DOI Number: 10.37506/v14/i1/2020/ijfmt/192925

Detection of ESBLs*CTX-M* Gene of *E.coli* Isolated from Clinical Cases in Maysan Province

Rabab Naeem Alag[1], ZahidSa′doon Aziz[1]

Department of Biology,College of Science, University of Misan, Maysan, Iraq

Abstract

The antimicrobial resistance are a worldwide increasing and important problem in health care domain. ESBLs represent a main group of β-lactamases enzymes that mostly produced by gram negative bacteria, and give resistance to β- lactam antibiotics, thus the detection of these enzymes are very important for optimal care of patients. The aim of this study was to determine the antibiotic profile with the prevalence of CTX-M gene producing *E. coli* isolates which recovered from clinical cases by phenotypic and genotypic methods. A total of (291) clinical samples (urine, wound swabs, blood and seminal fluids) were included in this study. All bacterial isolates were subjected to the cultural, microscopical, and biochemical examinations methods, confirmed by API 20E and Vitek 2 system. Where the results revealed that 105 of isolates were identified as *E. coli*. Antibiotic sensitivity was performed by using disk diffusion methods. Investigation of extended spectrum β-lactamase(ESBL) production for isolates was performed using Initial screening and double disc synergy method(DDST). The results showed that most isolates showed high resistance to ß-lactam and Cephalosporins antibiotics and vast majority of isolates were resistant to aminimum of three classes of antibiotics, which indicate that identified *E. coli* were multidrug resistant and ESBLs producer. While all isolates were sensitive to Imipenem and Amikacin. PCR technique was performed to detect ESBLs $bla_{\text{CTX-M}}$ gene, the results revealed that (100%) of *E.coli* isolates carried this gene.

Keyword: *Escherichia coli, Antibiotic Resistance, ESBL, CTX-M gene.*

Introduction

β-lactam antibiotics are the most common agents used for treatment of many bacterial infections caused by Gram-negative bacteria, but resistance versus these antibiotic groups occurred rapidly worldwide.[1] Extended spectrum beta-lactamases (ESBLs) are defined as enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin, they are therefore effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam.[2]ESBL-producing organisms are mostly resistant to other classes of antibiotics.[3] Therefore Patients who infected with ESBL producing organism not only have an increased risk of treatment failure and some times lead to death, but also require more health-care resources.[4]The CTX-M enzymes are belong to group of class (A) ESBLs that in general exhibit much higher levels of activity against cefotaxime and ceftriaxone than ceftazidime.[5]Additionally, the CTX-M enzymes have been recognized as the most prevalent among *Enterobacteriaceae* especially *in E.coli*.[6]

Corresponding author:
ZahidSa′doon Aziz
E-mail: dr.aziz_zoonobiology@uomisan.edu.iq, za_h_id1974@yahoo.com

Materials and Method

Bacterial samples collection and identification:

A total of 291 samples, were collected from different clinical cases (urine, wound swabs, blood and seminal fluid) from main Hospitals in Maysan Province during the period from October 2018 to the end of September 2018. Clinical samples immediately transferred to laboratory and culture according to the standard methods, the positive culture were identified by conventional techniques and confirmed by using API 20 E system and using VITEK®2 GN kit (BioMe'rieux, France) then the identified *E. coli* stored for molecular

study.

Antimicrobial susceptibility testing

Susceptibility testing was determined by the agar disk diffusion method,[7] and interpreted according to CLSI guidelines.[8]The following antibiotic disks were used: Ampicillin 10 µg, Pipracillin 100 µg, Amikacin 30 µg, Amoxicillin + Clavulanic acid 20/10 µg, Oxacillin 1 µg, Imipenem 10 µg, Ciprofloxacin 5 µg, Ceftazidime 30µg, Cefotaxime, 30 µg, Ceftriaxone 30 µg, Sulfamethoxazole+Trimethoprime 1.25/23.75 µg, Cefpodoxime 10 µg , Aztreonam 30 µg, Gentamicin 10 µg, Norfloxacin 10 µg, Nitrofurantoin 300 µg, Cefepime 30 µg, Cefoxitin 30 µg, (Bioanalyse, Turkey).

