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Full Length Research Paper

Fecal carriage of extended-spectrum β-lactamase-producing *Enterobactearicae*: a comparative study between hospitalized and non-hospitalized patients

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Recently the world has seen a surge in extended-spectrum β-lactamase (ESBL)-producing bacteria. However, data on the dissemination of ESBL-producing Enterobacteriaceae in the hospital and in non-hospitalized personal from systematically enrolled study subjects in Egypt remains few. Aim of study: To determine the prevalence, phenotypic resistance patterns and genetic characteristics of ESBL-producing Entearobacteracicea in fecal carriage in both hospital and in non-hospitalized personal. Material and methods: A total of 160 fecal samples from 60 non hospitalized coming from out patients clinics and100 hospitalized patients .The were screened for the presence of ESBL producing Enterobactearicae. A multiplex PCR assay was used to identify blaCTX-M blaSHV, blaOXA, blaTEM genes. Results: Out of 60 clinical isolates from non-hospitalized patients group (1) it was found that the 41 Escherichia coliisolates produced the different ESBLs types as follow:(58%) produced TEM gene, (95%) produced CTX-M, (54%) produced TEM combined with CTX-M. Out of 100 clinical isolates from hospitalized patients group (II) it was founded that the (50) Escherichia coliisolates produced the different ESBLs types as follow: (78%) produced TEM gene, (96%) produced CTX-M and (74%) produced TEM combined with CTX-M. Conclusion: The most affected age was 15-30 years; this may be due to the panting of this age group on fast food, which may be the reason in the transmission and spread of infection by these microbes. blaCTX-M was the most prevalent ESBL gene followed by blaTEM and finally, blaSHV, while the blaOXA gene was not detected in any of the isolated bacteria.

Keywords: Hospitalized, non hospitalized, ESBL ,fecal.

INTRODUCTION

β-Lactamase production is the most common mechanism of bacterial resistance to β-lactam antibiotics. Many new β-lactam antibiotics have been developed in the last few decades. However, with the introduction of each new β-lactam antibiotic a new β-lactamase class causing resistance to that drug has emerged (Medeiros 1997). One

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of these new classes is the extended-spectrum β-lactamases (ESBLs), which hydrolyze oximino-β-lactams, such as the expanded-spectrum cephalosporins, but not the carbapenems, and which are highly susceptible to inhibition by clavulanic acid and tazobactam (Livermore 1995). ESBLs were first reported in the mid-1980s, and most of them have been found in Klebsiellapneumoniae and Escherichia coli (Bradford 2001).

Many ESBLs arise from simple point mutations in existing plasmid-mediated β-lactamase genes, like TEM- and SHV-derived ESBLs; however, members of a new group of ESBLs (the CTX-M type), derived from chromosomal class A β-lactamases, have been identified in the past 10 years. The CTX-M enzymes are not related to TEM or SHV enzymes, as they share only 40% identity with these ESBLs (Bonnet 2004).

Patients with fecal carriage of ESBL-producing bacterial isolates have been investigated previously during nosocomial outbreaks (De Champs et al., 1989; Hollander et al., 2001; Peña et al., 1998), but the number of prospective longitudinal studies conducted in the hospital setting during non outbreak situations or in the community remains scarce (Bonomo et al., 2003; Millar et al., 2001; Mirellis et al., 2003; Abdul Rahman and El-Sherif 2011). Moreover, to the best of our knowledge, the rate of fecal carriage of ESBL-producing bacterial isolates during different and distant periods of time in the same location has not been investigated previously.

The aim of the present study was to investigate the prevalence, phenotypic resistance patterns and genetic characteristics of ESBL-producing Enterobactearicae in fecal carriage in both hospital and in non-hospitalized personal in a non outbreak period and during whole one year 2011.

MATERIAL AND METHODS

Detection of ESBL-producing isolates in fecal samples.

