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Prevalence and molecular characterization of extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella Pneumoniae isolated from Al Shifa hospital, Gaza, Palestine

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الملخص

الخصائص الجزيئية والوبائية للعزلات البكتيرية ايشيريشيا كولاي و كليبسيلا بنويموني المنتجة لانزيم بيتا لاكتاميز واسع الطيف بمستشفى الشفاء في قطاع غزة

تشكل الميكروبات المفرزة لإنزيم بيتا لاكتاميز واسع المدى(ESBL) ، وعلى الأخص القولونية مثل ايشيرشيا كولاي وكليبسيلا بنويموني مشكلة رئيسية في المستشفيات في جميع أنحاء العالم. تهدف هذه الدراسة إلى معرفة مدى وجود وانتشار الميكروبات المفرزة لانزيم بيتا لاكتاميز واسع المدى في مستشفى الشفاء بقطاع غزة بالطرق التقليدية والجزيئية.

حيث تم جمع 200 عزلة بكتيرية من مستشفى الشفاء في غزة منها 159(79.5%) ايشيريشيا كولاي و41(20.5%) كليبسيلا بنويموني، وقد تم الحصول على هذه العزلات من عينات بول وعينات قيحية. وقد تم إجراء العديد من الفحوصات على هذه العينات والتي تشمل الكشف عن الميكروبات المفرزة لإنزيم بيتا لاكتاميز واسع المدى باستخدام طريقة التآزر المزدوج(double synergy method) واختبار الحساسية للمضادات الحيوية، كذلك التحري الجزيئي عن مورثات البيتالاكتاميز ,CTX-M (SHV, TEM) باستخدام فحص تفاعل البولميريز المتسلسل (PCR).

أظهرت نتائج الدراسة بأن نسبة العزلات البكتيرية المنتجة لإنزيم بيتا لاكتاميز واسع المدى (ESBL 56) 28%عزلة بينما البكتيريا غير المنتجة للإنزيم 72% (122عزلة). ومن العدد الإجمالي لميكروبات الايشيرشيا كولاي 159 وكليبسيلا بنويموني 41 وجد أن نسبة العزلات المقاومة للبيتالاكتام فيما بينهم 27% و26% على التوالي. وتبين أن نسبة وجود هذه العزلات في عينات البول كانت 139/41 (29.4%) و في العينات القيحية 61/15 (24%).

وأظهرت نتائج الدراسة بأن العزلات البكتيرية المنتجة لإنزيم بيتا لاكتاميز واسع المدى وغير المنتجة له لديها مقاومة للمضاد الحيوي الأموكسيسيلين بنسبة 100%. ووجد أن العزلات البكتيرية المنتجة للإنزيم مقاومة للمضادات الحيوية التي تنتمي لمجموعة السيفلوسبورين مثل الكيفاليكسين، السيفتازيديم، السيفتريكسون، السيفوتكسيم بالنسب التالية (94.6%، 96.4%، 98.2%، 100%) على التوالي.

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ووجد من خلال الدراسة أن نسبة مقاومة البكتيريا المنتجة وغير المنتجة للإنزيم لعدد أربعة أو أكثر من المضادات الحيوية كانت 100%، 70% بالتتالي. كما وجد أن نسبة مقاومة البكتيريا المنتجة لإنزيم البيتالاكتاميز للمضاد الحيوي ميروبينيم كانت 10% بينما كانت البكتيريا الغير منتجة للإنزيم جميعها حساسة لهذا المضاد الحيوي.

وقد تم عرض 56 عزلة منتجة لإنزيم البيتالاكتاميز لفحص تفاعل البولميريز المتسلسل (PCR) للكشف عن جينات الميكروبات المفرزة لإنزيم بيتا لاكتاميز واسع المدى(CTX-M, TEM, SHV) وكانت نسب وجودهم(81.5%، 51.7%، 26.7%) بالتتالي. وكان معدل وجود جين واحد أو اثنين أو ثلاثة بالنسب التالية (44.6%، 46.4%، 8.9%).

وقد خلصت هذه الدراسة إلى وجود معدل عال للميكروبات المفرزة لإنزيم بيتا لاكتاميز واسع المدى في بمستشفى الشفاء في غزة وأن نسبة مقاومتها للمضادات الحيوية من عائلة السيفالوسبورين كانت مرتفعة وأن أكثر مورثات البيتالاكتاميز انتشاراً هو CTX-M.

الكلمات المفتاحية: البكتيريا المنتجة لإنزيم بيتا لاكتاميز واسع الطيف، ايشيريشيا كولاي، كليبسيلا بنويموني، مستشفي الشفاء، قطاع غزة.

