Egypt. J. Microbiol. **54,** pp. 91-101 (2019)

Egyptian Journal of Microbiology
http://ejm.journals.ekb.eg/

Prevalence of Extended Spectrum Beta- Lactamase Genes among *Escherichia coli* and *Klebsiella pneumonia*e Clinical Isolates

Rehab Mohamed M. Ali Amer[(1)#], Khaled Zakaria El-Baghdady[(2)], Iman K.F. Kamel[(3)], Hisham M. El-Shishtawy[(4)]

[(1)]*Central Lab, Egyptian Environmental Affairs Agency (EEAA), Cairo, Egypt;* [(2)]*Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt;* [(3)]*Clinical Pathology Department, Mataria Teaching Hospital, Cairo, Egypt;* [(4)]*Microbiology Department, Agricultural Genetic Engineering Research Institute, Giza, Egypt.*

EXTENDED spectrum beta lactamases (ESBLs) of *Escherichia coli* and *Klebsiella pneumoniae* became a major problem in the whole world. This work aimed to study the prevalence of ESBLs genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$) in *E. coli* and *K. pneumonia*e clinical isolates recovered from Al Kasr Al-Ainy Hospital, Cairo, Egypt, during the period between 2016 and 2018. One hundred sixty eight clinical isolates were screened for ESBLs production by double disc synergy test (DDST) and combination disc test (CDT). ESBLs genes were detected by PCR using specific primers. Out of 168 isolates, 113 (67.26%) were phenotypically positive ESBLs. Genotypically, only 108 (95.58%) were confirmed as ESBLs producer (61 *E. coli* and 47 *K. pneumoniae* isolates). The percentage of *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$ were 91.7%, 82.4% and 78.7%, respectively. The co-existence of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes were found in 47.54 and 72.34% for *E. coli* and *K. pneumoniae*, respectively.

Keywords: Enterobacteriacea, *Escherichia coli*, *Klebsiella pneumoniae,* ESBLs.

Introduction

Pathogenic bacteria and their resistance against antibiotics become a worldwide serious problem with challenge for the treatment of infectious diseases. The misuse of antibiotics in medicine, agriculture and veterinary is the main reason for dissemination of resistance genes (Alekshun & Levy, 2007; Lota & Latorre, 2013). Enterobacteriaeae family including more than 70 genera and all bacteria belonging to this family are bacilli, facultative anaerobic bacteria. Usually, their natural host is human and animal, e.g., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Morganella* spp., *Providentia* spp., *Enterobacter* spp. and *Serratia* spp. which cause infections of urinary tract, respiratory tract, blood stream and wounds (Tham, 2012; WHO, 2017).

Beta lactam antibiotics are those containing beta-lactam ring in their chemical structure including: pencillins; cephalosporins and carbapenems (Hamilton-Miller, 1999; Manneznhe et al., 2015). *Escherichia coli* and *Klebsiella pneumoniae* are the dominant extended spectrum β-lactamases (ESBLs) producing organisms isolated globally (Ashrafian et al., 2013; Shakya et al., 2017), that hydrolyze the amid bond in the β-lactam antibiotics and cause resistance to all pencillins, third generation cephalosporins (e.g. ceftazidime, cefotaxime and cefotriaxone) and monobactams (e.g. aztreoname) but not to cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (Bonnet, 2004; CLSI, 2014).

Most of ESBLs are derivatives of the narrow spectrum TEM and SHV type β-lactamases, with one or more amino acid substitutions surrounding their active site. *bla*$_{CTX-M}$, *bla*$_{SHV}$ and *bla*$_{TEM}$belong to class A of Ampler classification of ESBLs (Zhao & Hu, 2013). SHV (sulfhydryl variant), that originally identified in *K. pneumoniae* (Chaves et al., 2001) and it have greater hydrolytic activity against ceftazidime than other oxyimino-beta-lactams (Tzouvelekis

#Corresponding author email: rh_micro2010@yahoo.com Tel: 002- 01067740754
Received 1/9/2019; Accepted 10/10/2019
DOI: 10.21608/ejm.2019.16460.1113

& Bonomo, 1999; Shaikh et al., 2015b). TEM-1 (patient's name Temoneria), first reported from an *E.coli* isolates in 1965, has substrate and inhibition profiles similar to those of SHV-1 (Datta & Kontomichalou, 1965; Rice et al., 1990). CTX-M beta-lactamases (cefotaximase-Munich) are derived from *Kluyvera* spp. in 1989, **(**Bauernfeind et al., 1990**)**, and it is usually chromosomally coded, but in enterobacteriaceae, *E. coli* and *Klebsiella* spp., carry the gene of this beta-lactamase on plasmids **(**Decousser et al., 2001; Poirel et al., 2002; Bush & Jacoby, 2010**)**. Thus, key of this high spread among those bacteria (*E. coli* and *K. pneumoniae*) refers to these ESBLs encoded by plasmid-born genes and by which facilities its dissemination (Paterson & Bonomo**,** 2005; Pitout & Laupland, 2008). Now, these genes are the most prevalent type of ESBLs found in most areas of the world especially, $bla_{\text{CTX-M}}$ which are commonly identified among enterobacteriaceae, mainly *E. coli* and *K. pneumoniae* (Birbrair & Frenette, 2016; Chong et al*.,* 2018)

According to study conducted in 2004 including 28 countries, the rates of ESBL production was 10 and 17% among *E. coli* and *K. pneumoniae* isolates with the highest rates being in isolates from Latin America, Middle East, Africa, and Asia and lowest being in Europe and the United States (Rossi et al., 2006; Reinert et al., 2007). Unfortunately, the situation of the Middle East countries was most worrisome where this region seems to be the global ESBL pandemics according to many studies (Al-Agamy et al., 2006; Tawfik et al., 2011; Storberg, 2014). This is due to Many risk factors as poor hygiene and excessive consumption of antibiotics, even without a prescription, as well as non-adherence to the course of treatment, leads to increasing the resistance of bacteria and prevalent of ESBLs especially, in developing countries (Morgan et al., 2011; Ayukekbong et al., 2017)

Currently, the recommended therapy for infection caused by ESBL-producing organisms are carbapenems (e.g., imipenem and meropenem, ertapenem, doripenem) where they still the first choice of treatment for serious infections with ESBL-producing *E. coli* and *K. pneumoniae*. (Hodiwala et al., 2013; Baral et al., 2018).The aim of this work was to determine the prevalence of ESBLs genes (bla_{TEM}, bla_{SHV} and $bla_{\text{CTX-M}}$) in both *E. coli* and *K. pneumonia*e clinical isolates.

