

ESBL-Producing *E. Coli* and *Klebsiella* among Patients Treated at Minia University Hospitals

Ahmed A.Fadil Saedii^{1*}, Ahmed R. Abdelraheim², Ahmed A.Abdel-Aziz³ and Salwa H. Swelam⁴

¹Department of Clinical Pathology, Faculty of Medicine, Minia University School of Medicine, Egypt

²Department of Obstetrics and Gynecology, Faculty of Medicine, Minia University School of Medicine, Egypt

³Department of otorhinolaryngology, Faculty of Medicine, Minia University School of Medicine, Egypt

⁴Department of Pediatrics, Faculty of Medicine, Minia University School of Medicine, Egypt

*Corresponding author: Ahmed A Fadil Saedii, Department of Clinical Pathology, Faculty of Medicine, Minia University School of Medicine, Egypt, Tel: 00201005708462; E-mail: ahmedabdeladel@yahoo.com

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Abstract

Background: Extended spectrum beta-lactamases (ESBLs) are group of bacteria producing enzymes which can destroy one or more antibiotics. ESBL producing organisms are resistant to many types of antibiotics which would normally be used to treat such infections thus considered as multi-drug resistant (MDR). The emergence and spread of ESBL is a public health threat as it is usually associated with an increase of morbidity, mortality and healthcare costs. Aim of the work: Detecting the prevalence of nosocomial infections caused by ESBL-producing *E.coli* and *Klebsiella* species among patients treated at Minia University Hospitals.

Methods: Eighty-five isolates from nosocomial infections at Minia university hospital due to *E.coli* and *klebsiella* species were screened for ESBL production phenotypically and by PCR Results: Out of 85 isolates causing nosocomial infections at Minia university hospitals during the period of April 2014 - April 2015, the prevalence of *E.coli* was 52 isolates (61.1%) while *Klebsiella* spp. was 33 isolates (38.9%). The prevalence of ESBL among all the isolates of *E.coli* and *Klebsiella* species was 32.8% (28/85); with a prevalence of 16.4% among *E.coli* and 16.4% among *Klebsiella* spp. Isolates.

Conclusions: The prevalence of ESBL in Minia University Hospitals was 32.8% among the nosocomial infections. The predictors for the nosocomial acquired ESBL-EK infections were; old age, long hospital stay, mechanical ventilation, diabetes mellitus and prior antibiotics intake.

Keywords: ESBL; *E.coli*; *Klebsiella*; Nosocomial infection

Introduction

Enterobacteriaceae that produce extended-spectrum β -lactamases (ESBL-E) carry plasmid-encoded enzymes that can efficiently hydrolyze and confer resistance to a variety of β -lactam antibiotics. These enzymes are predominantly found in *Escherichia coli* (*E.coli*) and *Klebsiella* although present also in other members of the *Enterobacteriaceae* [1].

The emergence and spread of ESBL-E is a public health threat because these infections are associated with an increase of morbidity, mortality, and healthcare costs [2]. Curbing the spread of ESBL-E in healthcare facilities after their importation is important—as is controlling transmission in areas where they have become endemic—because they are associated with poor patient outcomes. Identifying the infection control measures that are effective is an important step in order to prevent patients from becoming colonized or infected with these multidrug-resistant organisms (MDROs) [1].

Methods

The current study was conducted at Minia University Hospitals over a period of 1 year from April 2014 to April 2015. Eighty-five isolates

from nosocomial infections at Minia University Hospitals, due to *E.coli* and *klebsiella* species, received at the microbiology laboratory and screened for ESBL production.

All isolates were obtained only from patients who met the CDC's criteria for nosocomial infection which was defined as any infection that develops during or as a result of an admission to our hospital and was not incubating at the time of admission.

Full clinical history was taken from all the selected patients with special emphasis on the risk factors predisposing for nosocomial infections which assessed by revising the patients' files.

All specimens were subjected to the following

Direct films stained with Gram stain, Routine culture on blood and MacConkey agar media, Identification of isolated colonies using: Gram stain morphology, Conventional biochemical tests, Antimicrobial susceptibility testing (CLSI guidelines, 2008).

Initial screening test was carried out using the commercial double-disk synergy test that was integrated in the routine susceptibility testing. Each isolated strain was considered a potential ESBL-producer when the susceptibility of the tested antibiotic was decreased as follow:

Ceftazidime ≤ 22 mm

Cefotaxime ≤ 27 mm

Phenotypic confirmatory test by using the double disk diffusion test for synergy between clavulanic acid and both ceftazidime and cefotaxime. An increase of ≥ 5 mm in zone diameter for either antimicrobial agent tested in combination with clavulanic acid compared with its zone when tested alone is a confirmation of the ESBL phenotype (CLSI, 2008).

Each phenotypically confirmed ESBL-EK was preserved, for subsequent molecular testing in 2 ml aliquot of tryptone soya broth (TSB) at -70°C.

Detection of bla-TEM, bla-OXA and bla-CTX-M genes in all collected ESBL-producing isolates (n=28) by real time PCR (Table 1).