Abstract

Extended spectrum beta-lactamase (ESBL) producing members of the Enterobacteriaceae most notably *Escherichia coli* and *Klebsiella pneumonia*e, are a major problem in hospitals worldwide. This study was undertaken to determine the frequency of ESBLs producing *E. coli* and *K. pneumoniae* in Al Shifa hospital in Gaza.

Two hundred bacterial isolates including 159(79.5%) *E. coli* and 41(20.5%) *K. pneumoniae* were collected from Al Shifa hospital – Gaza Strip. They were isolated from different clinical samples including urine and pus. The bacterial isolates were tested for ESBL production using Double Disc Synergy Test. Using PCR, ESBL producing *E. coli* and *K. pneumoniae* were examined for the presence of ESBL genes including CTX-M, TEM and SHV.

Out of 200 clinical isolates of *E. coli* and *K. pneumoniae*, 56(28%) were ESBL producers while 144(72%) were of non-ESBL phenotype. Out of 159 *E. coli*, 41 *K. pneumoniae* isolates ESBL production was detected in 45(27%),

11(26.8%) respectively. ESBL producing *E. coli* and *K. pneumoniae* were isolated from various clinical samples including urine 41/139 (29.4%) and pus 15/61 (24%). Both ESBL and non-ESBL isolates were resistant to amoxicillin (100%). ESBL producing isolates were resistant to cephalexin, ceftazidim, ceftriaxone and cefotaxime (94.6%, 96.4%, 98.2% and 100%) respectively. The resistance rate to four antibiotics or more among ESBL and non ESBL producing organisms (*E. coli* and *K. pneumoniae*) was 100% and 70% respectively. The resistance rate of ESBL producing isolates to meropenem reached 10%, while all non- ESBL producers were sensitive. Phenotypic positive ESBL producers were further tested by PCR, the prevalence of each of the following genes SHV, TEM and CTX-M were 26.7%, 51.7% and 81.7%, respectively. The prevalence of detection of one, two and three genes were 44.6%, 46.4% and 8.9% respectively.

In conclusion, this study revealed high prevalence of ESBL producing organisms in Al Shifa hospital and high levels of resistance to third generation cephalosporins. CTX-M was the predominant gene among ESBL producers. Our results emphasize the urgent need for formulation of an antibiotic policy in Gaza strip to prevent spread of these organisms.

Keywords: ESBL; *Escherichia coli*; *Klebsiella pneumoniae*; Al Shifa hospital; Gaza Strip.

Introduction:

Microbial resistance through extended-spectrum beta-lactamase (ESBL) was first detected in Western Europe in the mid-1980s and subsequently in the United States soon after the introduction of third-generation cephalosporins in clinical practice (Livermore, Hawkey 2005). ESBLs are enzymes capable of hydrolyzing 3rd and 4th generation cephalosporins and monobactams. ESBLs do not hydrolyze cephamycins (e.g., cefoxitin or cefotetan) or carbapenems (e.g., imipenem and meropenem), and are inhibited by β-lactamase inhibitors (clavulanic acid, sulbactam and tazobactam) (Paterson and Bonomo, 2005). The gene code for ESBL may be carried on integrons, which help in the dissemination of antimicrobial drug resistance in health care settings. Therefore, identification of ESBL producing organisms is important for implementation of appropriate use of

antibiotics and infection control measures (Robberts et al, 2009). Resistance to extended-spectrum beta-lactams among gram-negative pathogens is increasingly associated with ESBLs (Kimura et al, 2007). ESBL producing Enterobacteriaceae are becoming widespread throughout the world (Timko, 2004). The majority of ESBLs belongs to class A Ambler classification which includes the blaSHV, blaTEM, and blaCTX-M types and these enzymes are most often found in *E. coli* and *K. pneumoniae* (Paterson and Bonomo, 2005).

ESBLs are mostly encoded by large plasmids (up to 100 kb and even more) that are transferable from strain to strain and between bacterial species (AitMhand et al, 2002). Genes encoding ESBLs are frequently found on the same plasmid as genes encoding resistance for other classes of antibiotics such as aminoglycosides, tetracyclines, and sulfonamides (Livermore, 2012). Several studies showed that CTX-M-type ESBL-producing Enterobacteriaceae are endemic in most countries of Europe, Asia and South America with high rates of CTX-M-type ESBL-producers particularly among *E. coli* (30 to 90 %) and *K. pneumoniae* (10 to 60 %) (Canton and Coque, 2006; Rossolini et al, 2008)

The detection of ESBL-producing *E. coli* and *K. pneumoniae* is important because of its clinical implications and limited therapeutic options (Paterson, Bonomo 2005; Ramphal, Ambrose 2006). They become a major problem in various hospitals worldwide, causing outbreaks as well as sporadic infections (Canto´n et al, 2008). This may lead to increased patient mortality. Therefore, control of the initial outbreak of ESBL producing organisms in a hospital is of critical importance (Rupp and Fey, 2003; Paterson and Bonomo, 2005).