Material and Methods

Clinical isolates

One hundred and sixty-eight clinical isolates (85 *K. pneumoniae* and 83 *E. coli*) were kindly provided from Al Kasr Al-Ainy hospital during the period between 2016 and 2018. These isolates were recovered from different sources including urine, pus, blood, sputum, semen and stool. Patients' data (gender and ages) were recorded to obtain possible evidence of correlation between ages, genders and prevalence of ESBLs genes in *E. coli* and *K. pneumoniae*.

Bacterial identification

All bacterial isolates were identified by conventional microbiological methods including colony morphology on the MacConkey medium (Oxoid Ltd., Basingstoke, UK), Gram staining and biochemical testes according to Bergey's manual of systematic bacteriology (Holt & Krieg, 1984), and confirmed by API20E (Bio-Merieux, France) test.

Detection of ESBLs producers

Detection of ESBLs producers was done by phenotypic methods including double disk synergy test (DDST) and combination disk test (CDT) according to (EUCAST, 2013). DDST, carried out by add amoxacilin/clavulinic acid (AMC 20/10µg) at the center and around it add ceftziime (CAZ 30µg) or cefotaxime (CTX30µg) at distance 10-15mm, the positive result detected with the inhibition zone is augmented to the direction of AMC disk. CDT, occurred by add disk of CAZ 30µg and CTX 30µg with and without clavulinic acid (CV), the positive result detected if the inhibition zone increased by ≥ 5mm larger in clavulinic acid combination disk than without.

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were carried out using agar disc diffusion method and according to CLSI guidelines (CLSI, 2013, 2015). The antibiotics used were imipenem (IPM 10µg), meropenem (MEM 10µg), levofloxacin (LEV 5µg), norfloxacin (NOR 10µg), ciprofloxacin (CIP 5µg), ceftriaxone (CRO 30µg), cefotaxime (CTX 30µg), ceftazidime (CAZ 30µg), cefoxitin (FOX 30µg); nitrofurantoin (F 300 µg), amikacin (AK 30 µg), piperacillin/tazobactam (TPZ 110µg), Ampicillin (AP 10µg), amoxicillin/clavulinic acid (AMC 20/10µg) trimethoprim/sulfamethoxazole (SXT 1.25/23.75µg), ceftazidime/clavulinic acid

(CAZ/CV 30/10µg) and cefotaxime/clavulinic acid (CTX/CV 30/10µg).

Minimum inhibitory concentrations (MICs) was determined by broth dilution method for the selected isolates that showed positive ESBLs (NCCLS, 2003; EUCAST, 2003), using commercial cefotaxime and ceftazidime powder (GlaxoSmithKline).

Genotypic detection of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ by PCR

DNA of *E. coli* and *K. pneumoniae* isolates was extracted and purification by Quick-gDNA™ MiniPrep Kit (ZYMO RESEARCH).PCR was carried out using thermal cycler (applied ARKTIK, Germany) where the amplification reaction was as follow: 95ºC initial denaturation for 5min., 30 cycles of (denaturation 95ºC for 40 sec., annealing at 53ºC for bla_{SHV} and at 51ºCfor bla_{TEM} and $bla_{CTX\text{-}M}$ for 40sec., extension at 72ºC for 40sec.) and a final extension step at 72ºC for 7 min. The PCR products were analyzed by electrophoresis. Selected positive PCR products for *bla* genes (bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$) were sequenced by GIS research center, Giza, Egypt. The nucleotide sequences were submitted to GenBank to obtain accession No. The primers used for detection of ESBLs encoding genes bla_{SHV} (Schlesinger et al., 2005), blaTEM (Schmiedel et al., 2014) and $bla_{CTX\text{-}M}$ (Poirel et al., 2001) were listed in Table 1

Results

Out of 168 bacterial isolates, 113 were phenotypically ESBLs (67.26%) which confirmed by DDST and CDT. According to patients ages and gender among 113 positive ESBLs isolates, the gender was classified to: 52 (46.0%) female patients (including: 42 (37.2%) adult and 10 (8.8%) children); and 61 (54.0%) male patients (including: 57 (50.4%) adult and only 4 (3.5%) children) (Fig.1).

The isolates were 64 *E. coli* and 49 *K. pneumoniae* (confirmed by conventional identification methods and API20E). Figure 2 showed the different sources of positive phenotypic ESBLs clinical isolates where the highest source was urine with (53.1%); followed by pus, sputum, blood, and semen with 39.8, 4.4, 1.8 and 0.9 %, respectively.

The antimicrobial susceptibility among ESBLs isolates demonstrated that both *E. coli* and *K. pneumoniae* were 100% resistant to CRO and CTX, while for CAZ was 96.89 and 97.96% for *E. coli* and *K. pneumoniae*, respectively. Both bacteria were sensitive to carbapenems group including IPM with 100% and MEM with 96.88 and 95.92% for *E. coli* and *K. pneumoniae*, respectively (Fig. 3,4).

MICs values confirmed that all ESBLs producing isolates (*E. coli* and *K. pneumoniae*) were highly resistant against cephalosporines (CAZ and CTX).MIC for CTX were ≥512µg/ml for all isolates whereas MIC of CAZ ranged from 128 to > 512µg/ml.

PCR detection for *bla* genes demonstrated that only 108 (95.92%) isolates (out of 113 ESBLs isolates) were found to have one or more ESBLs genes. Among the positive isolates, the prevalence of bla_{SHV}, bla_{TEM} and $bla_{CTX\text{-}M}$ genes were 82.4%, 78.7% and 91.7%, respectively.

PCR was carried out for ESBLs isolates, including 61 *E. coli* and 47 *K. pneumoniae*. Single band of the right size was detected for each gene amplicon (Fig. 5). Out of 61 *E. coli* isolates, the prevalence of bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were 70.49, 77.05 and 91.8%, respectively. For *K. pneumoniae*, bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes were 97.87, 80.85 and 91.49%, respectively.

TABLE 1. Primers for ESBLs genes detection.