A total of 160 fecal samples from 100 hospitalized patients and 60 non hospitalized coming from out patients clinics selected according to no recent history of health care contacting. The samples prospectively collected during 2011 were screened for the presence of ESBL producing Enterobactearicae. None of the outpatients studied were residents of a skilled care facility or lived in a nursing home or a health care center. Samples were collected and processed within 4 h of sampling. A total of 0.5 g of each fecal sample was suspended in 5 ml of saline, and aliquots of 200 µl were seeded into blood and MacConkey agar plates (Mast Diagnostics, Merseyside, UK). Samples yielding bacteria that grew on MacConkey agar were identified by standard biochemical procedures(Schreckenberger et al., 2009). All isolates that grew were screened for ESBL production by using both the resistance phenotype and the standard CLSI combined disk method involving CTX with and without the inhibitor clavulanic acid (30 µg) (Mast Diagnostics, Merseyside, UK) was used to confirm the presence of ESBL (CLSI) 2009). ESBL production was indicated by an increase in zone size of more than 5 mm with and without clavulanic acid.

Genetic Characteristics of ESBL-Producing Isolates.

All confirmed ESBL-producing isolates were subjected to molecular testing to detect ESBL-encoding genes. A multiplex PCR assay was used to identify blaCTX-M blaSHV, blaOXA, blaTEM, genes. Genomic DNA from wild-type isolates was used as the template for PCR. ESBL amplification was performed with the appropriate primers and cycling conditions for ESBL types, as described previously (Elsherif and Maamoun 2012).

Ethics.

Oral consent was obtained from all patients included in our study. The research protocol was approved by the Research Ethics Committee of the Departments of Clinical pathology Cairo University Egypt.

Statistical analysis.

Statistical significance for comparison of proportions was calculated by the chi-square test (a P value <0.05 was considered statistically significant).

RESULTS

This study was conducted at Kasralainy hospital on 160 fecal samples, divided as 60 collected from out patients clinics (group I) and 100 collected from different patients in different hospital departments (surgical, gynecology, burn and internal medicine (group II). Among these isolates, all isolates (100%) had reduced susceptibility to one or more of extended spectrum cephalosporin antibiotics by screening test and all showed positive results to combined disc confirmatory test for ESBL in both group.

The age of patients subjected to this study in both groups was categorized each 15 years. The most affected category in the two groups were the youngest category (15-30) 37.1% of indexed patients, this effect decreased with age increase. E. coli was the predominant species which occurred between patients .

Table (1): Demonstrates the types, number and percentages of the studied isolates in the hospitalized patients in comparison with the hospitalized patients:

Table (2): The percentage of antimicrobial resistance in ESBL-producing organisms in both non hospitalized and hospitalized group.

Table 1. Demonstrates the types, number and percentages of the studied isolates in the hospitalized patients in comparison with the hospitalized patients.

Clinical sample	***No of Hospitalized***	***%***	***NO of Non Hospitalized***	***%***
E.coli.	50	50%	41	68.5%
Klebsiella spp.	44	44%	18	30%
Enterobacter spp.	6	6%	1	1.5%
Total	100	100%	60	100%

Table 2. The percentage of antimicrobial resistance in ESBL-producing organisms in both non hospitalized and hospitalized group.

Antibiotics		*E.coli*		*Klebsiella spp*.		*Enterobacter spp.*	
		NH	H	NH	H	NH	H
		No 41	No 50	No 18	No 44	No 1	No 6
Pencillins	P AMP	100	100	100	100	100	100
Combined penicillins	AUG SCF TZP	100	100	100	100	100	100%
Cephalosporins	C1 C2 C3 C4(CPM)	100 100 100 8	100 100 100 20	100 100 100 5	100 100 100 91	100 100 100 0	100 100 100 0
Carbapenim	IPM MEM	2	10	15	11	0	33
Aminoglycosides	AK GN	0	4	5	2	0	16
Quinolones	CIP LEV OFX	73	84	85	89	0	54
SulphTrimethoprims	SXT TM	96	100	95	95	0	100

