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Frequency and Molecular Characterization of β-lactamases Producing *Escherichia coli* Isolated from North of Palestine

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Authors' contributions

This work was carried out in collaboration between both authors. Author GA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed the literature searches. Authors GA and AAJ managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to determine the prevalence and molecular characterization of AmpC β-lactamases and extended-spectrum β-lactamases (ESβLs) among *E. coli* isolates.
Place and Duration of Study: Department of Biology and Biotechnology, An-Najah National University, Palestine, during February-April 2015.
Methodology: A total 52 isolates of *E. coli* were recovered from different hospitals and private labs in Jennin district-Palestine. These isolates were used to detect ESβLs and AmpC β-lactamases using phenotypic tests and molecular techniques.
Results: The prevalence of ESβLs and AmpC β-lactamases using conventional methods was 32.7% and 26.9%, respectively. Whereas, the prevalence using PCR technique was 67.3% and 5.8% for ESβLs and AmpC β-lactamases, respectively. *TEM* gene was the dominant (82.9%)

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among *E. coli* that carried ESβL genes. Other genes were (0.0%), (2.9%) and (15.4%) for *CTX-M*, *SHV* and *OXA* genes, respectively. Whereas, AmpC β-lactamases only *DHA* gene was detected and the prevalence was (5.8%). Molecular analysis by construction phylogenetic tree showed that all sequenced *TEM*, *SHV*, *OXA* and *DHA* genes were belonged to *TEM-1*, *SHV-1*, *OXA-1* and *DHA-1*, respectively. ERIC results showed that these strains were diverse and unrelated clones.

Conclusions: Our results showed high frequency of ESβLs and AmpC β-lactamases among *E. coli* isolates in Palestine. According to these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of Amp-C β-lactamases and ESβLs producing isolates within hospitals in Palestine.

Keywords: E. coli; AmpC β-lactamases; ESβLs; Palestine.

1. INTRODUCTION

Resistant β-lactamases pathogens are emerging worldwide as a threat to favorable outcomes of treatment of common infections in community and hospital settings [1]. *Escherichia coli* (*E. coli*) possess a naturally occurring chromosomally mediated β-lactamase or plasmid mediated β-lactamases evolved from penicillin binding proteins, may be due to selective pressure exerted by β-lactam producing soil organisms found in the environment [2]. Beta-lactamases are commonly classified according to two general schemes: the Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification [3,4]. The former functional scheme classifies these enzymes into four classes (A-D) according to the primary amino acids sequence. Enzymes of class A, C, and D possess an active site serine called serine β-lactamases, whereas enzymes of class B are metalloenzymes usually requiring a divalent cation (Zn^{+2}) for their activities. The later functional scheme is based on functional properties of enzymes, i.e. the substrate and inhibitor profiles. Beta lactamases, especially AmpC β-lactamases and/or extended-spectrum β-lactamases (ESBLs) are of particular concern, as a result, these enzymes-producing organisms pose a major problem for clinical therapeutics due to conferring resistance to many antibiotics and leaving a limited range of therapeutic agents [4,5].

In recent years, larger nosocomial outbreaks of clonally ESβL strains have been caused by a multi resistant CTX-M-15-producing *E. coli* strain [6]. In Europe, new TEM- and SHV-subtypes are still emerging, and distinct epidemic clones have been found, for example, in Spain TEM-52 gene was detected in *Salmonella* isolates [7], while in Italy SHV-12 gene was detected in *E. coli* and *K. pneumoniae* isolates [8]. The overall data on ESβL-producing *Enterobacteriaceae* in the countries of the Middle East are extremely worrisome, and this region might indeed be considered one of the major epicenters of the global ESβL pandemic [9]. In North of Palestine it was found that 85.4% and 60.1% of *E. coli* isolates were ESβL producers using phenotypic tests and multiplex PCR technique, respectively. For these positive with PCR, it was reported that the prevalence for CTX-M and TEM was 100% and 32%, respectively [10]. In Gaza, Palestine, it was found that 3.7% and 9% of *E. coli* isolates were ESβL producers [11,12]. A study carried out in Iraq, It was found that 62.2% of vaginal *E. coli* isolates were ESβL producers, the prevalence of CTX-M-type (50.8%), SHV-type (29.5%), OXA-type (11.4%) and TEM-type (1.6%) [13]. Investigation carried out in Jordan, it was found that 50.3% of the *E. coli* isolated from outpatients and diagnosed of having urinary tract infections were ESβL-producers, 80.7% had either *bla*CTX-M or *bla*TEM, or both [14]. Investigation conducted in Egypt from patients with urinary tract infections showed that 61% of *E. coli* produced ESβLs of the CTX-M-14, CTX-M 15, and CTX-M 27 types, and all of strains harbored the TEM enzyme [15]. Investigations carried out in Saudi Arabia in 2004–2005 showed that 10% of clinical urinary *E. coli* isolates from inpatients and 4% of such isolates from outpatients were ESβL producers [16]. Another study was conducted in the previous country, showed that 35.8% of *E. coli* were ESβL producers [17]. Moreover, data collected over three years in Kuwait showed that the levels of ESβLs were lower in community isolates of *E. coli* (12%) than in the corresponding hospital isolates (26%) [18]. In Bahrain, it was found that 73.9% of *E. coli* isolated produced ESβLs of both CTX-M or TEM in combination or CTX-M alone [19].

It was reported that isolates producing AmpC β-lactamases raise special concern as these isolates have been responsible for several nosocomial outbreaks and high rate of clinical failure among infected patients [20]. Investigation done in Iran, it showed that the prevalence bla-AmpC producers among *E. coli* isolates was 5.0% [21]. In Turkey, the prevalence of AmpC-producing strains among *E. coli* isolates was 10.9% using PCR technique, and CIT and MOX group genes were predominant type among these strains [22]. A study conducted in Egypt, it was found that the prevalence *AmpC* β-lactamase among *E. coli* isolates was 38.1% [23], and the most prevalent AmpC gene was that belonging to family CMY-1.

