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Detection of Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* and *Escherichia coli* of Environmental Surfaces at Upper Egypt

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ABSTRACT

This study was aimed to evaluate the rate of environmental contamination of Extended-spectrum β-Lactamase (ESBL) and non ESBL-producing *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) as well as the susceptibility to 13 antimicrobial agents. Moreover, this study documents two antibiotic resistance genes as well as highlights their important role in spreading ESBL-producing bacteria among environmental surfaces at upper Egypt. A total of 227 environmental isolates, *K. pneumoniae* (n = 102) and *E. coli* (n = 125) were recovered from 750 samples of 6 different environmental surfaces at upper Egypt. ESBL production was observed in 80 isolates, for overall prevalence of 56.25% with a predominance of *K. pneumoniae* (45/80), followed by *E. coli* prevalence of 43.75% with a predominance of (35/80). The resistance rate was higher among ESBL producers than non ESBL producers. All of *K. pneumoniae* and *E. coli* ESBL producers were found resistance (100%) to cephalothin, ampicillin, cefuroxime, cefataxine, ceftriaxone and ceftazidime. The resistance rate (%) was higher in aztreonam (93.3 and 100) and cefotaxime (95.5 and 91.42) then gentamicin (84.4 and 42.8) ciprpfloxacin (77.7 and 68.5) followed by cotrimoxazole (46.6 and 60) of ESBL-producing K and E strains, respectively. PCR analysis demonstrated that 80.3% of ESBL-K and 66.6% of ESBL-E isolates harbored SHV β-lactamase followed by 66.6% of ESBL-K and 0.0% of ESBL-E isolates harbored TEM β-lactamase.

Key words: ESBL, *K. pneumoniae, E. coli, bla*SHV, *bla*TEM, environmental surface

INTRODUCTION

Several reports have been published on ESBL-producing Enterobacteria, isolated from Intensive Care Units or their environment, or from clinical samples in Egypt (Ahmed *et al*., 2009; Al-Sweify and El-Zayat, 2008; Fam *et al*., 2011). However, not much information is available on β-lactamases of microbes of environmental origin at Out Patient Department. In addition, numerous studies have described ESBL-producing Enterobacteriaceae isolates in clinical environments at U.S. (Lewis *et al*., 2007; Mattar and Martinez, 2007). However, the importance of this colonization in patient-to-patient transmission has not been evaluated up to now. Moreover, there are some limitations in previous studies because many were performed during outbreak settings and few of them used molecular epidemiology techniques to definitively link environmental and patient isolates.

This study was aimed to evaluate the rate of environmental contamination of ESBL and non ESBL-producing *K. pneumoniae* and *E. coli* in a nonoutbreak setting and to assess their antibiotic resistance level. Moreover, this study documents two antibiotic resistance genes as well as highlights their important role in spreading ESBL-producing bacteria among environmental

surfaces at upper Egypt. These to until develop and define a strategy as rapidly as possible in prevention measures and control of multiresistant bacteria, both at a local and national level.

MATERIALS AND METHODS

Bacterial strains: A total of 750 environmental samples comprising of bathrooms, wash basin taps, wash basins, followed by drains, doors and handles were collected between June 2009 and June 2010 in Out Patient Department at the Al-Azhar University Hospital in Assuit. One hundred and twenty five samples were obtained from each surface. Sterile cotton swabs were moistened by a sterile physiological saline solution and used to collect the samples. Bacterial identification was performed by API 20E system (Biomeriux SA, Montalien Vercica and France).

Antimicrobial susceptibility testing: All isolates were tested for the antimicrobial susceptibility testing using NCCLS guidelines (NCCLS, 2003). The antimicrobial agents used in the susceptibility testing included β-lactams (cephalothin, ampicillin, cefotaxime, cefuroxime, cefataxine, ceftriaxone, ceftazidime, imipenem, aztreonam), aminoglycosides (amikacin, gentamicin), a fluoroquinolone (ciprofloxacin) and other (cotrimoxazole). Minimum Inhibitory Concentrations (MICs) were determined by the micro-broth dilution method for strains showing complete or decreased inhibition zone diameter in the disk diffusion test. Performance and evaluation of the MICs were followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006).

