ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Bacteriological and genetic study on *Escherichia coli* Causing Acute calculus cholecystitis for Diabetes patients in AL-Diwanyia City .

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Manuscript Info

Manuscript History:

Received: 17 April 2015
Final Accepted: 22 May 2015
Published Online: June 2015

Key words:

Nosocomial Infection, Neonatal Intensive care units, microorganisms

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Abstract

The aim of This study is identify the *E.coli* bacteria that cause acute calculus cholecystitis for patients with diabetes in the AL- Diwaniya city, genetically. The study include 110 patients (97 female, 13 male) undergoing cholecystectomy at the Diwaniya Teaching Hospital in from November 2013 to April 2014 .from each patien ,samples bile, a sample of the tissue of the gallbladder, and gall stone(if present).All samples were cultured on different media for full bacteriological identification The results showed that chronic cholecystitis is the most common ratio of 55.4%, followed by acute cholecystitis as the form of 44.6%. It is noted in this study that the age and gender effect on infection of bitterness as the highest injury rate recorded at the 45-36 year group among both sexes, and in the distribution of pathological cases, according to the sex of the patient turns out that there are greatly significant proportions infection bitterness between females and males reaching injury females 88.1%, while the injured were males 11.9% .The results showed that 41.5% of the total samples gave positive-growing bacteria on the different media .The bitterness of the fabric of bacterial isolation was 52%, and the bile 26.4%, and 21.6% gall stone. isolated *E.coli* bacteria were of the tissue of the gallbladder 40%, then the bile was 30.3%, and then gall stone 29.7%. Has been investigating the viability of these isolates to produce enzymes CTX-M, SHV, TEM and Ampc broad- spectrum β- lactames enzymes by revealing the presence of genes bla-Tem ,bla-CTX-M, bla-SHV and bla-Ampc among those isolates by using The polymerization chain reaction (PCR) It showed 23 (88.4%) isolates the bla-Ampc gene, the contain 7 (26.9%) isolates of the bla-SHV gene , and the contain 18(69.2%) isolates the of the bla-Tem gene, while 20 (76.9%), the isolates containing bla-CTX-M gene.

INTRODUCTION

Gall bladder diseases are two types ,first cholecystitis without the presence of stones cholecystitis the second type is the presence of stones in the gallbladder cholelithiasis this situation is more prevalent as an increase of 95% (Kumar *et al*., 2003). the second condition is more common in females than males, and be less in children and the elderly. The presence of stone in the gallbladder is one of the major health problems in many Countries in the world, especially in developing countries (Schirmer *et al*., 2005). The key component of gallstones is cholesterol may consist of calcium carbonat ,bilirubin and bile pigments (Irfan *et al*., 2007). Because there are several factors that lead to gallstones which are the deposition of bile. It is the other cofactors in the incidence of infection is Diabetes (Brody *et al*., 1998). The acute cholecystitis is caused by stay stone in the neck of the gallbladder and bile

duct we have this kind of inflammation common in females than males (Eslami *et al*., 2007). The chronic Cholecystitis is caused by the gallstones incidence of cirrhosis in the walls of bitterness and knows rheumatoid bitterness is the calculus, an inflammation of the sharp two types chronic affects the gallbladder without the grit, the sharp him type occurs as a result of the presence of microorganisms, either chronic caused by clotting in the blood vessels or specific injuries such as typhoid and parasitic diseases fever (Ruby and mohack, 2011). From more bacterial species isolated from gall bladder disease is gram negative bacteria intestinal particular *E.coli* and other types of bacteria, and this has been confirmed by many studies conducted in different parts of the the world (Darko and Archampong, 1994; Flores and Maguilnik *et al*., 2003; Capoor *et al*., 2008). Bacteria *E.coli* has the ability to adhesion and invasive areas of the body and causing many infections as a result of owning multiple vigorous ability to produce portfolio and secretion of enzymes Haemolysin, and Bacteriocin (Cornut *et al*., 2008). These factors help the bacteria to settle and invasion some areas of the body, the bacteria *E.coli* cause many infections, including urinary tract infection as it constitutes 80-90% of urinary tract infections at (Allou *et al*., 2009). also cause diarrhea, especially in children (Ansaruzzaman *et al*., 2007). and caused inflammation of the bile duct (Talaro, 1999). *E.coli* bacteria play an important role in the formation of stone between chromosome structure as microscopic examination contain these stones on a high percentage of bacteria (Karagin *et al*., 2010). the widespread use of others thoughtful of antibiotics has led to increase injury problems rheumatoid bitterness, as antibiotic resistance emergence is one of the outstanding problems that pose a significant threat to public Ahdeddasahh the reason for use of antibiotics for long periods of time led to the emergence of bacterial strains characterized by high Balthml antibiotics (Diense and Rolain., 2013) . The bacteria are resistant to antibiotics by several mechanisms, including the low permeability of the outer membrane, as well as the production of enzymes broken antibiotic such as β-lactamase enzymes It is the mechanism of production of ESBL enzymes of mechanisms important to resist the β-lactam antibiotics (penicillins, cephalosporins, Monobactam) discovered in negative bacilli and cationic dye (Poole, 2011). And encode β-lactamase enzymes chromomosomal or plasmid, the latter is characterized by its ability to self-replicate simultaneously with DNA chromosomal and the transition between bacterial species-mediated pairing bacterial process (Conjugation, and this explains why the spread of antibiotic resistance (Livermore and Brown, 2005). discovered in recent years, hundreds of β-lactamase enzymes different (Hsueh, and Luh, 2005). these enzymes have a significant and important impact in the spread of antibiotic resistance, and probably will determine the future choices of antibiotics used in the treatment of infections caused by strains of the *E.coli* producing these enzymes.