This study aimed to determine the prevalence and molecular characterization of ESβLs and AmpC β-lactamases producing *E. coli* using phenotypic tests and molecular techniques among clinical isolates recovered from North of Palestine.

2. MATERIALS AND METHODS

2.1 Sample Collection and *E. coli* Identification

Fifty-Two isolates of *E. coli* were isolated from inpatients and outpatients at The Martyar Dr. Khalil S. Hospital (n=23; 18 from urine samples, 5 from vaginal swabs), Al-Amal Hosptal (n=7; all from urine samples), AL-Razi Hospital (n=14; 11 from urine samples, 3 from vaginal swabs), Al-Shamal Lab (n=5; all from urine samples) and Hi Lab (n=3; all from urine samples), Jennin district-Palestine, during Fabruary-April 2015. In hospitals, the isolates were identified using API 20 E system, and also confirmed in Microbiology laboratories at An–Najah National University-Nablus, Palesine. For identification, these isolates were cultured on MacConkey or EMB agar, Gram stain and other biochemical tests were used such as IMViC Tests (Indole production, Methyl red test, Voges-Proskauer test and Citrate utilization) and H_2S production [24].

2.2 Antibacterial Susceptibility Test

Antimicrobial susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method [25]. Antibiotic disks (Oxoid) used were Ceftriaxone (CRO) 30 µg, Norfloxacin (NOR) 10 µg, Nalidixic acid (NA) 30 µg, Ciprofloxacin (CIP) 5 µg, Tetracycline (TE) 30 µg, Kanamycin (K) 30 µg, Trimethoprim/Sulfamethoxazole (SXT) 1.25/23.75 µg, Cefotaxime (CTX) 30 µg and Ceftazidime (CAZ) 30 µg. The plates were incubated at 37℃ for 18-24 hrs. The zone of inhibition were measured in millimetres using a caliper. Strains were classified as resistant or susceptible according to the criteria recommended by CLSI guidelines [25].

2.3 Detection of ESβLs and AmpC β-lactamases

The presence of ESβLs were tested by combination double disk test (CDDT) as a standard disk diffusion assay on Mueller Hinton agar (MHA). The test strains were heavily streaked on MHA plates. Four discs namely Ceftazidime (CAZ) 30µg, and Ceftazidime + Clavulanic acid (30/10 µg), Cefotaxime (CTX) 30 µg, and Cefotaxime + Clavulanic acid (30/10 µg) were placed at a distance of 20 mm (centre to centre) on plates containing the inoculum. The plates were incubated for 24 hrs at 37℃. An increase in the zone diameter ≥5 mm for both ceftazidime and cefotaxime tested in combination with clavulanic acid *vs.* its zone diameter when tested alone, confirmed the presence of an ESβL-producing organism [26].

AmpC phenotype was tested by combined disk method using Cefotaxime (CTX, 30 µg) and Ceftazidime (CAZ, 30 µg) disks alone, and in combination with boronic acid (400 µg) [26]. To prepare the combination disk, a total of 60 mg phenyl boronic acid was added to 1.5 ml Dimethyl sulfoxide (DMSO), the DMSO-boronic acid solution was diluted with 1.5 ml of sterilized distilled water, then 20 µl of this solution was added to Cefotaxime and Ceftazidime disks. The disks were used after incubation at room temperature for 1 h to dryness. Cefotaxime and Ceftazidime disks alone and in combination with boronic acid were placed on Mueller Hinton Agar plates inoculated with bacteria. Increased growth inhibition zone diameter around Cefotaxime and Ceftazidime disks in combination with boronic acid ≥5 mm is considered as AmpC β-lactamases producing organism in comparison with Cefotaxime and Ceftazidime disks alone [26].

2.4 PCR Amplification

E. coli DNA was prepared for PCR according to the method described previously [27]. Briefly,

cells were scraped off an overnight nutrient agar plate with a sterile loop, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was resuspended in 0.5 ml of sterile distilled H_2O, and boiled for 10-15 min. The cells then were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X *g* for 5 min. DNA concentration was determined using a spectrophotometer and the samples were stored at -20℃ until use for further DNA analysis.

Detection of plasmid mediated AmpC β-lactamase (*ampC*) genes in all isolates were carried out by multiplex PCR as described previously [28]. Primer nucleotide sequences and expected sizes of amplicons for AmpC β-lactamase (*ampC*) genes are presented in Table 1. Detection of ESβL gene sequences coding for the TEM, SHV, CTX-M and OXA enzymes were performed by the multiplex PCR. The oligonucleotide primer sets and expected amplicon sizes (bp) specific for the SHV, TEM, CTX-M and OXA genes are presented in Table 1. Briefly, PCR reactions were performed in a final volume of 25 µl of the amplification mixture containing 12.5 µL of PCR premix with MgCl2 (ReadyMixTM Taq PCR Reaction Mix with MgCl2, Sigma), 0.4 µM of each primer, 3 µl of DNA template. DNA amplification was carried out with a thermal cycler (Mastercycler Personal, Eppendorf) using the following conditions: 94℃ for 3 min; 94℃ for 30 sec, 64℃ (60℃ for detection of ESβL genes) for 30 sec and 72℃ for 1 min for 25 cycles; with a final extension at 72℃ for 5 min. Amplified PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

ERIC (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5`-ATG TAA GCT CCT GGG GAT TCA C-3 and Primer ERIC2: 5-AAG TAA GTG ACT GGG GTG AGC G-3. Each PCR reaction mix was performed in a final volume of 25 µL containing 12.5 µL of PCR premix with $MgCl_2$ (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 1 µM of each primer, 3 µL DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 0.4 mM, 3 mM MgCl2 and 1.5 U of Taq DNA polymerase. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94℃ was followed by 30 cycles of initial denaturation 94℃ for 50 s, 50℃ for 40 s and 72℃ for 1 min, with a final extension step at 72℃ for 5 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide. The gel images were scored using binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistics software version 20 (IBM).

