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Existence of ESBL genes in *Escherichia coli* and *Acinetobacter baumannii* isolated from different clinical specimens

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Abstract

Background: The antimicrobial resistance are a worldwide increasing and important problem in health care field . ESBLs represent a major group of β-lactamases enzymes that mostly produced by gram negative bacteria, and confer resistance to β- lactam antibiotics, so the detection of these enzymes are very important for optimal patients care.

Objective: The aim of this study was to determine the antibiotic profile for the isolates with the prevalence of ESBL producing *E. coli* and *A.baumannii* isolates which recovered from clinical specimens by phenotypic and genotypic methods.

Material and Methods: A total of 100 clinical samples (urine, sputum and swabs of wound, burn and ear) were included in this study. All bacterial isolates were subjected to the cultural, microscopical, and biochemical examinations methods, confirmed by VITEK-2 system. Antibiotic sensitivity was performed by using disk diffusion methods. Investigation of β-lactamase and extended spectrum β-lactamase (ESBL) production for both isolates was performed using rapid standard iodometric assay and double disc synergy method (DDST). PCR technique was conducted to detect β-lactamase genes, *TEM-2* and *SHV* and the gene *OXA-51 like* was used to confirm *A.baumannii* isolates.

Results: The results showed that out of 100 clinical samples, 15 isolates belonged to *E.coli* and 13 isolates belonged to *A. baumannii*. *E.coli* and *A.baumannii* isolates showed resistance to ß-lactam antibiotics and vast majority of isolates were resistant to a minimum of three classes of antibiotics, hence the isolates were considered to be multidrug resistant. Phenotypically, the results revealed that (100%) and (15.3%) of *E. coli* and *A.baumannii* isolates produced β-lactemases while Extended spectrum β-lactemases (ESBLs) production found in (100%) and (23%) of *E. coli* and *A.baumannii* isolaterespectively. PCR technique was performed to detect ESBLs genes *bla -TEM2* and *bla-SHV*, the results revealed that (86.6%) and (6.6%) of *E.coli* isolates carried *bla-TEM2*, and *bla-SHV*, while (15.3%) and (7.6%) of *A.baumannii* isolates carried *bla-SHV* and *bla-TEM-2*genes respectively.

Conclusion: There are a high resistance of bacterial isolates to most antibiotic especially to β –lactam antibiotics. ESBL genes distributed in clinical isolates of *E. coli*.

Keyword: ESBL, β-Lactamase, Multidrug-resistant, *E. coli*, *A.baumannii*

INTRODUCTION

Resistance of pathogenic organisms to antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to antibiotics resistance phenomenon(1). 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 (2). ESBLs are most common type of β-lactamase enzymes, have emerged as an important mechanism of resistance to β-lactam antibiotics in *Enterobacteriaceae* (3). In general, they are most frequently found in *K.pneumoniae* and *E.coli; however*, many publications have reported their growing occurrence in other organisms of the family *Enterobacteriaceae*, including: *Citrobacter spp., Proteus* spp., *Providencia spp., Serratia marcescens, Enterobacter spp.,* and others(4).

ESBL are mutant forms of broad spectrum β-lactamases like the *TEM-1*, *TEM-2* and *SHV-1* enzymes coded by genes placed on plasmids, which can easily spread from one bacteria to another. Since then, several other ESBL enzymes such as *CTX* and *OXA* have been reported in different parts of the world(5). ESBL enzymes capable of hydrolyzing and inactivating wide variety of β-lactams, including third generation cephalosporins , penicillins and monobactams(6). ESBL-producing organisms are also usually resistant to antibiotics of other classes such as, tetracyclines, aminoglycosides, fluoroquinolones, chloramphenicol and trimethoprim-Sulfamethoxazole(**7**).Patients infected with ESBL producing organism not only have an increased risk of treatment failure and sometimes lead to death, but also require more health-care resources(8).

This study was conducted to investigate the antibiotic resistance pattern and study the prevalence of ESBL among *E. coli* and *A.baumannii* isolates by using the phenotypic and genotypic method.

MATERIAL AND METHODS

Bacterial samples collection and diagnosis

A total of 100 clinical samples (burn,wound and ear swab, urine and sputum) were collected from patients attended hospitals (Al-Hilla Teaching Hospital and Babylon Hospital for Maternity and children) during a period of six months. The samples were immediately inoculated on blood agar and MacConkey agar, then incubated for overnight at 37°C under aerobic conditions. All *E. coli* and *A.baumannii* bacteria was isolated and identified according to their diagnostic characteristics and then compared with their being reported in referential references Collee *et.al*, (9)and MacFaddin(10). The isolates were confirmatively diagnosed by VITEK2 system by using VITEK®2 GN kit, then stored at maintenance medium until further tests.

