Zagazig Veterinary Journal **RESEARCH ARTICLE**
Volume 45, Number 1, p. 55-63, March, 2017

DOI: 10.21608/zvjz.2017.7687.

Molecular Characterization of β-lactamase Genes in Antibiotic Resistant Bacteria

Adel M. Attia, Mohammed N. Hassan and Shymaa E. Elwan*

Microbiology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

Article History: Received: 4/2/2017 Received in revised form: 1/3/2017 Accepted: 12/3/2017

Abstract

Beta-lactamases (bls) are implicated seriously in resistance to β - lactam antibiotics. β- lactam resistance related to Sulfa hydral variabl- beta lactamases (SHV- bls) were detected in *E. coli* and *Klebsiellae* isolated from human and chickens. In this work, resistance profile of the isolates, extended spectrum β-lactamases (ESBLs) activity and SHV genes sequencing were examined. *E. coli* was isolated from humans, broiler and day-old chicks samples, meanwhile, *K. pneumonia* was isolated only from human samples (88.8%). Sixty-four (88.8 %) of human isolates contained beta-lactamases (iodometric positive)**,** 28% were ESBLs and 35% contained SHV. In case of day-old chick isolates, 50% contained beta-lactamases (iodometric positive), 5.5% were ESBLs. Out of 45 broiler isolates, 37.7% contained beta-lactmases (iodometric positive)**,** 8.8% were ESBLs and 80% contained SHV. It was found that SHV were of SHV-5 and SHV-12. It could be concluded that, wide spread of ESBLs SHV-5 and SHV-12 in humans and chicken is a major concern in resistance to β -lactam drugs.

Keywords: Extended spectrum beta lactamases, Sulfa hydral variable (SHV), *E. coli*, *Klebsiella pneumoniae*

Introduction

Escherichia coli considered one of the major causes of nosocomial infections in humans. *E. coli* is a common inhabitant in the human and animal gut and an indicator of fecal contamination of food [1]. The β- Lactams are groups of antibiotics extensively used within human and veterinary medicine in treating *E. coli* infections.

In addition, *Klebsiella pneumoniae* is a member genus of *Enterobacteriaceae* and considered as one of normal flora of GIT of humans. It is the most prevalent and clinically important, hospital-acquired and occurred primarily in patients with impaired host defenses [2]. Emergence of resistance against β-lactam antibiotics began prior to the first β-lactam, penicillin, was developed. The first β-lactamase was identified in *Escherichia coli* before using penicillin in medical practices [3].

Bacterial genome can acquire resistance genes via integrons, transposons and plasmids because they are not fixed and resistance mechanisms are easily exchanged between different species of bacteria through their sophisticated ways sharing mechanisms essential for survival [4]. The β-lactam antibiotics are classified into several groups according to structural characteristics, and the presence of the four-membered β-lactam (azetidin-2-one) rings is the main structural feature of them. They include Penicillins, Cephalosporins, Cephamycins, Carbapenems, Monobactams and β -lactamase inhibitors. This category of antibiotics is subdivided into functional drug groups to facilitate understanding and prescribing practices, the antibiotics of each functional group differ in pharmacokinetics, safety, and duration of the clinical experience with their use [5]. There are three pathways which have an important role in clinical antibacterial resistance which include destruction of the antibiotics, altered antibiotics targets and decreased intracellular uptake of the drug [6]. Bacterial destruction of β-lactams by β -lactamases considered the most common method of resistance. β-lactamses open the β-lactam ring and the altered drug prohibits subsequent effective binding to penicillin binding proteins (PBP), consequently, cell wall synthesis is unable to continue [6].

β-Lactamases can be classified according to two main schemes: The Ambler molecular classification scheme and the Bush-Jacoby-Medieros functional classification system**.** The

***Corresponding author email: (shymoibrahim654@gmail.com), Microbiology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt.**

first scheme divides β- lactamases into four major groups (A to D). The main principle of this classification scheme depends upon protein homology (amino acid sequences) and not phenotypic characteristics. In the Ambler classification scheme the β-lactamases of classes A, C and D are serine β-lactamases. Meanwhile, the class B enzymes are metallo-β-lactamases [7]. There are other different β-lactamases which may be chromosomally encoded β-lactamases such as *Amp*C. There are also plasmid-borne genes coding for β-lactamases, such as *TEM*-1, *TEM*-2 and SHV-1. Mutations in the genes causing generation of the extended-spectrum β-lactamases (ESBLs), which can be divided into at least four groups. Those four groups mediate resistance to oxyimino cephalosporins, e.g. cefuroxime, cefotaxime and ceftazidime [8]**.**