2.5 Sequence Homology and Phylogenetic Analysis

Amplified PCR products of some β-lactamases genes were purified by Wizard® SV Gel and PCR Clean-Up System kit (Promega) and sequenced by dideoxy chain termination method using ABI PRISM sequencer, model 3130 (Hitachi Ltd, Tokyo, Japan), Bethlehem University, Bethlehem, Palestine. Sequence information were further submitted for accession number in primary bioinformatics web servers.

The comparison of the continuous sequences was done with previously available sequences of the AmpC β-lactamase genes and ESβL genes in NCBI (National Center for Biotechnology Information) using BLAST system. Multiple sequence alignment using ClustalW of the computer program MEGA software (version 5). The evolutionary distances were computed using the Tajima-Nei method. Phylogenetic analyses were based on alignments obtained from ClustalW of a 282 bp sequence. Phylogenetic tree was constructed using the program Neighbor-Joining in the same software. The robustness of the groupings in the Neighbor Joining analysis was assessed with 1000 bootstrap resamplings.

3. RESULTS

3.1 Antibiotic Resistance

The antimicrobial susceptibility pattern revealed that the least resistant antibiotics against these clinical *E. coli* strains were Ceftazidime (11.5%) and Norfloxacin (17.3%), while the most resistant antibiotics were Tetracycline (67.3%) and Trimethoprim/Sulfamethoxazle (55.76%). In addition, results showed that *E. coli* (40.4%) were multidrug resistant. Results are presented in Table 2.

Table 1. Beta-lactamases target genes for PCR amplification, amplicon size and primer sequences

Group	Targets	Primer sequence 5′→3′	Expected amplicon size (bp)	Primer mix	References
Plasmid mediated AmpC β-lactamase (Class C)	*MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11*	MOXM F 5-GCT GCT CAA GGA GCA CAG GAT-3 MOXM R 5-CAC ATT GAC ATA GGT GTG GTG C-3	520	1	[28]
	LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	CITM F 5-TGG CCA GAA CTG ACA GGC AAA-3 CITM R 5-TTT CTC CTG AAC GTG GCT GGC-3	462	1	[28]
	DHA-1, DHA-2	DHAM F 5-AAC TTT CAC AGG TGT GCT GGG T-3 DHAM R 5-CCG TAC GCA TAC TGG CTT TGC-3	405	1	[28]
	ACC	ACCM F 5-AAC AGC CTC AGC AGC CGG TTA-3 ACCM R 5-TTC GCC GCA ATC ATC CCT AGC-3	346	1	[28]
	MIR-1T, ACT-1	EBCM F 5-TCG GTA AAG CCG ATG TTG CGG-3 EBCM R 5-CTT CCA CTG CGG CTG CCA GTT-3	302	1	[28]
	FOX-1 to FOX-5b	FOXM F 5-AAC ATG GGG TAT CAG GGA GAT G-3 FOXM R 5-CAA AGC GCG TAA CCG GAT TGG-3	190	1	[28]
Extended spectrum β-lactamases (Class A)	*SHV*	SHV F 5-ATG CGT TATATT CGC CTG TG-3 SHV R 5-TGC TTT GTT ATT CGG GCC AA-3	747	2	[29]
	TEM	TEM F5-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3 TEM R5-ACG CTC ACC GGC TCC AGA TTT AT-3	445	2	[30]
	CTX-M	CTX-M F 5-ATG TGC AGY ACC AGT AAR GTK ATG GC-3 CTX-M R 5-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3	593	2	[31]
Extended spectrum β-lactamases (Class D)	*OXA*	OXA F 5-ATT ATC TAC AGC AGC GCC AGT G-3 OXA R 5-TGC ATC CAC GTC TTT GGT G-3	296	2	[32]

3.2 Detection of ESβL and AmpC β-lactamases

Results of this study showed that the prevalence of ESβL and AmpC β-lactamase using conventional techniques was (32.7%) and (26.9%), respectively. Whereas, the prevalence using multiplex PCR technique was (67.3%) and (5.8%) for ESβL and AmpC β-lactamase, respectively. This study showed that *TEM* gene was the dominant (82.9%) among *E. coli* that carried ESβL genes. Other genes were (0.0%), (2.9%) and (15.4%) for *CTX-M*, *SHV* and *OXA* genes, respectively. In case of AmpC β-lactamases, only *DHA* gene was detected and the prevalence was (5.8%). Data are presented in Table 3 and Fig. 1. A total of 13 strains were considered AmpC β-lactamases producers by conventional methods but were negative by PCR and a total of 24 strains were sensitive for Cefotaxime and Ceftazidime and considered as not ESβL producer strains by conventional methods, but they were positive with PCR technique (Table 3). In this study, all AmpC β-lactamases were coexisted with ESβL and coexistence of two β-lactamases in single organism was observed.

Molecular analysis by construction phylogenetic tree showed that all sequenced *TEM*, *SHV*, *OXA* and *DHA* genes were belonged to *TEM-1*, *SHV-1*, *OXA-1* and *DHA-1*, respectively (Fig. 2). The nucleotide sequences reported in this study were further registered at the GenBank database under the accession numbers (KT336739-KT336757).