Amplified gene	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
Bla TEM	TGAAGACGAAAGGGCCTCTCTG	TAATCAGTGAGGCACCTATCTC	770
Bla OXA-1	AATGGCACCAGATTCAACTT	CTTGGCTTTTATGCTTGATG	595
Bla CTXM	ATGGTTAAAAATCACTG	CCGTTTCCGCTATTACAAA	900

Table 1: Primers sequences used for detection of ESBL genes.

The reaction tubes were placed in the thermal cycler for 10mins at 95°C for initial activation of the Enzyme (Hot-start), followed by 30 successive cycles of denaturation, annealing, then by a final extension as follows:

Hot start: 95°C for 10 mins.

Denaturation: 95°C for 5 mins.

Annealing: 52°C for bla-TEM or blaOXA

57°C for bla-CTX-M for 45 sec

Extension: 72°C for 7 mins.

Detection of the amplified PCR products done by electrophoresis

Statistical Analysis

The collected data was revised, coded, tabulated, and analyzed using Statistical package for Social Science (SPSS 15). Data was presented and analysis was done according to the type of data obtained for each parameter.

Results

PCR analysis revealed that the overall prevalence of the detected ESBL genes among the studied isolates was 96.4% (27/28). The most prevalent ESBL-type was bla TEM as it was produced by 78.6% (22/28) of the ESBL-EK isolates, bla CTX-M was the second which detected in 78.6% (22/28), bla OXA was the third and detected in 75% (21/28).

Isolates	Total no of isolates		Prevalence of ESBLs among tested isolates	
	No	%	No	%
<i>E.coli</i> spp.	52	61.1	14	16.4
<i>Klebsiella</i> spp.	33	38.9	14	16.4
Total	85	100	28	32.8

Table 2: Prevalence of ESBLs among nosocomially acquired *E.coli* and *Klebsiella* spp. at Minia University Hospitals over a period of 1 year.

The following risk factors were significantly more common in the ESBL cases than in the non ESBL group (ESBLs vs. non-ESBLs): diabetes mellitus in 21.4% (6/28) vs. 8.8% (7/57), renal disease in 14.2% (4/28) vs. 8.8% (5/57), ICU admission in 21.3% (6/28) vs 15.8% (9/57), having surgery in the previous one month in 42.8% (12/28) vs.

15.8% (9/57), having mechanical ventilation in 14.3% (4/28) vs. 8.8% (7/57), having urinary catheter in place in 35.7% (10/28) vs. 17.5% (10/57), and longer hospital stay prior to infection (mean duration of hospitalization was 15.55 ± 9.89 vs. 6.30 ± 3.014) (Table 3).

Hospital location	ESBL-EK cases (n=28)		Non ESBL-EK cases (n=57)	
	No	%	No	%
Surgery wards	4	14.3	8	14.0
Gyn. & Obst. wards	2	7.1	4	7.0
Internal medicine wards	2	7.1	7	12.3

Internal medicine ICUs	6	21.4	13	22.9
Chest wards	10	35.7	17	29.8
Pediatric wards	4	14.4	8	14.0

Table 3: Distribution of ESBLs spp among different hospital wards.

Discussion

In our study, ESBLs was detected in 26.9% (14/52) of *E.coli* and in 42.4% (14/33) of *klebsiella* isolates and this percent is consistent with the study of Bouchillon, et al. [3] who carried out a survey that covered 38 medical centers in northern and southern European countries, Egypt, Lebanon, Saudi Arabia, and south Africa, they reported the highest rate of ESBL production in Enterobacteriaceae to be in Egypt with a prevalence of 38.5% (10/26) and Greece with a rate of 27.4% (96/343), and lowest in the Netherlands as the rate was 2% (9/454), and Germany with a rate of 2.6% (21/768), regarding the middle east they found the rate of ESBL production in Saudi Arabia was 18.6% (34/183) and 18.2% (33/181) in Lebanon. This is possibly; related to the less controlled use of antibiotics in Egypt, where many drugs are still available over the counter.

The high rates of ESBL production in Egypt was further supported by the study carried out by Fam and El-Damarawy [4], who examined 85 gram-negative bacterial isolates causing infection in the ICU of Theodor Bilharz Research Institute, Cairo, Egypt. They reported an ESBL rate of 65.8% (56 of 85 isolates). Their results are also supported by the study carried by Shaaban et al. [5] who reported that the rate of ESBLs among gram negative bacilli isolated from patients admitted to ICU at Assiut University Hospitals was 64.7% (33 of 51 isolates).

In the present study, no significant differences were demonstrated between the randomly selected cases of ESBL-EK as compared to the non-ESBL-EK cases regarding the distribution of patients among different hospital departments and the source of infection (Table 4).

Type of sample	ESBL-EK cases (n=28)		Non ESBL-EK cases (n=57)	
	No	%	No	%
Wound swab	4	14.3	8	14.0
Urine culture	8	28.6	19	33.3
Sputum culture	14	50	24	42.1
Blood culture	2	7.1	6	10.6

Table 4: Distribution of ESBLs spp. regarding the source of infection.