Materials and methods:

Collection of Samples :

110 patients were included in this study, which was extending from November 2013 till April 2014 The patients were attending the Al-Diwanyia Teaching Hospital in Al-Diwanyia province. All patients were undergoing cholecystectomy for acute or chronic cholecystitis, with or without cholelithiasis, werestudied. The patients were 97 (88.1%) females and 13 (11.9%) males. Bile fluid ,specimens of gallbladder and stone if present were collected aseptically from each patient using sterile universal bottles containing Brain Heart infusion broth as transport media. Specimens were culture on blood agar and MacConkey agar plates as soon as it delivered to the laboratory, and incubated overnight at 37 C° . Identification of bacterial growth was based on colonial morphology and standard biochemical tests (Collee *et al* ,1996).

Extraction and amplification DNA

Isolates producing ESBLs were subjected to polymerase chain reaction (PCR) targeting *bla*SHV, *bla*TEM, *bla*CTX-M and *bla*AmpC genes. Genomic DNA was extracted by phenol/chloroform method. PCR amplification was performed using theprimers listed in Table 1, the primers wereobtained from (Bioneer ,south korea).

PCR conditions were as follows:

reactions were carried out in MWG thermocycler in 25µl mixtures containing 12.5µl PCR Master Kit (Bioneer ,south korea), 9.5µl sterile deionized water, 1µl template DNA and 1µl of each oligonucleotide primer. Initial denaturation at 95°C for4min followed by 30cycles of denaturation at 95°C for 1min, annealing for 1min and at48°C for TEM, and 60°C for SHV, CTX-Mand AmpC, extension at 72°C for 1min. The final extension step was extended to 10min at 72°C for all genes.

The PCR products were separated on 1.5% agarose gels (Sigma, Co. USA) in TBE 1X (Tris/borate/EDTA) buffer. Bands were visualized under UV gel documentation after being stained with ethidium bromide and photographed. *E.*

coli carrying the AmpC gene CTX-M, SHV and TEM genes, DNA amplification of CTX-M, TEM, SHVand AmpC specific primers.

Table 1: **Primers used for amplification**

Primer	Primer sequence**(5'-3')**		Product size(bp)
blaCTX-M	F	AGCGATAACGTGGCGATGAA	247 bp
	R	TCATCCATGTCACCAGCTGC	
blaSHV	F	CCGCCATTACCATGAGCGAT	410 bp
	R	AATCACCACAATGCGCTCTG	
blaTEM	F	GGTGCACGAGTGGGTTACAT	531 bp
	R	TGCAACTTTATCCGCCTCCA	
blaAmpc	F	AAACGACGCTCTGCACCTTA	670 bp
	R	TGTACTGCCTTACCTTCGCG	

Results and Discussion :

The 110 patients studied were subdivided into two groups. The first one included 61 (55.4%) patients with chronic cholecystitis and the second group involved 49 (45.6%) patients with acute cholecystitis.

Isolation and identification of bacteria:

The results showed growth positive in 41.5% of the total, whether of bile or stone samples of tissue or bitterness and bacterial growth rate was positive samples from patients with chronic calculus cholecystitis (43 patients), 70.4%; while percentage of patients with samples acute calculus cholecystitis (41 patients) 83.6%. As our results showed that the isolated bacteria from patients with diabetes mellitus patients actually inflamed gall bladder, chronic calculus (18 patients), 41.8%, while the percentage in diabetes patients with acute calculus (23 patients), 56% . agree the results of the current study with have exceeded AL-Zuwaini (2013) that the percentage of positive bacterial growth amounted to 94.4% of the total samples (bile, stone, gall bladder tissue). Also do not agree with the results of the current study, a study conducted in Saudi Arabia indicated to implant bacterial positive rate of 25% of patients who underwent cholecystectomy process samples (Al Harbi *et al*., 2001) .also results of this study do not agree with (Hamdoon, Abdu-Allah, 2008). it indicated that the bacterial growth rate positive from samples taken from the gallbladder was 67.3%. Other studies have also indicated that bacterial growth positive samples bitterness between 55% −70.4% of samples (Ballal *et al*., 2001; Rerknimitr *et al*., 2002), and this difference is due to the sampling site (Manolis *et al*., 2008) as well as the use of patients to different treatments
(Csendes *et al*., 1996).
The number of isolates of *E.coli* from bile 8 isolates, 6 isolates from stones ,and 12 isolates from gallbladder tissue.