Phenotypic detection of ESBLs

All β-lactamase producing bacterial isolates were tested for ESBL production by initial screening test according to CLSI[8]. The isolates showing resistance to one or more 3rd generation cephalosporins (3GCs) were tested for ESBL production by Double Disc Synergy Test (DDST)[9].

Extraction of Bacterial Chromosomal DNA

DNA extraction was done toward all *E. coli* isolates according to Presto™Mini gDNA Bacteria Kit protocol (Geneaid, Taiwan). The integrity of extracted DNA was tested using Agarose Gel Electrophoresis. The chromosomal DNA then subjected to monoplex PCR.

Molecular detection of bla_{CTX-M} Gene Using PCR Technique

The protocol used depending on manufacturer's instructions(Bioneer, South Korea). The specific primer for bla_{CTX-M} gene have been chosen according to[10,11] where **F:** CGCTTTGCGATGTGCAG, **R:** ACCGCGATATCGTTGGT,which yield a product 550bp. PCR Program was performed as following: (1) cycles of (5 min.) initial denaturation at(94°C), followed by (34) cycles of denaturation at (94°C), (1min.) annealing at (55°C), (1 min.) extension at (72°C), (1 min.) and a final extension step of (5min.) at(72°C).then 5µl of amplified PCR product were loaded to the agarose gel wells with standard molecular weight of DNA ladder (Bionear, South Korea)and the Electrophoresis were ran as described by[12].

Results and Discussion

-Isolation and identification of Bacterial Isolates

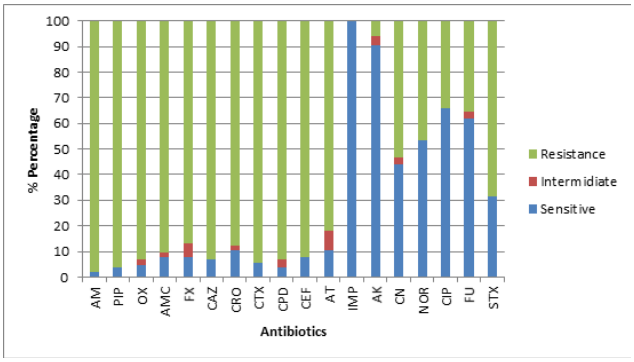
The results of this study showed that among 291clinical samples 235 gave positive growth and 105(44.7%)were identified as *E.coli*. As compared with other studies, our findings were compatible with study[13] where the result was(44.8%), also in India by[14]and in Southwestern Uganda by[15] who obtained results very similar to our results that were (44%) for both. While the results were contrary to the findings of many researchers as.[16,17]*E.coli* is the most gram negative bacteria found in clinical laboratories samples including the majority of urinary, wound, blood and peritoneal isolates.[18]

The antibiotic susceptibility pattern

The resistance patterns of *E.coli* towards various antibiotics were determined

using disc diffusion method. Data in (Figure 2) exhibited that isolates of *E. coli* have the highest level of resistance to Ampicillin where up to (98.1%) were resist to this antibiotic, followed by (96.2%) for Pipracillin and (93.3%) for Ceftazidime, Cefpodoxime and Oxacillin, and (92.4%) for Cefepime, and the resistance to Cefotaxime was (94.3%), while to Amoxicillin/Clavulanic acid the percent of resistance was(90.5%). The resistance to the Ceftriaxone was (87.6%), (86.7%) for Cefoxitin, Aztreonam (81.9%) and (68.6%) for Trimethoprim/Slfamethoxazole while the resistance was (53.3%) for Gentamicin. Whereas the resistance to antibiotic is less than a half for Norfloxacin , Nitrofurantion and Ciprofloxacin (46.7%), (35.2%) and (34.2%) respectively. Moreover this study recorded that all isolates (100%) were sensitive to Imipenem antibiotic followed by (90.5%) for Amikacin. Where these results were corroborated with findings of previous reports[19] which concluded that *E. coli* showed high resistance to Ampicillin and Pipracillin**,** also study conducted by[16] showed high resistance rates of *E.coli* to aztreonam, amoxicillin+clavulanic acid, which compatible with present study. Whilst[20]showed high resistance to third generation (Cephalosporins). The observed high resistance rates in most antibiotic may be due to uncontrolled consumption, consequence of easy access to inefficient and cheap antibiotics moreover could be justified by insufficient adherence to guidelines for infection control as well as inappropriate use of antibiotics. The members of *Enterobacteriaceae* has many mechanisms of resistance to β-lactam antibiotics