Table 2. Antibiotic resistance of 52 *E. coli* isolates recovered from different clinical samples, Jennin district-Palestine, during Fabruary-April 2015

Antibiotic	Resistant strains	
	No.	%
Ciprofloxacin	16	30.8%
Trimethoprim/Sulfamethoxazle	29	55.76%
Ceftriaxone	13	25.0%
Tetracycline	35	67.3%
Nalidixic acid	22	42.3%
Norfloxacin	9	17.3%
Kanamycin	13	25.0%
Cefotaxime	16	30.9%
Ceftazidime	6	11.5%

3.3 ERIC-PCR Analysis

ERIC-PCR analysis of 35 isolates which carried genes for ESβLs and/or AmpC β-lactamases were genetically diverse and comprised a heterogeneous population with a total 16 ERIC-PCR clusters at a (50%) similarity level. Data are presented in Figs. 3 and 4.

4. DISCUSSION

Beta-lactamases were more common in *E. coli* and *Klebsiella* species, but now are found in most members of *Enterobacteriaceae* and other Gram negative bacilli. A significant increase in the incidents of ESβL-related infections has been observed throughout the world in different researches [9]. Our studies showed that the prevalence of ESβL among *E. coli* in Palestine was (67.3%) using multiplex PCR and (32.7%) using phenotypic tests. Other countries of the Middle East, the prevalence of ESβL producers *E. coli* ranged from (3.7%-85.4%) [10,11,13-15, 17,19,21]. Finding of this research showed that TEM-type ESβL was most common in *E. coli* isolates and this result is consistent with other studies [33,34]. In contrast, other studies in Palestine, Iraq and Bahrain, showed that CTX-M-type ESβL was most common in *E. coli* [10,13,19]. Results showed that there were 24 strains tested positive for ESβL using PCR technique, but negative with phenotypic tests. The use of three distinct substrates in the combined disk tests increased the sensitivity of the test and cefotaxime and cefpodoxime performed the best, despite the occurrence of non-coincident results [35]. It was recommended the use of cefpodoxime alone or a combination of cefotaxime and ceftazidime as preferred substrates for detection ESβL producing clinical isolates of bacteria [36]. There were 6 strains tested positive for ESβL using phenotypic tests, but negative with PCR technique. This negative amplification in these phenotypic positive isolates may be due to these isolates carrying other ESβL genes, which could not be detected by these primers or could be chromosomally mediated β-lactamase. Tests based on the detection of ESBL by molecular techniques especially PCR are more conclusive in defining ESβL production [35]. A combination of different tests such as using both conventional tests and molecular techniques for all β-lactamase associated genes is the best for detection of β-lactamase producing organisms [33].

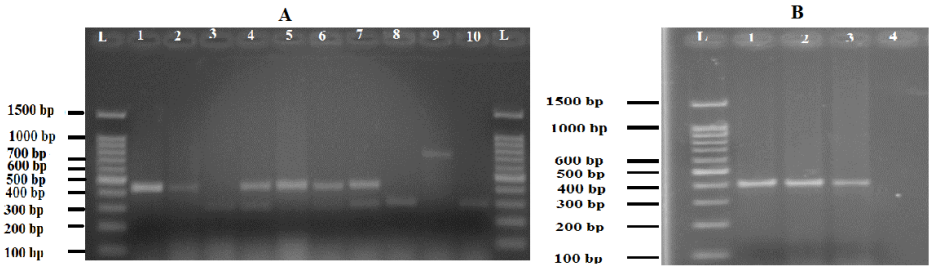

Fig. 1. A: Multiplex PCR profile specific for extended spectrum β-lactamases genes. L contained ladder ladder; Lanes1,2 and 5 for *TEM* gene; Lanes 3, 8 and 10 for *OXA* gene, Lanes 4, 6 and 7 for *TEM* and *OXA* genes and Lane 9 for *SHV* gene. B: Multiplex PCR profile specific for *AmpC* β-lactamase genes. Lane L contained ladder; Lanes 1, 2 and 3 for *DHA* gene and Lane 4 for negative control

