IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. VI (Nov. - Dec.2016), PP 108-112
www.iosrjournals.org

Bacteriological and Genetic Study of E-coli Isolated from different Infections in Diyala

Assistant lecturer Eman Abass Ali*,Assistant lecturer Ibtihal Hameed Mohsin *
** Department of Biology / College of Science/ Diyala University*

Abstract: *The goal of present study was to Isolation and Identification of Escherichia coli from different Infections and detection the sensitivity and resistance of Ecoli to antimicrobials Additionally detection of virulence factors . This study was conducted from the period from 1 / 6 / 2016 to 1/ 9 / 2016 in Baquba city in Iraq.. It included; Fiftin samples were collected from different infections from Baquba General Hospital and AL-Batool Hospital. Twenty isolates were found to be Escherichia coli .The susceptibility test was applied on these isolates against deferent antibiotics. The results revealed that the highest resistances were for Piperacilline(93.3%) and highest Sensitive Imipenem with 100%, While the lowest resistance were for Tobramycin (9%) . Moreover the results of virulence factors that had E. coli showed possession of all isolates many virulence factors and a high production of which increases the pathoginicity of it. All isolates were unable to produce urease and gelatinase, but heamolycin (35%) . As well as, Tow isolate (10%) were able to production Extended Spectrumβ-Lactamases enzyme. Furthermore, four isolate (20%) were able to production metalloβ-Lactamases Finally, six isolate(30%) were able to production Bacteriocin.*
Conclusion: E. coli isolates highest resistances were for Piperacilline and highest Sensitive Imipenem with 100%, While the lowest resistance were for Tobramycin . additionally some of isolates production Extended Spectrumβ-Lactamases enzyme, metallo β-Lactamases and Bacteriocin.

Keywords: *Escherichia coli , Extended Spectrumβ-Lactamases, Metallo β-Lactamases,Bacteriocin , Antibiotics*

I. Introduction

Escherichia coli (E. coli) bacteria normally live in the intestines of people and animals. Most E. coli are harmless and actually are an important part of a healthy human intestinal tract. However, some E. coli are pathogenic, meaning they can cause illness. (1)

E. coli is the most prevalent infecting organism in the family of gram-negative bacteria known as Enterobacteriaceae.(2) E. coli bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich.(3) E. coli is a facultative (aerobic and anaerobic growth) gram-negative organism, rod shaped, may or may not be motile. (Some rods are flagellated and some are not). (4) Bacteria that can be commonly grow best at 37 C. E. coli is a Gram-negative that cannot sporulate. Therefore, it is easy to eradicate by simple boiling or basic sterilization. (5) E. coli has only one circular chromosome, some along with a circular plasmid.(6)

Ecoli is a major human pathogen that occurs in many different types of infections of the human body, this due to Ecoli have different virulence factors as producing a wide variety of enzymes and toxins. (7) Nearly all strains of Ecoli secret a group of enzymes which includes heamolycin, Extended Spectrum β-Lactamases enzyme,Metallo β-Lactamases enzyme ,Bacteriocin, Catalase enzyme. The main function of these proteins may be convert local host tissues into nutrients required for bacterial growth.

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. these enzymes share the ability to hydrolyze third-generation cephalosporins and aztreonam and yet are inhibited by clavulanic acid. (8)

In addition, ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic option. Because of inoculum effect and substrate specificity, their detection is also a major challenge. (9)(10)

The increasing and rapid spread of metallo-beta-lactamase (MBL) producing Enterobacteriaceae, particularly Escherichia coli, Metallo-β-lactamases are a diverse set of enzymes that catalyze the hydrolysis of a broad range of β-lactam drugs including carbapenems. This diversity is reflected in the observation that the enzyme mechanisms differ based on whether one or two zincs are bound in the active site which, in turn, is dependent on the subclass of β-lactamase. (11) The

dissemination of the genes encoding these enzymes among Gram-negative bacteria has made them an important cause of resistance. In addition, there are currently no clinically available inhibitors to block metallo-β-lactamase action.(12)

Bacteriocins are bacterially produced peptides that are active against other bacteria and against which the producer has a specific immunity mechanism, Further, almost colicins are plasmid encoded, whereas microcin encoding genes are also found on the chromosome.[13],[14]

II. Materials and Methods

Samples collection:

Fiftin different clinical samples were collected from patients and carriers in Baquba General Hospital and Al-Batool Hospital over period from 1/6/2016 to 1/9/2016. The samples were included (14 from urin, 9 from umbilical cord, 15 from wound and 12 from burn).