Antimicrobial susceptibility testing:

The antimicrobial susceptibility pattern of bacterial isolates to different antibiotics was determined using disk diffusion test(Kirby-Bauer method)and interpreted according to CLSI guidelines CLSI (11). The following antibiotic disks were used: penicillin 10 U, 10 µg, cefixime 5 µg, ampicillin 10 µg, amikacin 10 µg, Amoxicillin + Clavulanic acid 30, Amoxicillin 25, Methecillin 10, Oxacillin 1, Piperacillin 30, Imipenem 10, cloxacillin 1µg, ciprofloxacin 10 µg, carbencillin 25 µg, Ceftazidime 10, Cefotaxime, 10 , Sulfamethoxazole+Trimethoprime 25, Tetracyclin 10, ceftriaxone 10 µg (Bioanalyse.UK).

Screening test for β-lactam resistance:
Ampicillin and amoxicillin (β-latams) were added, separately, from the stock solution to the cooled Muller-Hinton agar at final concentrations of 50 and 100 µg/ml, respectively. The medium was poured into sterilized Petri dishes and stored at 4˚C. Preliminary screening of bacterial isolates resistant to both antibiotics was carried out using pick and patch method on the above plates(12).

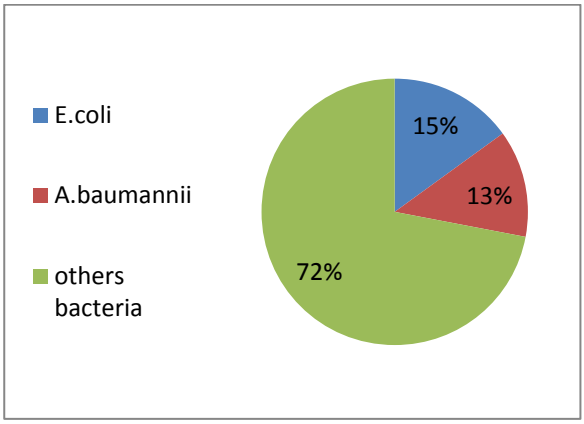
Detection of β-lactamases production
Production of β-lactamase test was done for bacterial isolates by using the rapid idometric method(9).

Phenotypic detection of ESBLs
All β-lactamase producing bacterial isolates were tested for ESBL production by initial screen test. The isolates showing resistance to one or more 3rd generation cephalosporins(3GCs) (i.e. cefotaxime and ceftazidime) were tested for ESBL production by Double Disc Synergy Test (DDST) according to CLSI guidelines(13).

Molecular detection of β-lactamases
DNA was extracted according to the genomic DNA purification kits supplemented by manufacturer company (promega, U.S.A.). ESBL genes (*bla TEM-2,bla-SHV*) were detected by PCR. PCR assay was also performed for detection of *bla OXA-51-like* gene for molecular confirmation of *A. baumannii* isolates at the species level. Each 25µl of PCR reaction mixture contained 1 µl of upstream primer, 1 µl of downstream primer, 9.5µl of nuclease free water, 1 µl of DNA extraction and 12.5 µl of master mix. The primers of *bla TEM-2, SHV,OXA-51* used to generated 374, 867and 501 bp fragments respectively. Thermal cycler conditions are shown in the table(1). The amplification products were separated in 1% agarose gels containing ethidium bromide. DNA ladder 100bp (Bionear, Korea) used for compare. After electrophoresis, the gel was photograhed under UV light.