The second prevalent type of β - lactamases is SHV-1, which was described primarily in *K. pneumoniae* and *E. coli*. The SHV-1 is responsible for plasmid mediated ampicillin resistance nad amino acids substitution causes change of enzyme structures and activities [9]. The aim of the present study was to identify ESBLs phenotypically and genotypically by PCR and sequence analysis of SHV genes.

Material and methods

Collection of samples

A total of 135 samples were collected from chickens (n=63) reared in poultry farms and humans (n=72) at Sharkia Governorate, Egypt. The chicken samples were obtained from livers and spleens of freshly dead and diseased broilers and one-day old chicks suspected to have colisepticemia. Urine samples were collected from humans suffering from urinary tract infections.

Isolation and identification

The samples were streaked directly onto the surface of nutrient agar according to the method of Calnek *et al.* [10]. All inoculated plates were incubated at 37ºC for 24 h and examined for bacterial growth. The isolated colonies were sub-cultured again onto MacConkey´s agar and examined for lactose fermentation [11]. The lactose fermenter colonies were then streaked onto Eosin Methylene Blue agar media (EMB) (Oxoid, UK). The characteristic greenish metallic sheen *E. coli* colonies on EMB media were sub-cultured on soft agar for further identification. The biochemical testes used for *E. coli* identification were indole, methyl red, Voges-Proskauer and Simmon's citrate (Oxoid, UK) [12,13]. The isolates which were Gram's negative bacilli, Indole +, Methyl red +, Voges-Proskauer and Simmon´s citrate negative were identified as *E. coli* strains and subjected to further tests. *K. pneuomonia* colonies were lactose fermenter and was confirmed as indole and Methyl red negative, while Voges-Proskauer and Simmon´s citrate positive [12,13].

Antibiogram testing

The identified isolates were purified again on nutrient agar and tested for antimicrobial susceptibility on 11 different antibiotics including β -lactams, aminoglycosides, penicillins and quinolones (Oxoid, UK). Antibiotic susceptibility profiles were determined using the Kirby-Bauer disk diffusion method and interpretation was based on the CLSI guidelines [14]. All isolates were tested for the detection of β -lactamases by iodometric tube method; briefly, 1 mL of crude or pure β-lactamase enzyme was used as a control positive and 8 bacterial colonies of tested bacteria were emulsified in 250 µL of penicillin solution in small test tubes. Penicillin solution alone was used as a negative control. After 30-60 min, 200 µL of 1% starch solution and 1 mL of iodine solution were added changing the color into blue. Rapid de-colorization of the blue color within 5-10 min was an indicator for β-lactamases production [15]. Isolates that were resistant to third generation cephalosporins and iodometric positive were tested for the presence of the extended spectrum β -lactamases using double disc diffusion (DDD) [14]. In brief, discs of cephalosporin (cefotaxime, ceftazidime and cefepime) (30 µg) alone and cephalosporin + clavulanic acid (30 µg/10 µg) were placed with a distance of 25 mm, center to center, on a Muller Hinton agar plate. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared to the zone around the disc with the cephalosporin alone. The test is positive if the inhibition zone is ≥ 5 mm larger with clavulanic acid than without) [14].

The isolates were also examined by PCR for detection of SHV resistance genes.

Genotypic detection of ESBL

The DNA from the purified colonies of ESBLs producing *E. coli* and *K. pneumonia* was extracted using Wizard® Chromosomal DNA purification kit (Promega company, USA), following the manufacturer's instructions. The SHV genes were detected as described previously [16]. Specific primers for the SHV genes including forward primer (5'-CACTCAAGGATGTATTGTG-3'); Reverse primer (5'-TTAGCGTTGCCAGTGCTCG-3') were used for PCR amplification that produced 885 bp amplicon. A reaction volume of 25 µL included 6 µL of Master Mix kits (Bioflux company). which consisted of (2.5 µL TAE 10X buffer + 2 µL dNTPs + 1.5 µL $Mgcl_2$ + 0.2 µL Taq polymerase), in addition,2 µL of each primer and 2 µL of DNA template were added and then distilled water was added to reach 25 µL total volume. The cycling conditions started with an initial 10 min denaturation at 94°C, followed by 35 cycles of 45 sec at 94°C, annealing at 42°C for 45 sec, extension at 72°C for 45 sec and a final extension for 10 min. The amplified products were visualized by electrophoresis in 2% agarose gel (BioRad) with ethidium bromide (Invitrogen) (0.5 µg/ mL) for 1 hour (6 Volts/cm), and visualized under UV light and results documented.