Primers	Oligoniculeotide sequence(5` - 3`)	Size of amplicons (bp)	References
SHV-F SHV-R	ATGCGTTATATTCGCCTGTG TGCTTTGTTATTCGGGCCAA	747bp	Schlesinger et al. (2005)
TEM-F TEM-R	ATGAGTATTCAACATTTCCG TTAATCAGTGAGGCACCTAT	851bp	Schmiedel et al. (2014)
CTX-M-F CTX-M-R	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550bp	Poirel et al. (2001)

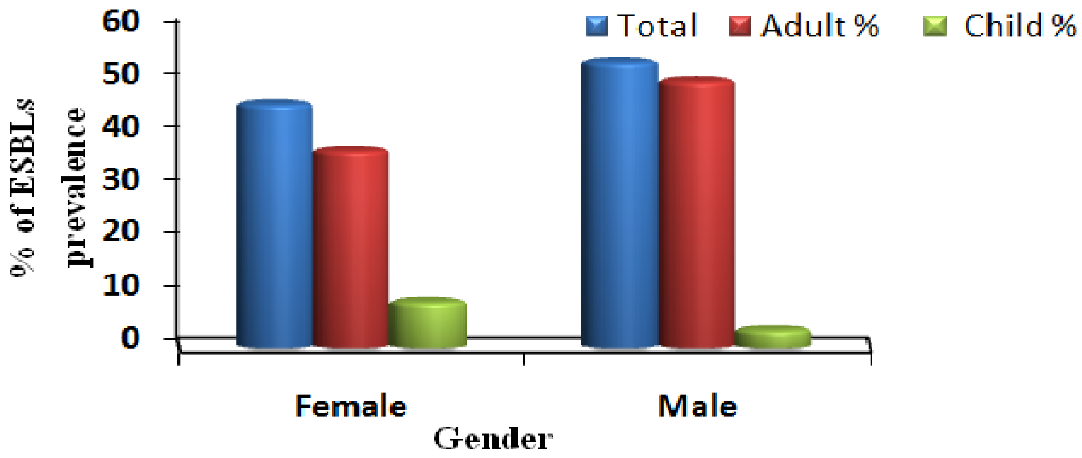

Fig. 1. Frequency of ESBLs isolates in patients' gender and ages.

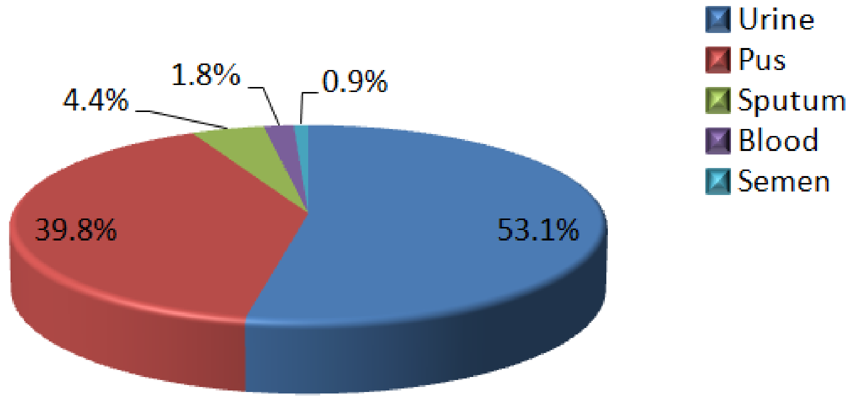

Fig. 2. Different sources of positive phenotypic ESBLs isolates.

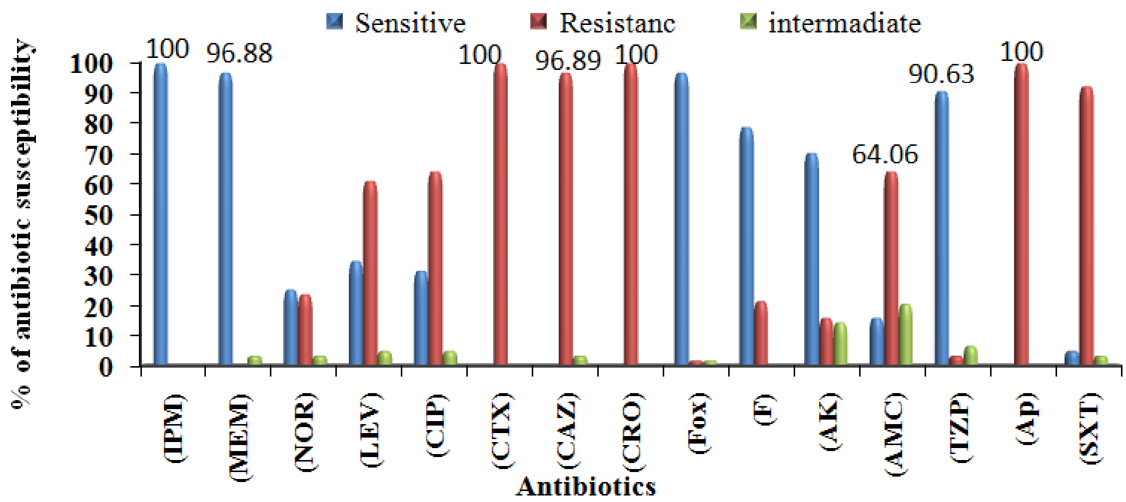

Fig. 3. Antibiotics susceptibility among ESBLs *E. coli* clinical isolates.