Many studies have attempted to identify different risk factors that predict true infections caused by ESBL-producing organisms not colonization so that effective strategies may be developed to limit outbreaks due to these infections, additionally, identification of the risk factors associated with higher rates of mortality also helps in ascertaining the prognosis of the patients with ESBL infections [6].

Carbapenems (imipenem and meropenem) were the only agents to which all the tested ESBL isolates did not demonstrate any resistance (their sensitivity is 100%) as carbapenems are highly stable to ESBL hydrolytic activity in addition their penetration to the outer bacterial membrane is excellent due to their compact molecular size [7]. Tazocin can be considered as a second choice of treatment as it was sensitive in 57% of the tested ESBL-EK.

Our findings are in agreement to the Egyptian study conducted by Fam and El-Damarawy [4], who reported high resistance rates to various antimicrobial classes as follows: aminoglycosides (90%), co-trimoxazole (90%), quinolones (100%), nitrofurantoin (50%). However meropenem was the only sensitive antibiotics that remained effective on all their ESBL isolates (100%) followed also with Tazocin as its sensitivity was 70% (Figure 1). These results are also supported by the Indian study conducted by Goyal et al. [8], who reported resistance rate of 68% to aminoglycosides, 79% to co-trimoxazole, and 94% to

quinolones. All their isolates were also sensitive only to imipenem (100%).

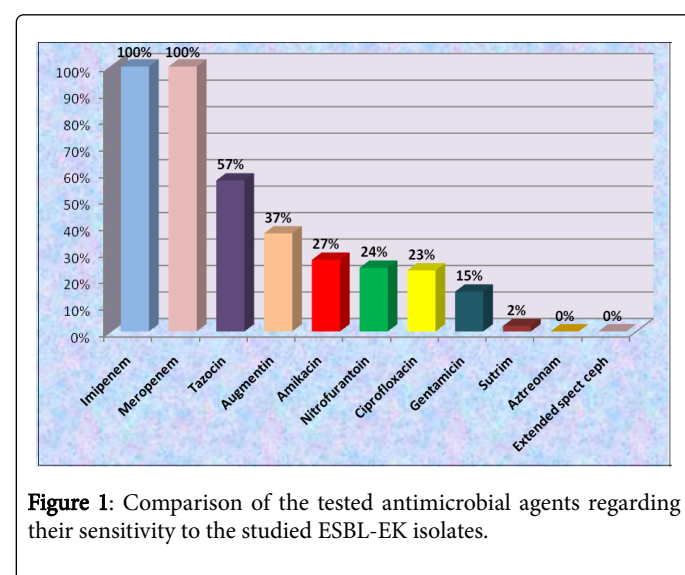
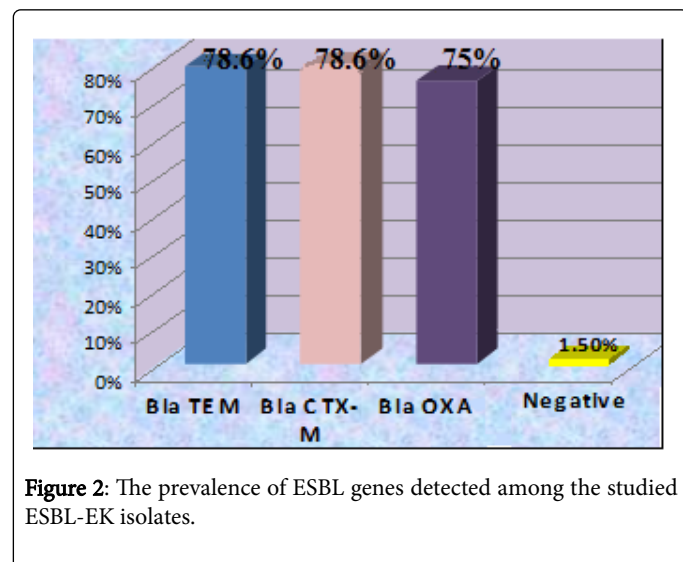


Figure 1: Comparison of the tested antimicrobial agents regarding their sensitivity to the studied ESBL-EK isolates.

On the contrary, in the study conducted by Marija et al. [9] from Croatia, the tested ESBL strains showed very low resistance rate for ciprofloxacin (10%), although they agreed with us regarding other antimicrobial profiles. Such discrepancy may be attributed to the difference in the prescription patterns of ciprofloxacin with respect to patient age. As most of ESBL isolates in their study originated from neonates and pediatric patients whom could not be treated with fluoroquinolones, indicating that the driving selection pressure within their hospital was probably not a fluoroquinolone.

Our findings regarding the PCR detection of ESBL genetic marker are in consistent to the Egyptian study conducted by Al-Agamy et al. [10] which revealed that the most prevalent ESBLs among their tested isolates were bla TEM (100%) and bla CTX-M (100%), that were produced simultaneously (Figure 2). These results are also supported by the Malaysian study conducted by Lim et al. [11] who showed that analysis of the ESBL-encoding genes indicated that the majority of ESBL-positive isolates harbored bla TEM (88%), followed by bla CTX-M (20%), followed by bla SHV (8%), followed by bla OXA (5%), and only two isolates did not harbor any of the tested ESBL-encoding genes.



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