Molecular Detection of β- lactamases

Due to the lack of information available on the extent of the presence and frequency of extended spectrum β-lactamases enzymes and AmpC in some isolates of the bacteria *E.coli* isolated cases of acute calculus cholecystitis for patients with diabetes in Iraq in general and in particular the city of Diwaniyah, as there are no specialized and in-depth study to these enzymes at the molecular level, so it felt the current study to investigate the viability of these isolates to produce these enzymes using molecular interaction technique monomer PCR what characterized by the technique of very high precision and sensitivity in the investigation of genetic factors controlling the production of enzymes ESBL enzymes. It is through the use of specialized primers for β-lactamases genes, which included all of the genes blaTEM, blaSHV, blaAmpC, and blaCTX-M, as we relied on DNA to bacteria *E.coli* extract, which we got used to him using the kit. The investigation of ESBL enzymes of 26 isolates of pure isolates *E.coli*..

showed 18/26 (69.2%) isolates of *E.coli* have blaTEM gene under study, Figure (1-1) . These results were consistent with have exceeded Hadi (2008) found that as the frequency of this gene 66.6% 14 isolation in the Najaf city These results are contrary to that reached by the Al-Hilali (2010) found that as the frequency of this gene. As well as 18.8% disagreed with the findings of the Harran,(2012), as was found that the rate of recurrence of this gene is 44.4%, and also disagreed with the results of this study (Davoud Kalantar *et al*., 2010) as it was found that the percentage of repeat gene Tem 43.5% ,. As mentioned Bradfford,(2001) that more than 90% of the *E.coli* bacteria resistant to Ampicillin The reason for this is to produce enzymes Tem-1, which has the ability to penicillins and cephalosporins such as cephalothin analysis.

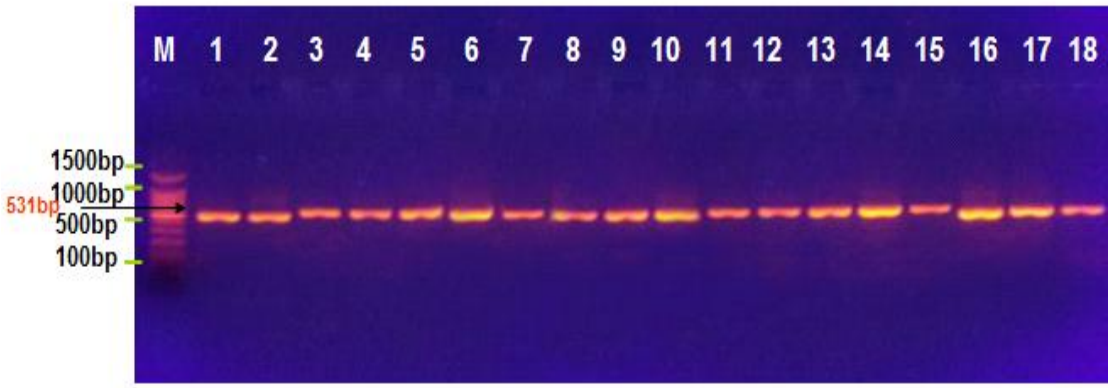

Figure (1-1): Electric deportation of gene bla-TEM Isolates bacteria *E coli*. Multiplier using the PCR technique electrically stage on agarose gel (1.5%) and voltage (100) volt teams effort (80) amp for a period of one hour. Where M represents (Marker ladder 100-2000bp) as the isolation of 18 isolates showed a positive result for the TEM gene of 531 bp**.**

Showed 7/26 (26.9%) isolates of *E.coli* have blaSHV gene under study ,Figure (1-2) . The results of this study agreed with the findings, in the study by Harran (2012) found that as the frequency of this gene 2/9 (28.6%), while the results of the study do not agree with (Davoud Kalantar *et al.*, 2010) found that the proportion of repeat this gene 48 isolates (34.8%). So was contrary to his findings Al-Hilali (2010), as he found that all isolates containing this gene is 100% and the longer these enzymes prevalent in strains of *E.coli* within certain geographic areas spread globally, in a study by (Davoud *et al*.,2010) in Iran that more legacies prevalent among clinical isolates of *E.coli* bacteria were blaSHV, blaTEM and frequency by 41% and 33% respectively.