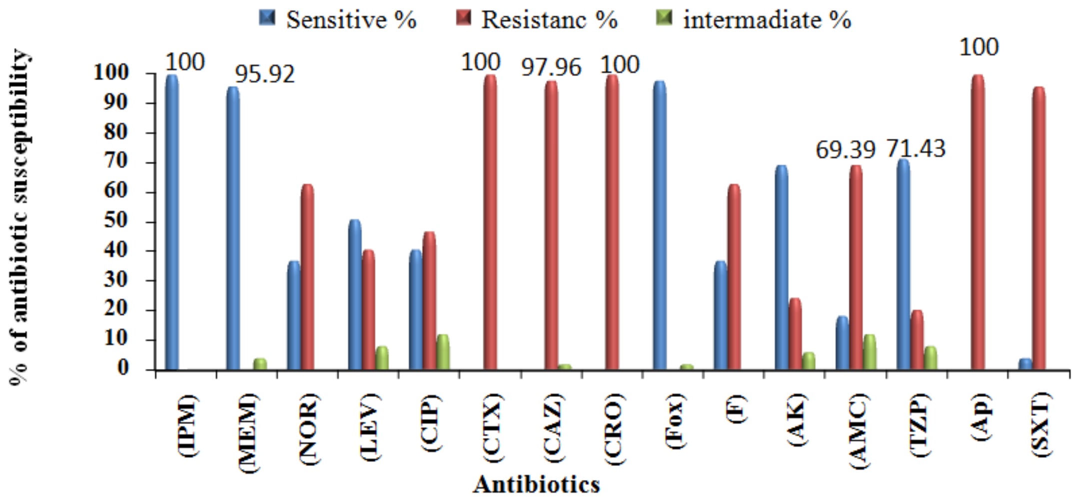

Fig. 4. Antibiotics susceptibility among ESBLs *K. pneumoniae* clinical isolates.

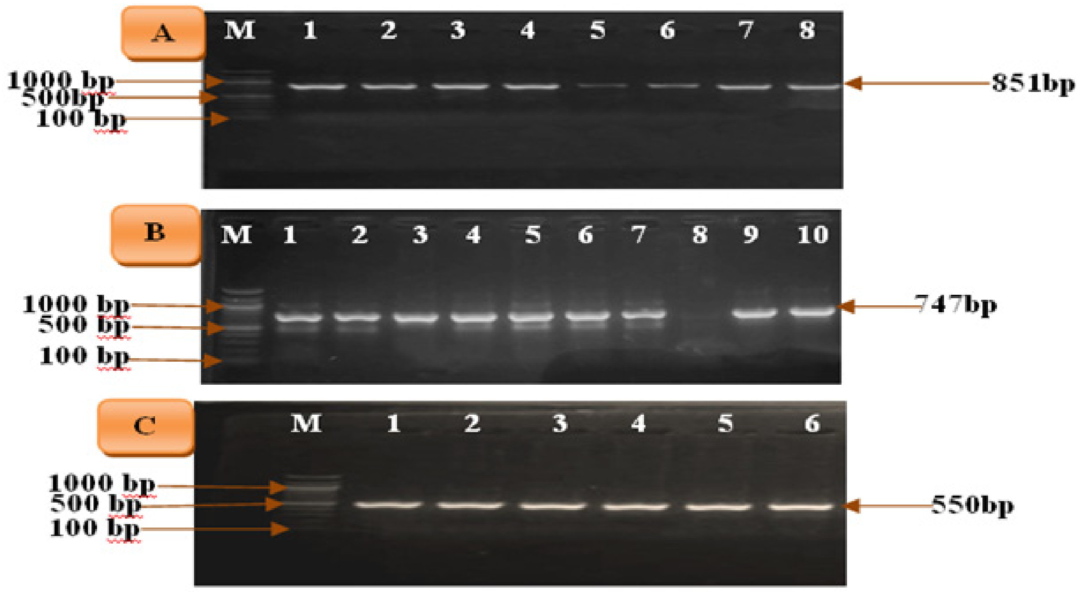

Fig. 5. Agarose gel electrophoresis of PCR ESBLs genes of clinical isolates [Lane M: DNA Marker (100-1500bp), A) PCR amplification of *bla*$_{TEM}$ gene showing single band at 851bp (lanes No. 1, 3 and 4 positive *bla*$_{TEM}$ of *K. pneumoniae;* lanes No. 2, 5, 6, 7 and 8 positive *E. coli*), B) PCR amplification of *bla*$_{SHV}$ gene, showing single band at 747bp (lanes No. 1, 3, 4, 5, 6, 7, 9 and 10 positive *bla*$_{SHV}$ genes of *K. pneumoniae*; lane 2 showed positive and lane 8 negative *bla*$_{SHV}$ of *E. coli*), C) PCR amplification of *bla*$_{CTX-M}$ gene, showing single band at 550bp (lanes No. 1, 2, 5 and 6 positive *bla*$_{CTX-M}$ gene of *K. pneumoniae*; lanes No. 3 and 4 positive *bla*$_{CTX-M}$ gene of *E. coli*)].

Moreover, the coexistence of the 3 genes was detected in 47.54 and 72.34% of *E. coli* and *K. pneumonia*e, respectively. In addition, detection of two genes was found in many isolates, where for *E. coli* isolates were 13.11% (8/61); 19.67% (12/61); 8.2% (5/61) for *bla*$_{CTX-M}$& *bla*$_{SHV}$; *bla*$_{CTX-M}$ & *bla*$_{TEM}$; *bla*$_{TEM}$ & *bla*$_{SHV}$, respectively. While for *K. pneumoniae* isolates were 17.02% (8/47); 2.13% (1/47); 6.38% (3/47) for *bla*$_{CTX-M}$ & *bla*$_{SHV}$ genes; *bla*$_{CTX-M}$ & *bla*$_{TEM}$; *bla*$_{TEM}$ & *bla*$_{SHV}$ (Fig. 6, 7).

Nucleotide sequences of selected ESBLs genes (based on highly purified positive phenotypic and Genotypic ESBLs results) were submitted to *GenBank* under the accession numbers MN096660, MN96662 and MN096664 were for 1K *bla*$_{TEM}$, 98K *bla*$_{CTX-M}$, 107K *bla*$_{SHV}$, respectively, of *K. pneumoniae*. While for *E. coli*, the accession numbers were MN096661, MN096663 and MN096665 for 3E *bla*$_{TEM}$, 97E *bla*$_{CTX-M}$ and 69E *bla*$_{SHV}$, respectively.