Determination of CTX-M, TEM and SHV genes by molecular techniques in ESBL producing bacteria and their pattern of antimicrobial resistance can supply useful data about their epidemiology and risk factors associated with these infections (Jain and Mondal, 2005).

Few studies from the Middle East area have focused on prevalence and molecular characterization of ESBL producing bacterial isolates (Moubareck et al, 2005; Ahmed et al, 2008; Chmelnitsky et al, 2008)
Previous studies in Gaza have shown the prevalence rate of ESBL was (22% and 37.5%) (Astal and Ramadan, 2008; Tayeh et al, 2016). Moreover, very scarce data are available, so far, on molecular characterization of ESBL-producing bacteria in Gaza strip and West bank, Palestine (Hussein et al, 2009; Adwan et al, 2014; Tayeh et al, 2016).

In this follow up study, we report the prevalence and molecular characterization of ESBL producing *E. coli* and *K. pneumoniae* in Al Shifa hospital, which is the largest and referral hospital in Gaza strip. Furthermore, we describe the antibiotic susceptibility patterns of ESBL and non-ESBL producing bacterial isolates. The results of the study will help in formulating an antibiotic policy and standard treatment guidelines for appropriate use of antibiotics.

Materials & Methods:

Study design:

A cross sectional study involving 200 was conducted in Al Shifa hospital in Gaza strip. To collect dada about prevalence and molecular characterization of Extended spectrum beta- lactamase producing Escherichia coli and klebsiella pneumonia. The study period was from February to July 2015. The sample sources of these isolates were urine and pus. The study was approved by the department of human resources and development in the Ministry of Health – Gaza.

Culture of clinical isolates:

Upon receipt of *E. coli* and *K. pneumoniae* isolates from Al Shifa hospital, they were subcultured on MacConkey agar and incubated overnight at 37 °C aerobically. All isolates were identified by conventional microbiological methods (colonial morphology, Gram staining and

biochemical tests like IMViC test and API20E) (Engelkirk and Duben-Engelkirk, 2007)

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing of clinical isolates was performed by modified Kirby Bauer disc diffusion method as recommended by CLSI guidelines (CLSI, 2011) using Mueller-Hinton agar. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standards. The antibiotics used in this study were Amikacin(30 µg), Amoxicilin(30 µg), Amoxicillin-Clavulanic (20/10 µg), Ceftazidime(30µg), Ceftriaxone(30 µg), Cefotaxime(30 µg), Cephalexin(30 µg), Chloramphenicol(30 µg) , Ciprofloxacin(5 µg), Gentamicin(10 µg), Kanamycin(10 µg) and meropenem(10 µg), Trimethoprim(23.75 µg). Zone of inhibition for each antimicrobial agent was interpreted, reporting the organism as resistant, intermediate or susceptible.

Phenotypic detection of ESBLs using Double Disk Synergy test:

Phenotypic confirmatory test for ESBL producers was done using double disc synergy test. The organism to be tested was spread onto a Mueller–Hinton agar plate. The antibiotic discs used were Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), and Amoxicillin /Clavulanic acid (20/10 µg) (Figure 1). The three antibiotics were placed at distances of 20 mm (edge to edge) from the Amoxicillin/Clavulanic acid disc that was placed in the middle of the plate. After 24-h incubation, if an enhanced zone of inhibition between either of the Cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disc occurred, the test was considered positive. This indicated synergistic activity with Clavulanic acid and the presence of an ESBL. Positive and negative controls are included in each run of the experiments.

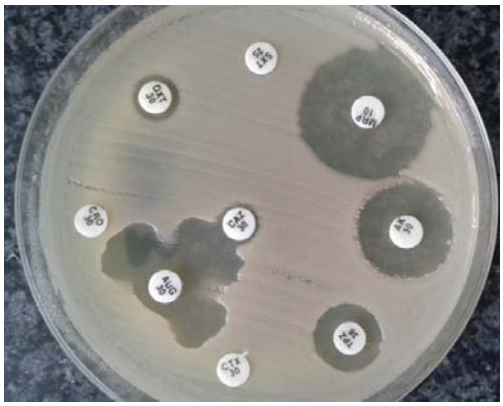

Figure 1 Double disc synergy test showing ESBL production

DNA extraction:

DNA was extracted from cultured isolates by alkaline lysis as previously described. Briefly, one bacterial colony was suspended in 20 µl of lysis buffer (0.25% sodium dodecyl sulfate, 0.05 N NaOH) and heated at 95 °C for 15 min. The cell lysate was diluted by 180 µl of distilled water. The cell debris was pelted by centrifugation at 16000 xg for 5 min. and the supernatants were used for PCR or frozen at -20 °C until further use (El Aila et al, 2009).