RESULTS AND DISCUSSION

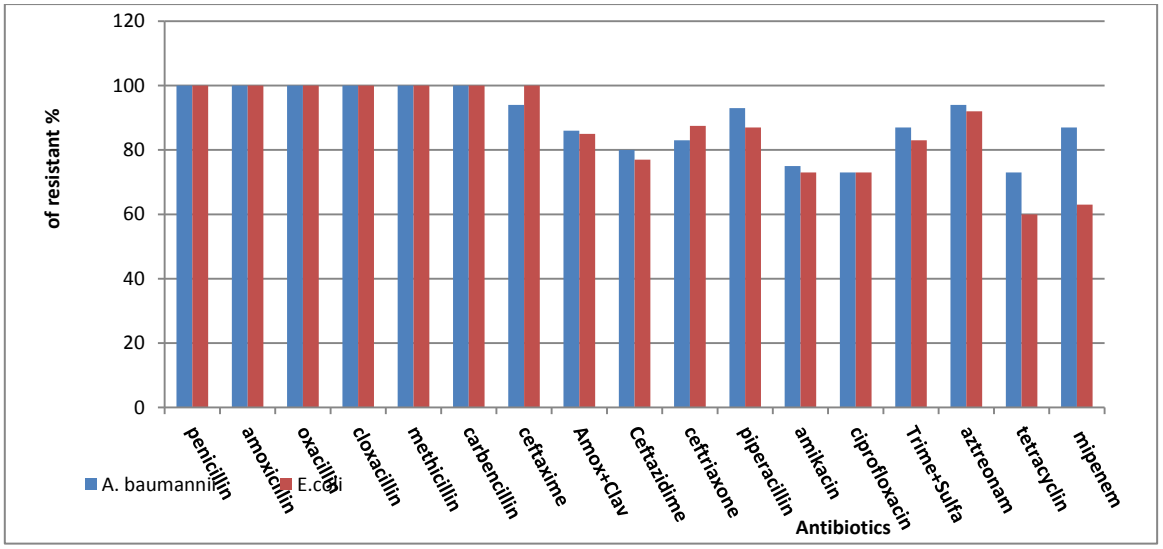
Isolation and identification of Bacterial Isolates
The results of this study showed that among 100 clinical specimens, 15(15%) isolates were identified as *E.coli* and 13(13%) isolates were identified as *A.baumannii* (Fig1).
Compared with other studies, these finding which is in concordance with other reports, who isolated *E. coli* at similar rate (13.8%)(14). kibret *et.al.*,(15) and Gautam (16) also reported similar findings. This finding was lower than a studies of (5,17,18). *E.coli* most gram negative bacteria found in clinical laboratories samples including the majority of urinary, ,wound, blood and peritoneal isolates (19). *E.coli* is a common inhabitant of the human and animal, intestine, but can also be detected in water, soil and vegetation. It is the leading pathogen causing urinary tract infections and important pathogens causing blood stream infections, otitis media, wounds infections, and other complications in humans. It's also the common cause of food and water-borne infections(18).
Regarding *A.baumannii*, similar results found by(20,21) that frequency of *A.baumannii* was (16.6%) and (15%) respectively. But, this finding was lower than others studies (22,23,24,25) recorded lower finding .
A.baumannii is widely distributed in nature (26). Although, it has always been considered a low virulence microorganism , sometimes it can be highly pathogenic and cause invasive diseases. Its common nosocomial pathogens worldwide. It's one of the most difficult pathogens to treat and features contributing to *A. baumannii* pathogenicity are resistance to a wide range of antimicrobial agents and to environmental conditions, persistence in the hospital environment, and the tendency for epidemic spread. *A. baumannii* tolerance desiccation better than other *Acinetobacter* spp. with its capacity to form biofilm that involved in attachment of the cell on epithelial cell and smooth surfaces of medical instruments such as urinary catheters and lung tubes(24).



Fig(1) Percentage of *E.coli* and *A.baumannii* isolated from clinical samples

Table(1) Primers sequences and thermal cycler conditions

Genes	Primer sequence (5′-3′)	Size of product bp	PCR condition	Reference
TEM2 **F** TEM2 R	ACT GCG GCC AAC TTA CTT CTG CGG GAG GGC TTA CCA TCT G	374	95°C/ 5 min 1X 94°C /30s 62°C/30 s 30X 72°C/30 s 72°C/10min 1X	(Kaye *et al.*, 2004)
SHV **F** SHV **R**	GGT TAT GCG TTA TAT TCG CC TTA GCG TTG CCA GTG CTC	867	96/5 min 1X 96/1 min 60/1 min 35 X 72/1 min 72/10 min 1X	(Ferreira *et al.*, 2011)
*OXA51-ike*gene **F** *OXA51-ike* gene **R**	TCGACCGAGTATGTACCTGC TTGAGGCTGAACAACCCATC	501 bp	94 5min. 1X 94 1min 58 /45sec. 35 X 72 1min. 72 5min. 1X	AL-Masoudi.,2015)



Figure(2) The resistant rate of *E.coli* and *A. baumannii* to the antibiotics

The antibiotic susceptibility pattern

Resistance of gram- negative bacteria isolated from clinical samples to antibiotics has been increased worldwide. The resistance patterns of *E.coli* and *A. baumannii* towards various antibiotics were determined using disc diffusion method. Data in (figure 2)exhibited that all isolates of *E.coli* were fully resistant (100%) to penicillin, amoxicillin, methicillin, oxacillin,cloxacillin, carbencillin, ceftaxime. Also, the isolates showed high resistance to aztreonam (92%), amoxicillin+clavulanic acid (86%), ceftriaxone (87.5%), piperacillin(87%), Trimethoprim+Sulfamethoxazole (83%), ceftazidime (77%), amikacin (73%), ciprofloxacin (73%), and mipenem (63%), tetracyclin(60%).

These results was corroborated with findings of previous reports that *E. coli* isolates showed high resistant to penicillins and most cephalosporins antibiotics(27). Almohana and Al-Salamy reported high resistance rates among *E.coli* isolates for ampicillin, cephalothin, carbenicillin, ciprofloxacin and trimethoprim, while intermediate resistance rates obtained for ceftriaxone, kanamycin and nitrofurantoin (28).

E. coli showed a high resistance rate (73%) to amikacin , which is in agreement with the findings of other researchers (29) ,while other studies(30,31) revealed a lower resistant. The resistance to ciprofloxacin in the present work was observed in 31% of the isolated *E. coli* which was in agreement with other studies (27). Flouroquinolones are particularly used for the treatment of UTI because a high drug concentration in the urine can be obtained (29).