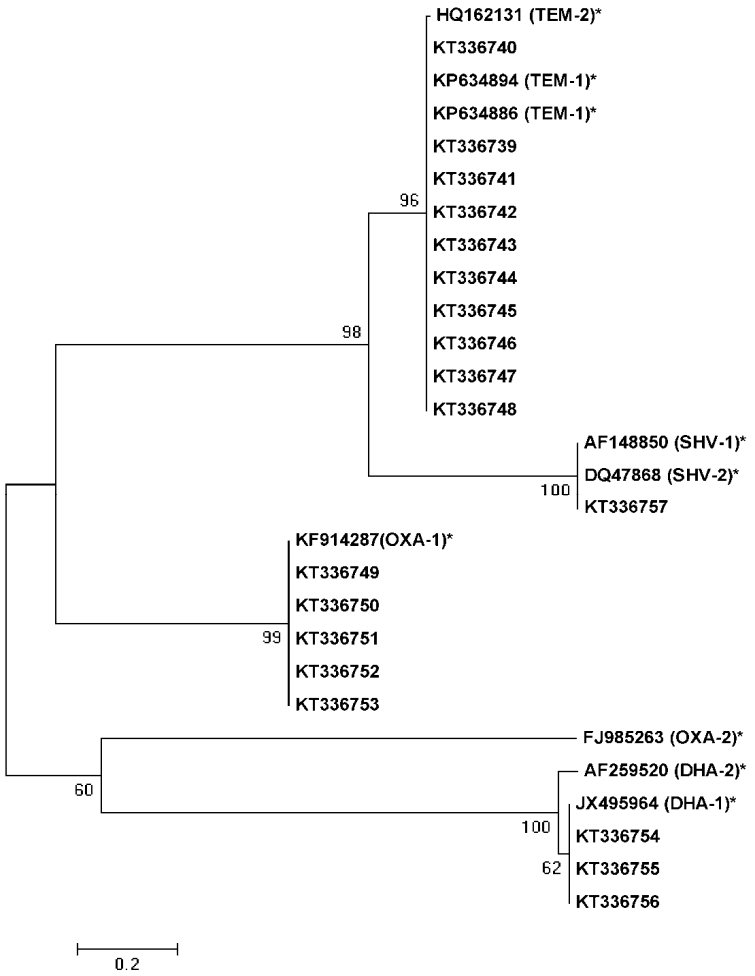

Fig. 2. Phylogenetic analysis by Neighbor-Joining method based on the partial *OXA-1*, *DHA-1*, *SHV-1* and *TEM-1* gene nucleotide sequences. Reference sequences for the *OXA-1*, *DHA-1*, *SHV-1* and *TEM-1* gene nucleotide sequences were denoted by asterisk. The tree was bootstrapped with 1000 replicate, and the genetic distance corresponding is shown by the bar. The evolutionary distances were computed using the Tajima-Nei method. All positions containing gaps and missing data were eliminated. There were a total of 282 positions in the final dataset. Evolutionary analyses were conducted in MEGA5

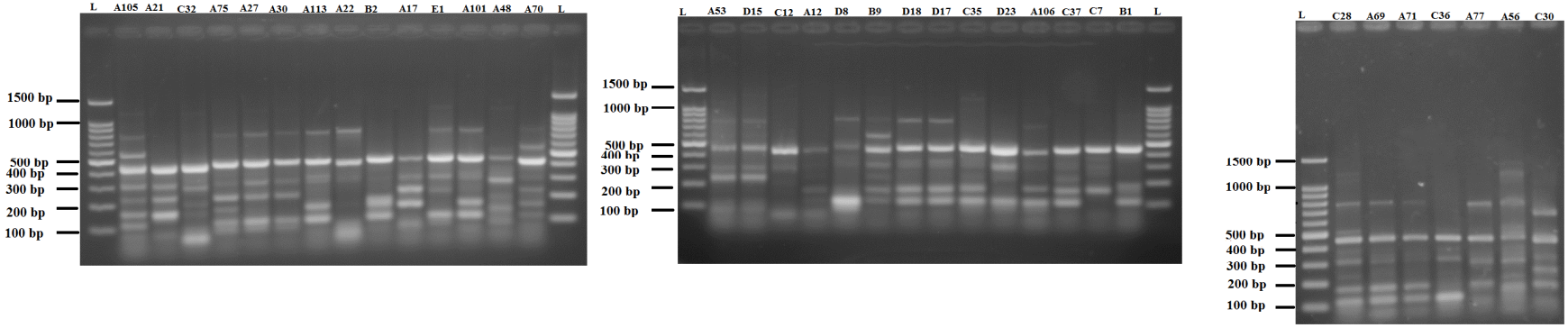

Fig. 3. DNA fingerprints generated by ERIC-PCR analysis of 35 clinical *E. coli* isolates carried genes for ESβLs and/or AmpC β-lactamases recovered on 1.5% agarose gel. L: Lanes contained ladder while other lanes for ERIC-PCR products

Table 3. Prevalence of extended spectrum β-lactamases and AmpC β-lactamase among 52 clinical *E. coli* using conventional techniques and multiplex PCR technique

Technique	β-Lactamases										
	Extended spectrum β-lactamases no. (%)					AmpC β-lactamase no. (%)					
	Class (A)				Class (D)	Class (C)					
	SHV	TEM	CTX-M	TEM and OXA	OXA	MOX-M	DHA-M	ACC-M	CIT-M	FOX-M	EBC-M
PCR Technique	1 (1.9)	26 (50)	0 (0)	3 (5.8)	5 (9.6)	0 (0)	3 (5.8)	0 (0)	0 (0)	0 (0)	0 (0)
	Total 35 (67.3)					Total 3 (5.8)					
Conventional technique	17 (32.7)					14 (26.9)					
Distribution according to methods	Positive by both techniques: (n=11)					Positive by both techniques: (n=1)					
	Positive by PCR only: (n=24)					Positive by PCR only: (n=2)					
	Positive by conventional only: (n=6)					Positive by conventional only: (n=13)					