Detection of ESBLs

Screening for ESBL-production: Isolates that exhibited by reduced susceptibility to one or more of ceftazidime, aztreonam, cefotaxime or ceftriaxone were considered as potential producers of ESBL (NCCLS, 2003).

Confirmatory tests

The combined disk method or Inhibitor potentiated disc diffusion test: Ceftazidime (30 μg) versus ceftazidime/clavulanic acid (30/10 μg), (Oxoid, UK), were used as a phenotypic confirmatory test where a greater than or equal to 5 mm increase in the zone diameter for the antimicrobial agent tested in combination with β-lactamase inhibitor versus its zone when tested alone indicates ESBL production (NCCLS, 2003).

Double-disk synergy (DDS) test: Where any enhancement in the zone of inhibition between a β-lactam disk and one containing the β-lactamase inhibitor was indicative of the presence of an ESBL (Coudron *et al*., 1997).

ESBL-E-test: According to the manufacturer, a ceftazidime MIC/ceftazidime clavulanic acid MIC ratio which is equal to or greater than 8 indicates the presence of ESBLs (positive test) (Cormican *et al*., 1996).

Polymerase chain reaction (PCR): Screening for genes of resistance to β-lactams genes (SHV and TEM) (Invitrogen, Germany) was performed by PCR after extraction of genomic DNA. Conditions for PCR used were those described by Rayamajhi *et al*. (2008). Two different oligonucleotide primer sets were used to amplify 1074-bp and 1016-bp fragments that spanned the entire *bla*SHV and *bla*TEM genes, respectively. Primer sets were described by Perez-Perez and Hanson (2002) (Table 1). Each PCR mix was totaled 50 μL: 5 μL of 10X Ex *Taq* buffer (Mg^{2+} free), 1 μL extracted template genomic DNA extracted 0.1 mM $MgCl_2$, 0.015 mM of each

Table 1: PCR primer sequences specific for ESBL subgroups and their product sizes used in this study

Targets	Primer	Sequence (5'-3')	Product size
TEM	TEM-F	TCG GGG AAA TGT GCG	1074
	TEM-R	TGC TTA ATC AGT GAG GCA CC	
SHV	SHV-F	GCC GGG TTA TTC TTA TTT GTC GC	1016
	SHV-R	ATG CCG CCG CCA GTC A	

dNTP 50 pmol of each primer and 2.5 units of *Taq* polymerase (Qiagen, Germany). After 5 min of initial denaturation at 94°C, PCRs were consisted of 35 cycles of denaturation for 30 sec at 94°C, annealing at 60°C for 30 sec and extension for 3 min at 72°C. A final extension was performed for 10 min at 72°C. The PCR reaction was performed on thermal cycler (Biometra, TProfessional Thermocycler, Germany). The PCR products were electrophorised in a 1.5% agarose gel (Biometra, Compact XS/S Horizontal Gel Electrophoresis Apparatus, Germany).

Statistical analysis: The Chi-square (χ^2) test was performed with SPSS 12.0 statistical software for Windows and a probability value of 0.05 or less, was considered to be significant.

RESULTS

A total of 227 isolates of six environmental surfaces, *K. pneumoniae* (n = 102) and *E. coli* (n = 125) were recovered from 750 environmental samples comprising of bathrooms, wash basin taps, wash basins, drains, doors and handles. These isolated bacterial strains were divided into a ESBLs-producing *K. pneumoniae* and *E. coli* group and a non ESBLs-producing *K. pneumoniae* and *E. coli* group according to the ESBL production.

The results confirmed that ESBL production was observed in 80 isolates, for overall prevalence of 56.25% with a predominance of *K. pneumoniae* (45/80), followed by *E. coli* prevalence of 43.75% with a predominance of (35/80). ESBL-K and E isolates were distributed as follows: Bathrooms (30/80), wash basin taps (13/80), wash basins (12/80), followed by drains (11/80), doors (7/80) and handles 7/80). Moreover, non ESBL-KE production was observed in 147 isolates, for overall prevalence of 38.77% with a predominance of *K. pneumoniae* (57/147) and *E. coli* prevalence of 61.22% with a predominance of (90/147). Moreover, non ESBL-KE were distributed as follows: Bathrooms (56/147), wash basin taps (30/147), wash basins (23/147), followed by drains (22/147), doors (12/147) and handles (4/147) (Table 2). There was a significant correlation between the overall prevalence of ESBL and non ESBL producers in the six environmental surfaces except wash basins was non significant ($p>0.05$).

