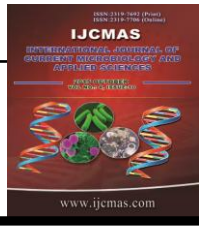
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 48-59**
http://www.ijcmas.com



Original Research Article

Study of Extended Beta Lactamase Producing Gram Negative Bacilli in Mansoura University, Hospital in Egypt

Maysaa El Sayed Zaki* and Walaa Othman El Shabrawy

Clinical Pathology Department, Mansoura Faculty of Medicine, Egypt

**Corresponding author*

Keywords

Entero-bacteriaceae, hospital acquired infections, ESBLs

A B S T R A C T

Enterobacteriaceae hospital acquired infections are common. Extended spectrum beta lactamases (ESBLs) producing species is an emerging health problem. The aims of the present study were to assess the prevalence of ESBL-positive species among *Enterobacteriaceae* and non *Enterobacteriaceae* gram negative bacilli recovered from hospital acquired infections and to evaluate the susceptibilities of ESBL-positive isolates to other compounds. Over two years period (September 2012 to September 2014), our task in the clinical microbiology laboratory in Mansoura University Hospital, Egypt was to evaluate phenotypic susceptibility to beta-lactams of *Enterobacteriaceae* recovered from hospitalized patients. All isolates positive in ESBL screening test were subjected to testing to detect the possible presence of SHV, TEM and CTX-M genes by conventional PCR. A total of 232 5isolates of the family *Enterobacteriaceae* were studied during two years period. The double-disk method was positive in 37.9% PCR detection showed that TEM-type ESBLs were more prevalent than SHV-type and CTX-M enzymes (30 versus20 and 11 respectively) and that about 5.7% of ESBL-positive *Enterobacteriaceae* had mixed TEM and SHV genotypes, and 22 (25%) isolates failed to show the presence any of the studied genotypes of ESBLs. From this study it is concluded that ESBLs is common among Enterobacteriaceae species isolated from hospital acquired infections. TEM-bla was the most common genotype followed by SHV and CTX-M. The isolates with ESBLs production retain susceptibility to carbapenem compounds and to amikacin.

Introduction

Enterobacteriaceae family has been accounted to deliver augmented beta lactamase enzymes that are in charge of around of half non response to beta lactams antibiotics [1]. These catalysts have the limit of hydrolyzing betalactams mostly affecting cephalosporins but not carbapenem and cephamycins [2]. These enzymes are ordinarily created by distinctive sets of qualities placed either in plasmid like TEM-1 and TEM-2 or in bacterial chromosomes like SHV-1 [3]. Different qualities may be in charge of betalactamase phenotypes like the vicinity of chromosomal AmpC [2],

hyperproduction of TEM enzymes, creation of inhibitor-resistant TEM (IRT), generation of oxacillinases, or obtaining of plasmid-interceded cephalosporinase [2,4-6]. Notwithstanding the established TEM- and SHV-inferred compounds, betalactamases of the CTX-M gathering and VEB-1 have been accounted for [7-11]. Consequently, the routine identification of betalactam phenotypes of *Enterobacteriaceae* is vital for recognizing and observing the spread of the different sorts of betalactamases.

In *Enterobacteriaceae* species, ESBLs have been accounted for in *Klebsiella* spp. *E. coli, Citrobacter, Enterobacter, Morganella, Proteus, Providencia, Salmonella* and *Serratia* [3,12-15]. Infections brought about by ESBL-positive organisms frequently reported in immunocompromised patients particularly in health care facility procured diseases making it hard to treat these organic entities in high-hazard wards [16-18].

Treatment of patients infected with *Enterobacteriaceae* species producing betalactamase is at most vitality and obliges full joint effort from clinical microbiology labs [19].

Reduced susceptibility or resistance to extended-spectrum cephalosporins and/or monobactams represents the a clue for the presence of ESBL production, however confirmatory tests of using combined clavulanate and selected betalactams are required like double-disk method and E-test specific strips [20-22]. Routine screening of isolates by minimal inhibitory concentration (MIC) tests or disk diffusion method does not accurately define the expression of an extended-spectrum enzyme [23]. ESBL-positive strains are reported as resistant with confirmatory tests even if they were susceptible by MICs break points for cephalosporins and aztreonam. This is well established for for *Klebsiella* spp. and *E. coli* but has not been established for other *Enterobacteriaceae species*.