Isolation and Identification of Escherichia coli:

The collected samples were inoculated on the blood agar, incubated at 37ºC for 24 hours. The isolates were examined for their shape, size, colour, pigments, and haemolytic activity. Then transferred and streaked on MacConky agar for detecting the ability of each isolate to ferment lactose. All plates were incubated at 37ºC for 24 hours then a single pure then transferred to Eosin methylene blue agar (EMB) appearing as a metallic green sheen. pure isolated colony was transferred to Nutrient broth medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates. The isolates were identified according to the Bergey's Manual. [15],[16] As the following: gram stain and biochemical tests Standard biochemical tests were used for detecting *Ecoli* strains.

Antimicrobial susceptibility test:

The sensitivity and resistance of *Ecoli* to antimicrobials was tested by the disc diffusion method on Muellar-Hinton agar using antibiotic discs according to Clinical and Laboratory Standards Institute (CLSI) guidelines. [17] Twelve antibiotics were tested: Ampicillin (30Mg), Imipenem (10Mg), Cefixime (30Mg), Ciprofloxacin (5Mg), Cefotaxime (10Mg), Augmentin (10Mg), Nitrofourantoin (15Mg), Ceftazidime (15Mg), Tobramycin (30Mg), Gentamicin (10Mg), Piperacillin (5Mg) and Co-trimoxazle (30Mg). Interpretation of inhibition zones was carried out based on the manufactures and CLSI guidelines .[18] Then the plates are incubated overnight at 37^{oC}, and the zone of inhibition of bacterial growth is used as a measure of susceptibility, where large zones of inhibition indicate that the organism is susceptible, while small or no zone of inhibition indicate resistance. An interpretation of intermediate is given for zones which fall between the accepted cutoffs for the other interpretations**.** [19]

Detection of virulence factors &Biochemical test :

The *Ecoli* ability to produce some of virulence factors (enzymes and toxins) were recognized and tests were applied on 20 isolates that identified. it included: Haemolysin production, urease production and identified by Biochemical test such as Triple Sugar Iron Agar (TSI) , IMViC test (indole, methyl red, Voges-Proskauer, and citrate). [20]

Detection of Extended-spectrum □-lactamases (ESBLs):

Extended-spectrum β-lactamases production was tested by Disc approximation. using double disc synergy test.Briefly, a sterile Mueller-Hinton agar was prepared and a 0.5 MacFarland equivalent standard of the test organisms was streaked on the surface of the agar with a sterile loop and allowed for 15-20 mins to pre-diffuse. An Augmentin which is a combination of clavulanic acid 20 (μg) and amoxicillin (10 μg) was placed at the center of the petri-dish and cefotaxime (30 μg), ceftaxidime (30 μg), aztreonam (30 μg) ciprofloxacin (30 μg) were placed15mm apart center to centre on the plates with a sterile forceps.These were incubated at 35oC for18-24 h. An enhanced zone of inhibition from 5 mm above in the presence of Augmentin is regarded as positive for phenotypic production of ESBL enzyme. [21]

Detection of Metallo- □-lactamases (MBLs):

MBL production was detected by performing combined disc test described by Franklin et al. in all carbapenemase screening positive isolates. In this test, two imipenem discs (10 μg), one containing 10 μl of 0.1 M (292 μg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO) were used. They were placed on a MHA plate inoculated with 0.5 McFarland suspension of the test isolate. Plates were incubated for 16–18 hours at 35°C. After incubation, the diameter of inhibition zones was measured. An increase in zone diameter of >4 mm around the imipenem-EDTA disc compared to that of the imipenem disc alone was considered positive for MBL production. [22]