The resistance rate was higher among ESBL producers than non ESBL producers. Moreover, all of *K. pneumoniae* and *E. coli* ESBL producers were found resistance (100%) to cephalothin, ampicillin, cefuroxime, cefataxine, ceftriaxone and ceftazidime (Table 3). The resistance rate (%) was higher in aztreonam (93.3 and 100) and cefotaxime (95.5 and 91.42) then gentamicin (84.4 and 42.8) and ciprpfloxacin (77.7 and 68.5) followed by cotrimoxazole (46.6 and 60) of ESBL-producing K and E strains, respectively (Table 3).

On the other hand, the resistance rate (%) of non ESBL producers was high in ampicillin (98.2 and 76.6), followed by cephalothin (38.5 and 46.6), then cotrimoxazole (24.5 and 52.2), cefotaxime (29.8 and 6.6) and aztreonam (21.0 and 8.8) and the lowest resistance rate was determined in gentamicin (19.22 and 16.6), ceftriaxone (17.5 and 11.1), ciprofloxacin (15.7 and 17.7) then amikacin (10.5 and 1.1) of non ESBL-producing K and E strains, respectively. Significant correlations were found between the total number of resistant ESBL and non ESBL producers in each antimicrobial used except cephalothin found non significant ($p>0.05$) (Table 3).

Table 2: Distribution of ESBL[a] producers and non ESBL producers in different environmental sample

Environmental surface	Total No. of samples (n = 750)	ESBL producers (n = 80) No. of isolates[b] K n = 45/80 (56.25%)	E n = 35/80 (43.75%)	Total	Non ESBL producers (n = 147) No. of isolates[b] K n = 57/147 (38.77%)	E n = 90/147 (61.22%)	Total	p-value[c]
Bathrooms	125	20(16)	10(8.0)	30/80	23(18.14)	33(26.4)	56/147	0.005
Wash basin taps	125	6(4.8)	7(5.6)	13/80	14(11.20)	16(12.8)	30/147	0.010
Wash basins	125	4(3.2)	8(6.4)	12/80	7(5.60)	16(12.8)	23/147	0.086
Drains	125	7(5.6)	4(3.2)	11/80	6(4.80)	16(12.8)	22/147	0.056
Doors	125	5(4)	2(1.6)	7/80	5(4.00)	7(5.6)	12/147	0.025
Handles	125	3(2.4)	4(3.2)	7/80	2(1.60)	2(1.6)	4/147	0.036

[a]ESBL-extended-spectrum β-lactamase, [b]K: *K. pneumoniae*; E: *E. coli*; [c]carried out between the total No. of ESBL and non ESBL producers in each environmental surface

Table 3: Antibiotic resistant patterns of ESBL producers and non ESBL producers

Antimicrobials	ESBL producers (n = 80) No. of isolates resistant[a] K isolates (n = 45)	E isolates (n = 35)	Non ESBL producers n = 147) No. of isolates resistant[a] K isolates (n = 57)	E isolates (n = 90)	p-value[b]
Cephalothin	45(100)	35(100)	22(38.5)	42(46.6)	0.182
Cotrimoxazole	21(46.6)	21(60)	14(24.5)	47(52.2)	0.004
Ampicillin	45(100)	35(100)	56(98.2)	69(76.6)	0.003
Cefotaxime	43(95.5)	32(91.42)	17(29.8)	6(6.6)	0.000
Cefuroxime	45(100)	35(100)	0(0.0)	10(11.1)	0.000
Cefataxine	45(100)	35(100)	0(0.0)	10(11.1)	0.000
Ceftriaxone	45(100)	35(100)	10(17.5)	10(11.1)	0.000
Ceftazidime	45(100)	35(100)	0(0.0)	9(10.0)	0.000
Gentamicin	38(84.4)	15(42.8)	11(19.2)	15(16.6)	0.002
Amikacin	0(0.0)	0(0.0)	6(10.5)	1(1.1)	0.000
Imipenem	0(0.0)	0(0.0)	4(7.0)	0(0.0)	0.002
Aztreonam	42(93.3)	35(100)	12(21.0)	8(8.8)	0.000
Ciprofloxacin	35(77.7)	24(68.5)	9(15.7)	16(17.7)	0.033