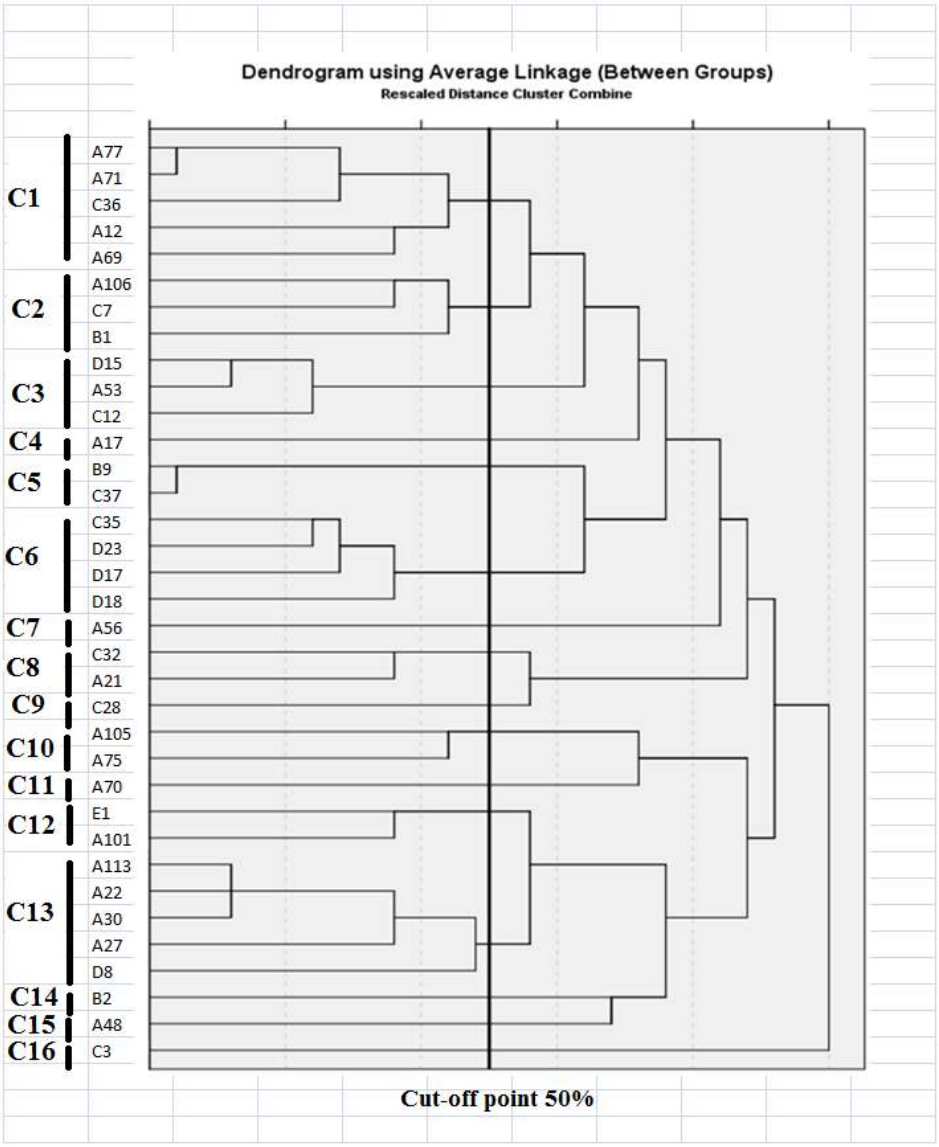

Fig. 4. Dendrogram of 35 *E. coli* isolates carried genes for ESβLs and/or AmpC β-lactamases based on the UPGMA method derived from analysis of the ERIC-PCR profiles at a 50% similarity level

C: Cluster

Our study showed that the prevalence of AmpC β- lactamases among *E. coli* isolates was 26.9% and 5.8% using conventional technique and multiplex PCR technique, respectively. The prevalence of *AmpC* β- lactamases among *E. coli* isolates reported from various countries in Middle East ranged from (5%-38%) [21-23], other various parts of the world such as United Kingdome, Pakistan and India, showed high prevalence and ranged (45%-71%) [37-40]. This research showed that only DHA-1 subtype of AmpC β-lactamases detected among clinical isolates of *E. coli*. Whereas, several other studies from various parts of the world reported the presence of other subtypes among *E. coli* isolates [21,37,41]. There were 13 strains considered AmpC β-lactamases producers by conventional methods but negative with PCR technique. This may be due to hyperproduction of endogenous or non-plasmid-derived (chromosomal) AmpC activity [42].

Coexistence of two β-lactamases in single isolate was observed. This was an alarming finding, that is, 3 isolates (5.8%) producing both ESβL (Class A and Class D) and all AmpC β-lactamases were coexisted with ESβLs. The coexistence of different classes of β-lactamases in a single bacterial isolate may pose treatment challenges, this will seriously limited therapeutic options. In addition, may pose diagnostic challenge that high-level expression of certain β-lactamases such as the AmpC β-lactamases may mask the recognition of the ESβLs and it may result in a fatal and an inappropriate antimicrobial therapy [43]. The presence of AmpC β-lactamases and ESβLs in a single isolate decreases the effectiveness of the β-lactam-β-lactamase inhibitor combinations [44]. Coexistence more than one type of β-lactamases or multiple ESβLs was reported from different species of *Enterobacteriaceae* including *E. coli* [10,13,19, 33,43,45].

The high percentage of β-lactamases producing isolates may be due to several risk factors such as long term exposure to antibiotics in hospitals as in intensive care unit, prolonged hospitalization, inappropriate therapy, nursing home residency, severe illness, instrumentation or catheterization and movement of health workers between wards in the hospital and can migrate ESβL producers from ward to ward leading to dissemination throughout the hospital [46,47]. Geographical differences in the rates of β-lactamases production from country to country and even within countries from hospital-to-hospital, were reviewed previously [48,49].

The ERIC-PCR typing of AmpC β-lactamase and ESβL-producing isolates showed various DNA banding profiles. This clonal diversity suggests that most of the strains have been unable to be maintained or spread in different settings of hospital. This observation challenges many conventional thoughts about the nosocomial epidemiology of antibiotic resistance including β-lactamase. These isolates recovered mostly from urine of patients treated mainly in hospitals, sharing significant patient demographics (all patients from Jenin Governorate) and isolate characteristics including *Amp-C* β-lactamases, ESβL enzymes and other antibiotic resistance profiles differed. It is clearly indicates that multiple clones of these β-lactamases producing isolates were widespread in these hospitals but not sporadic. This supporting the suggestion that the high rate and extensive inappropriate use of antibiotic especially cephalosporins in the country could be the only major cause [50].

In this study, transcunjugation experiments have to be carried out to confirm that these β-lactamases genes are located on a transferable element such as a conjugative plasmid or native to the chromosomes.

5. CONCLUSION

In conclusion, our results showed high occurrence of ESβLs and AmpC β-lactamases among *E. coli* isolates in Palestine. Based on these results we recommend the continuous monitoring and surveillance of the prevalence, proper control and prevention practices and effective antibiotic use will limit the further spread of AmpC β-lactamases and ESβLs producing isolates within hospitals in this country.