Detection of TEM, SHV and CTX-M genes by PCR:

The sequences of primers used for detection of SHV gene were 5'-GCC CGG GTT ATT CTT ATT TGT CGC-3'as a forward primer and 5'- TCT TTC CGA TGC CGC CGC CAG TCA-3' as a reverse primer. The two primers included a 1016bp fragment (Nuesch-Inderbinen et al, 1996) . For detection of CTX-M gene, the sequences of primers used were 5'-ACC GCG ATA TCG TTG GT-3'as a forward primer and 5'-CGC TTT GCG ATG TGC AG-3' as a reverse primer. The two primers included a 550bp fragment (Bonnet et al, 2001). For detection of TEM gene, the sequences of primers used were 5'- ATG AGT ATT CAA CAT TTC CG-3'as a forward primer and 5'- CCA ATG CTT AAT CAG TGA GG-3' as a reverse primer. The two primers included a 858bp fragment (Arlet et al, 1995).

The reactions were performed in 25µl final volumes in the presence of 1µM of each primer, 2µl DNA and 1X of the GoTaq Green MMX (Promega, USA).

The thermal cycling program for detection of CTX-M, TEM and SHV genes was as follows: one cycle of initial denaturation at 95°C for 5 min; 34 cycles of denaturation at 95°C for 30s, the difference in the thermal cycling program was in their annealing temperature (54°C, 55°C and 68°C) respectively for 30s, and extension at 72°C for 1min followed by a final extension at 72°C for 5 min. The amplified products were resolved on a 2% agarose gel. The fragments were stained with ethidium bromide and visualized and photographed using gel documentation system. A 100bp ladder was run as a molecular weight marker.

Statistical Analysis:

The results were tabulated and analyzed using version 20 of the Statistical Package for the Social Sciences (SPSS) program. Frequencies, cross tabulation and appropriate statistical tests as Chi-square were performed. A *P*-value of less than 0.05 was considered statistically significant.

Results:

A total of 200 bacterial isolates of *E. coli* and *K. pneumoniae* were obtained during the study from different clinical samples. Of these, 159 (79.5%) were identified as *E. coli* and 41 (20.5%) as *K. pneumoniae* (Table 1).

Table 1. The prevalence rate of ESBL according to the type of bacterial isolate

Organism	ESBL(56)		Non-ESBL(144)		Total	Frequency	P value
	N	%	N	%	N	%	
E. coli	45	80.3	114	79.1	159	79.5	0.851
K. Pneumoniae	11	19.6	30	20.8	41	20.5	

Legend: ESBL: Extended Spectrum Beta Lactamase, Non-ESBL: Non Extended Spectrum Beta Lactamase, P value < 0.05 statistically significant.

All 200 Gram negative bacilli were phenotypically screened for ESBL production. 56 (28%) of them were ESBL producers, while 144 (72%) were non ESBL producers. Among 56 ESBL producers, *E. coli* showed high rate

of ESBL production 45/56 (80.3%) in comparison with *K. pneumoniae* 11/56 (19.6%). Out of 159 *E. coli* and 41 *K. pneumoniae* isolates, ESBL production was detected in 45(27%) and 11(26.8%) respectively.

These bacterial isolates were obtained from different clinical samples including urine 139(69.5%) and pus 63(30.5%). Among 56 ESBL producers, 41(73.2%) were isolated from urine samples whereas 15(26.7%) were isolated from pus samples. The rate of ESBL production according to the total number of urine and pus samples was 29.4% and 24% respectively (Table 2).

Table 2. The prevalence rate of ESBL according to the type of sample

Sample	ESBL(56)		Non-ESBL(144)		Total	Frequency	P value
	N	%	N	%	N	%	
Urine	41	73.2	98	68	139	69.5	0.667
Pus	15	26.7	46	31.9	61	30.5	

Legend: ESBL: Extended Spectrum Beta Lacatmase Non ESBL: Non Extended

Spectrum Beta Lacatmase, P value < 0.05 statistically significant- All ESBL and non-ESBL Gram-negative bacterial isolates were resistant to amoxicillin(100%). ESBL producing isolates were resistant to cephalexin, ceftriaxone, ceftazidim and cefotaxime and (94.6%, 96.4%, 98.2%, 100%) respectively, whereas non-ESBL producers were resistant to the preceding antibiotics (73%, 64%, 60% and 73%) respectively (Table 3).