Sequencing and sequence analysis

The PCR products of were purified from agarose gel using QIAquick® PCR Purification kit (Qiagen) , and then sequenced by Sanger dideoxy sequencing method (Sigma Company). The nucleotide sequences were identified using NCBI-BLAST and submitted to the GenBank (accession numbers KU096850, *E. coli* EGY20151// KU096851, *E. coli* EGY20152// KU096852, *E. coli* EGY20153// KU096853, *E. coli* EGY20154// KU319567, *E. coli* EGY20155 // KU319568, *E. coli* EGY20156 // KU096848, *E. coli* EGY20157 // KU319569, *E. coli* EGY20158 // KU096849, *E. coli* EGY20159 // KU319570, *E. coli* EGY201510 // KU319571, *K. pneumoniae* EGY201511 were provided) The sequences were then aligned with other sequences in the GenBank by ClustalW method using MegAlign of DNAStar software (Lasergene version 7.2; DNASTAR, Madison, WI, USA).

Results and Discussion

A total of 135 samples from different sources were subjected to bacterial isolation. All the samples were positive for *Enterobacteriacea.* After bacterial culture on MacConkey's and EMB media and biochemical identification using IMVC tests most of the isolates were Lactose fermenters. *E. coli* and *Klebsiella* spp. were isolated from 123 and 12 examined samples, which were used for antimicrobial studies.

Table 1: Resistance percentage of each antimicrobial agent against *E. coli* and *K. pneumoniae* isolates

Antimicrobial agents		Resistance pattern of each antimicrobial agent	
		***E. coli* (n=123)**	***K. pneumoniae* (n=12)**
Penicillin	**Amoxicillin**	119/ 123 (96%)	12/12 (100%)
	Amoxicillin/clavulanic acid	90/ 123 (73%)	9/ 12 (75%)
β-lactamase inhibitors	**Cefepime/ tazobactam**	0.0	0.0
1st generation	**Cephalexin**	27/ 123 (96%)	10/ 12 (83%)
2nd generation	**Cefuroxime**	94/ 123 (76 %)	8/ 12 (66.6%)
3rd generation	**Cefotaxime**	39/ 123 (31 %)	5/ 12 (41%)
	Ceftriaxone	85/ 123 (30%)	5/ 12 (41%)
4th generation	**Cefepime**	33/ 123 (26%)	4/ 12 (33%)
Non β-lactam antibiotics	**Trimethoprime/ Sulfamethazole**	83/ 123 (67%)	7/ 12 (58%)
	Gentamicine	10/ 123 (8%)	2/ 12 (16%)
	Chloramphenicol	45/ 123 (36%)	3/ 12 (25%)

Concerning antimicrobial resistance of *E. coli* isolates to the β- lactam antibiotics, the results revealed that 96% of *E. coli* isolates were resistant to amoxicillin. This was compatible with the findings obtained by Jorgensen and Turnidge [17], who reported that 92.8% of *E. coli* isolates were resistant to amoxicillin. In addition, 90% of *E. coli* isolates were resistant to amoxicillin/ clavulanic acid (Table 1). Similar results were obtained by Jorgensen and Turnidge [17].

Concerning the resistance to extended-spectrum cephalosporins, *E. coli* isolates showed low resistance to third generation cephalosporins including cefotaxime (31%) and ceftriaxone (30%). Similar results were reported by Li *et al.* [18], who recorded that 44% of *E. coli* isolates were resistant to cefotaxime. In contrary, Daniel *et al.* [19] reported that 2.4% of *E. coli* isolates were resistant to ceftriaxone (Table 1).