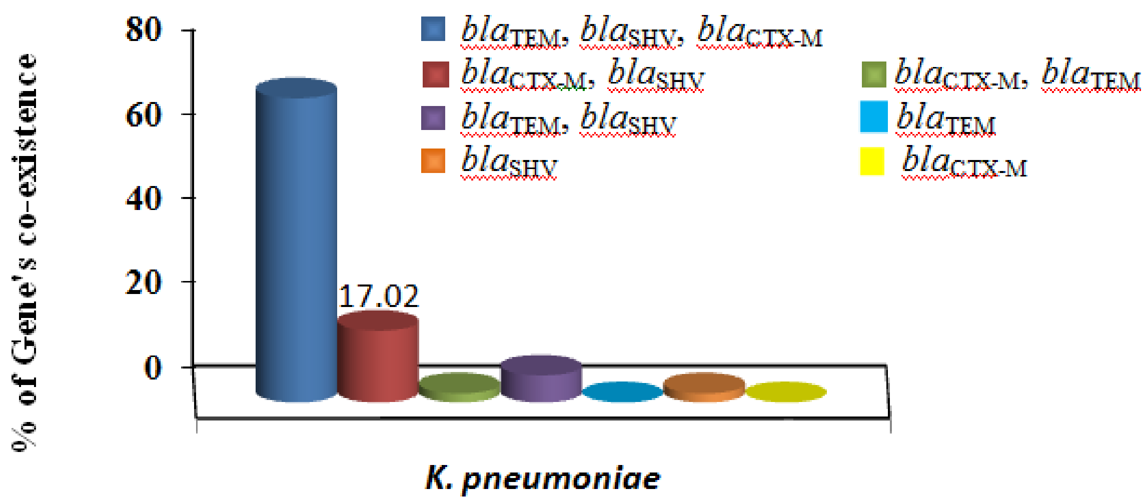

Fig. 6. Prevalence of ESBLs genes coexistence among *K. pneumoniae* clinical isolates.

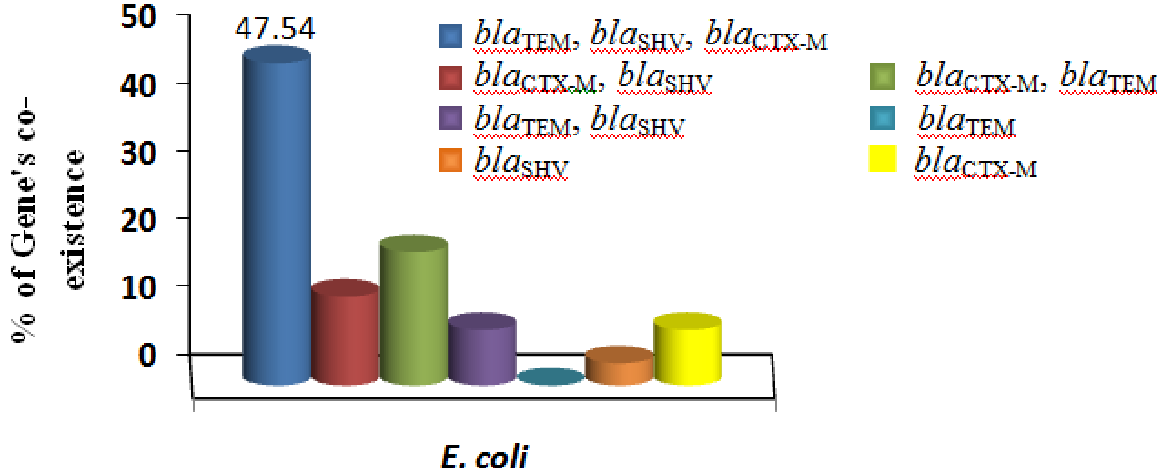

Fig. 7. Prevalence of ESBLs genes coexistence among *E. coli* clinical isolates.

Discussion

The continued emergence of ESBLs is a serious problem in hospitals as well as, community setting (Shakil et al., 2010). Unfortunately, it is recognized that Egypt has an extremely high rate of ESBL producers (Ahmed et al., 2009; Saied et al., 2011). In this study, out of 168 *E. coli* and *K. pneumonia*e clinical isolates, 67.26% were phenotypically positive ESBLs. This percentage is high compared to previous studies in Egypt which was 53.3% (Khater & Sherif, 2014), 42.9% (Storberg, 2014) and 57.8% (Amer et al., 2017). In addition, it was higher than percentage in other countries, Ahmed et al. (2013a) in Sudan and Wadekar et al. (2013) in India that was 59.6%.

ESBLs producers were higher in males (54%) than females (46%) which agreed with the study done by Ben-Ami et al. (2009). While Gibold et al. (2014) found no notable significant difference between the two genders among ESBLs producer's isolates. In our work, the highest infection was in adult 87.6% while in children was12.4% which agreed with Gibold et al. (2014) who confirmed higher relative risk in adult patients. Infection in children may be due to chronic medical conditions, prior immunosuppressive therapy, beside similar risk factors to adults (Logan et al., 2014). The highest percent of ESBLs producers was detected in the clinical isolates from urine (53.1%), followed by that from pus, sputum, blood, and semen with

39.8%, 4.4%, 1.8% and 0.9%, respectively. These results agreed with Elsherif & Maamoun (2012) and Shaikh et al. (2015a). In contrast, Ouedraogo et al. (2016) study, recorded that blood cultures was the highest.

Susceptibility test showed that all isolates were resistant to cephalosporins (CAZ, CTX and CRO), while sensitivity was highest to Carbapenems (imipenem; meropenem). This results agreed with Ahmed et al. (2013b) and Ouedraogo et al. (2016) studies who found that ESBL-producing bacteria were resistant to almost all generations of cephalosporins but remained highly susceptible to carbapenems (imipenem and meropenem) which is consistent with the resistance pattern of organisms with ESBLs (Baral et al., 2018). The most effective ß-lactam/ß-lactamase inhibitor combination against *E. coli* was TZP (90.62%) followed by AMC (15.63%); whereas against *K. pneumonia*e were, 71.42 and 18.37% for TZP and AMC, respectively. These results are consistent with other local and global studies done by Ahmed et al. (2013a), Amer et al. (2017). The MIC for *E. coli* and *K. pneumoniae* ranged from 128 to > 512µg/ml for CAZ; and >512µg/ml for CTX, these results were higher than that demonstrated by Bostanoğlu et al. (2013) but agreed with Peerayeh et al. (2016).