[a]K: *K. pneumoniae*; E: *E. coli*; [b]carried out between the total No. of resistant ESBL and non ESBL producers for each antimicrobial agent

An interesting but notable observation was that 10 isolates among non ESBL-E were resistant to cefuroxime, cefataxine and ceftriaxone followed by the same number (n = 10) of non ESBL-K to ceftrixone. All ESBL producer and almost all of non ESBL producer were not resistant to amikacin and imipenem (Table 3).

Table 4 gives the MICs of selected 12 multi resisistant ESBL-producing strains toward 11 antimicrobial agents. All these strains were showed simultaneous resistance (>32) to ceftriaxone, (>16) to cephalothin, ampicillin, cefuroxime and aztreonam. In addition, the strain number K89 was exhibited the lowest rate of MIC (≤0.5) to ciprofloxacin, followed by (≤1) to cotrimoxazole similar to strain numbers E117 and E121.

PCR analysis demonstrated that 80.3% of ESBL-K and 66.6% of ESBL-E isolates harbored SHV β-lactamase followed by 66.6% of ESBL-K and 0.0% of ESBL-E isolates harbored TEM β-lactamase. From these results, the most frequent extended-spectrum β-lactamases in this group were *bla*SHV.

Table 4: The MICs values of selected 12 multi resisistant ESBL-producing strains in different environmental sample

		MIC (μg mL^{-1})[c]											Type of genes[d]	
Surface[a]	Strains[b]	CEP	COT	AMP	CEFO	CEFU	CEFA	CEFE	CEFT	GEN	AZT	CIP	SHV	TEM
B	K60	>16	>4	>16	>16	>16	>16	>32	>16	>8	>16	>2	SHV	-
WBT	K67	>16	>4	>16	16	>16	>16	>32	>16	>8	>16	>2	SHV	-
WB	K81	>16	>4	>16	>16	>16	>16	>32	>16	≤2	>16	>2	SHV	TEM
DR	K89	>16	≤1	>16	>16	>16	>16	>32	>16	>8	>16	≤0.5	SHV	TEM
DO	K95	>16	>4	>16	≤4	>16	>16	>32	16	>8	>16	>2	SHV	TEM
HA	K101	>16	>4	>16	16	>16	>16	>32	>16	>8	>16	>2	SHV	TEM
B	E96	>16	>4	>16	≤4	>16	>16	>32	>16	>8	>16	>2	SHV	-
WBT	E100	>16	>4	>16	8	>16	>16	>32	>16	>8	>16	>2	-	-
WB	E105	>16	>4	>16	≤4	>16	>16	>32	16	>8	>16	>2	SHV	-
DR	E115	>16	>4	>16	≤4	>16	>16	>32	8	>8	16	>2	-	-
DO	E117	>16	≤1	>16	8	>16	>16	>32	8	≤2	4	>2	SHV	-
HA	E121	>16	≤1	>16	>16	>16	>16	>32	8	≤2	16	>2	SHV	-

[a]B-bathrooms, WB: Wash basin taps, WB: Wash basins, DR: Drains, DO: Doors, HA: Handles, [b]K: *K. pneumoniae*; E: *E. coli*; [c]CEP: Cephalothin; COT: Cotrimoxazole, AMP: Ampicillin, CEFO: Cefotaxime, CEFU: Cefuroxime, CEFA: Cefataxine, CEFE: Ceftriaxone, CEFT: Ceftazidime, GEN: Gentamicin: AZT: Aztreonam, CIP: Ciprofloxacin; [d]Betalactamase gene, MIC[c]: Minimum inhibition concentration

Most the selected multiresistant isolates (n = 12) in this study were harbored genes encoding SHV and TEM β-lactamase genes, either alone or in combination (Fig. 1, 2; Table 2). Among the selected multi resistant ESBL-producing *K. pneumoniae* (K60 and K67) were showed the presence of SHV and the absence of TEM. *K. pneumoniae* (K81, K89, K95 and K101) were contained amplified SHV and TEM β-lactamases genes. Additionally, *E. coli* (E96, E105, E117 and E121) were contained amplified SHV and not contained amplified TEM β-lactamase gene. MICs values and the amplified β-lactamases of almost isolates are listed in Table 2 and represented in Fig. 1, 2.