Concerning fourth generation cephalosporins, 26% of *E. coli* isolates were resistant to cefepime (Table 1). This result was compatible with that reported by Pai *et al.* [20], who found that the activity of cefepime was not significantly impaired by *Amp*C and more than 90% susceptibility had been reported in *E. coli* isolates from animals. The low resistance rate to cefepime could be explained by the co-presence of ESBL isolates [21]. However, the results revealed no resistance to Cefepim/ tazobactam (Table 1).

E. coli isolates showed low resistance rate to gentamicin (8%) which is considered the second most active antibiotic (Table 1). This result coincides with Zahar *et al.* [22] who reported the highest susceptibility to imipenem followed by gentamicin. The results revealed that trimethoprim/ sulfamethoxazole and chloramphenicol resistance rates were 67 % and 78 %**,** respectively (Table 1). These results were compatible with those obtained by Al-Agamy [23], who reported that 61.9% and 85.7% of *E. coli* isolates were resistant to trimethoprim/ sulphamethoxazole and chloramphenicol, respectively.

Table 2: Clinical finding of SHV gene among *E. coli* and *K. pneumoniea* isolates by PCR

Isolates	β-lactamases detected by I.M.	ESBLs detected by DDD	SHV	SHV- Inhibitor resistant	SHV – non inhibitor resistant
H14	**+ve**	**+ve**	**-**	**-**	**-**
H15	**+ve**	**+ve**	**-**	**-**	**-**
H16	**+ve**	**+ve**	**-**	**-**	**-**
H17	**+ve**	**+ve**	**-**	**-**	**-**
H20	**+ve**	**+ve**	**-**	**-**	**-**
H22	**+ve**	**+ ve**	**-**	**-**	**-**
H23	**+ ve**	**+ ve**	**-**	**-**	**-**
H24	**+ ve**	**+ ve**	**-**	**-**	**-**
H28	**+ ve**	**+ ve**	**-**	**-**	**-**
H31	**+ ve**	**+ ve**	**-**	**-**	**-**
H39	**+ ve**	**+ ve**	**+ ve**	**+ve**	**-**
H40	**+ ve**	**+ ve**	**+ve**	**-**	**+ve**
H44	**+ ve**	**+ ve**	**-**	**-**	**-**
H48	**+ ve**	**+ ve**	**-**	**-**	**-**
H63	**+ ve**	**+ ve**	**-**	**-**	**-**
H68	**+ve**	**+ve**	**+ve**	**+ve**	**-**
H69	**+ve**	**+ve**	**+ve**	**+ve**	**-**
H70	**+ve**	**+ve**	**+ve**	**+ve**	**-**
H71	**+ve**	**+ve**	**+ve**	**+ve**	**-**
H72	**+ve**	**+ve**	**+ve**	**+ve**	**-**
D3	**+ve**	**+ve**	**-**	**-**	**-**
Br42	**+ve**	**+ve**	**+ve**	**+ve**	**-**
Br43	**+ve**	**+ve**	**+ve**	**+ve**	**-**
Br44	**+ve**	**+ve**	**+ve**	**+ve**	**-**
Br45	**+ ve**	**+ ve**	**+ve**	**-**	**+ve**

H: Human samples D: Day old chicks Br: Broilers I.M : Iodometric method ESBLs = Extended spectrum β- Lactamase DDD : double disc diffusion
SHV: sulfa hydral variable