Regarding *A.baumannii*, isolates were highly resistant(100%) to penicillin, amoxicillin, methicillin, oxacillin, cloxacillin and carbencillin, While the resistance toward ceftaxime (94%), aztreonam (94%), piperacillin (93%), ceftriaxone (83%), Amoxicillin+ Clavulanic acid (85%), ceftazidime (80%), , mikacin(75%), 87 % to Trimethoprim-Sulfamethoxazole(87%),mipenem 87%), tetracyclin(73%), ciprofloxacin (73%)(Figure2).

These data were compatible with a previous local studies, like (32) that *A. baumannii* showed high resistance to most antibiotic:100% resistance to amoxicillin, amoxicillin-clavulanic acid, cefotaxime and (97.3%) to aztreonam, (97%) to ceftriaxone, (89.5%) to ceftazidime, (83.4%) to ciprofloxacin, (86%) to trimethoprime-sulphamethoxazole ,(72.1%) to amikacin and (58.2%) were resistant to both imipenem and meropenem(7). Alkasaby stated that *A. baumannii* were highly resistant to imipenem, ceftazidime, sulfamethoxazole/trimethoprim, piperacillin/ tazobactam, ciprofloxacin, tetracycline, amikacin and ciprofloxacin(33). The increasing prevalence of drug-resistant *A. baumannii* has caused concern in global medicine and this could possibly be due to the ability of bacteria to resist many disinfectant and antibiotics or could possibly be due to selective pressure or misuse of the drugs in the hospital(34).

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(6).

In current study, most bacterial isolates were resistant in at least one agent in ≥3 antimicrobial agents class, which means they were MDR. In a large number of previous studies, MDR strains have a high percentage of *E.coli and A.bumannii* isolated from clinical specimens. The appearance of MDR isolates of *E.coli and A.bumannii* has caused many problems in the treatment of these isolates(7). This finding can be justify by inadequate adherence to infection control guideline and also inappropriate use of antibiotics.

Phenotypic Detection of β-lactamase Production

ESBLs have been found worldwide and they are forming a leading contributor of drug resistance in many *Enterobacteriaceae*. Bacterial isolates were surveyed phenotypically and genotypically for ESBL production. Phenotypically, β-lactamase production were observed in (100%) and (15.3%) of *E. coli* and *A.baumannii* isolates respectively, while the results of the combined disk test showed that (100%) and (23%) of *E. coli* and *A.baumannii* isolates produce ESBL(table 2).

Table (2) Number and percentage of β-lactamase and ESBLs- produced isolates

Bacterial isolates	No. of tested isolates	β-lactamase positive isolates	ESBLs positive isolates
E.coli	15	15(100%)	15(100%)
A.baumannii	13	2(15.3%)	3(23%)

χ^2=18.6198, Pvalue=0.002

Table (3) Number and percentage of ESBL genes among isolates

Bacterial isolates	No. of tested isolates	No. (%) of *TEM2* gene positive isolates	No. (%) of *SHV* positive isolates
E.coli	15	13 (86.6%)	1 (6.6%)
A.baumannii	13	2(15.3%)	1(7.6%)

χ^2=30.867, Pvalue=0.0009

Kadhim *et.al.*, in his study found that β-lactamase test was positive for (91.9%) of *E.coli* isolates, while (4.8%) of isolates showed positive result for ESBL(30).

In study done by Al-Hilla, it's found that (96.4%) of *E.coli* isolates were able to produce extended-spectrum β-lactamases (ESBLs)(31). A number of previous studies have showed the high prevalence of ESBLs producing *E. coli*(36)(37).Current results showed high incidence of ESBL producing *E. coli* isolates in compare with published data from countries such as Kuwait, Lebanon, Iran, France, Spain ,Thailand and Pakistan(38). The high incidence of ESBL-producing isolates 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.

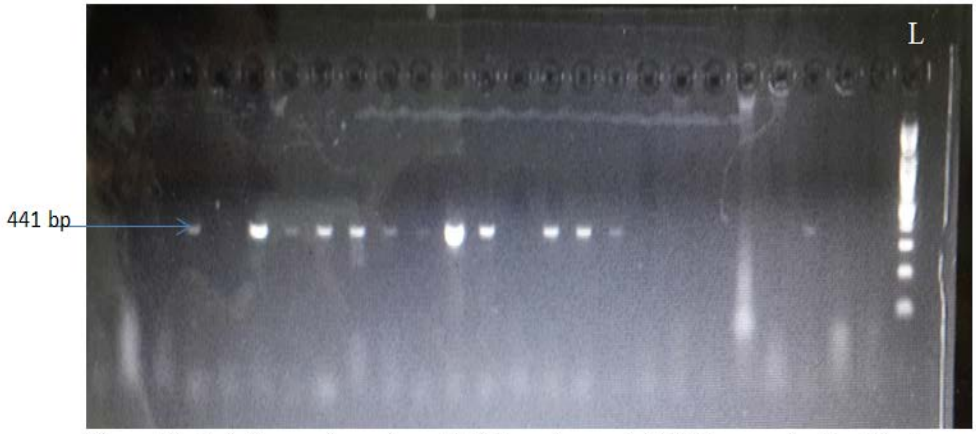
It has been noticed that ESBL producers *E. coli* bacteria are more likely to resist other non β-lactam antimicrobial agents. This MDR may be due to plasmid carrying many genes coding multiresistance which are transferred from one bacteria to another(27).