Genotypic characterizations of ESBLs genes showed the prevalence of bla_{CTX-M} genes where it was dominant in almost isolates followed by bla_{SHV} and bla_{TEM}. These findings agree with other studies which reported the prevalence of CTX-M (Abdallah et al., 2015; Ouedraogo et al., 2016; Kpoda et al., 2018). On the other hand, Ahmed et al. (2009) reported that bla_{TEM} was the most frequent β-lactamase encoding gene. This study revealed those ESBLs genes presented 56.48% in *E. coli* and 43.93% in *K. pneumoniae* this data agree with Ouedraogo et al. (2016) and Amer et al. (2017) who reported that ESBL producers were more often found in *E. coli* than *K. pneumonia*e isolates.

The high percentage of bla_{SHV} (97.87%) in *K. pneumoniae* indicated the origin of bla_{SHV} is *Klebsiella* spp (Bush & Fisher, 2011), which accounts for up to 90% resistance in *K. pneumoniae* (Shaikh et al., 2015b).

The co-productions of ESBLs genes were recorded in most of the isolates either in *K. pneumoniae* or *E. coli*. The coexistence of ESBL genes were reported in many studies such as Daef et al. (2009), Salah et al. (2016) and Amer et al. (2017).

Conclusion

The results of our study concluded that the production of ESBLs in Egypt increased in *E. coli* and *K. pneumoniae*. Resistance towards 3rd generation cephalosporins especially cefotaxime, ceftazidime confirmed with high MIC for cefotaxime and ceftazidime. The co-existence of ESBLs genes was also recorded. Challenges have to be taken to control outbreaks caused by *K. pneumonia*e and *E. coli*.

References

Al-Agamy, M.H.M., Ashour, M.S.E.D., 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.

Abdallah, H.M., Wintermans, B.B., Reuland, E.A., Koek, A., Al Naiemi, N., Ammar, A.M. (2015) Extended spectrum β-actamase and carbapenemase producing *enterobacteriaceae* isolated from Egyptian patients with suspected blood stream infection. *PLoS ONE,* **10**(5), e0128120.

Ahmed, S.H., Daef, E.A., Badary, M.S., Mahmoud, M.A., Abd-Elsayed, A.A. (2009) Nosocomial blood stream infection in intensive care units at Assiut University hospitals (Upper Egypt) with special reference to extended spectrum beta-lactamase producing organisms. *BMC Res. Notes*, **2,** 76.

Ahmed, O.B., Omar, A.O, Asghar, A.H., Elhassan, M.M. (2013a) Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella* spp. urinary isolates from Sudan with confirmed ESBL phenotype. *Life Sci. J.* **10**(2), 191-195.

Ahmed, O.I., El-Hady, S.A., Ahmed, T.M., Ahmed, I.Z. (2013b) Detection of bla_{SHV} and bla_{CTX-M} genes in ESBL producing *Klebsiella pneumoniae* isolated from Egyptian patients with suspected nosocomial infections. *EJMHG*, **14**, 277-283.

Alekshun, M.N., Levy, S.B. (2007) Molecular mechanisms of antibacterial multidrug resistance. *Cell*, **128**(6), 1037-1050.

Amer, S.A., El-Hefnawy, A.M., Abouseada, N.M., Elshehy, E.R. (2017) Detection of extended spectrum beta-lactamase producing strains among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Alexandria using Chrom-ID ESBL agar and molecular techniques. *EJMM*, **26** (2), 9-17.

Ashrafian, F., Askari, E., Kalamatizade, E., GhabouliShahroodi, M.J., Naderi-Nasab, M. (2013) The frequency of extended spectrum beta lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*. *J. Med Bacteriol.* **2**(1,2), 12-19.

Ayukekbong, J.A., Ntemgwa, M., Atabe, A.N. (2017) The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrob. Resist. Infect. Cont.* **6,** 47.

Baral, B.P., Sharma, B.K., Gurung, K., Gurung, R.R., Supram, H.S., Bhattachan, B. (2018) Fecal carriage of extended spectrum β-lactamases (ESBL) producing *Escherichia coli* and *Klebsiella* spp. among school children in Pokhara, Nebal. *NJB*, **6**(1), 25-32.

Bush, K., Jacoby, G.A. (2010) Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother*, **54,** 969-976.

Bauernfeind, A., Schneider, I., Jungwirth, R., Sahly, H., Ulmann, U.A. (1999) Anovel type of AmpC β-lactamase, ACC-1, produced by a *Klebsiella pneumoniae* strain causing nosocomial pneumonia. *Antimicrob Agents Chemother*, **43**(8), 1924-31

Ben-Ami, R., Rodri`guez-Ben`o, J., Arslan, H. (2009) A multinational survey of risk factors for infection with extended spectrum beta-lactamase producing *enterobacteriaceae* in non hospitalized patients.*CID,* **49**, 682-690.

Birbrair, A., Frenette, P.S. (2016) Niche heterogeneity in the bone Marrow. *Ann N. Y. Acad. Sci.* **1370**(1), 82-96.

Bonnet, R. (2004) Growing group of extended spectrum beta-lactamases: The CTX-M enzymes. *Antimicrob Agents Chemother*, **48**(1), 1-14.

Bostanoğlu, E., Demirbilek, M., Alişkan, H.E., Çolakoğlu, Ş., Göçmen, J.S. (2013) Interpretation of minimal inhibitory concentration of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* spp. strains according to new clinical laboratory and standards institute (CLSI) criteria. *Afr. J. Microbiol. Res.* **7**(6), 498-503.

Bush, K., Fisher, J.F. (2011) Epidemiological expansion, structural studies and clinical challenges of new β-lactamases from gram-negative bacteria. *Annu. Rev. Microbiol*. **65**, 455-478.

Chaves, J., Ladona, M.G., Segura, C., Coira, A., Reig, R., Ampurdanés, C. (2001) SHV-1 beta-lactamase is mainly a chromosomally encoded species-specific enzyme in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, **45,** 2856-61.

Chong, Y., Shimoda, S., Shimono, N. (2018) Current epidemiology, genetic evolution and clinical impact of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect. Genet. Evol*. **61,** 185-188.

Clinical and Laboratory Standards Institute (CLSI) (2013) Disk diffusion supplemental tables, performance standards for antimicrobial susceptibility testing. CLSI document M100-S23 (M02-A11). Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI) (2014) Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplements. CLSI document, M100-S24.Wayne, PA.

Clinical and laboratory standards institute (CLSI) (2015) Guidelinefrom antimicrobial susceptibility testing. CLSI supplement M100 S25. Wayne, PA.