+ ve: positive

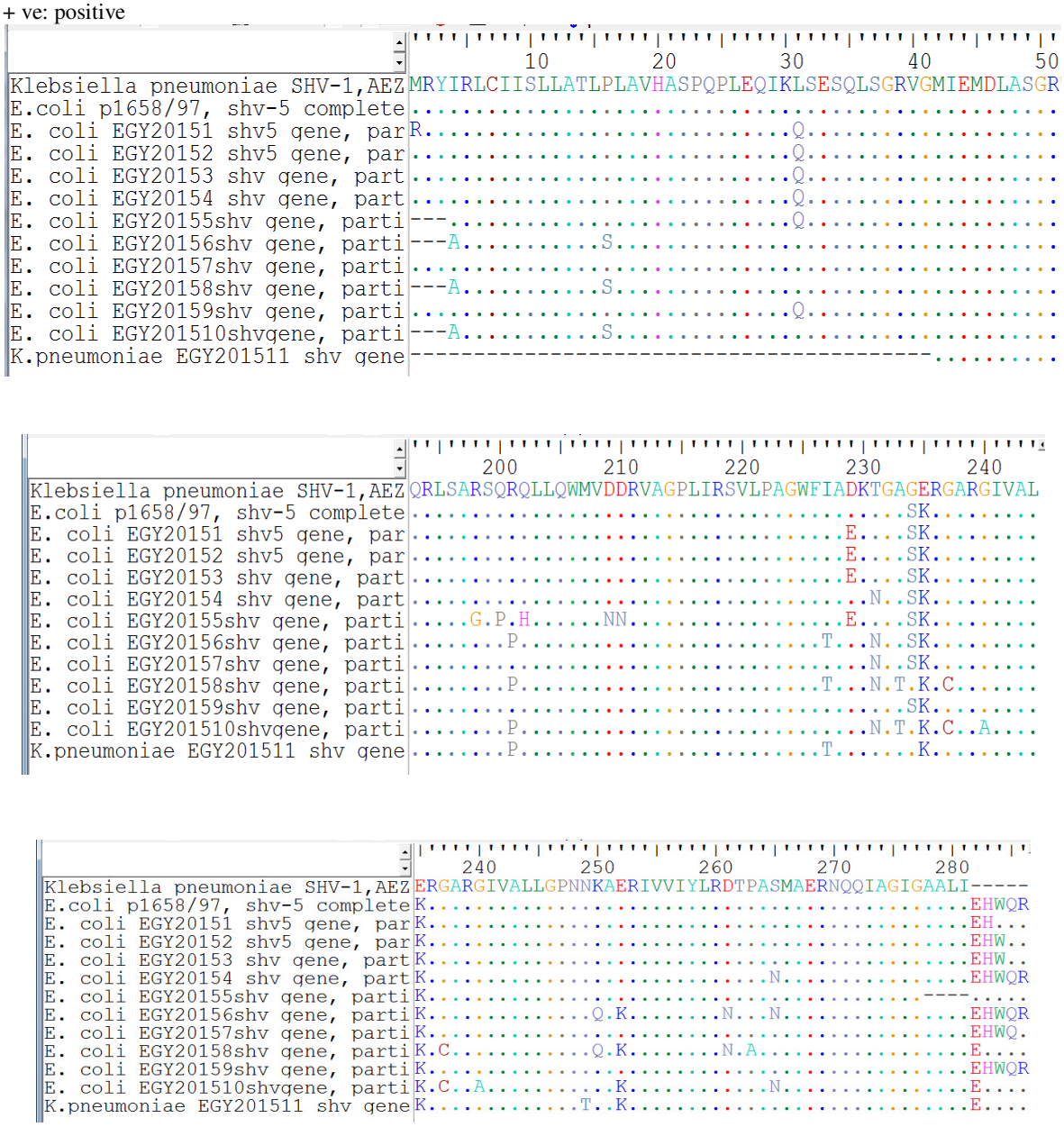

Figure 1: Multiple sequence alignment of SHV-1 and SHV-5 done by BioEdit software against similar Amino acid sequence from GenBank.

This study showed a co-resistance for sulfonamide with amoxicillin and chloramphenicol, as reported in other studies [19]. In the present investigation, resistance to gentamicin, chloramphenicol, and trimethoprim/ sulfamethoxazole in β-lactamase producing isolates especially those containing SHV associated genes was high. Similar results were reported in Egypt by Al-Agamy [24] and Al-Agamy *et al*. [25].

Large number of β-lactamases (BLs) has been discovered such as ESBL. The detection of ESBL producing microorganisms is a challenge requiring both phenotypic and genotypic tests for all genes associated with β-lactamase production. According to the majority of epidemiological studies on ESBL, *K. pneumoniae* and *E. coli* are the most common species implicated in this type of resistance. In this study, *E. coli* and *K. pneumonia* were found carrying detectable ESBL. The results agreed with a study done in Rio Grande do Sul by Freitas and co-workers who observed that these two species were the most prevalent among ESBL producing microorganisms [15,26,27]. Concerning the crude β-lactamase assay, iodometric method was sensitive for β- lactamase detection by measuring the rate of penicillin hydrolysis to penicilloic acid by β-lactamase enzyme [15]. In this method, iodine binds with penicilloic acid rather than penicillin and this led to rapid de-colorization of blue color of starch iodine complex, which indicated the presence of β-lactamase producers (positive result). The isolates showed differences in the de-colorization time from those with less inhibition diameters in penicillin sensitivity test than the other, were the faster in the reduction of iodine. These results agreed with Lianes *et al*. [28].