Our study showed that (15.3%) and (23%) of *A. baumannii* isolates were β-lactamase and ESBL producing respectively. This finding is comparable with results reported by (39,40,34). ESBL production by *A.baumannii* identified in this study lower than reported in India (41). The first mechanism of resistance in *Acinetobacter* to β-lactam drug by β-lactamases production that carried on plasmids or chromosome and protect bacteria from the effects of antimicrobial agents(42). The prevalence of ESBLs among clinical bacterial isolates varies widely in different geographical areas and are quickly changing over time. This variation may be due to difference in antibiotics use between different localities especially β-lactam antibiotics and also different method used for detection(2)(4).

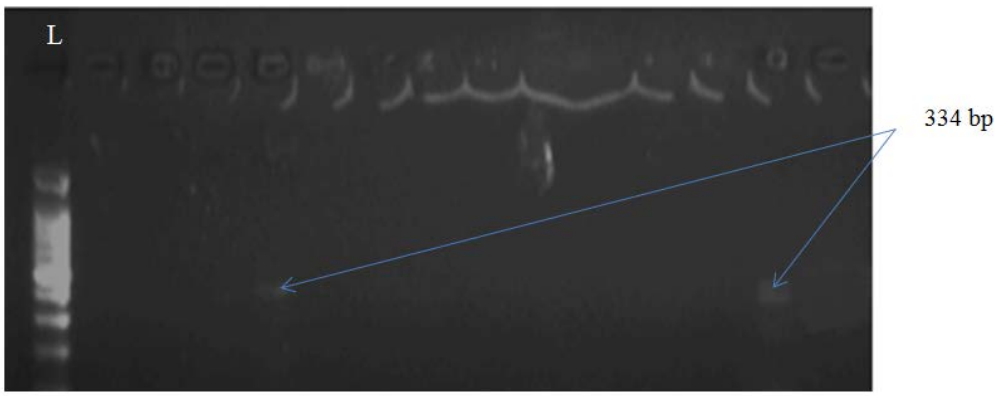
Molecular Detection of ESBL Genes by PCR

The ESBL phenotype of bacterial isolates was correlated with the genotyping results. Genotypically, 13(86.6%) and 1(6.6) of *E.coli* isolates were harboring *blaTEM* and *blaSHV* genes respectively(figure3&4) (table3).

Our finding consist with Rastegar Lari study regarding *TEM-2* gene, in which 85.6% of ESBLs producing *E. coli* had *TEM-2* gene, but *SHV* gene prevalence (69.2%) was much higher than our results(2).



Figure(3) Gel electrophoresis of PCR product of *TEM-2* gene in *E.coli* isolates, L= DNA molecular size markers (100 bp), 1 to 15 represent number of isolates



Figure(4) Gel electrophoresis of PCR product of *SHV* gene in isolates, L= DNA molecular size markers (100 bp), 1 to 15 represent number of isolates

In study done in Thailand, found that 78% of . *E. coli* isolates had *bla TEM-2*, another study in German found similar results(3). *TEM2* gene has high frequency compared to SHV gene ; a fact which is similar to previous studies (43) but it was different compared to Taşli *et al* and Ramazanzadeh's results (44). Zaniani mentioned in his study that the SHV gene was not detected in ESBLs producing *E. coli* isolates(2).

The *blaTEM* -2 was the most prevalent β-lactamase gene in *E. coli*. The *TEM* β-lactamases spread worldwide and it is found in many *Enterobacteriaceae*. This may be due to its presence on highly mobile genetic elements that promote its spread among bacteria and that it is one of first genes mediate drugs resistance and decreased susceptibility to first and second generation cephalosporins(36).

Regarding *A.baumannii* isolates, *blaTEM* -2 was detected among (15.3%) of isolates followed by *bla-SHV* (7.6%), *A.baumannii* strains were identified by amplifying blaOXA-51 gene by PCR technique. The *Oxa-51 like* gene detected in all of the isolates. Fazeli *et.al*., supported the findings from this study regarding prevalence of *bla-SHV* among *A. baumannii*(45). Previous research done by Safari *et.al*., detected *SHV* and *TEM-2* among (58%)and (20%) of *A.baumannii* isolates respectively (46). Compared with other studies, this finding was lower than a study of (42), who found that *TEM-2* expressed highly *A. baumannii* isolates, also most isolates were harboring *HSV* genes. ESBLs identified in *Acinetobacter spp*. were *TEM-2*, *SHV*, *PER* and *CTX*, and the most popular was also *TEM*. *TEM* could be mediated by plasmid and it's recorded to be associated with resistance to sulbactam in *A. baumannii* (47).