Detection of Bacteriocin (colicin) :

The frequency of colicin production was determined using the agar overlay method with indicator strain E. coli CL173. Briefly, agar plates were stab inoculated with the test strains and incubated overnight at 37°C. Colonies were lysed for 15 min using cellulose pads impregnated with chloroform. To eliminate residual chloroform vapour the plates were then exposed to air and overlaid with soft agar containing an indicator strain and incubated overnight at 37°C**.** [(23)]

III. Results and Discussion

Isolation and Identification:

Fifteen samples were collected from patients and carriers, the samples comprised from (urine, umbilical cord , wound and burn). Twenty isolates (25%) have the ability to grow on the MacConky agar which considered selective and differential media for gram negative bacteria [(24)].All 20 isolates had ability to ferment Lactose and form large Pink colonies, smooth. They are grow on Eosin methylene blue agar (EMB) (Selective and Differential media) appearing as a metallic green sheen .Microscopic examination was used to all 20 isolates after staining by gram stain and cells appeared as Gram-negative rods. For further identification some of the biochemical tests was performed on 20 isolates, included: catalase test was all 20 isolated gave positive results ,While 20 isolates gave the negative result for all of oxidase test, H_2S production test and Gelatin laquification test . Also all 20 isolates were positive to Indol test and Methyl red but Negative result for Voges-Proskauer test and Citrate Utilization Test . Additionally , nitrate reduction test was not applied for further identification because the *Ecoli* often unable reduce nitrate to nitrite**.** [(25)] 7(35%) isolated can be produced Haemolysin.Ecoli can be production haemolysin that enzyme imported play role in against immune cells for host. [(26)]

E-Coli	**Testes**
-	**Gram stain**
-	**Oxidase test**
+	**Catalase test**
+	**Haemolysin production**
+	**Indol production test**
+	**Methyl red test**
-	**Voges proskauer test**
-	**Citrate utilization test**
-	**H_2S production test**
-	**Urea hydrolysis (urease test)**
-	**Gelatin laquification test**
+	**Lactose fermentation test**

Susceptibility test of *Ecoli*:

The sensitivity of 20 isolates were tested against 12 antibiotics. The susceptibility test was applied according to the Kirby-Baure Method (antibiotic disc diffusion method).

Table (1): Rate sensitivity and resistance of different antibiotics for *Ecoli*

Ecoli			**المضادات الحيوية**
R	**I**	**S**	
%67.5	**0**	**%32.5**	**Ampicillin**
0	**0**	**%100**	**Imipenem**
%66.66	**%13.33**	**%20**	**Cefixime**
%40	**%13.33**	**% 60**	**Cefotaxime**
%73.3	**%20**	**%6.66**	**Augmentin**
%86.66	***%6.66***	***%6.66***	***Nitrofourantoin***
%46.6	**%6.66**	**%46.6**	**Ceftazidime**
%9	**0**	**%91**	**Tobramycin**
%40	**%13.33**	**%46.6**	**Gentamicin**
%33.33	**%6.66**	**60%**	**Ciprofloxacin**
% 93.3	**0**	**%6.6**	**Piperacillin**
%75	**0**	**%25**	**Co-trimoxazole**