Awareness of ESBL production by *K. pneumoniae* and *E. coli* is very important clinically. Reliable laboratory method is now available, double disc diffusion (DDD), by which ESBL production can be detected. This method has also been promoted by the CLSI [15]. Isolates suspected to produce ESBLs should not be reported as susceptible to third-generation cephalosporins or cefepime until follow-up confirmatory tests are performed. The results in Table 3 show that 20 out of 72 (27%) human isolates, 1 out of 18 (5%) day old chicken isolates and 4 out of 45 (8.8%) broiler isolates were ESBLs according to the results of DDD test. Concerning studied gene in this investigation the SHV gene was recovered from *E. coli* and *Klebsiella* isolates (Table 2). This result agreed with Kola *et al.* [29] who found that the most predominant β-lactamase-encoding genes identified among the *Enterobacteriaceae* isolates were TEM, followed by CTX-M, and SHV.

Sequencing of amplified SHV genes was performed for ten β-lactamase resistant *E. coli* isolates and one β-lactamase resistant *K. pneumoniae* isolate. Alignment of the sequences with wild type of SHV-5, SHV-12 and SHV-1 in *E. coli* and *K. pneumoniae* isolates on the GenBank with accession numbers (*E. coli* NC_004998.1, *E. coli* NC_024967.1 and *K. pneumoniae* X98098.1), respectively, was 90-100% (Figure 1). This matched with Newire *et al*. [30], who explained that comparison of the corresponding amino acid sequence of the 11 SHV positive isolates demonstrated the predominance of SHV-12, while SHV-5 and SHV-2a was the most closely related sequencing match in the remaining isolates. SHV-12 is thought to have evolved from SHV-2a, and SHV-5 from SHV-2.

Conclusion

There is a high prevalence of β-lactamase genes in our clinical isolates that are responsible for such resistance. Therefore, it must report β-lactamases production along with routine sensitivity reports, which will help the clinician in prescribing proper antibiotics. Also, the sequence analysis of amplified genes showed differences between multiple SNPs in the same gene among different local isolates and with internationally published sequences. In the end, it has been felt that there is a need to formulate strategies to detect and prevent the emergence of β-lactamases producing strains for the effective treatment of infections which are caused by them.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Livermore, D. M. (1998): Beta-lactamase-mediated resistance and opportunities for its control. J Antimicrob Agents, 41(Suppl 4):25-41.

[2] Shahab Qureshi, M.D. (2016): Klebsiella Infections . Michael Stuart Bronze, Medscape. Updated: Nov 07.

[3] Ahmad, M.; Urban C.; Mariano, N.; Bradford, P. A.; Calcagni, E.; Projan, S. J.; Bush, K. and Rahal, J. J. 1999: Clinical characteristics and molecular epidemiology associated with imipenem-resistant *Klebsiella pneumoniae*. Clin Infect Dis, 29(2):352-355.

[4] Sullivan, A.; Edlund, C. and Nord, C.E. (2001): Effect of antimicrobial agents on the ecological balance of human microflora, Lancet Infect Dis, 1(2): 101-104.

[5] Calderwood, S.P. (2007): Overview of the beta-lactam antibiotics. In/l Hopper D C edi.up to date.

[6] Forbes, B.A.; Sahm, D.F. and Weissfeld, A.S. (2007): *Enterobacteriaceae*. In: Bailey and Scott's diagnostic microbiology12th ed.Allen A, Wilson L and Wurm E(editors), Mosby Elsevier:323-333

[7] Stürenburg, E.; Lang, M.; Horstkotte, M.A.; Laufs, R. and Mack, D. (2004): Evaluation of the MicroScan ESBL plus confirmation panel for detection of extended-spectrum beta-lactamases in clinical isolates of oxyimino-cephalosporin-resistant Gram-negative bacteria. J Antimicrob Chemother, 54(5):870-875.

[8] Larson, L.L and Ramphal, R. (2002): Extended spectrum beta-lactamases. In Seminar Respiratory Infections, 17(3): 189-194.

[9] 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(4):933-951.