The *OXA-51-like* gene was detected in all *A.baumannii* isolates. This is accordance with others reports like (33,45,48,49). In the present study the existence of the *OXA-51-like* gene was investigated to prove *A.baumannii* strains. *OXA-51-like* genes are one of the most important means of resistance to carbapenem in *Acinetobacter* (which occurs inherently in all *A.baumannii* strains and is chromosomal coded. The trace of this gene is an accurate and sensitive way for detection of *A.baumannii* bacteria compared to the widely used biochemical tests(28).

This high antibiotic resistance could be explained by the fact that easily available of drugs in our locality, which taken from pharmacy without doctor instruction and are relatively inexpensive antibiotics. Also, inadequate quantity of these agents are sometimes taken for treatment of many infections types which may result in the development of high rate of resistance.

CONCLUSION

E. coli and *A.baumannii* important cause of nosocomial infection. Both bacteria showed high levels of resistance to most antibiotics, therefore, spreading of multidrug resistant bacteria represent a major problem in the area of infection disease. *E. coli* produced β-actamase and ESBL in high percentage. *TEM-2* genes are predominant gene in *E.coli* followed by *SHV* genes, while *A.baumannii* isolates showed low incidence of β-lactamase genes. The alarming situation with dissemination of ESBL producing *E. coli* isolates highlights the need for strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing this resistance.

REFERENCES

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

2- Zaniani, F.R.; Meshkat, Z.; Nasab, M.N.; Khaje-Karamadini, M.; Ghazvini,K;, A; Esmaily, H; Nabavinia, M.S. and Hoseini, M.D. (2012). The Prevalence of TEM and SHV Genes among Extended-Spectrum Beta-Lactamases Producing *Escherichia Coli* and *Klebsiella Pneumoniae*. Iran J Basic Med Sci;15(1):654–660.

3- Al-Hamadani, A.H.; Al-Rikabi,A.M. and Al- Fatlawi, A.F. (2013). Detection of TEM and SHV genes in *Escherichia coli* and *Klebseilla* species isolated from cancer patients in Al-Diwaniya Governorate. QMJ. 9(16).

4- Jabar , F.A.A.; Abbod, L.S. and Alutbi, D.A.K.(2017). Detection of *Escherichia Coli* and *Proteus Mirabilis* Extended –Spectrum β-Lactamase Isolated from Urinary Tract Infections. Diyala Journal For Pure Science 13(3)part 1:1-10

5- Mohammed, Y.; Gadzama, G.B.; Zailani, S.B. and Aboderin, A.O. (2016). Characterization of Extended-Spectrum Beta-lactamase from *Escherichia coli* and *Klebsiella* Species from North Eastern Nigeria. J Clin Diagn Res. ; 10(2): DC07–DC10.

6- Jabbar, A. (2013).Phenotypic and Genotypic Detection of Extended-Spectrum β-Lactamases (ESBL) among *Escherichia coli* Isolated from Symptomatic Female's Genital Tract Infection. Journal Of Wassit For Science & Medicine; 6(1):59-67.

7- Ahmadikiya, B.F.; Mosadegh, A.; Moradi, M. and Hossieni-Nave, H.(2017). Antimicrobial Resistance Patterns and Frequency of Extended-Spectrum Beta-Lactamase Genes among *Acinetobacter Baumannii*. J Babol Univ Med Sci.19(7):28-34.

8- Tolbert, s.; Happiness, k.; Marco, v.; Michael, a.; Ole, L.; Gibson,K.; Aarestrup, F.M. (2016). Meta-analysis of proportion estimates of Extended-Spectrum-Beta-Lactamase producing *Enterobacteriaceae* in East Africa hospitals. Antimicrobial Resistance and Infection Control. 5:18

9- Collee,J.G.;Fraser,A.G.;Marmino,B. P. and Simons,A.(1996). Mackin and McCartney Practical Medical Microbiology. 14thed. The Churchill Livingstone,USA.

10- MacFaddin,J.F.(2000). Biochemical tests for the identification of medical bacteria. 3rded., The Williams and Wilkins-Baltimor,USA.

11- CLSI, Clinical and Laboratory Standards Institute.(2012). Performance standard for antimicrobial susceptibility testing. Approved Standard-Eleventh Edition. M02-S11. 32(1).

12- National Committee for Clinical Laboratory Standards. (2007). Performance standards for antimicrobial disc susceptibility testing. Disc diffusion. 12 ed. Informational supplement.M100-s17. NCCLS.Wayne, pa.

13- Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing, Seventeenth informational supplement. 27 (1); 2010.

14- Mulu, W.; Abera, B.; Yimer, M.; Hailu, T.; Ayele, H. and Abate,D. (2017). Bacterial agents and antibiotic resistance profiles of infections from different sites that occurred among patients at Debre Markos Referral Hospital, Ethiopia: a cross‑sectional study. BMC Res Notes;10:254.