ESBL producing *E. coli* and *K. pneumoniae* were sensitive to amikacin, chloramphenicol, gentamicin (66%, 67.8% and 62%) respectively, whereas the sensitivity rate against non- ESBL producers were (82.6%, 77% and 65.9%) respectively (Table 3).

Resistant profile to cephalosporins, meropenem, trimethoprim, tertracycline and ciprofloxacin was greater for ESBL producers than non-ESBL producers (Figure 1).

All ESBL-producers displayed multiple resistance to four or more antimicrobial agents and (80.95%) were resistant to nine antibiotics. In non-

ESBL producers, (70%) were resistant to four antibiotics, whereas (36.4%) were resistant to nine or more agents. 10% of ESBL producing bacterial isolates were resistant to meropenem whereas all non-ESBL producers were sensitive to meropenem.

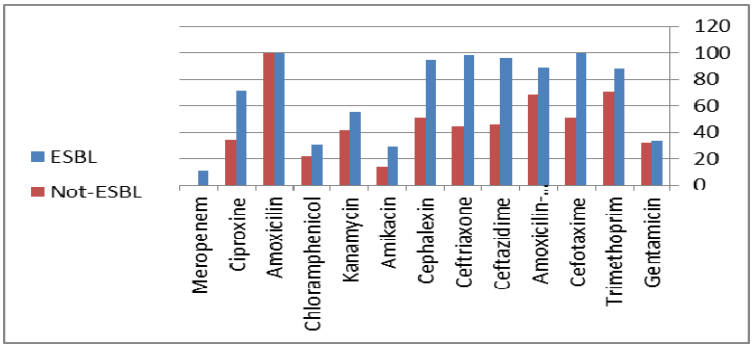

Figure 1: Antibiotic resistance profile between ESBL and non ESBL producing bacteria.

Genotypic detection:

Phenotypic ESBL producers(56) were further analysed by PCR, the prevalence of each of the following genes CTX-M, TEM and SHV was (81.7%, 51.7%, 26.7%) respectively (Figure 2). The prevalence of detection of one, two and three genes was (44.6%, 46.4% and 8.9%) (Figure 3).

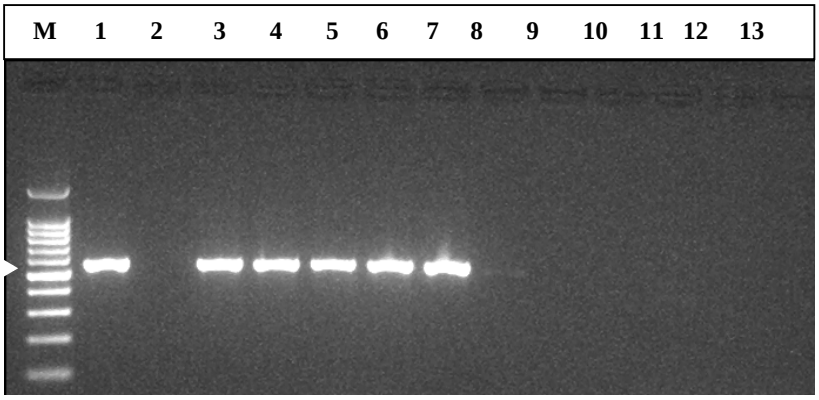

Figure 2. A representative result of CTX-M PCR

Agarose gel of CTX-M PCR products; lane M: marker of molecular weight (GeneRuler 100 bp DNA ladder); lane 1: positive control; lane 2: negative control, lanes 3-7 are tested isolates with positively amplified CTX-M genes; lanes 9-12 are negative. Lane 13: blank