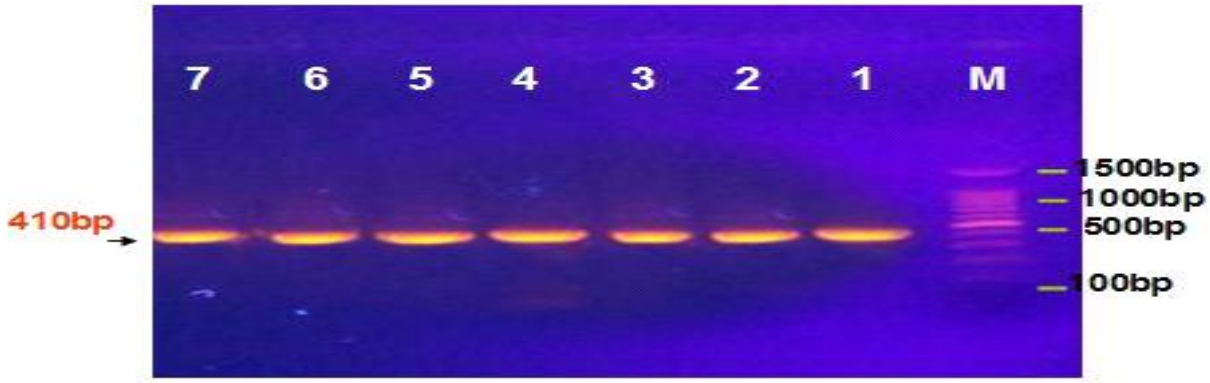

Figure (1-2): Electric deportation of gene bla-SHV Isolates bacteria *E coli*. Multiplier using the PCR technique electrically stage on agarose gel (1.5%) and voltage (100) volt teams effort (80) amp for a period of one hour. Where M represents (Marker ladder 100-2000bp) as the isolation of 7 isolates showed a positive result for the SHV gene of 410 bp**.**

Showed 20 (76.9%) isolates of *E.coli* under study have blaCTX-M gene, Figure (1-3). These results were contrary to that reached the study by (Mirzaee *et al*., 2007), which found that the frequency of gene Tem 15.9% rate .while These results were contrary to that reached the study by (Jemima and susan vergese, 2007) because they found that the proportion of Tkarhzh gene was 42/21 isolation,(50 %.) While these results agreed with the study conducted in southern India indicated that 72(77.4%) isolates out of 93 isolated *E.coli* has blaCTX-M gene by using PCR technique (Shahid *et al*., 2006). Also conducted another study in the same area indicated to acquire 14(35.8%) isolation of 39 clinical isolates to gene (Sekar *et al.*, 2006) blaCTX-M boards enzymes are instabilmente for antibiotic resistance especially third generation of cephalosporins since it was found that 72% percent of the gram negative bacteria be resistant to all antibiotics (Suhkla *et al.*, 2004). It is resistant bacteria *E.coli* to antibiotics such as ampicillin, carbenicillin, oxacillin and broad-spectrum antibiotics through hydrolysis by producing ESBL enzymes including CTX-M, TEM, and SHV which are genes mounted plasmids (Paterson, Bonomo, 2005).

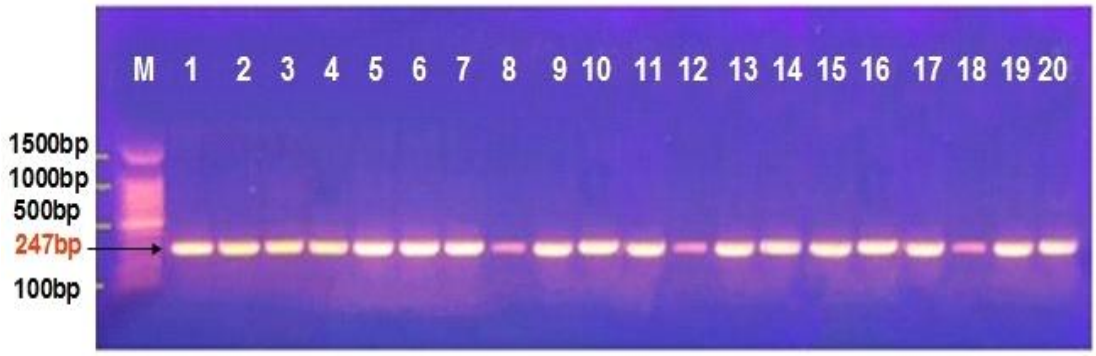

Figure (1-3): Electric deportation of gene bla-CTX-M Isolates bacteria *E coli*. Multiplier using the PCR technique electrically stage on agarose gel (1.5%) and voltage (100) volt teams effort (80) amp for a period of one hour. Where M represents (Marker ladder 100-2000bp) as the isolation of 20 isolates showed a positive result for the CTX-M gene of 247bp **.**

showed 23/26 (88.4%) isolates of *E.coli* under study have ampc gene, Figure (1-4).these results Disagreed with the results of the current study have exceeded (Eftekhar *et al*., 2005) which indicated that the proportion of gene frequency blaAmpc amounted to 4% of the *E.coli* bacteria isolated from urine. As a study conducted in Algeria pointed to acquire isolates of *E.coli* gene Ampc by frequency reached 1% (Messai *et al*., 2006) .while another study

in Britain (Potz *et al*.,2006) that the percentage of possession of *E.coli* bacteria Ampc gene amounted to 7.15%. The determination of the production of β- lactamases enzymes type Ampc by bacteria that produce them have difficult things because these enzymes donot inhibit by extended spectrum β-lactamase inhibitors such as Clavulanic acid and Sulbactam (Jacoby, 2009). And also have the ability to analysis Monobactams, Cephamycins antibiotics (Sundin, 2009).