15- kibret, M. and Abera, B.(2011). Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. Afri Health scis;11(l):S40–S45.

16- Gautam, R.; Chapagain, M.L.; Acharya, A.; Rayamajhi, N.; Shrestha, S.; Ansari, S.; Upadhaya, G. and Nepal, HP). (2013). Antimicrobial susceptibility patterns of *Escherichia coli* form various clinical sources. Journal of Chitwan Medical College; 3(3):14-17.

17- Nepal, N.K.; Pant, N.D.; Neupane, B.; Belbase, A.; Baidhya, R.; Shrestha, R.K.; Lekhak, B.; Bhatta, D.R. and Jha, B. (2017). Extended spectrum beta‑lactamase and metallo beta‑lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandun. Clin Microbiol Antimicrob, 16:(62).

18- Saravanan, R. and Raveendaran, V. (2013). Antimicrobial resistance pattern in a tertiary care hospital: An observational study.J Basic Clin Pharm;4(3): 56–63.

19- Omololu-Aso, J,; Oluwatoyin, O.; Omololu-Aso; Egbedokun, A.; Olufunmilayo, O.; Otusanya; Owolabi, A.T. and Oluwasanmi, A.V. (2017). Antibiotic Susceptibility Pattern of *Escherichia coli* Isolated from Out-patient Individuals Attending the University College Hospital (UCH), Ibadan, Nigeria. Journal of Infectious Diseases and Treatment. 3 (1).

20- Mohammed, H.J. (2013). Physicochemical Factors Affected the Partial Purified Lipase Activity of *Acinetobacter baumannii* "local isolates". Iraqi J Pharm Sci, Vol.22(1).

21- Al-Hamadani, A.H.; Al-Mohana, A.M. and Al-Khazaali, A.S. (2014). Emergence of plasmid mediated *aac(6´)-Ib-cr* Gene in Flouroquinolon-resistant *Acinetobacter* spp. QMJ;10(17).

22- Jafari, R. and Karbasizade, V. (2014). Frequency and Antimicrobial Susceptibility of *Acinetobacter baumannii* in Burn infections in Isfahan, Iran Adv. Biores.,5(2):148-152

23- Nassar, R.M. ; Lafi M. A. K. and Salman S. M. (2013). The incidence of pan-drug resistance in a sample of *Acinetobacter baumannii* clinical isolates obtained from Al-Ramadi Teaching Hospital. Iraqi J. Comm. Med.; (3):262-266.

24- Hussein, N.H.; Al-Mathkhury, H.J.F. and Sabbah, M.A. (2013).Serological relatedness among clinical and environmental *Acinetobacter baumannii* isolates isolated from hospitals in Baghdad. Iraqi Journal of Science, 54(3):553-559.

25- Musa, A.H. and Abdullah,SH.N.(2012). Study the MIC effect for some antibiotics against adhesive factors of *Acinetobacter baumannii* isolated from burn and ;18(76):813-826.

26- Wen, J-T.; Zhou, Y.; Yang, L. and Xu, Y. (2014). Multidrug-resistant genes of aminoglycoside-modifying enzymes and 16S rRNA methylases in *Acinetobacter baumannii* strains. Genet. Mol. Res.13 (2): 3842-3849.

27- Hamdoon, A.M.(2011). Detection of extended spectrum B-lactamase in *E.coli* from clinical samples. Annals of the College of Medicine 37 (1 & 2).

28- Almohana, A.M. and Al-Salamy, A.K. (2013). Dissemination of TEM and SHV Extended-Spectrum Beta-Lactamases in Clinical Isolates of *Escherichia coli*. Magazin o f alkufa university for biolog ;5(2).

29- Shanthi, B.; Selvi, R. and Madhumathy, A. (2018). Antimicrobial susceptibility pattern of *Escherichia coli* from patients with urinary tract infections in a tertiary care. Int.J.Curr.Microbiol.App.Sci;7(1): 289-294.

30- Kadhim, S.R.;. Hassa, A,M, and Shoukat, D.S. (2011). Antimicrobial susceptibility patterns against *Escherichia coli* and prevalence of extended–spectrum β-lactamases. Mustansiriya Medical Journal; 10 (1).

31- Al-Hilali, S. A. M. (2010): Occurrence and Molecular characterization of Enteropathogenic *Escherichia coli* (EPEC) Serotypes isolated from children with diarrhea in Najaf. M. Sc. Thesis. University of Kufa, College of Medicine.

32 - Hussein, N. H.; Al-Mathkhury H.J.F. and Sabbah M.A. (2013). Imipenem-Resistant *Acinetobacter baumannii* isolated from patients and hospitals environment in Baghdad. Iraqi Journal of Science, 54, (4):803-812.