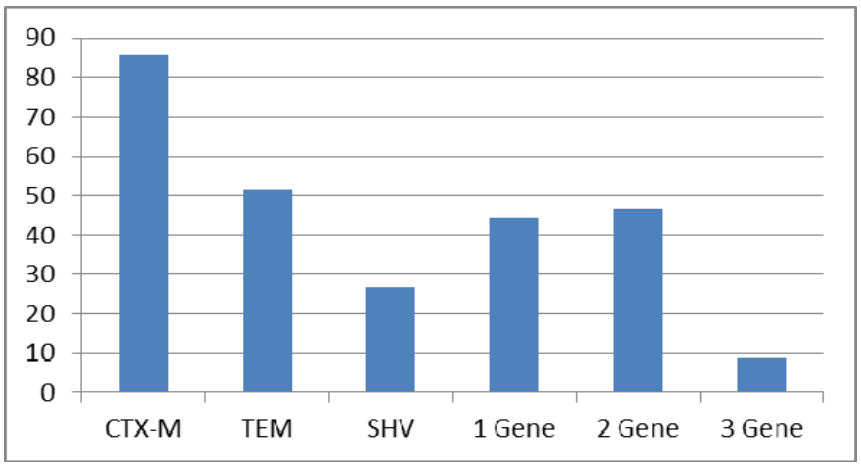

Figure 3: The prevalence rate of ESBL genes (CTX-M, TEM and SHV) and the rate of detection of one, two and three gens.

The most prevalent gene in *E. coli* was CTX-M 84.4% followed by TEM 55.5% then SHV 17.7% . on the other hand, the prevalence of TEM, SHV and CTX-M genes among *K. pneumoniae* was (36.3%, 63.6%, 90% respectively) (Figure 4) .

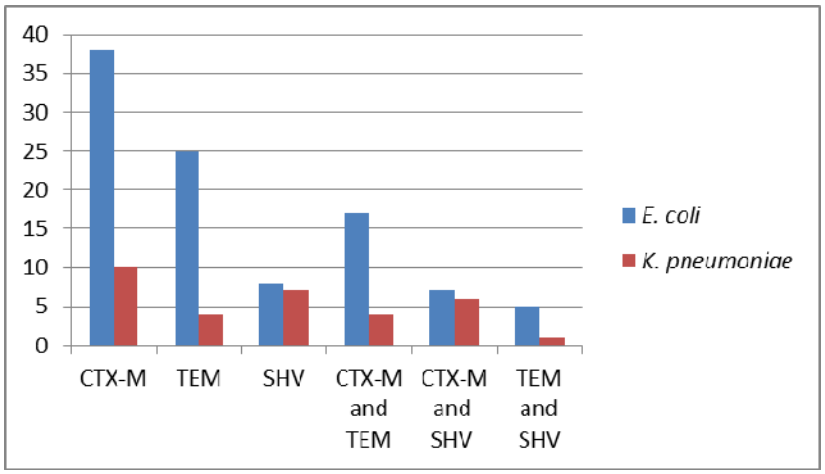

Figure 4: The rate of detection of ESBL genes among *E. coli* and *K. pneumoniae*

Discussion:

An extensive use of β-lactam antibiotics in hospitals and communities has created major problems leading to increased morbidity, mortality and health care costs. Knowledge on local antimicrobial resistance trends among bacterial isolates is important in guiding clinicians to prescribe appropriate antibiotics (Blomberg et al., 2005).

This study described the antimicrobial resistance rates, in addition to phenotypic and genotypic detection of ESBL among *E. coli* and *K. pneumoniae* clinical isolates.

All 200 Gram negative bacilli were screened for ESBL production. 56 (28%) of them were ESBL producing while 144 (72%) were non-ESBL producers.

In the present study, the prevalence level of ESBL-producing *E. coli* and *K. pneumoniae* was (27%, 26.8%) respectively. Very few studies have addressed this issue in Gaza. The published information in 2008 indicates that prevalence of ESBL among 200 bacterial isolates was 22%. The rate of ESBL production among *E. coli* and *Klebsiella* spp. was (9% and 35%)

respectively (El Astal and Ramadan, 2008). Another recent study showed that ESBL production among 40 clinical isolates from burn unit in Al Shifa hospital in Gaza was 37.5% (Tayeh et al. 2016). These data assure the importance of establishing national surveillance and control programs to combat antibiotic resistance in Palestine.

High prevalence of ESBL was reported in Asian countries which varied from 66.7% in India (Hawkey, 2008), 54.7%-61% in Turkey (Gur et al, 2007; Perez et al, 2007), 41% in United Arab Emirates (Al-Zarouni et al, 2008), and 72.1% in Iran (Feizabadi et al. 2010).

Most researchers in Egypt have used phenotypic methods and have reported prevalence ranging from 11.6 to 70% (Eiman and Rasha 2011; Borg et al. 2006). One survey found that Egypt, Lebanon, Saudi Arabia and South Africa had the highest rates of ESBLs (Bouchillon et al. 2004). The prevalence of confirmed ESBL-positive isolates in the USA, Europe, Latin America, the Middle East, and Asia Pacific was 3, 5, 10, 13, and 17% for *E. coli* and 7, 11, 14, 20, and 18% for *K. pneumoniae* (Paterson et al, 2003).