H= Hospitalized NH= Non hospitalized P= pencillin, AMP= Ampicillin ,AUG= Amoxicillin-Clavulenic acidSCF=cephoprazone+sulbactam ,TZP= Piperacillin+Taobactam ,C1= First Generation Cephalosprins (Cephalothin, Cephalexin, Cefadroxil and Cefazolin) ,C2= Second Generation Cephalosporins (Cefaclor, Cefoxitin, Cefuroxime and Cefixime)\ ,C3= Third Generation Cephalosporins (cefoperazone, Ceftaidime, Cefotaxime and Ceftriaxone) ,C4= Fourth Generation Cephalosporins, (CPF, CPM)= Cefepime ,IPM= Imipenem ,MEM= Meropenem ,AK= Amikacin ,GN= Gentamicin ,CIP= Ciprofloxacin ,LEV= Levofloxacin ,OFX= Ofloxacin ,SXT= Sulphameth-Trimeth ,TM= Trimethoprim

Table (3): The types of ESBLs genes and prevalence among the non-hospitalized patients group (I) ESBLs producing Enterobacteriaceae:

Out of 60 clinical isolates from non-hospitalized patients group (1) it was found that the 41 Escherichia coliisolates produced the different ESBLs types as follow:(58%) produced TEM gene, (95%) produced CTX-M, (54%) produced TEM combined with CTX-M. The (18) Klebsiella spp. isolates produced the different ESBLs types as follow: (100%) of klebsiella spp. Produced SHV gene,(72%) produced TEM gene ,(94%) produced CTX-M, (5%) SHV combined with TEM,(28%) SHV combined with CTX-M, (67%) SHV combined with TEM and CTX-M .And only one Enterobacter spp. produced the different ESBLs types as follow: TEM with CTX-M.

Table 3. The types of ESBLs genes and prevalence among the non-hospitalized patients group (I) ESBLs producing Enterobacteriaceae.

ESBLs genes	**SHV**		**TEM**		**CTX-M**		**SHV+TEM**		**SHV+ CTX-M**		**TEM+CTX-M**		**SHV+TEM+ CTX-M**	
Clinical isolates	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E.coli	0	0	24	58	39	95	0	0	0	0	22	54	0	0
Klebsiella spp.	18	100	13	72	17	94	1	5	5	28	0	0	12	67
Enterobacter spp.	0	0	1	100	1	100	0	0	0	0	1	100	0	0

Out of 60 clinical isolates from non-hospitalized patients group (1) it was found that the 41 *Escherichia coli*isolates produced the different ESBLs types as follow:(58%) produced TEM gene, (95%) produced CTX-M, (54%) produced TEM combined with CTX-M. The (18) *Klebsiella* spp. isolates produced the different ESBLs types as follow: (100%) of *klebsiella* spp. Produced SHV gene,(72%) produced TEM gene ,(94%) produced CTX-M, (5%) SHV combined with TEM,(28%) SHV combined with CTX-M, (67%) SHV combined with TEM and CTX-M .And only one *Enterobacter* spp. produced the different ESBLs types as follow: TEM with CTX-M, (100%).

Table 4. The types of ESBLs genes and prevalence among hospitalized group (II) ESBLs producing *Enterobacteriaceae.*

ESBLsgenes	**SHV**		**TEM**		**CTX-M**		**SHV+TEM**		**SHV+ CTX-M**		**TEM+CTX-M**		**SHV+TEM + CTX-M**	
Clinical isolates	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
E.coli	0	0	39	78	48	96	0	0	0	0	37	74	0	0
Klebsiella spp.	36	82	31	70	39	89	4	9	10	23	6	14	21	48
Enterobacter spp.	2	33	6	100	5	83	0	0	0	0	4	67	1	17

Out of 100 clinical isolates from hospitalized patients group (II) it was founded that the (50) *Escherichia coli*isolates produced the different ESBLs types as follow: (78%) produced TEM gene, (96%) produced CTX-M and (74%) produced TEM combined with CTX-M. The (44) *Klebsiella* spp. isolates produced the different ESBLs types as follow: (82%) of klebsiella spp. Produced SHV gene, (70%) produced TEM gene, (89%) produced CTX-M, (9%) SHV combined with TEM, (23%) SHV combined with CTX-M, (48%) SHV combined with TEM and CTX-M and (14%) TEM with CTX-M.

