA
Sbjct  131  ALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGSGGYGTTNDIA  180
```

Klebsiella pneumoniae strain F14 plasmid insertion sequence ISEcp1 insertion sequence IS1, complete sequence; and CTX-M-15 (CTX-M-15) gene, complete cds

Sequence ID: gb|KF055400.1|

Score	Expect	Identities	Gaps
928 bits(502)	0.0	507/509(99%)	2/509(0%)

```
Query  14    TTAT-CCCCACAACCCAGGAAGCAGGCAGTCCAGCCTGAATGCTCGCTGCACCGGTGGTA  72
             |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2815  TTATCCCCCACAACCCAGGAAGCAGGCAGTCCAGCCTGAATGCTCGCTGCACCGGTGGTA  2756

Query  73    TTGCCTTTCATCCATGTCACCAGCTGCGCCCGTTGGCTGTCGCCCAATGCTTTACCCAGC  132
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2755  TTGCCTTTCATCCATGTCACCAGCTGCGCCCGTTGGCTGTCGCCCAATGCTTTACCCAGC  2696
```

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.7/ NO.1/ Year : 2015
URL: http://www.kufabiojournal.org/

```
Query  133   GTCAGATTCCGCAGAGTTTGCGCCATTGCCCGAGGTGAAGTGGTATCACGCGGATCGCCC  192
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2695  GTCAGATTCCGCAGAGTTTGCGCCATTGCCCGAGGTGAAGTGGTATCACGCGGATCGCCC  2636

Query  193   GGAATGGCGGTGTTTAACGTCGGCTCGGTACGGTCGAGACGGAACGTTTCGTCTCCCAGC  252
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2635  GGAATGGCGGTGTTTAACGTCGGCTCGGTACGGTCGAGACGGAACGTTTCGTCTCCCAGC  2576

Query  253   TGTCGGGCGAACGCGGTGACGCTAGCCGGGCCGCCAACGTGAGCAATCAGCTTATTCATC  312
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2575  TGTCGGGCGAACGCGGTGACGCTAGCCGGGCCGCCAACGTGAGCAATCAGCTTATTCATC  2516

Query  313   GCCACGTTATCGCTGTACTGTAGCGCGGCCGCGCTAAGCTCAGCCAGTGACATCGTCCCA  372
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2515  GCCACGTTATCGCTGTACTGTAGCGCGGCCGCGCTAAGCTCAGCCAGTGACATCGTCCCA  2456
Query  373   TTGACGTGCTTTTCCGCAATCGGATTATAGTTAACAAGGTCAGATTTTTTGATCTCAACT  432
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2455  TTGACGTGCTTTTCCGCAATCGGATTATAGTTAACAAGGTCAGATTTTTTGATCTCAACT  2396
Query  433   CGCTGATTTAACAGATTCGGTTCGCTTTCACTTTTCTTCAGCACCGCGGCCGCGGCCATC  492
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2395  CGCTGATTTAACAGATTCGGTTCGCTTTCACTTTTCTTCAGCACCGCGGCCGCGGCCATC  2336
Query  493   ACTTTACTGGTGCTGCAC-TCGCAAAGCG  520
              |||||||||||||||| ||||||||||
Sbjct  2335  ACTTTACTGGTGCTGCACATCGCAAAGCG  2307
```

beta-lactamase CTX

Sequence ID: gb|AGB07538.1|

Score	Expect	Identities	Positives	Gaps
337 bits(863)	2e-117	164/164(100%)	164/164(100%)	0/164(0%)

```
Query  509  CSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQ  330
            CSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQ
Sbjct  27   CSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTMSLAELSAAALQ  86
Query  329  YSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQ  150
            YSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQ
Sbjct  87   YSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQ  146
Query  149  TLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVG  18
            TLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVG
Sbjct  147  TLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVG  190
```

Appendix(2): electrophorogram of amplicons:

CTX-Mz-3-CTX-M. docx

CTX-Mz-3-CTX-M2 .docx