Similar results were obtained in Saudi Arabia, the overall proportion of ESBL-producing enterobacterial isolates was 30.6%. The proportion of ESBL producers was higher in *E. coli* (35.8%) than in *K. pneumoniae* (25.2%) (Hassan et al, 2014). ESBL production among *K. pneumoniae* in our study 11/56 (26.8%) was low in comparison with a study conducted in Jordan, where the ESBL production was 60/84 (72.4%) (Batchoun et al, 2009).

Recently, in Egypt, a study showed high prevalence of ESBL-producing *E. coli*, where 52% of the collected isolates were ESBL producers (Abdel-Moaty et al, 2016).

On the other hand, alower level of ESBL producing *E. coli* isolates in the United States and Europe was reported. Bhusal, et al (2011) investigated that out of 443 *E. coli* isolated from cancer patients at a cancer center in USA, only 41 (9.2%) isolates were ESBL producers (Bhausal et al, 2011).

Also alow prevalence level of ESBL-producing *E. coli* was reported in many African countries such as Morocco (1.3%) (Barguigua, et al, 2011), Nigeria (12.8%) (Aibinu et al, 2012), and South Africa (7.6%) (Brink et al, 2012).

Likewise, Hawser et al. (2011) examined 3160 isolates of *E. coli* collected from 44 hospitals in different European countries (i.e. France, Germany, Greece, Romania, Spain, Turkey, Estonia, Italy, Latvia, Lithuania, Portugal, and UK), and found that only 11% of isolates were ESBL producers (Hawser et al. 2011).

The global overall prevalence of ESBL producers varied greatly in different geographical areas. This may be due to differences in the type and volume of consumption of antibiotics and differences in the time of specimen's collection.

It is necessary to identify the genes involved in ESBL production for the surveillance and epidemiological studies of their transmission in hospitals. A total of 56 ESBL bacterial strains were screened by PCR, the prevalence of each of the following genes SHV, TEM and CTX-M was (26.7%, 51.7%, 81.7%) respectively.

Of the 45 *E. coli* isolates, 84.4% harbored CTX-M gene, 55.5% harbored TEM gene and 17.7 % harbored SHV gene. Of 11 *K. pneumoniae*, 90% harbored CTX-M gene, 36.3% harbored TEM gene and 63.6 % harbored SHV gene. Comparable results were obtained by Kiratisin et al. who found increasing trends in the prevalence of CTX-M and SHV among *K. pneumoniae* isolates (99.2%, 87.4%) respectively (Kiratisin et al. 2008).
We reported the prevalence of CTX-M among *E. coli* and *Klebsiella* spp. was (84.4%, 90%) respectively. Comparative results were obtained by Vaida et al, who reported CTX-M encoding genes in the majority of *E. coli* (96%) and *K. pneumoniae* (71%) isolates showing ESBL phenotype (Vaida et al, 2010). Different results obtained by El sherif et al. in Egypt, who found the prevalence rate was (11.2 and 44%) respectively (El sherif et al, 2013). In another study in Egypt, Ahmed et al. reported the prevalence rate

of CTX-M among 30 clinical isolates of *Klebsiella* spp. was 53.3% (Ahmed et al, 2013).

Several studies reported that the CTX-M gene is the most prevalent ESBL-encoding gene in many European and Asian countries (Livermore et al, 2007; Bali et al, 2010).

In our study, 55% of *E. coli* and *K. pneumoniae* isolates carried more than one gene. A Similar finding was obtained by Goyal et al, who reported that the majority of strains (57.3%) harbored two or more ESBL genes (Goyal et al, 2009). Lower level was reported by Bali et al. who have demonstrated 19.2% of ESBL isolates carried more than one type of β-lactamase genes (Bali et al., 2010). Likewise, another study reported Eighteen (9.9 %) out of 118 isolates carried more than one type of β -lactamase genes (El sherif et al, 2013).

In our study, out of 56 ESBL positive isolates of *E. coli* and *K. pneumoniae*, six (10%) isolates carrying TEM and SHV and 13 (23%) isolates harbouring blaSHV and blaCTX-M genes. Only 51% of ESBL isolates possessed TEM gene and 26% SHV gene alone.

Lal and colleagues reported the prevalence rate of TEM and SHV genes among 95 *Klebsiella* isolates. Isolates having both TEM and SHV genes were common (67.3%) whereas only 20% isolates possessed TEM gene and 8.4% SHV gene alone (Lal et al, 2007). Another study in India showed that bla TEM and bla SHV alone or together were present in 88.8% of the *Klebsiella* isolates (Grover et al, 2006).