Daef, E.A., Aly, S.A., Seif El-Din, S.A., El Sherbiny, N.M., El Gendy, S.M. (2009) Phenotypic and genotypic detection of extended spectrum beta-lactamase *Klebsiella pneumoniae* isolated from intensive care units in Assiut university hospital. *EJMM,* **18**(2), 29-40.

Datta, N., Kontomichalou, P. (1965) Pencillinase synthesis controlled by infectious R factors in *enterobacteriaceae*. *Nature,* **208,** 239-241

Decousser, J.W., Poirel, L., Nordmann P. (2001) Characterization of a chromosomally encoded extended-spectrum class A beta-lactamase from Kluyvera *cryocrescens*. *Antimicrob Agents Chemother*, **45**, 3595-8.

Elsherif, R.H., Maamoun, H.A. (2012) Molecular characteristics of extended-spectrum beta-lactamases among Gram-negative isolates collected in Cairo University hospital. *Comp. Clin. Pathol.* **22**(4), 733-739.

European Committee for Antimicrobial SusceptibilityTesting of the European Society of Clinical Microbiology and Infectious diseases (EUCAST) (2003) Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clinical Microbiology and Infection,* **9**(8), ix-xv.

European Committee for Antimicrobial Susceptibility Testing (EUCAST) (2013) Guidelines for detection of resistance mechanisms and specific resistances of clinicaland/orepidemiologicalimportance. Version1.0http://www.eucast.org/resistance_mechanisms

Gibold, L., Robin, F., Tan, R.N., Delmas, J., Bonnet, R. (2014) Four-year epidemiological study of extended spectrum beta-lactamase producing *enterobacteriaceae* in a French teaching hospital. *Clin. Microbiol. Infect.* **20**, 020-026.

Hamilton-Miller, J.M.T. (1999) Beta-Lactams: Variations on a chemical theme, with some surprising biological results. *J. Antimicrob. Chemother.* **44**, 729-34.

Hodiwala, A., Dhoke, R., Urhekar, A.D. (2013) Incidence of metallobeta-lactamase producing *Pseudomonas*, *Acinetobacter* & *Enterobacterial* isolates in hospitalized patients. *Int. J. Pharm. Bio. Sci.* **3,** 79-83.

Holt, J.C., Krieg, N.R. (1984) "*Bergey's Manual of Systematic Bacteriology*", Volume 1, William and Wilkins Co., *Baltimore*, U.S.A.

Khater, E.S., Sherif, H.W. (2014) Rapid detection of extended spectrum β-lactamase (ESBL) producing strain of *Escherichia coli* in urinary tract infections patients in Benha University hospital, Egypt. *Br. Microbiol. Res. J.* **4**, 443-53.

Kpoda, D.S, Ajayi, A., Somda, M., Traore, O., Guessennd, N., Ouattara, A.S., Sangare, L., Traore, A.S., Dosso, M. (2018) Distribution of resistance genes encoding ESBLs in *enterobacteriaceae* isolated from biological samples in health centers in Ouagadougou, Burkina Faso. *BMC Res. Notes*, **11,** 471.

Logan, L.K., Braykov, N.P., Weinstein, R.A., Laxminarayan, R. (2014) For the CDC epicenters prevention program. Extended spectrum β-lactamase producing and third-generation cephalosporin resistant *enterobacteriaceae* in children: Trends in the United States, 1999–2011. *J. Pediatr. Infect. Dis. Soc*. **3**(4), 320-8.

Lota, M., Latorre, A. (2013) A retrospective study on extended spectrum β-lactamase bacteria in the Philippines from 1999–2013. *Acta Med. Philipp.* **48**(1), 28-35.

Manneznhe, R.I., Zar, H.J., Nicol, M.P., Kaba, M. (2015) The spread of carbapenemase producing bacteria in Africa: a systematic review. *J. Antimicrob. Chemother.* **70**, 23-40.

Morgan, D.J., Okeke, I.N., Laxminarayan, R., Perencevich, E.N., Weisenberg, S. (2011) Non-prescription antimicrobial use worldwide: A systematic review. *Lancet Infect. Dis*. **11**, 692-701.

National committee for clinical laboratory standards (NCCLS) (2003) "*Methods for Dilution Antimicrobial Susceptibility test for Bacteria that Grow Aerobically*", 6th ed. Approved standard M7-A6, Wayne, PA.

Ouedraogo, A.S., Sanou, M., Kissou, A., Sanou, S., Solaré, H., Kaboré, F., Poda, A., Aberkane, S., Nicolas Bouzinbi, N., Sano, I., Nacro, B., Sangaré, L., Carrière, C., Decré, D., Ouégraogo, R., Jean-Pierre, H., Godreuil, S. (2016) High prevalence of extended spectrum ß-lactamase producing *enterobacteriaceae* among clinical isolates in Burkina Faso. *BMC Infect. Dis.* **16,** 326.

Paterson, D.L., Bonomo, R.A. (2005) Extended spectrum beta-lactamases: aclinical update. *Clin. Microbiol. Rev.* **18**, 657-686.

Peerayeh, S.N., Rostami, E., Eslami, M., Rezaee, M.A. (2016) High frequency of extended spectrum β-lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* isolates from male patients urine. *Arch.Clin. Infect. Dis.* **11**(2), e32696.

Pitout, J.D.D., Laupland, K.B. (2008) Extended-spectrum beta-lactamase-producing *enterobacteriaceae*: An emerging public-health concern. *Lancet Infect Dis*. **8**, 159-66.

Poirel, L., Naas, T., Le Thomas, I., Karim, A., Bingen, E., Nordmann, P. (2001) CTX-M type extended spectrum β-lactamase that hydrolyzes ceftazidime through a single amino acid substitution in the omega loop. *Antimicrob Agents Chemother*, **45**(12), 3355-3361.

Poirel, L., Kämpfer, P., Nordmann, P. (2002) Chromosome-encoded Ambler class A beta-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum beta-lactamases. *Antimicrob Agents Chemother,* **46**, 4038-40.

Reinert, R.R., Low, D.E., Rossi, F., Zhang, X., Wattal, C., Dowzicky, M.J. (2007) Antimicrobial susceptibility among organisms from the Asia/ Pacific Rim, Europe and Latin and North America collected as part of TEST and the *in vitro* activity of tigecycline. *J. Antimicrob. Chemother.* **60**(5), 1018-1029.