In the presence of ESBL-positive strains, microbiology research lab should provide the clinician with reliable therapeutic selection for successfully treating infected patients. Since ESBL distribution has been show to differ among geographical neighborhood [23,24], monitoring of the prevalence and the types of extended spectrum enzyme may contribute to defining the problem.

The aims of the present study were to assess the prevalence of ESBL-positive species among *Enterobacteriaceae* and non *Enterobacteriaceae* Gram negative bacilli recovered from hospital acquired infections and to evaluate the susceptibilities of ESBL-positive isolates to other compounds.

Materials and Methods

The study was carried out in Mansoura University Hospital. It is tertiary regional hospital with 500 beds serving surgical, medical and obstetric and gynaecological departments in Dakhlia government. Over two years period (September 2012 to September 2014), the task of the clinical microbiology laboratory was to evaluate susceptibility to beta-lactams of *Enterobacteriaceae* recovered from hospitalized patients.

Antimicrobial susceptibility testing and ESBL detection

In vitro susceptibility testing of all isolates to a wide range of antimicrobials, including both beta-lactams and non-beta-lactams, was performed using the automated MicroScan WalkAway system (Siemens HealthCare Diagnostics, formerly Dade Behring, USA)

and Microscan® Gram Negative Breakpoint Combo panels.

Isolates reported as ESBL positive, using the automated system, were designated as ESBL screen-positive and were further subjected to a confirmatory test. Confirmation of the ESBL phenotype was performed using the combination disk method based on the inhibitory effect of clavulanic acid according to the CLSI criteria

Detection of ESBL genes by PCR

All isolates positive in ESBL screening test were subjected to testing to detect the possible presence of *SHV, TEM* and *CTX-M* genes by conventional PCR. The primers and a list of the detectable genes of each gene group are listed in table 1. A single colony of the isolated bacteria was emulsified in the 50 µl reaction mix, which contained 10 pmol of each primer, 10mM dNTPs mix (Qiagen, Hilden, Germany) and 2.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) in 1x Taq polymerase buffer.

Amplification reactions were performed under the following conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds with an extension at 72°C for 50 seconds, and a final extension for one cycle at 72°C for 5 minutes. The PCR product was then run on a 1.5 % agarose gel for detection of the amplified fragment [24].

Results and Discussion

During the period of the study the laboratory received 600 different samples diagnosed as hospital acquired infections according to CDC criteria. Among those samples, 232 samples culture yielded *Enterobacteriaceae* species.

The commonest sites of infections were wound infections (41.4%), gastrointestinal tract infections (26.7%) and sepsis (18.9%) table 2.

The commonest *Enterobacteriaceae* isolates were *E. coli* in 40.5%, *Pseudomonas aeruginosa* in 13.8% and *Klebsiella* species in 12.9%, table 3.

Antibiotics susceptibility patterns of the isolated *Enterobacteriaceae* species revealed high resistance pattern for trimethoprim (69.8%) cefepime (67.2%), piperacillin (67.2%), cephalothin (62.1%) and ampiciilin (60%) while lowest resistance was demonstrated toward ticarcillin (13.8%), table 4

Occurrence of ESBL-producing organisms. A total of 232 5isolates of the family *Enterobacteriaceae* were studied during two years period. The double-disk method showed that 88 out of 232 isolates (37.9%) were characterized by synergy between clavulanate and at least one of the tested beta lactams. As shown in table 5, the most common ESBL-producing strain was *E. coli* (*n* =28), followed by *K. pneumoniae* (*n* =12), and *Pseudomonas aeruginosa* (*n* =12).