like loss of porin and efflux pumps etc, however, β-lactamases enzyme most common and clinically significant mechanism of resistance to β-lactam antibiotics among these bacterial family.[21] The most effective β-lactam antibiotics against *E. coli* was Imipenem where (100%) of *E. coil* were susceptible to this antibiotic, followed by Amikacin. Similar pattern of results were obtained by[22] which appeared high degree of effectiveness of these antibiotics against *E. coli*, also corresponded with the recent study for[23]who considered the antibiotic Imipenem and Meropenem should be preferred drugs for *E. coli* infection isolated from clinical samples.



Figure(1): The percentage of resistance rate of *E.coli* to different antibiotics.

Phenotypic Detection of ESBLs

ESBLs have been found worldwide and they are considered a leading causer of drug resistance in many *Enterobacteriaceae*. One hundred and five of *E.coli* isolates in present study were examined phenotypically and genotypically for ESBL production. The findings of screening test concluded that (82.9%) of *E. coli* were suspected to be ESBLs producers whereas the results of Double disk synergy test was (21.8%) as showed in figures (3 and 4).The results of screening test were similar to local studies of [16,21] who found that screening test was positive in (100%) of *E.coli.* The high occurrence of ESBLs producing *E. coli* obtained in this study was probably due to the consumption of large amount of third-generation cephalosporins, trend of self-medication and the extensive prophylactic misuse of antimicrobials by Iraqi patients and physicians. Additionally a number of previous studies have showed the low prevalence of ESBLs producing *E. coli* when used confirmatory test which in concordance with present study such as the study implemented by[24,25]where the results were(23.8%) and (29.8%) respectively. Many researcher such as[26,27] concluded in their studies that there is incompatible between the results of the phenotypic observation and genotypic analysis and showing that the presence of ESBL-associated β-lactamase genes may be undetected when using the conventional phenotypic approach and mutation in these unexpressed genes may be the leading cause to ESBL antibiotic resistance, suggesting that the unexpressed and undetected genes may serve as reservoir for ESBL genes, therefore phenotypic screening should not be used as monitoring of ESBLs because there had been studies showing the discrepancy in the phenotypic and genotypic detection .

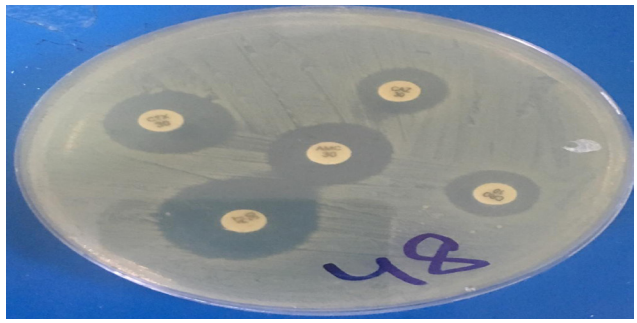
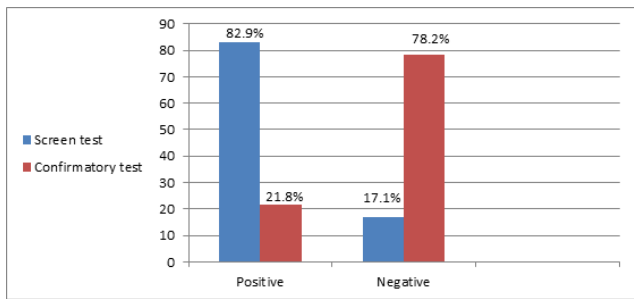