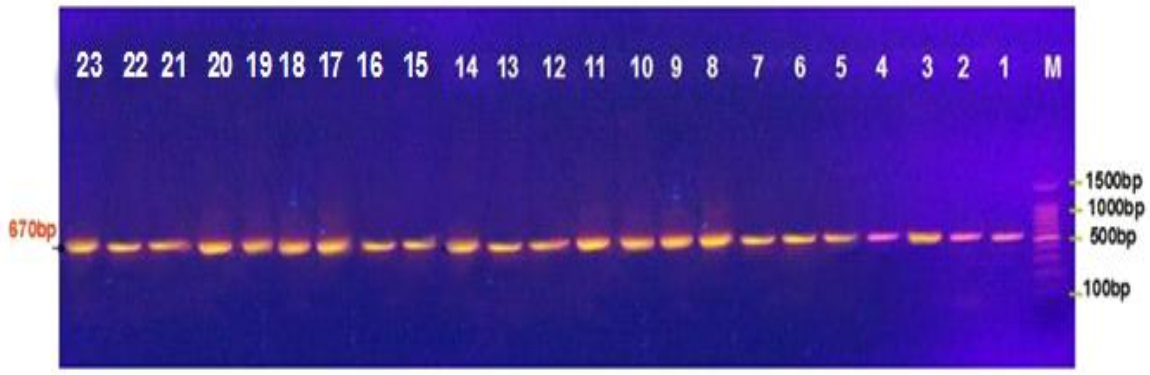

Figure (1-4): Electric deportation of gene bla-Ampc Isolates bacteria *E coli*. Multiplier using the PCR technique electrically stage on agarose gel (1.5%) and voltage (100) volt teams effort (80) amp for a period of one hour. Where M represents (Marker ladder 100-2000bp) as the isolation of 23 isolates showed a positive result for the Ampc gene of 670bp **.**

The production of extended spectrum β-lactamases enzymes mechanism of the most important resistance mechanisms that have been discovered in the gram negative bacilli (Poole, 2004). Thus, these bacteria are resistant to different types of antibiotics, including the β-lactam antibiotics .as indicated studies to the existence of a strong relationship between infection with bacteria *E.coli* and *K.pneumoniae* producing extended spectrum β-lactamases enzymes and the indiscriminate use of to antibiotics (Lautenbach *et al*., 2001; Grover *et al*., 2006). This results recorded by Other studies have shown that reducing the use of antibiotics and particularly cephalosporins such ceftazidim will lead to a lower incidence of bacteria producing β- lactamase enzyme (Lautenbach *et al*., 2001; Graffunder *et al*., 2005). Detection of β-lactamases genes is one of the main reasons for resistant bacteria *E.coli* to antibiotics have Manmalanh may help reduce the emergence of bacterial strains most resistant as well as get the results can be inferred from which the doctor to give appropriate treatment . .

A study conducted in Iran by(Aminzadeh *et al.*,2008) that ratio the production of β – lactamase enzymes and a wide spectrum by the bacteria *E.coli* 45.2%, also recorded another study in the same area by(Fazly *et al*.,2009) results did not differ much from the previous study, as the production rate of these enzymes 59.5% of by the bacteria *E.coli* and this Maevsrlna reason resistant bacteria *E.coli* treatments .az that the mechanism of production of extended spectrum β – lactamase enzymes is one of the most important mechanisms used by these bacteria to resist the antibiotics. At the same time, we find that the production of these enzymes varies from one region to another and from one country to another as the proportion of production of these enzymes in Australia recorded a 2.1% ,1.59% in Italy and in the United States 2.8% (Rossi *et al*., 2006; Zong *et al*., 2008, and other studies conducted, one in Turkey before (Tasli, 2005) indicated that ratio the production of extended spectrum β-lactamase enzymes 17%, and another study conducted in Egypt by (Al-Agamy *et al*., 2006) found that produce β-lactamase enzymes by the bacteria *E.coli* 60.9%. the most common is an enzyme CTX-M. these enzymes as noted Mirzee and his group (2007) in a study conducted in Tehran that all isolates of *E.coli* showed the result is positive for the enzyme CTX-M. have described the diverse nature of these enzymes by (Al.Hashem *et al*., 2011; Khanna *et al*., 2012). The Ampc enzymes it may play an important role in the pathogenesis of bacteria as noted (Shahid *et al*., 2009) that the enzymes Ampc aberrations are found in many pathogenic bacteria such as *E.coli*, *Enterobacter spp, Proteus.rettegri* which is responsible for pathognicity. Subramaniam and his group (2006) that the enzyme SHV-5, which is a type of enzyme SHV responsible for infections in hospitals in many countries and there is an enzyme SHV in isolates producing extended spectrum β-lactamase enzymes in many countries, including Italy, Australia, the United States of America (Nuesch *et al.*,.1997). Grover and his group (2006), in a study conducted in India owning bacteria *Klebsiela* spp

enzymes bla-Tem and bla SHV singly or doubly in 88.8% of the isolates. As study by(Umadevi *et al*.,2011) recorded that the percentage of bacteria *E.coli* producing extended spectrum β-lactamase enzymes 47.83%, this result was similar to the findings of the Hague and salam (2010) that the percentage of bacteria *E.coli* producing these enzymes 43.9%, while the results the study by(Mathur *et al.,*2005) violation of these studies since concluded that the proportion of bacteria *E.coli* producing these enzymes 62%. Paterson has said and his group (2005) that the spread of these enzymes and attributes phenotypic between clinical isolates may Tngarahsp geographical areas. as Other studies have found that the bacteria *E.coli* producing extended spectrum β-lactamase enzymes be sensitivity high antibiotic Meropenem (Subha *et al*., 2002; Rodrigues *et al.,* 2004).