Distribution of ESBL gene types in different members of the family *Enterobacteriaceae*. PCR detection of *bla*TEM, *bla*SHV and CTX-M was performed for 88 isolates revealed positive by double disk screening test. The assay showed that TEM-type ESBLs were more prevalent than SHV-type and CTX-M enzymes (30 versus20 and 11 respectively) and that about 5.7% of ESBL-positive *Enterobacteriaceae* had mixed TEM and SHV genotypes, and 22 (25%) isolates failed to show the presence any of the studied genotypes of ESBLs.

TEM-type ESBLs appeared to be particularly prevalent equal to or above 50% for the following species: *E. coli, K. pneumoniae* and *M. morganii* SHV-type enzymes, in contrast, were widely diffused for *Acinetobacter* and *S. fonticola.*

It is noteworthy that *Salmonella* species isolated had CTX-M genotypes whereas non-TEM, non-SHV enzymes were found in most species but were particularly frequent in *Pseudomonas species, Enterobacter and Citrobacter.*

Susceptibility pattern of isolated *Enterobacteriaceae* producing ESBLs revealed susceptibility to amikacin, imipenem and meropenem (50% each) with reduced susceptibility to ciprofloxacin (23.9%) (Table 6).

In general, hospital-acquired infections (HAIs) are most commonly associated with invasive medical devices or surgical procedures. Lower respiratory tract and bloodstream infections are the most lethal; however, urinary tract infections are the most common [25]. Hospital-acquired infections are considered the sixth leading cause of death in the United States [26] and Europe [27]. Among HAIs, those infections caused by Gram-negative bacteria are of special concern. These organisms are highly susceptible to acquire antibiotics resistance especially in the presence of antibiotic selection pressure. Moreover, they have multiple mechanisms against the same antibiotic. In the present study, the commonest HAIs that yielded *Enterobacteriaceae* were wound infections (41.4%), gastrointestinal tract infections 26.7% and sepsis (18.9%).
Mansoura University Hospital mainly serves surgical departments and internal medicine departments. Usually, the type of hospital acquired infections depends on the services offered by the health care institute. Moreover, the increase rate of gastrointestinal tract infections in this study may denotes the source of *Enterobacteriaceae* to be feco-oral.

The commonest isolated *Enterobacteriaceae species were E. coli* in 40.5%, *Pseudomonas aeruginosa* in 13.8% and *Klebsiella* species in 12.9%,

The presence of suitable portal of enter any Gram-negative organism can cause HAIs, however, the most common organisms include *Klebsiella* species, *Escherichia coli*, and *Pseudomonas aeruginosa* [25].

It worth noticing the high prevalence of ESBLs among isolated *Enterobacteriaceae* (37.9%).The production of ESBLs by enterobacteria is a well known resistance mechanism against β-lactams. The prevalence rates of ESBLs in *Enterobacteriaceae* vary according to the geographical region of the study from 9.1% up to 90% [26, 28-30]. Previous study carried in Egypt reported prevalence rate 34.5% [31].

The ESBLs enzymes spread rapidly throughout the world and become the common resistance mechanism once established in a region [32, 33]. Poor hand hygiene and lack of food hygiene are common predisposing factors for the acquiring infection with this resistant bacteria in hospitals [34-37]. Other well known risk factors are improper antibiotic use, prolonged hospital stay, severe underlying illness, recent surgery and the use of invasive medical devices [38-43].

Among ESBL-positive strains, the prevalence of TEM-*bla* type (34.1%) type enzymes was higher than that of SHV- type (22.7%) type enzymes and CTX-M (12.5%). Other researchers also reported high

prevalence rates of the TEM-*bla* 92 % [44], 82 % [41], 72% [45] and 70% [46].

Usually most ESBLs evolved from gene mutations in classical ß-lactamases (TEM-1, TEM-2 and SHV-1), giving rise to ESBL various forms of the TEM and SHV types. Another family of ESBLs, CTX-M, has emerged over recent years, especially in *E. coli*. This family has become one of the most important families of ESBL enzymes in many countries [47-50]. However, apparently this is not the case in the present study.