DISCUSSION

The existence of ESBL-producing KE is of serious concern as it stands for a pathogen with the capacity to acquire resistance to a wide range of clinically important antimicrobials and it can lead to numerous infectious processes. This may lead to an increase in the percentage participation of antibiotic resistant bacteria in various environments causing problems in therapy by selecting resistant bacteria.

In addition, the infections caused by ESBL-producing *E. coli* and *K. pneumoniae* are considered an increased risk associated with the treatment failure (Livermore and Brown, 2001). Thus, this study was aimed to assess the prevalence of ESBL and non ESBL producers, especially isolates associated with environmental contamination around carriers of Out Patient Department at Al-Azhar University Hospital in Assuit.

Detection of ESBL production was done with ceftazidime, aztreonam, cefotaxime or ceftriaxone and showed that 35.24% (80/227) of the environmental isolates were considered as potential producers of ESBLs which was higher to environmental isolates reported (Ahmed *et al*., 2009). In this study, the author used DDS and E-tests for confirmation of ESBL production although the former being the relatively cheaper method can be used effectively in hospital setting. Similarly, ESBL production was detected by the double disk method (Jayapradha *et al*., 2007;

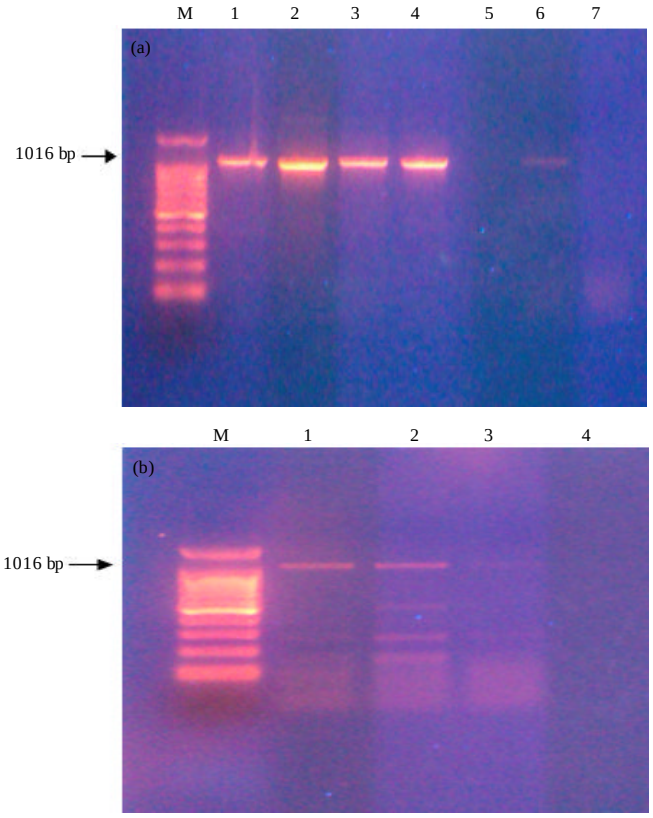

Fig. 1(a-b): PCR amplification of β-lactamases genes, (a): PCR amplification of *bla*SHV and *bla*TEM genes. Lane M: 1 kb DNA marker (Qiagen, Germany), lanes 1, 2, 3 and 4: *bla*SHV gene of *K. pneumoniae*/K60, K67, K81 and K89, respectively, lane 5: negative control, lanes 6 and 7: *bla*TEM gene of *E. coli* /E96 and E100, respectively, (b): PCR amplification of *bla*SHV gene. Lane M: 1 kb DNA marker (Qiagen, Germany), lanes 1, 2 and 3: *bla*SHV gene of E coli /E96, E105 and E115, respectively, lane 4: negative control