The results in table (1) showed that isolates were resistance for β-lactamases antibiotic suchas: Piperacillin (93.3%), Ampicillin (67.5%) ,Cefixime (66.66%) ,Cefotaxime (40%),ceftazidime (46.6%) this results was agreed with studies [(27)], who reported (61.1%) to Ampicillin and (42.8%) to Cefotaxime, [(28)] but disagrees with the work of Bonomo *etal*. (2003) for the resistance of Cefotaxime (13.3%) [(29)] due to change in penetration of outer cell membrane because has protein called burin the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic**.** [(30)] While aminogiycosides antibiotics group such as Gentoamycin(9%),Tobramycin (13.33%) this result Gentoamycin agreed with studies conducted in Ethiopia (57.8%).[(31)]Because this antibiotics can able inhibition of syntheses protein by linked with small ribosome unite (30S).[(32)] Quinoloes antibiotics such as Ciprofloxacin (33.3%)this result agreed with study by Mavroidi.*etal*.(2012) . [(33)] Because this antibiotics can able inhibition of DNA syntheses and super coiling. [(34)] Reported result Resistance for Co-trimoxazole (75%) ,Augmentin (73.3%) this study agreed with the work of Drawz and Bonomo(2010). [(35)]Furthermore, Resistance result of Nitrofourantion higher (86.66%) this result agreed with study by Gums (2005). [(36)] While imipenem sensitive higher 100% this result agreed with Livadariu *etal*.(2006).[(37)] In general the resistance to different antibiotics may be due to the type of antibiotics and how much that used among the patients in the community. In addition to that the resistance to ward any antibiotics was depended on the amount of PBP2a or β-Lactamase enzyme that produced by each strain of *Ecoli* . All these reasons could create variations in the rate of resistance.

Table (3): Some of virulence factors of *Ecoli* results

Virulence factor	**Isolate NO.**	***Ecoli* positive (%)**	***Ecoli* negative (%)**
Extended-spectrum β-lactamases	2	10%	90%
Metallo- β-lactamases	4	20%	80%
Bacteriocin(colicin)	6	30%	70%

The results in table (3) showed that 2 (10%) isolates were production for Extended-spectrum β-lactamases , in this study also agreed with the findinds of Babypadmini, and Appalaraju (2004).(38) Who reported 41% ESBL positivity E. coli and 40% was reported by Jayapradha etal.(2007) . (39)

Isolates can be explained in most cases to production of β- Lactamase enzyme that destroyed the β-Lactam ring and inactivated the penicillin and this enzyme was encoded by plasmid that easy to transfer among strain. While 4(20%) isolated can be produced of Metallo β-lactamases,this result ageeed with Several recent studies from other parts of Asia such as (18.98%) about this study Khanal etal.(2013) (40) Also demonstrated increasing incidence of MBL production in Enterobacteriaceae isolates. (41).(42) Production of MBL in Enterobacteriaceae isolates currently follows an increasing prevalence pattern and the prevalence rate may vary greatly in different geographical areas and from institute to institute. (43)

6 (30%) isolated can be produced Bacteriocin (colicin) , As noted previously, variable levels of susceptibility to colicins could be due to variability in the number of colicin receptors per cell or due to shielding of receptors by the lipopolysaccharide O-antigenic. (44)

References

[1]. Hudault S, Guignot J, Servin AL (Jul 2001). "Escherichia coli strains colonising the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection". Gut. 49 (1): 47–55. doi:10.1136/gut.49.1.47. PMC 1728375. PMID 11413110 .

[2]. Nataro, J. P., Bopp, C. A., Fields, P. I., Kaper, J. B., & Strockbine, N. A. (2007). Escherichia, Shigella and Salmonella. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology (9th ed., pp. 670-687). Washington, DC, USA: ASM press.

[3]. cookes1985, Feng, Peter, et al., "Enumeration of Escherichia coli and the Coliform Bacteria," in BACTERIOLOGICAL ANALYTICAL MANUAL (8th Ed. 2002), available online at http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods /Bacteriological Analytical Manual BAM/ucm064948.ht .

[4]. "Escherichia coli". CDC National Center for Emerging and Zoonotic Infectious Diseases. Retrieved 2012-10-02.

[5]. Wilson, W. R., Sande, M. A., & Drew, W. L. (2001). Current diagnosis & treatment in infectious diseases. New York: Lange Medical Books/McGraw-Hill.

[6]. Reed, Jennifer Leanne. "Model Driven Analysis of Escherichia coli Metabolism." 2005.

[7]. Nataro, J. P., Bopp, C. A., Fields, P. I., Kaper, J. B., & Strockbine, N. A. (2007). Escherichia, Shigella and Salmonella. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology (9th ed., pp. 670-687). Washington, DC, USA: ASM press.