Twenty two (25%) isolates of *Enterobacteriaceae* strains had ESBLs other than TEM-*bla*, SHV and CTX-M derived enzymes; among these, *Pseudomonas species Enterobacter* and *Citrobacter* were particularly notable. This finding confirms the importance given to the emerging problem of non-TEM, non-SHV enzymes that are spreading worldwide [51-54]. Though many of these unusual enzymes have been detected only in small number of isolates (SFO-1, TLA-1, VEB-1, and BES-1), and PER-type enzymes have been found in Turkey, France, Italy, and Argentina [55, 56].

Table.1 List of primers and the detectable ESBL genes in each gene group

Gene	primer	Amplicon	detectable genes*
SHV	SHV-F:CGCCTGTGTATTATCTCCCT SHV-R: CGAGTAGTCCACCAGATCCT	294 bp	1- 2, 2A, 5,8-9,11-13, 18, 24-27, 29-31, 33-38, 41-42, 44-46, 48, 50-52, 55, 57, 59- 60, 62-67, 69-83, 85- 86, 89, 92- 93, 95-97, 101-105, 108, 110, 120-123, 128-129, 133-137, 140-142, 145, 147-163, 165, 167
TEM	TEM-F:TTTCGTGTCGCCCTTATTCC TEM-R: ATCGTTGTCAGAAGTAAGTTGG	404 bp	1, 10, 15, 28, 30, 34, 47, 68, 70, 76-77, 79, 88, 95, 102, 104-107, 109, 124, 126-130, 132, 140, 143-144, 148, 158, 162, 166, 176, 186, 198, 201
CTX-M	CTX-M-F: CGCTGTTGTTAGGAAGTGTG CTX-M-R: GGCTGGGTGAAGTAAGTGAC	754 bp	1, 3, 10-12, 15, 22-23, 28-30, 32, 34, 36, 42, 52, 54-55, 57-58, 60-62, 71-72, 79-80, 82, 88, 96, 101, 108, 114, 117, 123, 132-133

Table.2 Sites of hospital acquired infections

	Frequency	%
Sepsis	44	18.9
Wound Infections	96	41.4
Pneumonia	24	10.3
Gastrointestinal tract infections	62	26.7
Urinary tract infections	6	2.6
Total	232	100.0

Table.3 *Enterobacteriaceae* and non *Enterobacteriaceae* Gram negative bacilli isolates

Enterobacteriaceae species	**Frequency**	**%**
Achromobacter	16	6.9
Aeromonas hydrophila	2	0.9
bordetella bronchiseptica	2	0.9
Citrobacter	10	4.3
Enterobacter spp.	10	4.3
E. coli	94	40.5
Klebsiella spp.	30	12.9
Morganella morganii	12	5.2
Pseudomonas aeruginosa	32	13.8
Plesiomonas shigelloides	4	1.7
Proteus vulgaris	4	1.7
Serratia fonticola	8	3.4
Salmonella spp.	4	1.7
Shigella spp.	4	1.7
Total	232	100.0

Table.4 Antibiotics resistance pattern for isolated Gram negative bacilli species

Antibiotics	**No.**	**%**
amikacin	41	35.3
amoclav	50	43.1
Ampicillin/sulbactam	66	56.9
ampicillin	68	58.6
aztreonam	8	6.9
cefazolin	62	53.4
cefepime	78	67.2
cefotaxime	9	7.8
cefox	57	49.1
ceftazidime	61	52.6
ceftriaxone	54	46.6
cefur	68	58.6
cephalothin	72	62.1
ciprofloxacin	48	41.4
gentamycin	48	41.4
imipenem	43	37.1
levofloxacin	55	47.4
meropenem	30	25.9
Piperacillin/tazobactam	55	47.4
piperacillin	78	67.2
tetracycline	74	63.8
ticarcillin	16	13.8
tobramycin	66	56.9
trimethoprim	81	69.8
Total	116	100