33- Alkasaby, N.M. and Zaki, M.E. (2017). Molecular Study of *Acinetobacter baumanii* isolate for Metallo- *β*- lactamase and Extended- Spectrum- *β*-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt Int J Microbiol. 3925868.

34- Solomon, F.B.; Wadilo, F.; Girma Tufa, E. and Meseret Mitiku, M. (2017). Extended spectrum and metalo beta-lactamase producing airborne *Pseudomonas aeruginosa* and *Acinetobacter baumanii* in restricted settings of a referral hospital: a neglected condition. Antimicrobial Resistance and Infection Control 6:106

35- Shamki, J.S.; Al-Charrakh, A.H. Al-Khafaji, J.K. (2012). Detection of ESBLs in Enteropathogenic *E. coli* (EPEC) Isolates Associated with Infantile Diarrhea in Kut City. Med. J. Babylon, 9(2), 403- 412.

36- Muhammad, M.H. and Swedan, S. (2015). Molecular and phenotypic characterization of carbapenem resistance and extended spectrum beta-lactamases among urinary *Escherichia coli* isolates. Internal J Scie Tech; 5(9).

37- Al-Attar, Z.I. (2015).The prevalence and antimicrobial sensitivity of Esbl *Escherichia Coli*. in clinical isolates. Al-Kindy College Medical Journal 10 (2), 96-99

38- Al- Mayahi, F.S. and Almohana, A.M. (2014). Incidence of Extended-spectrum β- lactamases ESBLs *Escherichia coli* in patients with urinary tract infection. AL-Qadisiyha J Sci.,19(2): 37-55.

49- Sacha, P.;Wieczorek, P. and Ojdana ,D. (2012). Susceptibility, phenotypes of resistance, and extendedspectrum beta-lactamases in *Acinetobacter baumannii* strains. Folia Histochemica et Cytobiologica. 50(1).46–45.

40- Owlia, P.,;Azimi, L.; Gholami, A.; Asghari, B. and Lari, A.R.(2012). ESBL- and MBL-mediated resistance in *Acinetobacter baumannii* :a global threat to burn patients. Infezioni in Medicina 20(3) 182–187..

41- Ibrahimagici, A.; Kamberovic, F.; Ecular; Uzunovici, S.; characteristics and Bedenic, B. .(2017). Molecular characteristics and antibiotic resistance of *Acinetobacter baumannii* beta-lactamase-producing isolates, a predominance of intrinsic *bla*OXA-51, and detection of *TEM* and *CTX-M* genes.Turk J Med Sci 47:715-720.

42- Tuwaij,N.S.S. (2016). Molecular Screening of Some β-Lactam Resistance Genes Producing Clinical Isolates of *Acinetobacter baumannii*. J. Babyl. Univery/Pure Appl.Sci. 24(7): 1799- 1809.

43- Shahcheragh, F.; Nasiri, S. and Noveiri , H.(2009). Detection of extended-spectrum β-lactamases (ESBLs) in Escherichia coli. Iran J Clin Infect Dis . 4:63–70.

44- Ramazanzadeh, R. (2010). Prevalence and characterization of extended-spectrum beta-lactamase production in clinical isolates of *Klebsiella* spp. Afr J Microbiol Res.;4:1359–1362.

45- Fazeli, H.; Taraghian, A,; Kamali, R.; Poursina, F.; Esfahani, B.N. and Moghim, S. (2014). Molecular identification of ESBL genes bla-SHV, bla-VEB and bla-PER, in *Acinetobacter baumannii* strains isolated from patients adamitted to a university hospital in Isfahan,IRAN. Indian Journal of Fundamental and Applied Life Sciences;4 (4):448-454.

46- Safari, M.; Nejad, A.S.M.; Bahador , A.; Jafari e, R. and Alikhani, M.Y. (2015). Prevalence of ESBL and MBL encoding genesin *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU). Saudi Journal of Biological Sciences; 22:424–429.

47- Yang, H.; Huang, L.; Barnie, P.A.; Su, Z.; Mi, Z.; Chen, J.; Aparna, V.; Kumar, D. and Huaxi Xu, H. (2015). Characterization and distribution of drug resistanceassociated *β-lactamase*, *membrane porin* and*efflux pump* genes in MDR *A. baumannii* isolated from Zhenjiang, China.;Int J Clin Exp Med;8(9):15393-15402

48- Al-Agamy, M.H.; Khalaf ,N.G.; Tawfick, M.M.; Shibl, A.M.; El Kholy, A.(2014). Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt. Int J Infect Dis.;22:49-54.

49- Aryann R.G.; Delshad M.M; Isam Y.M.; Dik J.K.; Juliette A.S; Henri A.V.; Deborah,K.; Fatah, M. H.; Alnakshabandi, A. A.; Asad, D.; Anette, M.H.; Kim, N. and Wil, G. (2016). OXA-Carbapenemases Present in Clinical *Acinetobacter baumannii-calcoaceticus* Complex Isolates from Patients in Kurdistan Region, Iraq. Microbial Drug Resistance; 22(8): 627-637).