COMPETING INTERESTS

Authors have declared that no competing interests exist

REFERENCES

1. Chaudhary U, Aggarwal R. Extended spectrum β lactamases (ESBL): An emerging threat to clinical therapeutics. Indian J Med Microbiol. 2004;22:75-80.
2. Kumar D, Singh AK, Ali MR, Chander Y. Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli from Various Clinical Samples. Infect Dis (Auckl). 2014;7:1-8. DOI: 10.4137/IDRT.S13820
3. Ambler RP. The structure of β-lactamases. Philos Trans R Soc Lond B Biol Sci. 1980; 289:321-31.
4. Bush K. Bench-to-bedside review: The role of β-lactamases in antibiotic-resistant Gram-negative infections. Crit Care. 2010; 14:224. DOI: 10.1186/cc8892
5. Al-Muharrmi Z, Rafay A, Balkhair A, Jabri AA. Antibiotic combination as empirical therapy for extended spectrum Beta-lactamase. Oman Med J. 2008;23:78-81.
6. Alsterlund R, Carlsson B, Gezelius L, Haeggman S, Olsson-Liljequist B. Multi resistant CTX-M-15 ESBL-producing *Escherichia coli* in southern Sweden: description of an outbreak. Scand J Infect Dis. 2009;41:410-5.
7. Fernandez VM, Munoz BJL, Garcia GMI, Garcia RJA. Salmonella enterica serovar Enteritidis producing a TEM-52 β-lactamase: First report in Spain. Diagn Microbiol Infect Dis. 2006;55:245-56.

8. Perilli M, Segatore B, Mugnaioli C, Celenza G, Rossolini GM, Stefani S, et al. Persistence of TEM-52/TEM-92 and SHV-12 extended-spectrum β-lactamases in clinical isolates of *Enterobacteriaceae* in Italy. Microb Drug Resist. 2011;17:521-4.
9. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci. 2014;22:90-101.
10. Adwan K, Jarrar N, Abu-Hijleh A, Adwan G, Awwad E. Molecular characterization of Escherichia coli isolates from patients with urinary tract infections in Palestine. J Med Microbiol. 2014;63:229-34.
11. Astal ZY, Sharif FA, Abdallah SA, Mona I. Fahd MI. Detection of extended spectrum β-lactamases in *Escherichia coli* isolated from the community in Gaza Strip, Palestine. J Al-Aqsa Unv. 2004;8:10-8.
12. El Astal Z, Ramadan H. Occurrence of extended-spectrum beta-lactamases in isolates of *Klebsiella pneumoniae* and *Escherichia coli*. Int J Integ Biol. 2008;2: 122-8.
13. Al-Mayahie SMG. Phenotypic and genotypic comparison of ESBL production by Vaginal *Escherichia coli* isolates from pregnant and non-pregnant women. Ann Clin Microbiol Antimicrob. 2013;12:7.
14. Nimri LF, Azaizeh BA. First report of Multidrug-Resistant ESBL Producing urinary *Escherichia coli* in Jordan. Brit Microbiol Res J. 2012;2:71-81.
15. Al-Agamy MH, Mohamed El-Din Ashour MS, Wiegand I. First description of CTXM β-lactamase-producing clinical *Escherichia coli* isolates from Egypt. Int J Antimicrob Agents. 2006;27:545-8.
16. Khanfar HS, Bindayna KM, Senok AC, Botta GA. Extended spectrum beta lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: Trends in the hospital and community settings. J Infect Dev Ctries. 2009;3:295-9.
17. Hassan H, Abdalhamid B. Molecular characterization of extended-spectrum beta-lactamase producing *Enterobacteriaceae* in a Saudi Arabian tertiary hospital. J Infect Dev Ctries. 2014; 8:282-8.
18. Al Benwan K, Al Sweih N, Rotimi VO. 2010. Etiology and antibiotic susceptibility patterns of community and hospital acquired urinary tract infections in a general hospital in Kuwait. Med Princ Pract. 2010;19:440–6.
19. Bindayna KM, Murtadha M. High prevalence of blaCTX-M in *Enterobacteriaceae* isolates from the Kingdom of Bahrain. Asian Pac J Trop Med. 2011;4:937-40.
20. Adler H, Fenner L, Walter P, Hohler D, Schultheiss E, Oezcan S, et al. Plasmid-mediated AmpC β-lactamases in *Enterobacteriaceae* lacking inducible chromosomal ampC genes: Prevalence at a Swiss university hospital and occurrence of the different molecular types in Switzerland. J Antimicrob Chemother. 2008;61:457-8.
21. Dallal MS, Sabbaghi A, Aghamirzaeie HM, Lari AR, Eshraghian MR, Mehrabad JF, et al. Prevalence of AmpC and SHV β-Lactamases in clinical isolates of *Escherichia coli* from Tehran Hospitals. Jundishapur J Microbiol. 2013;6:176-80.
22. Yilmaz NO, Agus N, Bozcal E, Oner O, Uzel A. Detection of plasmid-mediated AmpC β-lactamase in *Escherichia coli* and *Klebsiella pneumoniae*. Indian J Med Microbiol. 2013;31:53-9.
23. El-Hady SA, Adel LA. Occurrence and detection of AmpC β-lactamases among *Enterobacteriaceae* isolates from patients at Ain Shams University Hospital. Egypt J Med Hum Genet. 2015;16:239–44.
24. Bailey and Scott. *Enterobacteriaceae*. In: Tille PM, Ed. Diagnostic Microbiology, Elsevier, Mosby. 2014;307-328.
25. Clinical Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. Wayne, PA, USA: CLSI: M100-S22; 2012.
26. Mansouri S, Kalantar Neyestanaki D, Shokoohi M, Halimi S, Beigverdi R, Rezagholezadeh F, et al. Characterization of AmpC, CTX-M and MBLs types of β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* producing Extended Spectrum β-lactamases in Kerman, Iran. Jundishapur J Microbiol. 2014;7:e8756.
DOI: 10.5812/jjm.8756
27. Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. Prevalence of *seg, seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. Br Microbiol Res J. 2013;3:139-49.
28. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase

genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40:2153-62.

29. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, the International *Klebsiella* study group. Extended spectrum beta-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: Dominance and widespread prevalence of SHV-and CTX-M type beta-lactamases. Antimicrob Agents Chemother. 2003;47: 3553-60.
30. Monstein H, Ostholm-Balkhed A, Nilsson M, Nilsson M, Dornbusch K, Nilsson L. Multiplex PCR amplification assay for the detection of *bla*SHV, *bla*TEM and *bla*CTX-M genes in *Enterobacteriaceae*. *APMIS:* Acta pathologica, microbiologica, et immunologica Scandinavica. 2007;115: 1400-08.
31. Boyd DA, Tyler S, Christianson S, McGeerA, Muller MP. The Canadian nosocomial infection surveillance program, Health Canada. Complete nucleotide sequence of a 92-kilobase plasmid harbouring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother. 2004;48:3758-64.
32. Kim J, Jeon S, Lee B, Park M, Lee H, Lee J, et al. Rapid detection of extended spectrum β-lactamase (ESBL) for *Enterobacteriaceae* by use of a multiplex PCR-based method. *Infect Chemother*. 2009;41:181-4.
33. Kaftandzieva A, Trajkovska-Dokic E, Panovski N. Prevalence and molecular characterization of extended spectrum beta-lactamases (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae*. Prilozi. 2011;32:129-41.
34. Chaudhary M, Payasi A. Molecular characterization and in vitro susceptibilities of β-lactamase producing *Escherichia coli*, *Klebsiella* species, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to CSE1034 and other β-lactams. Asian Pac J Trop Med. 2014;7(Suppl 1):S217-S23.
35. de Oliveira CF, Salla A, Lara VM, Rieger A, Horta JA, Alves SH. Prevalence of extended-spectrum beta-lactamases-producing microorganisms in nosocomial patients and molecular characterization of the shv type isolates. Braz J Microbiol. 2010.;41(2):278-82.
36. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Effects of phenotype and genotype on methods for detection of extended-spectrum-β-lactamase-producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Norway. J Clin Microbiol. 2007;45:199-205.
37. Woodford N, Reddy S, Fagan EJ, Hill RL, Hopkins KL, Kaufmann ME, et al. Wide geographic spread of diverse acquired AmpC β-lactamases among *Escherichia coli* and *Klebsiella* spp. in the UK and Ireland. J Antimicrob Chemother. 2007;59: 102-5.
38. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Frequency and antibiotic susceptibility pattern of Amp C beta-lactamase producing bacteria isolated from a tertiary care hospital of Rawalpindi, Pakistan. Pak J Med Sci. 2011;27:578-81.
39. Taneja N, Singh G, Singh M, Madhup S, Pahil S, Sharma M. High occurrence of blaCMY-1 AmpC lactamase producing *Escherichia coli* in cases of complicated urinary tract infection (UTI) from a tertiary health care centre in North India. Indian J Med Res. 2012;136:289-91.
40. Vandana KE, Honnavar P. AmpC beta lactamases among ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*- If you don't look, you won't find. J Clin Diag Research. 2009;3:1653-6.
41. Barua T, Shariff M, Thukral SS. Detection and Characterization of AmpC B-Lactamases in Indian Clinical Isolates of *Escherichia coli, Klebsiella pneumoniae* and *Klebsiella oxytoca*. Univer J Microbiol Res. 2013;1:15-21.
42. Tan TY, Ng LS, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. Antimicrob Agents Chemother. 2009;53:146-9.
43. Oberoi L, Singh N, Sharma P, Aggarwal A. ESBL, MBL and Ampc β-lactamases producing superbugs - Havoc in the intensive care units of Punjab India. J Clin Diagn Res. 2013;7:70-3.
44. Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN. Isolation of MRSA, ESBL and AmpC - β -lactamases from neonatal sepsis at a Tertiary Care

Hospital. J Clin Diagn Res. 2014;8: DC24-7. DOI: 10.7860/JCDR/2014/8597

45. Pokhrel RH, Thapa B, Kafle R, Shah PK, Tribuddharat C. Co-existence of beta-lactamases in clinical isolates of *Escherichia coli* from Kathmandu, Nepal. BMC Res Notes. 2014;7:694. DOI: 10.1186/1756-0500-7-694
46. Waiwarawooth J, Jutiworakul K, Joraka W. The prevalence and susceptibility patterns of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* in Chonburi hospital. J Infect Dis Antimicrob Agents. 2006;23: 57-65.
47. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing *Enterobacteriaceae* in samples collected from patients in various wards of Mulago Hospital, Uganda. BMC Pharmacol Toxicol. 2015;16:14. DOI: 10.1186/s40360-015-0013-1
48. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev. 2005;18: 657-86.
49. Livermore DM. Current epidemiology and growing resistance of Gram-negative pathogens. Korean J Intern Med. 2012; 27:128-42.
50. Akpaka PE, Legall B, Padman J. Molecular detection and epidemiology of extended-spectrum beta-lactamase genes prevalent in clinical isolates of Klebsiella pneumoniae and E coli from Trinidad and Tobago. West Indian Med J. 2010;59(6): 591-6.

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