Table.5 ESBLs among Gram negative bacilli species

Gram negative bacilli species	Pheno types No.	TEM type No. (%)	SHV type No. (%)	CTX-M No. (%)	mixed TEM and SHV genotypes No. (%)	Negative for the studied genotypes of ESBLs No. (%)
Acinetobacter	12	2(16.7%)	5(41.7%)	3(25%)	0(0%)	2(16.7%)
Aeromonas hydrophila	2	0(0%)	0(0%)	0(0%)	2(100%)	0(0%)
bordetella bronchiseptica	0	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Citrobacter	4	0(0%)	0(0%)	0(0%)	0(0%)	4(100%)
Enterobacter	8	2(25%)	1(12.5%)	0(0%)	0(0%)	5(62.5%)
E. coli	28	18 (64.3%)	6 (21.4%)	2(7.1%)	1(3.6%)	1(3.6%)
K. pneumoniae	12	6 (50%)	2(16.7%)	2(16.7%)	0(0%)	2 (16.7%)
Morganella morganii	4	2(50%)	1(25%)	0(0%)	0(0%)	1 (25%)
P. aeruginosa	12	0(0%)	1(8.3%)	2(16.7%)	2(16.7%)	7(58.3%)
Serratia fonticola	4	0(0%)	4(100%)	0(0%)	0(0%)	0(0%)
Salmonella spp.	2	0(0%)	0(0%)	2(100%)	0(0%)	0(0%)
Total	88	30 (34.1%)	20(22.7%)	11(12.5%)	5(5.7%)	22(25%)

Table.6 Susceptibility pattern of ESBLs Gram negative bacilli species (88) to carbapenem, ciprofloxacin and aminoglycosides

Antibiotics	Susceptibility	
	No.	%
Amikacin	44	50%
Ciprofloxacin	21	23.9%
Gentamycin	24	27.3%
Imipenem	44	50%
Levofloxacin	25	28.4%
Meropenem	44	50%
Tobramycin	40	45.5%
Trimethoprim	18	20.5%
Piperacillin/tazobactam	8	9.1%

Increase prevalence of hospital outbreaks due to ESBL-producing *Enterobacteriaceae* have been observed over the last few years [26, 28], The responsible strains are usually also have resistance to multiple antibiotics, including but not limited to ciprofloxacin, gentamycin, and aminoglycosides [5,57].

The present study confirms the presence of multiple antibiotics resistance to non-betalactams, showing a marked resistance to ciprofloxacin among ESBLs producing strains. This may be explained by the location of ESBL genes on integrons containing promoters for the coordinated

expression of multiple resistance gene cassettes [58].

In our study, 50% of ESBL producing *Enterobacteriaceae* maintained susceptibility to imipenem. On the whole, resistance to aminoglycosides did not appear to be associated with the type of produced enzyme(s). Our data indicate that a valuable option for treatment is represented by amikacin, a bactericidal drug effective against 50% of strains.

However, lactam–betalactamase inhibitor combinations remained quite inactive against most isolates. The reduced activity of the combination against *E. coli* in hospitalized patients has already been reported [59].

The present study on *Enterobacteriaceae* assesses, for the first time, the breadth of the ESBL problem in Egypt by using classical bacteriological methods and molecular techniques for extended types of antibiotics in use. The finding of ESBLs resistance phenotypes and genotypes supports the hypothesis that clinical microbiology laboratory plays an important role in eradicating infections caused by ESBLs producing *Enterobacteriaceae*. So, it is clear from the results of our study that the use of amikacin alone or in combination with imipenem is effective in HAIs due to ESBLs producing *Enterobacteriaceae*.

References

1.Livermore DM (1998) Betalactamase-mediated resistance and opportunitiesfor its control. J. Antimicrob. Chemother. 41:25–41.

2.Livermore D M (1995). Betalactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. 8: 557–584.

3.Cao V, Lambert T, Nhu DQ, Loan H K, Hoang NK, Arlet G, and Courvalin P (2002) Distribution of extended-spectrum Betalactamases in clinicalisolates of *Enterobacteriaceae* in Vietnam. Antimicrob. Agents Chemother. 46:3739–3743.

4.Bradford P A (2001) Extended-spectrum Beta lactamases in the 21st century: characterization, epidemiology, and detection of this important resistancethreat. Clin. Microbiol. Rev. 14:933–951.

5.Leflon-Guibout V., Speldooren V, Heym B., and Nicolas-Chanoine M H (2000) Epidemiological survey of amoxicillin-clavulanate resistance and correspondingmolecular mechanisms in *Escherichia coli* isolates in France: newgenetic features of *bla*TEM genes. Antimicrob. Agents Chemother. 44:2709–2714.