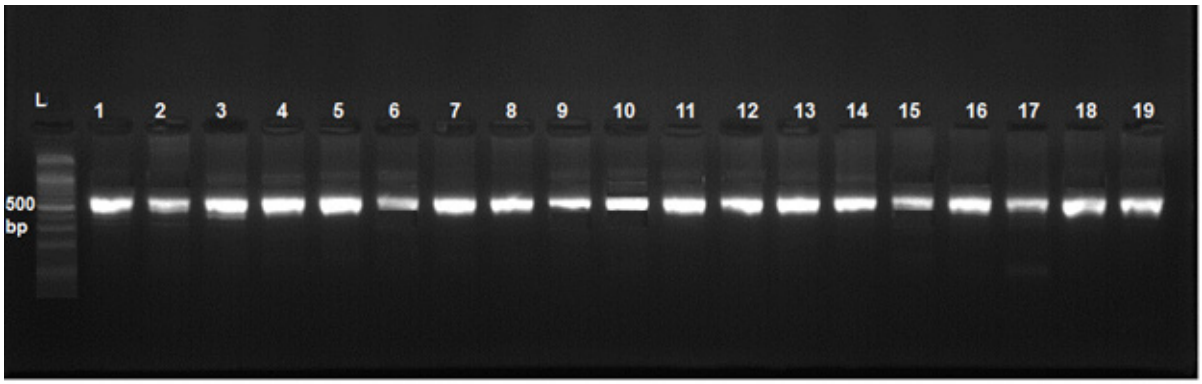
Figure (2): Show the positive result of *E. coli* (n=48) for Double disk synergy test (DDST).



Figure(3): Percentage of screening and confirmatory tests for Identified *E.coli*.

Molecular Detectionof ESBL Genes

The CTX-M type β-lactamases constitute a novel group of enzymes that have a typical ESBL resistance phenotype, are capable of hydrolyzing broad-spectrum cephalosporins and are inhibited by clavulanic acid, They also confer a high level of activity against ceftazidime.[28]As shown in figure (5)the results of molecular study revealed that (100%) of *E. coli* carry the *CTX-M* gene with amplified product 550 bp. The findings were directly in line with previous studies as[29,22,30]who reported that majority of CTX-M ESBL producers were *E. coli*. The CTX-M was considered as a common type of ESBLs that detected at Asia, Europe, North and South America among multidrug-resistant *E.coli*.[31] The important reason for the prevalence of CTX-M β-lactamases gene in Maysan Province may be due to the use of certain



third generation cephalosporins which led to high resistance in our study. Whereas, the results of current study went beyond previous reports[19,32,33] that results were **(56.7%), (28.9%), (10%)** respectively**.**

Figure (4): Agarose gel electrophoresis of PCR CTX-M amplicon (550bp), where L: ladder (100bp) , Lane(1-19) positive results ,the gel stained by ethidium bromide 3µl (0.5 µg/ml) and ran at (65) Volts for one hour.

Conclusion

In this study isolated *E.coli* showed high levels of resistance to most antibiotics especially Beta-lactam group and considered as multidrug resistant bacteria. Furthermore, *E. coli* produced CTX-M in high rate of occurrence reached to (100%). This terrifying situation with the spread of ESBL isolates highlights the need to adopt strict antibiotics using in internal hospitals to assess the effect of high resistance in bacteria and to take steps to reduce this resistance.

Acknowledgment: We would like to thank the staff of Al-Sader Teaching, Birth and Child, and Al-Zahrawi Hospitals for helping us in collection of specimens, and also thank to Heart disease center for facilitating our work.

Financial Support and Sponsorship: Nil.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics of Experimentation: Permission to conduct this study was issued by the Health institutional committee at Al-Sader Teaching, Birth and Child, and Al-Zahrawi Hospitals, Maysan province; and the samples were taken from patients under the supervision of professional health care workers.

References

1- Zaniani F R, MeshkatZ, NasabM N, Khaje-Karamadini M, Ghazvini K, Rezaee A, Hoseini M D. The prevalence of TEM and SHV genes among extended-spectrum beta-lactamases producing Escherichia coli and Klebsiellapneumoniae. Iran JBasic medsci,2012; 15(1): 654-660.

2- GhafourianS, Sadeghifard N, Soheili S, Sekawi Z. Extended spectrum beta-lactamases: definition, classification and epidemiology. Curr Issues MolBiol, 2014; 17(1): 11-22.……

3- Ahmadikiya BF,Mosadegh A, Moradi M. Hossieni-Nave H. Antimicrobial Resistance Patterns and Frequency of Extended-Spectrum Beta-Lactamase Genes among AcinetobacterBaumannii. J BabolUniv Med Sci, 2017; 19(7):28-34.

4- Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Lund O, Kibiki G, Aarestrup F M. Meta-analysis of proportion estimates of Extended-Spectrum-Beta-Lactamase-producing Enterobacteriaceae in East Africa hospitals. Antimicrob Resist. In,2016; 5(1), 18.

5- Shaikh S, Fatima J, Shakil S, Rizvi S M D, Kamal M A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi JBiolsci, 2015; 22(1): 90-101.

6- Day M J, Rodríguez I, van Essen-Zandbergen A, Dierikx C, Kadlec K, Schink A K, Helmuth R. Diversity of STs, plasmids and ESBL genes among Escherichia coli from humans, animals and food in Germany, the Netherlands and the UK. J AntimicrobChemother, 2016; 71(5): 1178-1182.

7- Bauer A W, Kirby W M M, Sherris J C, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol,1966; 45(4_ts): 493-496.

8- CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100.Wayne, PA: Clinical and Laboratory

Standards Institute; 2017.

9- BedenićB, Vraneš J, Bošnjak Z, Marijan T, Mlinarić-Džepina A, Kukovec T, Knežević J, Anušić M, Beader N, Barl P, Leskovar V,Kalenić S. Emergence of CTX-M group 1 extended-spectrum ß-lactamase–producing Klebsiellapneumoniaestrains in the community. Med. Glas,2010;7(1): 32-39.

10- PatersonDL,HujerKM,HujerAM,YeiserB,Bonomo M D, Rice L B, Bonomo R A. Extended-spectrum β-lactamases in Klebsiellapneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV-and CTX-M-type β-lactamases. AntimicrobAgentsChemother, 2003; 47(11): 3554-3560.

11- HujerK M, Hujer A M, Hulten E A, Bajaksouzian S, Adams J M, Donskey C J, Thomson J M. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. AntimicrobAgentsChemother, 2006, 50(12): 4114-4123.

12- Mishra A K, Sharma K, Misra R S. Isozyme and PCR-based genotyping of epidemic Phytophthoracolocasiae associated with taro leaf blight. Arch Phytopathology Plant Protect,2010; 43(14): 1367-1380.

13- Al-Dulaimi T H, Aziz H W, Al-Marzoqi A H, Al-Aziz S A, Mohsin S AA. Molecular characterization and antibiotic susceptibility of diarrheagenicEscherichia coli from Children. Med. J. Babylon,2015; 12(2): 541-550.

14- Singh N, Pattnaik D, Neogi D K, Jena J, Mallick B. Prevalence of ESBL in Escherichia Coli isolates among ICU patients in a tertiary care hospital. JclilDiagncRes,2016; 10(9): DC19-DC22.

15- Andrew B, Kagirita A, Bazira J. Prevalence of extended-spectrum beta-lactamases-producing microorganisms in patients admitted at KRRH, Southwestern Uganda. Int. J. Microbiol, 2017;2017: Article ID 3183076: 5 pages.

16- Al-Hasnawy H H, Saleh R H, Hadi B H. Existence of a ESBL genes in Escherichia coli and Acinetobacterbaumannii isolated from different clinical specimens. J. Pharm. Sci.Resh, 2018;10(5): 1112-1117.

17- Gashe F, Mulisa E, Mekonnen M, Zeleke G. Antimicrobial resistance profile of different clinical isolates against third-generation cephalosporins. J. pharm,2018;Vol 2018, Article ID 5070742, 7 pages.

18- Omololu-Aso J, OluwatoyinO,Omololu-Aso,Egbedokun, A,Olufunmilayo, O, Otusanya, Owolabi A T. Oluwasanmi A V. Antibiotic Susceptibility Pattern of Escherichia coli Isolated from Out-patient Individuals Attending the University College Hospital (UCH), Ibadan, Nigeria. J Infect Dis, (2017); 3(1).

19- Al-Hilali S A M. Genetic affinities of multiple drug resistant uropathogenic Escherichia coli isolated from patients with urinary tract infection in Najaf. Ph.Dthesis Najaf, Faculty of Medicine Department of Microbiology, University of Kufa. 2015.