[8]. Lachmayr KL, Lee J, Kerkhof LJ, DiRienzo AG, Cavanaugh CM, Ford T E.Quantifying Nonspecific TEM β-Lactamase (blaTEM) Genes in a Wastewater Stream Appl. Environ. Microbiol, 2009; 75(1): 203-211.

[9]. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1989;33:1131–6.

[10]. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005;18:657–86.

[11]. Bebrone C: Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol. 2007, 74: 1686-1701.

[12]. Cornaglia G, Giamarellou H, Rossolini GM: Metallo-β-lactamases: a last frontier for β-lactams. Lancet Infect Dis. 2011, 11: 381-393 .

[13]. Cotter PD, Hill C, Ross RP. 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3:777–788. Cross .

[14]. Klaenhammer TR. 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12:39–85.

[15]. Holt, J.G.; N.R. Krieg; P.H.A. Sneath; J.T. Staley; and S.T. Williams. 1995. Bergy,s Manual of Determinative Bacteriology. 9th.ed. Williams &Wilkins, USA. P.532-551.

[16]. MacFaddin J F. Biochemical tests for identification of medical bacteria. 1st Ed. Williams and Wilkins. Baltimore, USA, 2000.

[17]. Clinical and Laboratory Standards Institute (CLSI). (2013). Performance Standards for Antimicrobial Susceptibility Testing; twenty- second information supplement; CLSI Document M100-S22. Clinical and Laboratory Standards Institute: Wayne, PA, USA.

[18]. ALan MD, Partin PH, McConnell DH.Campbell-Walsh urology. 9th ed. Saunders, 2006, pp. 1119-1125.

[19]. Jorgensen JH and JD Turnidge. Susceptibility test methods: dilution and disk diffusion methods, p. 1152–1172. In PR Murray, E J Baron, JH Jorgensen, M L Landry, and M.A Pfaller (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC , 2007.

[20]. Collee, J.G.; R.S. Miles; and B. Watt. 1996.Test for The Identification of Bacteria. P.131-149. In, J.G. Collee; A.G. Fraser; B.P. Marmion; and A. Simmons (eds.). Mackie and McCarteny Practical Medical Microbiology. 14th.ed. Churchill Livingstone, New York .

[21]. Jarlier, V.; Nicolas, M.; Fournier, G.; and Philippon, A. (1988). Extended broad-spectrum β-Lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis. Vol.10,No. 4 :pp. 867-78.

[22]. Franklin C, Liolios L, Peleg AY: Phenotypic detection of carbapenem susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol. 2006, 44: 3139-3144.

[23]. Pugsley AP (1985) Escherichia coli K12 strains for use in the identification and characterization of colicins. J Gen Microbiol 131: 369–376.

[24]. Holt ,J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.A.; & Williams, S.T.(1994). Bergy, s Manual Of Derminative Bacteriology. (9th) ed. Williams & Wilkins.

[25]. .Friedrich , A.W.; Koch , R.; Bielasz ewska , M.; Zhang , W.; Karch , H.and mathys , W. (2005) . Distribution of the urease gene cluster amongand urea activity of enterohemorrhagic Escherichia coli 0157 lsolateds from humans . J. of Clinical Microbiology .Vol.43,No. 2 : pp.546-50 .

[26]. Dhakal ,B . K.and Mulvey . M . A.(2012). The UPEC Pore-Formin Toxin α- Hemolysin Triggers Proteolysis of Host Proteins to Disrupt Cell Adhesion, Inflammatory, and Survival Pathways. Cell Host & Microbe, Vol.11, No. 1, pp.58- 69.

[27]. Olowe OA, Eniola KIT, Olowe RA, Olayemi AB. Starch paper technique is easy to detect beta lactamase detection from cases of diarrheagenic Escherichia coli in Osogbo. Life Sci J. 2007:4.

[28]. Al-Chalabi, R .;Al- Ibadi, M. andAl –Ubaidy,A .(2010). Detection of Urovirulence Genes (eae,E-hly,α-hly) of Uropathogenic Escherichia coli by Specific PCR. Journal of Biotechnology Research Center (special edition). Vol.4, No.1.pp.