Rossi, F., Baquero, F., Hsueh, P.R. (2006) *In vitro* susceptibilities of aerobic and facultative 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**, 205-210.

Rice, L. B., Willey, S.H., Papanicolaou, G.A., Medeiros, A.A., Eliopoulos, G.M., Moellering, R.C.J., Jacoby, G.A. (1990) Outbreak of ceftazidime resistance caused by extended- spectrum beta- lactamases at a Massachusetts chronic-care facility. *Antimicrob Agents Chemother*, **34**(11), 2193-2199.

Salah, M., Azab, M., Halaby, H., Hanora, A. (2016) Mutations in β-lactamases detected in multidrug resistant gram negative bacteria isolated from community acquired urinary tract infections in Assiut, Egypt. *Afr. J. Microbiol. Res.* **10**(46), 1938-1943.

Saied, T., Elkholy, A., Hafez, S.F. (2011) Antimicrobial resistance in pathogens causing nosocomial bloodstream infections in university hospitals in Egypt. *Am. J. Infect. Control*, **39**, e61-5.

Schlesinger, J., Navon-Venezia, S., Chmelnitsky, I., Hammer-Münz, O., Leavitt, A., Gold, H.S., Schwaber, M.J., Carmeli, Y. (2005) Extended-spectrum beta-lactamases among *Enterobacter* isolates obtained in Tel Aviv, Israel. *Antimicrob Agents Chemother*, **49**(3), 1150-1156.

Schmiedel, J., Falgenhauer, L., Domann, E., Bauerfeind, R., Prenger-Berninghoff, E., Imirzalioglu, C., Chakraborty, T. (2014) Multiresistant extended-spectrum β-lactamase producing *enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol*. **14**(1), 187-200.

Shaikh, S., Fatima, J., Shakil, S., Danish Rizvi, S.M., Kamal, M.A. (2015a) Risk factors for acquisition of extended spectrum beta-lactamase producing *Escherichia coli* and *Klepsiella pneumoniae* in North-Indian hospitals. *Saudi J. Biol. Sci*. **22**(1), 37-41.

Shaikh, S., Fatima, J., Shakil, S., Danish Rizvi, S.M., Kamal, M.A. (2015b) Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. *Saudi J. Biol. Sci*. **22,** 90-101.

Shakil, S., Akram, M., Ali, S.M., Khan, A.U. (2010) Acquisition of extended-spectrum beta-lactamase producing *Escherichia coli* strains in male and female infants admitted to a neonatal intensive care unit: molecular epidemiology and analysis of risk factors. *J. Med. Microbiol*. **59,** 948-954.

Shakya, P., Shrestha, D., Maharjan, E., Sharma, V.K., Paudyal, R. (2017) ESBL production among *E. coli* and *Klebsiella* spp. causing urinary tract infection: A hospital based study. *Open. Microbiol. J*. **11,** 23-30.

Storberg, V. (2014) ESBL-producing *enterobacteriaceae* in Africa a non-systematic literature review of research published 2008- 2012. *Infect. Ecol. Epidemiol*. **4,** 50-55.

Tawfik, A.F., Alswailem, A.M., Shibl, A.M., Al-Agamy, M.H.M. (2011) Prevalence and genetic characteristics of TEM, SHV, and CTX-M in clinical *Klebsiella pneumoniae* isolates from Saudi Arabia. *Microb. Drug Resist*. **17**(3), 383-388.

Tham, J. (2012) Extended-spectrum β-lactamase-producing *enterobacteriaceae:* Epidemiology, risk factors and duration of carriage. Sweden: Media-Tryck, Lund University. p. 11

Tzouvelekis, L.S., Bonomo, R. A. (1999) SHV-type β-lactamases. *Curr. Pharm. Des.***5**, 847-864.

Wadekar, M.D., Anuradha, K., Venkatesha, D. (2013)

phenotypic detection of ESBL and MBL in clinical isolates of *enterobacteriaceae*. *Int. J. Curr. Res. Aca. Rev.* **1**(3), 89-95.

World Health Organization (WHO) (2017) Global priority list of antibiotic resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva

Zhao, W.H., Hu, Z.Q. (2013) Epidemiology and genetics of CTX-M extended spectrum β-lactamases in Gram-negative bacteria. *Crit. Rev. Microbiol*. **39** (1), 79-101.

انتشارجينات البيتالاكتاميز الممتدةالطيف في الكليبسيللا نيمونيا والإيكولاي المعزولة اكلينيكيا"

رحاب محمد محمد على عامر[1]، خالد زكريا البغدادي[2]، إيمان كامل فهمي كامل[3]، هشام محمد الشلشتاوي[4]

[1]المعمل المركزي- وزارة البيئة المصرية- القاهرة- مصر، [2]قسم الميكروبيولوجي- كلية العلوم- جامعة عين شمس- القاهرة- مصر، [3]قسم الباثولوجيا الأكلينيكية- مستشفي المطرية التعليمي- القاهرة- مصر، [4]قسم الميكروبيولوجي- معهد بحوث الهندسة الوراثية الزراعية- الجيزة- مصر.

تم جمع عدد مائة وثمانية وستون عينة إشتملت علي كليبسيللا نيمونيا وإي كولاي وبعد عمل التحاليل الأولية بالمعمل(DDST & CDT) للكشف عن العينات المنتجة لل(ESBLs) وجدت أن مائة وثلاثة عشر عينة تحتوي على ال (ESBLs) بنسبة 67.26% وبعد التأكيد بتحليل ال PCR للمائة وثلاثة عشر تأكدت العينات المنتجة للجينات في مائة وثمانية عينة أي بنسبة 95.58% والتي اشتملت على واحد وستون عينة إي كولاي وسبعة وأربعون عينة من الكليبسيللا نيمونيا. كانت نسبة انتاج الجينات في العينات كالأتي: 91.7%, 82.4%, 78.7% للجينات سي تي اكس-إم , إس إش في , تي إي إم بالتواللي. أيضا تم رصد عينات تحتوى على أكثر من جين بل كانت في معظم الحالات تحتوى على الثلاث جينات معا حيث وجدت 72.3% من عينات الكليبسيللا تنتج الثلاث جينات معا بينما كانت 47.54% في حالة ال إي كولاي.