The production of extended spectrum β-lactamase enzymes of mechanics important resistance to third generation of Cephalosporins. These enzymes are a major group of β-lactamase enzymes diagnosed in a large number of countries around the world, which is mainly produced by negative bacteria gram. Identifying these enzymes is very important to provide optimal care for patients.

Conclusions and Recommendations :

PCR technique revealed *E.coli* bacteria ability to produce extended spectrum β-lactamase enzymes CTX-M, TEM, SHV, AMPC. Emphasis on the use of antibiotics when needed and sensitivity test before giving treatment in order to select an appropriate counter for the treatment of infection caused by the bacteria *E.coli*. Avoid indiscriminate use of antibiotics, but consult a doctor to avoid the emergence of strains of antibiotic resistance. therefor we suggest use of polymerase chain reaction (PCR) in the diagnosis of types of β-lactamase enzymes for her money from a role in the monitoring of antibiotic resistance and to reduce the spread.

References :

Talaro, K. P. and Talaro, A. (1999). Foundations in Microbiology. 3rd ed., McGraw–Hill Companies, Inc., U. S. A.

A. Subha , S. Ananthan .(2002) . Extended spectrum betalactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumonia in Chennai. Ind. J Med Microbiol.;20:5-9.

Al-Harbi, M .; Osoba, A.O.; Mowallad, A. and Al-Ahmadi, K.(2001). Tract microflora in Saudi patients with cholelithiasis. Trop. Med. Int. Health;6(7):570-4.

Al Hashem G,Al Sweih N,Jamal W,Rotimi VO.(2011). Sequence analysis of bla(CTX-M) genes carried by clinically significant *Escherichia coli* isolates in Kuwait hospitals.Med princ pract.;20(3):9-13.

Al-Agamy MHM, Ashour MSED, Wiegand I.(2006). First description of CTX-M β-lactamase-producing clinical Escherichia coli isolates from Egypt. Int J Antimicrob Agents.27: 45-54.

Al-Hilli, Z. B. A. (2010). Dissemination of β- lactamases in Escherichia coli and Klebsiella spp. Isolated from Merjan Teaching Hospital in Hilla City. M.Sc. Thesis. College of Science. Kufa University.

Allou, **N. ; Cambau, E. ; Massias, L. ; Chau, F. ; & Fantin, B. (2009).** Impact Low-Level Resistance to Fluoroquinolones Due to *qnrA1* and *qnrS1* Genes or a *gyrA* Mutation on Ciprofloxacin Bactericidal Activity in a Murine Model of *Escherichia coli* Urinary Tract Infection. Antimicrob. Agents Chemother. 53(10):4292-4297

and gallstones. Iran. J. Clin. Infect. Dis ., 2(3):139-141

Ansaruzzaman, M. ; Bhulyan, N.A. ; Begum, Y.A. ; Kuhn, I. ; Nair,G.B. and Sack, D.A.(2007). Characterization Enterotoxigenic *Escherichia coli* from diarrhoeal patients in Bangladesh using phenotyping profiling . J. Med. Microbiol., 56:217-222 .

AL-Zuwaini , Sama Jawad Kadhim.(2013). Bacteriological, Immunological and Histological study on inflammed gallbladder in Babylon province. Messaging Master, College of Science for Women**,** Babylon University.

Ballal, M.; Jyothi, K. N.; Antony, B.; Arun, C. and Prabhun, T.(2001 .(Bacteriological spectrum of cholecystitis and its antibiogram. Ind. J. Med. Microbiol., 19:212-214.

Bradford, P.A.(2001) . Extended-spectrum β- lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev*.14: 933-51.

C. Rodrigues , P. Joshi , S.H. Jani, et al.(2004). Detection of beta-lactamases in nosocomial gram negative clinical isolates.Ind. J Med Microbiol ., 22: 47-50.

Capoor, M.R.; Nair, D.; Rajni, K.G.; Krishna S.V.; Chintamani M.S. and Aggarwal, P.(2008). Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: a tropical experience.Braz.J. Infect. Dis.2008;12(3):222-5.

Collee, J.; Garald, G.; Fraser, F.; Andrew, G.; Marmion, M.; Barrie, P.; Simmon, S. and Anthong, N. (1996). Practical medical micro-biology. 14th ed. Churchill Livingstone. Newyork., P:131-150.