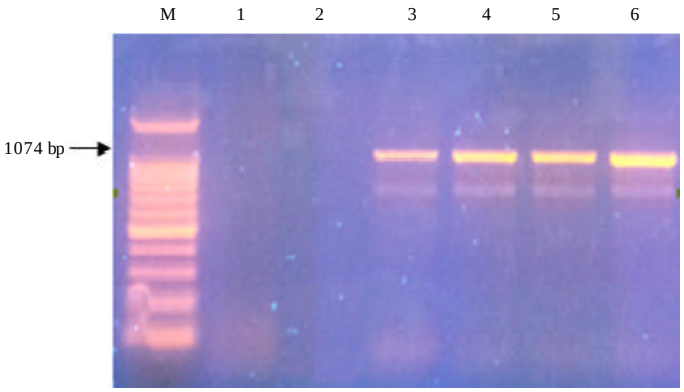

Fig. 2: PCR amplification of *bla*TEM gene. Lane M: 1 kb DNA marker (Qiagen, Germany), lanes 1, 3, 4, 5 and 6: *bla*TEM gene of *K. pneumoniae*/K60, K81, K89, K95 and K101, respectively, lane 2: negative control

Mansouri *et al.*, 2011). Nosocomial outbreaks involving ESBL-PE are frequently described worldwide. Patient-to-patient transmission by means of environmental surfaces has been reported several times (Van't Veen *et al.*, 2005).

The results of this study suggest that the environmental contamination is more frequent in the environment of ESBL-producing *K. pneumoniae* than in the environment of ESBL-producing *E. coli*. Where, Gram negative pathogens *K. pneumoniae* were higher on the rank of organisms and included 45/227 (19.8%) which was the most commonly isolated Gram negative organism, followed by *E. coli* 35/227 (15.4%) which was higher to isolates reported (Arabaci *et al.*, 2009), where, ESBL positivity was found 31.86% (65/204) of *E. coli* strains and 33.33% (31/93) of *K. pneumoniae* strains. This could suggest that ESBL-producing *Klebsiella* has a higher ability to spread and/or persist in the environment than ESBL-producing *E. coli*. *Klebsiella* spp. are known to form biofilms which may be a way of surviving during long periods in the environment (Jones and Bradshaw, 1997). These results are higher to those of other contaminated surfaces where these organisms have been among the leading Gram-negative pathogens (Ahmed *et al.*, 2009; Guet-Revillet *et al.*, 2012). These results confirm the serious role of the hospital environment in the transmission and spread of infections.

The results of this study show that the prevalence of environmental contamination with a strain identical to that isolated from the doors and handles of clinic (i.e., 5.6%) is rather low compared with the environmental contamination observed from other contaminated surfaces. This observation might be due to the clinic doors usually opened by nurses from the inside and/or the number of patients who touch the handles and door are a few.

In this study, the results showed the high level of ESBL and non ESBL contaminations represented in bathrooms, wash basin taps, wash basin as well as the drains. This observation might be due to the reason that these places are used for the collection of urine and stool specimens too. This gives a chance to the number of carriers, who touch the wash basin taps and wash basins with ESBL and non ESBL strains. However, this observation might also highlights the quality of surface cleaning at the hospital and the efficacy of the disinfectants used in addition to specify several hygiene strategies to prevent the spread of Enterobacteria mainly ESBL and non ESBL-producing strains. Thus, further study is needed to evaluate the action of the antiseptics used on various surfaces contaminated with different Enterobacteria. For instance, studies have shown that patient-to-patient transmission decreases after the implementation of measures such as single room, hand hygiene, cohorting, patient screening and decontamination of the carriers (Cooper *et al.*, 2004). Moreover, a place dedicated to collecting urine samples far from wash basins should be designed in Out Patient Department to limit the spread of infection among people at the University Hospital. However, most of these studies concerned ESBL-KE transmission.

Consequently, most of them lack methodology because of retrospective comparison of different strategies with or without a bundle of measures. The impact of each measure alone is thus difficult to evaluate. Therefore, there are no universal guidelines concerning the isolation of ESBL-KE carriers. The measures implemented are usually those applied for their carriers. They consist in geographical isolation of the patient (single department), hand hygiene and use of gloves and gowns by the health care worker when they are in contact with the patient or his environment (FHCPH, 2010) and subsequently the existence of the pathogen in the food chain as well as in public health (Frederick, 2011) but some differences could appear between different geographical areas because nosocomial incidences depend on multiple factors, including the colonization pressure (Shanthi and Sekar, 2010; Kohlenberg *et al.*, 2012).