[10] Calnek, B.W.; Barnc, H.J.; Charles, W.B.; Larry, R. M. and Saif, Y.M. (1997): Diseases of Poultry, 10th Edition, Iowa State University Press, U.S.A.

[11] Difco and BBL Manual (2003): BD Diagnostic Systems; Manual of Microbiological Culture Media by Power D.A. and Zimbro, M.J. Australia & New Zealand.

[12] Koneman, E.W.; Allen, S.D.; Dowell, V.R. and Sommers, H.N. (1983): Color Atlas and Text Book of diagnostic Microbiology. 2nd edition J.P.Lippincott Company, N.Y.; London, P.57-125.

[13] Gradwohl, R.B.H.; Sonnenwirth, A.C. and Jarett, L. (1980): Gradwohol's Clinical Laboratory Methods and diagnosis. Vol.2,8th edition Sonnenwirth, A.C.; Jarett,L. (eds.), The C.V. Mosby Co. London,Toronto.

[14] Clinical and Laboratory Standards Institute (2012): Performance standards for antimicrobial susceptibility testing; 21st informational supplement M100–S22, CLSI, Wayne, PA.

[15] Thornsberry, C. (1980): Detection of beta-lactamase in *Neisseria gonorrhoeae* and *Haemophilus influenzae*, Laboratory Training and Consultation Division Services, Centers for Disease Control, p. 1-8.

[16] Briñas, L.; Zarazaga, M.; Sáenz, Y.; Ruiz-Larrea, F. and Torres, C. (2002): β-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, human, and healthy animals. Antimicrob agents Chemother, 46(10):3156-3163.

[17] Jorgensen, J.H. and Turnidge, J.D. (2007): Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods. In: Murray P.R., Baron E.J., Jorgensen J.H., Landry M.L., Pfaller M.A. eds. Manual of Clinical Microbiology. 9th ed. Washington, DC: American Society for Microbiology, p:1152-72.

[18] Li X.Z.; Mehrotra M.; Ghimire S. and Adewoye L. (2006): Extended spectrum b-lactamase-producing bacteria from food and companion animals: an emerging issue. 2006 Health Canada Science Forum Book of Abstracts, Ottawa, Canada, P3: 42.

[19] Daniel A. Tadesse; Shaohua Zhao; Emily Tong; Sherry Ayers; Aparna Singh, Mary J. Bartholomew and Patrick F. McDermott (2007): Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing;

seventeenth Informational supplement. CLSI document M100-S17 (ISBN 1-56238-625-5), 940 West Valley Road, Suite 21400, Wayne, Pennsylvania 19087-1898 USA. Vol. 27 No. 1.

[20] Pai, H.; Kang, C.I.; Byeon, J.H.; Lee, K.D.; Park, W.B.; Kim, H.B.; Kim, E.C.; Oh, M.D. and Choe, K.W. (2004): Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother, 48(10): 3720-3728.

[21] Kang, C.I.; Pai, H.; Kim, S.H.; Kim, H.B.; Kim, E.C. and Oh, M.D. (2004): Cefepime and the inoculum effect in tests with Klebsiella pneumoniae producing plasmid-mediated AmpC-type-β-lactamase. J Antimicrob Chemother, 54(6): 11301133.

[22] Zahar, J.R.; Lortholary, O.; Martin, C.; Potel, G.; Plesiat, P. and Nordmann, P. (2009): Addressing the challenge of extended-spectrum β-lactamases. Curr Opin Investig Drugs, 10(2): 172-180

[23] Al-Agamy, M.H. (2013): Phenotypic and molecular characterization of extended-spectrum β- lactamases and AmpC β-lactamases in *Klebsiella pneumoniae*. Pak J Pharm Sci, 26(2): 291-298.

[24] Ben-Ami, R.; Rodríguez-Baño, J.; Arslan, H.; Pitout, J.D.D.; Quentin, C.; Calbo, E.S.; Azap, O.K.; Arpin, C.; Pascual, A.; Livermore, D.M.; Garau, J. and Carmeli, Y. (2009): A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis, 49(5):682-690.

[25] Al-Agamy, M.H.; Ashour, M.S.E. and Wiegand, I. (2006): First description of CTX-M β-lactamase-producing clinical *Escherichia coli* isolates from Egypt. Int J Antimicrob Agents, 27(6): 545-548.