6-Philippon A, Arlet G., and. Jacoby G A (2002) Plasmid-determined AmpCtype Betalactamases. Antimicrob. Agents Chemother. 46:1–11.

7.Bou, G., Cartelle M, Tomas M, Canle D, Molina F, Moure R, Eiros J M, and Guerrero. A (2002) Identification and broad dissemination of the CTXM--lactamase in different *Escherichia coli* strains in the northwest areaof Spain. J. Clin. Microbiol. 40:4030–4036.

8.Girlich, D., Poirel L, Leelaporn A, Karim A., Tribuddharat C, Fennewald M, and.Nordmann P. (2001). Molecular epidemiology of the integronlocated VEB-1 extended-spectrum Beta lactamase in nosocomial enterobacterialisolates in Bangkok, Thailand. J. Clin. Microbiol. 39:175–182.

9.Chanawong A F 'Zali H M, Heritage J, Xiong J H, and. Hawkey P M (2002) Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among*Enterobacteriaceae* in the

People's Republic of China. Antimicrob. Agents Chemother. 46:630–637.

10.Moland E S., Black J A, Hossain A, Hanson N D, Thomson K S, and Pottumarthy S. (2003) Discovery of CTX-M-like extended spectrum beta-lactamasesin *Escherichia coli* isolates from five U.S. states. Antimicrob. AgentsChemother. 47:2382–2383.

11. Saladin MV, Cao T, Lambert T, Donay JL, Herrmann Z. Ould-Hocine,C, Verdet F, Delisle, Philippon A, and Arlet G. (2002) Diversity of CTX-M Beta lactamases and their promoter regions from *Enterobacteriaceae*isolated in three Parisian hospitals. FEMS Microbiol. Lett. 209:161–168.

12.Coudron, PE. Molland ES. and Sanders CC (1997) Occurrence and detection of extended-spectrum Betalactamases in members of the family *Enterobacteriaceae*at a Veterans Medical Center: seek and you may find. J. Clin. Microbiol. 35:2593–2597.

13.Franceschini N., Perilli M, Segatore B, Setacci D, Amicosante G, Mazzariol A, and Cornaglia G (1998) Ceftazidime and aztreonam resistance in*Providencia stuartii*: characterization of a natural TEM-derived extendedspectrum beta-lactamase, TEM-60. Antimicrob. Agents Chemother. 42:1459–1462.

14.Jacoby G A, and. Medeiros. A A (1991) More extended-spectrum Betalactamases. Antimicrob. Agents Chemother. 35:1697–1704.

15.Tzelepi E G, Panagiota D, Sofianou V, Loukova A., Kemerglou, and Tsakris A (2000) Detection of extended-spectrum Betalactamases in clinical isolatesof *Enterobacter cloacae* and *Enterobacter aerogenes*. J. Clin. Microbiol. 38:542–546.

16.Babini G., and Livermore D M (2000) Antimicrobial resistance amongst*Klebsiella* spp. from intensive care units in southern and western Europe in1997–1998. J. Antimicrob. Chemother. 45:183–189.

17.Kaye K S., Fraimow H S, and Abrutyn E (2000) Pathogens resistant toantimicrobial agents. Epidemiology, molecular mechanisms, and clinicalmanagement. Infect. Dis. Clin. North Am. 14:293–319.

18.Luzzaro F M. Perilli R., Migliavacca G, Lombardi P, Micheletti A, Agodi S, Stefani G, Amicosante, and Pagani L (1998) Repeated epidemics causedby extended-spectrum beta-lactamase-producing *Serratia marcescens* strains. Eur. J. Clin. Microbiol. Infect. Dis. 17:629–636.