Multidrug resistance was higher among β-lactamase producers than in non β-lactamase producers. Thus, antibiotic resistance is a growing problem in hospitals everywhere. In the present study, all *K. pneumoniae* and *E. coli* ESBL producers were found resistance (100%) to cephalothin, ampicillin, cefuroxime, cefataxine, ceftriaxone and ceftazidime. An interesting but notable observation was that 10 isolates among non ESBL-E were resistant to cefuroxime, cefataxine and ceftriaxone. On the other hand, 10 isolates among non ESBL-K were resistant to cefetriaxone. In a previous study conducted in Assiut in 2002, lower rate of cefotaxime resistance (33.3%) was recoded (Mandour, 2002) till it reached 61.1% in 2009 (Daef *et al*., 2009).

In this study, the MICs values of selected 12 multi resisistant ESBL producer strains were high and usually ranged from >16 to >32 of cephalothin, ampicillin, cefuroxime, cefataxime, ceftazydiem and aztreonam, reflecting a wide use of antibiotics groups in this hospital. In line with my finding, the study included 10 strains of ESBL-K collected randomly MIC values for cefuroxime, cefotaxime, ceftazidime, cefepime were recorded as >256, >16, >32 and >256 mg mL^{-1}, respectively (Grover *et al*., 2006). But for cefoxitin MIC values of eight resistant strains was >32 mg mL^{-1} and two strains showed relative lack of resistance to cefoxitin as MIC 8 mg mL^{-1} which may be considered as biological resistant to cefoxitin (Pagani *et al*., 2000).

My finding demonstrated that none of all the ESBL-producing and non ESBL-producing strains were resistant to amikacin and imipenem as in previously reported (Zaoutis *et al*., 2005; Pascual *et al*., 2007; Yu *et al*., 2007; Daef *et al*., 2009; Gururajan *et al*., 2011; Murugan *et al*., 2011; Starlander and Melhus, 2012). On the contrary 22.2-33.3% of isolates were resistant to amikacin in Egypt (Abdel-Hady *et al*., 2008; Daef *et al*., 2009) and in USA only 11% of isolates were resistant to amikacin (Zaoutis *et al*., 2005) thus justifying its use as empiric therapy and indicating that these agents can still be used in the treatment of infections caused by these pathogens in Egypt.

Resistance to extended-spectrum β-lactams mediated by ESBLs and SHV and TEM β-lactamase enzymes is an increasing problem worldwide. Determination of their prevalence is essential to formulate an effective antibiotic policy and hospital infection control measures. In this study, PCR analysis demonstrated that 80.3% of ESBL-K and 66.6% of ESBL-E isolates harbored SHV β-lactamase followed by 66.6% of ESBL-K and 0.0% of ESBL-E isolates harbored TEM β-lactamase. followed by 82.6% (Daef *et al*., 2009). These findings suggest that ESBL-K SHV seems to be representative to of those circulating in Egypt.

In the present study, the type of β-lactamase gene was determined among *K. pneumoniae* and *E. coli* strains by using a polymerase chain reaction which showed that SHV was the main type of β-lactamase, followed by TEM. Similar results were reported (Bradford *et al*., 1997; Ahmed *et al*., 2009), they observed that TEM-and SHV-type ESBLs remain more common at upper Egypt and North America, respectively. Other studies confirmed that the strain isolated from the environmental surface was identical to the strain isolated from clinical samples (Ahmed *et al*., 2009; Guet-Revillet *et al*., 2012).

CONCLUSION

This study revealed the high prevalence of ESBL-producing KE in environmental samples. Most of theses isolates are multidrug resistant and harbored SHV genotype than TEM type. This means that the environment is a potential reservoir of pathogens such as ESBL-producing KE. This is to the author knowledge the first study in which a contaminated surface at Out Patient Department

has been the source of ESBL-producing *K. pneumoniae* and *E. coli*. Reinforcing hygiene measures around ESBL-producing *K. pneumoniae* and *E. coli* might be necessary to reduce the spread of ESBL-KE in hospital environments.

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