Cornut, G.; Fortin, C. and Soulieres, D. (2008). Antineoplastic properties of bacteriocin: revisiting potential active agents. *Am. J. Clin. Oncol.* 31:399-404.

Csendes A, Burdiles P,Maluenda F,Diaz JC, Csendes P, Mitru-N.(1996). Simultaneous bacteriogic assessment of bile frome gallbladder and common bill duct in control subjects &patients with gall stones and duct stones-Ar Ch surg. 131:389-394.

Diens, S. ; and Rolian, J. (2013). Investigation of antibiotic resistance in the genomic are of multidrug-resistance gram-negative bacilli, enterobacteriaceae especially *Pseudomonas* and *Acinetobacter*. Anti. Infication therapy. 11(3):277-296.

Dorak R and Archampong EQ .(1994). The microflora of bile in Ghanians. West Afr. J. Med.13(2):113-115.

Eftekhar F, Hosseini-Maziani SM, Ghandili S, Hamraz M, Zamani S. (2005). PCR detection of plasmid mediated TEM,SHV and AmpC β-lactamases in community and nosocomial urinary isolates of *Escherichia coli* . *Iranian* J Biotech . 3(1):48-54.

Eslami, G.; Nowruzi, J.; Fllah, F.; Goudarzi, H.; Hakemivala, M. and Jahangiri, S.(2007). Detection of bacteria responsible for gallbladder inflammation

Fazly Bazzaz BS, Naderinasab M, Mohamadpoor AH, Farshadzadeh Z, Ahmadi S, Yousefi F. (2009). The prevalence of extended-spestrum β-lactamase producing *Escherichia. coli* and *Klebsiella* . *pneumonia* among clinical isolates from ageneral hospital in Iran. Acta Microbiol Immunol Hung .;56(1):89-99.

Flores C, Maguilnik I, Hadilick E *et al.*,(2003).Microbiology for chledocal bile in patints with choledocholithiasis admitted to atertiary hospital. J. Gastroenterol. Hepatol .18(3): 333-336.

Graffunder EM, Preston KE, Evans AM, Venezia RA.(2005). Risk factors associated with extended-spectrum β-lactamase-producing organisms at a tertiary care hospital. J Antimicrob chemother .; 56: 45-139.

Grover SS, Sharma M, Chattopadhya D, Kapoor H, Pasha ST, Singh G. (2006). Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in *Klebsiella pneumoniae* :emergence of high resistance against cefepime, the fourth generation cephalosporin. J Infect .;53:88-279.

Hadi, Z.J. (2008). Detection of Extended-Spectrum Beta-lactamases of *Escherichia coli* and *Klebsiella spp.*isolated from patients with significant Bacteriuria in Najaf. M.Sc. Thesis. College of S. Kufa University.

Hamdoon Ansaam M . and Abdul-Allah Zen Al-Abdeen , Department of microbiology College of Medicine University of Mosul **,(2008).** Sero-bacteriolocal detection of bacteria in the gall baldder blood and stool of patients having cholecystectomy; J. Ann.coll. Med. Mosul ,34(2):118-125.

Haque R, Salam MA. (2010). Detection of ESBL Producing nosocomial gram negative bacteria from atertiary care hospital in Bangladesh Pak J Med Sci .;26(4):887-891.

Harran, Omar Hussein (2012) . Detection of **β**-lactam Resistance Genes in Bacterial Isolates from Some Clinical Infections in Al_Diwaniyah City. Messaging Master, College of Science - University of Al-Qadisiya

Hsueh, P.P.;Chen,W.H.and Luh , K.T.(2005). Relationships between antimicrobial use and antimicrobial resistance in Gram- Negative bacteria causing nosocomial infections from 1991-2003 at a university hospital in Taiwan.Int.J.Antimicro.;Agents .; Nov .6.

http://www.textbookofbacteriology.net/e.coli.htm

Irfan S. , Adnan A. , Shahid R.,Zahid M. and Asadullah K .(2007). Freguency of infection in cholelithiasis .GCPSP . 17(1): 48-50.

Jacoby GA. (2009). AMPC β-lactamases. Clin Microbiol Rev .;22:82-161.

Jemima ,S.A. and Verghese .S.(2008). Department of Microbiology ,Dr.K.M.Cheriani Heart Foundation,Chennai,India.Multiplex PCRfor the blactx-m and blaSHV in the extended spectrum beta lactamase (ESBL) producing Gram- negative isolates .;Indian J Med Res 128 : pp313-317.

Kalantar D, Mansouri S.(2010). Emergence of multiple β-lactamases produced by Esherichia coli clinical isolates from hospitalized patient in Kerman, Iran. Jundishapur J Microbiol 3(4):45-50.

Karagin, P.H.; Stenram, U.; Wadstrom, T. and Ljungh, A. (2010). *Helicobacter* species and common gut bacterial DNA in gallbladder with cholecystitis. *World J. Gastroenterol* ., 16 (38) : 4817-4822.

Khanna N, Boyes J, Lansdell PM, Hamouda A, Amyes SG,(2012). Molecular epidemiology and antimicrobial resistance pattern of ex-tended-spectrum-beta-lactamase-producing Enterobacteriaceae in Glasgow, Scotland. J Antimicrob chemother.;67(3):230-235.