[29]. Bonomo , R.A.; Donskey , C.J.; Blumer , I.L; Hujer , A.M.; Hoyenm , C.K.; Jacob , M.R.; whalen , G.G. and Salata, R.A. (2003) Cefotaxime resistant bacteria colonizing older people a dmitted to an acute care hospital . (2003) .J. AM. Geriatr . Soc . Vol. 51 ,No. 4 : 519 –22 .

[30]. Spanu, T.; Luzzaro, F.; Perilli, M.; Amicosanti, G.; Toniolo, A.; Fadda, G. and the Italian ESBL study group .(2002). Occurance of extended-spectrum-B. lactamas and other antimicrobial drug. Antimicrobial Agent and Chemotherapy. Jun. Vol .46 ,No. 1 :pp. 196-202.

[31]. Tesfaye G, Asrat D, Woldeamanuel Y, Gizaw M. Microbiology of discharging ears in Ethiopia. Asian Pac J Trop Med. 2009;2(91):60–67.

[32]. Heritage, J. (2003). Antibiotics. University of Leeds.

[33]. Mavroidi A, Miriagou V, Liakopoulos A, Tzelepi E, Stefos A, Dalekos GN, et al. Ciprofloxacin-resistant Escherichia coli in Central Greece: mechanisms of resistance and molecular identification. BMC Infect Dis.2012;12 [PMC free article] [PubMed]

[34]. (Hardy etal., (2009).Virulence factors of avian pathogenic Escherichia coli (APEC)) Pesq. Vet. Bras. vol.29 no.7 Rio de Janeiro July

[35]. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev.2010;23(1):160–201. [PMC free article] [PubMed]

[36]. Gums J.G., Comparison of National and Regional Non Susceptibilities of S. aureus,E. coli and P. aeruginosa to commonly prescribed antibiotics: results of the antimicrobial resistance management program 1997-2004, 434, 43rd IDSA, San Francisco, 6-9 oct. 2005.

[37]. Livadariu M., Cristea V., Frunzoi M., Studiul infecţiilor cutanate în cazuistica laboratorului Synevo Bucureşti, Zilele Medicale Medicover-Synevo, Bucureşti, 2006.

[38]. Babypadmini, S. and Appalaraju, B. (2004). Extended spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae –Prevalence and susceptibility pattern in atertiary care hospital. Indian Journal of Medical Microbiolology. 22(3):172-174.

[39]. (Jayapradha, R., Murugesh, S., Mahesh, N and Brahatheeswaran, D. (2007). Prevalence of ESBL Producing Strains in Tuberculosis Patients. Research(Journal of Microbiology, 2:491-495).

[40]. Khanal S, Joshi DR, Bhatta DR, Devkota U, Pokhrel BM: β-lactamase-producing multidrug-resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at national institute of neurological and allied sciences. Nepal ISRN Microbiol. 2013, 2013: 847569.

[41]. Kumar S, Bandyopadhyay M, Mondal S, Pal N, Ghosh T, Bandyopadhyay M, Banerjee P: Tigecycline activity against metallo-β-lactamase-producing bacteria.Avicenna J Med. 2013, 3: 92-96.

[42]. Datta S, Wattal C, Goel N, Oberoi JK, Raveendran R, Prasad KJ: A ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli & Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Res. 2012, 135: 907-912.

[43]. Yong D, Choi YS, Roh KH, Kim CK, Park YH, Yum JH, Lee K, Chong Y: Increasing prevalence and diversity of metallo-beta-lactamases in Pseudomonas spp., Acinetobacter spp., and Enterobacteriaceae from Korea. Antimicrob Agents Chemother. 2006, 50: 1884-1886.

[44]. Bradley DE, Howard SP, Lior H (1991) Colicinogeny of O15 enterohemorrhagic Escherichia coli and the shielding of colicin and phage receptors by their O- antigenic side chains. Can J Microbiol 37: 97–104