19.Schiappa DA., Haiden M K, Matushek M G, Hashemi F N, Sullivan J, Miyashiro K Y, Quinn J P, Weinstein R A, and Trenholme G M (1996) Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstreaminfection: a case-control and molecular epidemiologic investigation. J. Infect. Dis. 174:529–536

20.Emery C L, and Weymouth L A (1997) Detection and clinical significance of extended-spectrum Beta lactamases in a tertiary-care medical center. J. Clin. Microbiol. 35:2061–2067

21.National Committee for Clinical Laboratory Standards. (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7–A5. National Committee for Clinical LaboratoryStandards, Wayne, Pa.

22.Tenover F C, Mohammed M J, Gorton T S, and. Dembek Z F (1999) Detection and reporting of organisms producing extended-spectrum Beta lactamases: survey of laboratories in Connecticut.

J. Clin. Microbiol. 37:4065–4070.
23.Jacoby G. A., and. Medeiros A A (1991) More extended-spectrum Beta lactamases. Antimicrob. Agents Chemother. 35:1697–1704.
24.Rahal J J (2000). Extended-spectrum Beta-lactamases: how big is the problem? Clin. Microbiol. Infect. 6(2):2–6.
25.Peleg AY, Hooper DC (2010) Hospital-Acquired Infections Due to Gram-Negative Bacteria.*The New England journal of medicine* ;362(19):1804-1813. 26-Kung HC, Hoyert DL, Xu J, Murphy SL (2008). Deaths: final data for 2005. Natl Vital Stat Rep. ;56:1–120.
27.Chopra I, Schofield C, Everett M, et al (2008). Treatment of health-care-associated infections caused by Gram-negative bacteria: a consensus statement. Lancet Infect Dis.;8:133–139.
28.Sader HS, Gales AC, Pfaller MA, Mendes RE, Zoccoli C, Barth A, et al (2001) Pathogen frequency and resistance patterns in brazilian hospitals: summary of results from three years of the SENTRY Antimicrobial Surveillance Program. Braz J Infect Dis; 5:200-214.
29.Freitas ALP, Machado DP, Soares FS, Barth AL (2003). Extended-Spectrum β-lactamases in *Klebsiella* spp. And *Escherichia coli* obtained in a Brazilian teaching Hospital: detection, prevalence and molecular typing. Braz J Microbiol; 34:344-348.
30.Jones RN, Pfaller MA (2003) Antimicrobial activity against strains of *Escherichia coli* and *Klebsiella* spp. with resistance phenotypes consistent with extended-spectrum β-lactamases in Europe. Clin Microbiol Inf; 9:708-712.
31.Bouchillon SK, Johnson BM, Hoban DJ, Johnson JL, Dowzicky MJ, Wu DH, Visalli MA, Bradford PA (2004) Determining incidence of extended spectrum beta-lactamase producing *Enterobacteriaceae*, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries: the PEARLS study 2001-2002. Int J Antimicrob Agents.; 24(2):119-24.
32.Bradford PA (2001). Extended-spectrum-β-lactamases in the 21st century: caracterização, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev; 14:933-951
33.Paterson DL, Bonomo RA (2005). Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev; 18:657-686.
34.Hidron AI, Edwards JR, Patel J, et al (2009). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007.Infect Control Hosp Epidemiol. 2008; 29:996–1011
35.Gaynes R, Edwards JR (2005) Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis.; 41:848–854.
36.Jarvis WR (2007). The Lowbury Lecture: the United States approach to strategies in the battle against healthcare-associated infections, 2006: transitioning from benchmarking to zero tolerance and clinician accountability Hosp Infect. ; 65 (2):3–9.
37.Souli M, Galani I, Giamarellou H (2008). Emergence of extensively drug-resistant and pan drug-resistant Gram-negative bacilli in Europe. Euro