[26] Perilli, M.; Caporale, B.; Celenza, G.; Pellegrini, C.; Docquier, J. D.; Mezzatesta, M.; Rossolini, G.M.; Stefani, S. and Amicosante, G. (2007): Identification and characterization of a new metallo-beta-lactamase, IND-5, from a clinical isolate of *Chryseobacterium indologenes*. Antimicrob Agents Chemother, 51(8): 2988-2990.

[27] Wikler, M.A.; Cockerill, F.R.; Craig, W.A.; Dudley, M.N.; Eliopoulos, G.M. and Hecht, D.W. (2009): Performance standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute, 29 (3): 32- 44.

[28] Lianes, R.; Gonzales, M.; Martines, I.; Sosa, J.; Guzman, D.; Gutierrez, O.; Liop, A. and Sanches, L. (2003): Evolution of four methods for detecting the beta-lactamase activity in *Neisseria gonorrhoeae* isolated in Cuba. Memórias do Instituto Oswaldo Cruz, 98 (8):1089-1091.

[29] Kola, A.; Kohler, C.; Pfeifer, Y.; Schwab, F.; Kühn, K.; Schulz, K.; Balau, V.; Breitbach, K.; Bast, A.; Witte, W.; Gastmeier, P. and Steinmetz, I. (2012): High prevalence of extended-spectrum-β-lactamase-producing Enterobacteriaceae in organic and conventional retail chicken meat, Germany. J Antimicrob Chemother. 67(11):2631-2634.

[30] Newire, E.A.; Ahmed, S.F.; House, B.; Valiente, E. and Pimentel, G. (2013): Detection of new SHV-12, SHV-5 and SHV-2a variants of extended spectrum Beta-lactamase in *Klebsiella pneumoniae* in Egypt. Ann Clin Microbio Antimicrob, 12(1):16.

الملخص العربى

دراسات عن التقنيه وتسلسل العقد النوويه لجينات إنزيمات البيتا لاكتاماز فى البكتيريا المقاومه للمضادات الحيويه

عادل عطيه محمد أحمد، محمد نبيل حسن و شيماء ابراهيم علوان ابراهيم
قسم الميكروبيولوجيا – كليه الطب البيطرى – جامعه الزقازيق – جمهوريه مصر العربيه

إن انزيمات البيتالاكتامز دائما ما تتهم بأنها المتسبب الرئيسى فى المقاومه ضد المضادات الحيويه من مجموعه البيتالاكتام.وتعزى مقاومة البيتالاكتامزإلى وجود جينات السلفاهيدريز والتى تم اكتشافها فى ميكروبات الإشيرشيا كولاي والكلبسيلا.وفى هذا العمل توضيح بروفيل المقاومه للبكتريا المعزولة من خلال رصد الأنشطة المتعددة المجالات لانزيمات البيتالاكتامز وجينات السلفاهيدريز قد تم فحص تتابعها الجينى. وقدعزلت الإشيرشيا كولاي من عينات الإنسان والدواجن بينما الكلبسيلا عزلت فقط من الإنسان. أربعة وستون (٨٨.٨%) من عينات الإنسان كان موجود بها جينات البيتالاكتامز (عن طريق اختبار اليود), ٢٨% كانت ايجابيه لإختبار تعدى الجينات و ٣٥% من عينات الإنسان تحتوى على جين السلفاهيدريز. بينما العزلات من الكتكوت عمر يوم كان ٥٠% موجود بها جينات البيتالاكتامز (عن طريق اختبار اليود), ٥.٥% كانت ايجابيه لإختبار تعدى الجينات. من ٤٥ عزله من دواجن التسمين، ٣٧.٧% كان موجود بها جينات البيتالاكتماز (عن طريق اختبار اليود), ٨.٨% كانت ايجابيه لإختبار تعدى الجينات و ٨٠% تحتوى على جين السلفاهيدريز. ووجد أن جينات البيتالاكتماز كانت من النوع ٥,١٢ ومن ذلك يمكننا معرفه ان الإنتشار الواسع للإنزيمات المتعدده الجينات من نوع السلفاهيدريز ٥,١٢ فى الإنسان والدواجن تعتبر ذات صله كبيره بالمقاومه ضد المضادات من مجموعه البيتالاكتام.