Kumar, V.; Cotran, R. S. and Robbins , S. l. (2003). Basic Pathology. 7th ed. Philadelphia. London. pp:628-629.

Lautenbach E, Patel JB, Bilker WB, Edelstein NO.(2001). Extended-spectrum β-lactamase-producing Escherichia coli and klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes.Clin Infect Dis .;32:1162-71.

Livermore, D. M. and Brown. D. F. J.(2005). "Detection of β-lactamase-mediated resistance". Retrieved 31.
Manolis EN, Filippou DK, Papadopoulos VP, Kaklamanos I, Katostaras T, Christianakis E, et al.(2008). The culture site of the gall bladder affects recovery bactria in symptomatic cholelithiasis. J Gastroitestin liver Dis. 17 :179-182.
Mathur P, Kapil A, Das B, Dhawan B.(2002). Prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res*; *115* : 153-7.
Messai Y, Benhassine T, Naim M, Paul G, Bakour R.(2006). Prevalence of β-lactams- resistance among *Escherichia coli* clinical isolates from a hospital in Algiers. Rev Esp Quimioterap. 19(2): 144-51.
Mirzaee M, Owlia P, Mansouri S.(2007). Distribution of CTX-M β-lactamase genes among Escherichia coli strains isolated from patients in Iran.Lab Medicine.;40(12):724-727.
Nuesch-Inderbinen MT, Kayser FH.(1997). β-lactamases in Enterobacteriaceae in Switzerland: two novel enzymes, SHV-11 and SHV-12. Antimicrob Agents Chemother;41:943-9.
Paterson, D. L. and Bonomo, AR. (2005). Extended-spectrum β-lactamases: a clinical update. Clin. Microbiol. Rev. 18 : 657-686.
Poole K.(2004). Resistance to β-lactam antibiotics. CMLS,Cell Mol Life Sci.61: 22-23.
Poole, K.(2011). *Pseudomonas aeruginosa*: resistance to the max. Frontiersin Microbio . cellular infect. Microbio.,2.(65).
Potz NA, Hope R, Warrner M, Johnson AP, Livermore DM.(2006). Prevalence and mechanisms of cephalosporin resistance in *Enterobacteriaceiae* in London and South-East England . J Antimicrob Chemother. 58:320-6.
Rerknimitr, R.; Fogel, E.L.; Kalayci, C.; Esber, E.; Lehman, G.A.and Sherman,S. (2002). Microbiology of bile in patients with and without plastix biliary endoprosthesis. *Gastrointest. Endosc* ., 56:885-9.
Rossi F, Baquero F, Hsueh PR, et al.(2006). In vitro susceptibilities of aerobic and facultatively anaerobic Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: 2004 results from SMART (Study for Monitoring Antimicrobial Resistance Trends). J Antimicrob Chemother.; 58:205-10.
Schirmer, B.D.; Winters, K.L. and Edlich, R.F.(2005). Cholelithiasis and cholecystitis. J. Long Term Eff. Med. Implants.;15(3):329-38.
Sekar B ,Shwetha R, Arunagiri K , Menaka K , Lalitha P Aparna V ,*et al*.(2006). Detection and Characterization of blaCTX-M gene by PCR-RFLP analysis among third generation cephalosporin resistant Gram negative isolates. Proceedings of MICROCON.XXX National Congressof Indian Association of Medical Microbiologists ,2006 October 27-29; Government Medical College ,Nagpur. MICROCON 2006;OB -17:P27.
Shahid M, Farrukh S, Anuradha S, *et al* (2009). AMPC β-lactamases and bacterial resistance: an updated mini review. Rev Med Microbiol; 20(3): 41-5.
Shahid M, Singhal M , Malik A ,Shukla I, Khan HM .(2006). ESBL phenotypes and prevalent genotype of CTX-M type β-lactamase in clinical isolates of *E.coli* in North Indian tertiary care hospital. Proceedings of MICROCON 2006.XXX National Congressof Indian Association of Medical Microbiologists , 27-29; Government Medical College,Nagpur. MICROCON 2006;PB -17:P-70.
Shukla I,Tiwari R, Agrawal M.(2004). Prevalance of extended Spectrum-Lactamase producing Klebsiella pneumonia in a tertiary care hospital. Indian J Med Microbial;22:87-91.
Sundin DR, (2009). Hidden beta- lactamase in *Enterobacteriaceae* dropping The extra disks for detection ,part II . Clin Microbial Newsletter .31(7): 47-52.
Tasli H, Bahar IH.(2005). Molecular characterization of TEM –and SHV – derived extended –spectrum beta –lactamases in hospital –based Enterobacteriaceae in Turkey .Jpn J Infect Dis., 58:162-7.
Zong Z, Partridge S, Thomas L, Iredell JR. (2008). Dominance of the blaCTX-M within an Australian extended-spectrum β--lactamase gene pool. Antimicrob Agents Chemother.,52:198-202.