Surveill.;13(47):19045.
38.Colodner R, Rock W, Chazan B, et al (2004) Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. Eur J Clin Microbiol Infect Dis.; 23(3):163–167.
39.Tham J, Odenholt I, Walder M, Brolund A, Ahl J, Melander E (2010) Extended-spectrum beta-lactamase-producing Escherichia coli in patients with travellers' diarrhea. Scand J Infect Dis.; 42(4):275–280.
40.Tangden T, Cars O, Melhus A, Lowdin E (2010). Foreign travel is a major risk factor for colonization with Escherichia coli producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. Antimicrob Agents Chemother. ; 54(9):3564–3568.
41.Pitout JD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis.; 8(3):159–166.
42.Ben-Ami R, Rodriguez-Bano J, Arslan H, et al (2009) A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in nonhospitalized patients. Clin Infect Dis.; 49(5):682–690.
43.Lytsy B, Sandegren L, Tano E, Torell E, Andersson DI, Melhus A (2008) The first major extended-spectrum beta-lactamase outbreak in Scandinavia was caused by clonal spread of a multiresistant Klebsiella pneumoniae producing CTX-M–15. APMIS. ;116(4):302–308.
44.Fang H, Ataker F, Hedin G, Dornbusch K (2008) Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J Clin Microbiol; 46:707-712.
45.Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH (2007) First Report of the Emergence of CTX-M-Type Extended-Spectrum β-Lactamases (ESBLs) as the Predominant ESBL Isolated in a U.S. Health Care System. Antimicrob Agents Chemother; 51:4015-4021.
46.Andersson H, Lindholm C, Iversen A, et al (2012) Prevalence of antibiotic-resistant bacteria in residents of nursing homes in a Swedish municipality: healthcare staff knowledge of and adherence to principles of basic infection prevention.Scand J Infect Dis. ;44(9):641–649
47.Bonnet R, Dutour C, Sampaio JLM, Chanal C, Sirot D, Labia R, et al (2001) Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-2403Gly. Antimicrob Agents Chemother; 45:2269-2275.
48.Livermore DM, Brown DFJ (2001) Detection of β-lactamases-mediated resistance. J Antimicrob Chemother; 48:59-64.
49.Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey MR, et al (2007). Molecular epidemiology of CTX-M-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob Agents Chemother; 51:1281-1286.
50.Canton R, Coque TM (2006) The CTX-M β-lactamase pandemic. Curr Opin Microbiol; 9:466-475.
51.Bonnet R, Sampaio JLM, Labia R, Champs C, Sirot D, Chanal C, et al

(2000). A novel CTX-M b-lactamase (CTX-M-8) in cefotaxime resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob Agents Chemother; 44:1936-1942.

52.Stone PW, Hedblom EC, Murphy DM, Miller SB (2005). The economic impact of infection control: making the business case for increased infection control resources. Am J Infect Control. ; 33:542–547

53.Yokoe DS, Mermel LA, Anderson DJ, et al.(2008) A compendium of strategies to prevent healthcare-associated infections in acute care hospitals. Infect Control Hosp Epidemiol. ; 29 (1):S12–S21.

54.Boucher HW, Talbot GH, Bradley JS, et al (2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis.; 48:1–12.

55.Bauernfeind, A. I, Stemplinger R., Jungwirth P, Mangold S, Amann E, Akalin O, Ang C, Bal, and Casellas J M (1996) Characterization of _-lactamasegene *bla*PER-2, which encodes an extended-spectrum class A β- -lactamase. Antimicrob. Agents Chemother. 40:616–620.

56.Pereira, M., Perilli M, Mantengoli E, LuzzaroF, Toniolo A, Rossolini GM, and Amicosante G (2000) PER-1 extended spectrum _-lactamaseproduction in an *Alcaligenes faecalis* clinical isolate resistant to expandedspectrumcephalosporins and monobactams from a hospital in northernItaly. Microb. Drug Res. 6:85–90

57. PatersonD L, Mulazimoglou L, Casellas J M, Ko,W, Goossens H, Von Gottberg A, Mohapatra S, Trenholme G M, Klugman K P,. McCormack J G and Yu. V L (2000) Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β--lactamase production in *Klebsiellapneumoniae* isolates causing bacteremia. Clin. Infect. Dis. 30:473–478.

58.Poirel, L., Thomas I., Naas T, Karim A., and Nordmann P (2000) Biochemical sequence analysis of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 44:622–632.

59.Kaye K S., Harris A D, Gold H., and Carmeli Y (2000) Risk factors for recovery of ampicillin-sulbactam-resistant *Escherichia coli* in hospitalized patients. Antimicrob. Agents Chemother. 44:1004–1009.