The extensive use of third generation cephalosporins and fluoroquinolones has been widely reported as a risk factor for infection with ESBL-producing bacteria (Hsieh et al,, 2010; Goulenok et al, 2013). In our study, ESBL producing *E. coli* and *K. pneumoniae* showed ahigh rate of resistance to cephalexin, cefotaxime, ceftriaxone and ceftazidime (94.6%, 100%, 98.2% and 96.4%) respectively. Our results are in concordance with those of Chong et al. which found the ESBL-producing bacteria were resistant to almost all generations of cephalosporins (Chong et al, 2011).

In our study, the resistance level of trimethoprim and the fluoroquinolone (87.5%, 71.4%) respectively was highly significant in ESBL producing organisms in comparison with non-ESBL producers. This is in agreement with other studies which showed ahigh rate of resistance to fluoroquinolones and co-trimoxazole and less resistance degree to gentamicin among ESBL producing isolates and non-ESBL producers (Chander and Shrestha, 2013; Somily et al, 2014).

No significant difference in the resistance level to gentamicin was found between ESBL- producing and non-SBL producing isolates. In contrast to our results; other studies reported asignificant difference of resistance level to gentamicin between ESBL producing and non ESBL producing Isolates (Cagan Aktas et al, 2014).

In this study, only 10% of ESBL producing isolates were resistant to meropenem whereas all non-ESBL producing isolates were sensitive to this antibiotic (Table 3). This suggests that meropenem may be the proper antibiotic used for treating serious infections caused by ESBL producing isolates. Many studies reported high susceptibility rate to imipenem (100%) among ESBL producing *E. coli* (Chong et al, 2011; Hawser et al, 2011)

In our study, the high level of resistance among ESBL producing isolates is most likely due to overuse or misuse of these antimicrobials agents, lack of an antibiotic policy and the availability of antibiotics sold over the counter in Palestine.

In conclusion, our results showed ahigh prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolated from urine and pus samples in Al Shifa hospital in Gaza strip. Among ESBL genes, CTX-M was the predominant one. Moreover, ESBL producing bacterial isolates showed ahigh rate of resistance to cephalosporins.

In view of this emerging drug resistance, routine ESBL detection should be mandatory along with conventional antibiogram. This will help in the

proper treatment of patients and also prevent further development of bacterial drug resistance.

Continued surveillance, appropriate use of antibiotics, and implementation of strict infection control measures are recommended to decrease ESBL frequency. Molecular detection and identification of β- lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. Further large-scale studies involving molecular characterization of ESBLs are also recommended.

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Table 3: Antibiotic susceptibility pattern of ESBL and Non-ESBL producing bacterial isolates

Antibiotics	ESBL (56)						Non-ESBL (144)						P Value
	S		I		R		S		I		R		
	%	N	%	N	%	N	%	N	%	N	%	N	
Gentamicin	62	35	33.9	19	3.5	2	65.9	95	2	3	31.9	46	0.789
Trimethoprim	12.5	7	0	0	87.5	49	27.7	40	1.3	2	70.8	102	0.044
Cefotaxime	0	0	0	0	100	56	41.6	60	7.6	11	50.6	73	0.000
Amoxicilin-cluvanic	5.3	3	5.3	3	89.2	50	18.7	27	13.1	19	68	98	0.009
Ceftazidime	1.7	1	1.7	1	96.4	54	36.8	53	17.3	25	45.8	66	0.000
Ceftriaxone	1.7	1	0	0	98.2	55	52	75	3.4	5	44.4	64	0.000
Cephalexin	1.7	1	3.5	2	94.6	53	36.8	53	12.5	18	50.6	73	0.000
Amikacin	66	37	5.3	3	28.5	16	82.6	119	4.1	6	13.1	19	0.030
Kanamycin	28	16	16	9	55.3	31	45.1	65	13.1	19	41.6	60	0.099
Chloramphenicol	67.8	38	1.7	1	30.3	17	77	111	1.3	2	21.5	31	0.404
Amoxicilin	0	0	0	0	100	56	0	0	0	0	100	144	NS
Meropenem	51	10	0	0	5	10	100	144	0	0	0	0	0.000
Ciproxine	35.7	20	5.3	3	71.4	40	55.5	80	4.8	7	34.7	50	0.001

Legend: ESBL: Extended Spectrum Beta Lacatmase Non-ESBL: Non Extended Spectrum Beta Lacatmase
N: number of isolates, S: susceptible, I: intermediate, R: resistant, NS: Not statistically computed
P value < 0.05 statistically significant