20- NepalK, Pant N D, Neupane B, Belbase A, Baidhya R, Shrestha R K, Jha B. Extended spectrum beta-lactamase and metallo beta-lactamase production among Escherichia coli and Klebsiellapneumoniae isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal. Ann ClinMicrobiolAntimicrob,2017; 16(1): 62.

21- Jabbar A D. Phenotypic and Genotypic Detection of Extended-Spectrum β-Lactamases (ESBL) among Escherichia coli Isolated from Symptomatic Female's Genital Tract Infection. J.Wassit. Sci. Med,2013; 6(1):59-67.

22- Alfatlawi, A. S., Alsaidi, M. A., Almaliky, N. K., &Alsherees, H. A. A. Molecular characterization of extended spectrum â-Lactamases (ESBLs) producing Escherichia coli isolated from Cholecystitis. EurAsiJ BioSci, 2019; 13(1): 113-120.

23- Cebeci T, Keskin D, Gökal A A. Comparison of Antibiotic Sensitivity Pattern of Escherichia coli which Produce Extended Spectrum Beta-Lactamase Strains Isolated from Various Clinical Specimens in Intensive Care Unit. J HEALTH SCI,2019; 5(1): 33-38.

24- Mohammed Y, Gadzama G B, Zailani S B, Aboderin A O. Characterization of extended-spectrum beta-lactamase from Escherichia coli and Klebsiella species from North Eastern Nigeria. *J ClinDiagnRes*,2016; 10(2): DC07-DC10.

25- Lhwak N S, Abbas Y A. Detection of Extended Spectrum β–Lactmase GeneeCTX-M-1 in

Escherchia coli and *Klebseilla pneumonia* Isolated from Urinary Tract Infection of Pregnant Women in Al-Nassyriah City. *University of Thi-Qar J Sci*,2018; 6(4): 92-96.

26- Xu L, Zhai Y, Lyu Y, Wang Q, An S, Chen J, Gao Z. Identification of *Klebsiellapneumoniae* strains harboring inactive extended-spectrum beta-lactamase antibiotic-resistance genes. *Chin. Med. J.*, (2014); 127(17): 3051-3057.

27- Cruz M C, Hedreyda C T. Detection of Plasmid-Borne β-Lactamase Genes in Extended-Spectrum β-Lactamase (ESBL) and Non-ESBL-Producing *Escherichia coli* Clinical Isolates. *Philipp J. Sci*., 2017; 146(2): 167-175.

28- Bush K, Jacoby G A. Updated functional classification of β-lactamases. *Antimicrob Agents Chemother*,2010; 54(3): 969-976.

29- Jubrael J M. Molecular Characterization of Extended Spectrum B-lactamase Producing *Escherichia coli* Isolated from Urine in Kurdistan Region-Iraq.*Int. j. chem. biol. sci*.,2016;2(2): 51-55.

30- Al-Jelehawy A H j. Molecular Characterization of Antibacterial Resistant Genes in *Escherichia coli* Isolated from Clinical Samples. Thesis Submitted to the Council of the College of Medicine . University of Al-Qadisiyah(2016).

31- Feizabadi M M,Delfani S,Raji N , Majnooni A ,Aligholi M , Shahcheraghi F, Parvin M. YadegariniaD. Distribution of bla(TEM), bla(SHV), bla(CTX-M) genes among clinical isolates of *Klebsiellapneumoniae*at Labbafinejad Hospital, Tehran, Iran.). *MICROB DRUG RESIST J*.,2010; 16(1): 49-53.

32- Al-Mijalli S HS. Extended-Spectrum β-Lactamase Enzymes (ESBLs) Produced by *Escherichia coli* Urinary Pathogens at Riyadh, Saudi Arabia. *J Antimicrob Agents*, 2016; 2(3): 125.

33- AlBarraq A A, El-Badawy M F, Al-Qarni M A, Al-Saedi S S, Al-Sufyani W. M, Osaimi F E, Shohayeb M M. Prevalence of blaTEM, blaSHV and blaCTX-M among β-lactamase producing Escherichia coli isolated from tertiary care hospitals in Taif, Saudi Arabia.*Int. J. Pharm. Biol. Sci*.,2017; 12(1): 11-16.