The 6 *Enterobacter*spp. produced the different ESBLs types as follow: (33%) Produced SHV gene, (100%) produced TEM gene, (83%) produced CTX-M, (17%) SHV combined with TEM and CTX-M and (67%) TEM with CTX-M.

Table (4) The types of ESBLs genes and prevalence among hospitalized group (II) ESBLs producing Enterobacteriaceae:

Out of 100 clinical isolates from hospitalized patients group (II) it was founded that the (50) Escherichia coliisolates produced the different ESBLs types as follow: (78%) produced TEM gene, (96%) produced CTX-M and (74%) produced TEM combined with CTX-M.

The (44) Klebsiella spp. isolates produced the different ESBLs types as follow: (82%) of klebsiella spp. Produced SHV gene, (70%) produced TEM gene, (89%) produced CTX-M, (9%) SHV combined with TEM, (23%) SHV combined with CTX-M, (48%) SHV combined with TEM and CTX-M and (14%) TEM with CTX-M.

The 6 Enterobacterspp. produced the different ESBLs types as follow: (33%) Produced SHV gene, (100%) produced TEM gene, (83%) produced CTX-M, (17%) SHV combined with TEM and CTX-M and (67%) TEM with CTX-M.

DISCUSSION

In order to investigate the molecular epidemiology of carriage with ESBL-producing Enterobacteriaceae, we systematically enrolled 100 hospitalized patients from different departments at kasralainy hospital and 60 non hospitalized patients from the outpatient clinics. Kasralainy Hospitals, Cairo University

We found that among non-hospitalized patients there is 68.5% were carriers of ESBL-producing E. coli, 30% were klebsiella and 1.5 % was enterobacter.

There is limited data on the carriage prevalence of community acquired ESBL-producing Enterobacteriaceae

from Africa The carrier prevalence in our study was higher than but comparable to a similar study in Cameroon that reported a prevalence of 23.1% in a study conducted on the outpatients.

In our study the prevalence was high in youngest age groups, also among the youngest category (15-30) 37.1% of index patients. This indicates that colonization with ESBL-producing bacteria often occurs early in life in this population.

We did not observe an increased risk of ESBL carriage among study participants with a history of health care contact orantibiotic usage.

The vast majority of our ESBL producing E. coli 95% have the blaCTX-M gene, and that the dominating gene group was the same in other studies that were performed in fecal carriage studies from Madagascar, Niger, Cameroon, Tanzania and South Africa [15,16,17], highlighting the importance of these genes in the ongoing global dissemination of ESBLs (18).

Multidrug-resistance (MDR) was very common, out of the 100 ESBL-producing isolates in hospitalized patients it was 87% while in the non hospitalized it was 60% out of 60 ESBL isolates and it is being classified as MDR according to the definition proposed by Magiorakos et al (19)

Quinolone resistance in hospitalized patients was high in ESBL-producing E. coli (84%) and K.pneumoniae (89%) isolates and Enterobacterspp (84%). This can be compared to reports from other West African settings with 52–67% resistance prevalence reported from Accra, Ghana (20).

In our study in non-hospitalized patients with E.coli ESBL- producing isolates, it was found that there were co-resistant to (73%) ciprofloxacin, (2%)carpabenem and (96%)trimethoprim-sulfamethoxazole.

This is alarming since apart from cephalosporins these drugs are the only easily available antibiotic agents to treat infections with gram negative bacteria in Egypt.

A recent issue that concerns the extended-spectrum beta lactamases (ESBL) is the global dissemination of the CTX-M beta-lactamases since the mid 1990s, (21). Unlike the TEM and SHV variants, CTX-M-producing isolates are increasingly recovered from patients with community-onset infections, especially among urinary Escherichia coli and from populations with minimal or absent healthcare risks. CTX-M-15 was the most prevalent ESBL in ESBL-Ec (58%) and ESBL-Kp (70%) in the index patients (22).

In conclusion ESBLs occur at an alarming rate among Enterobacteriaceae isolates from the study site which can result in outbreak of nosocomial infections that may be difficult to treat. The most affected age was 15-30 years; this may be due to the panting of this age group on fast food, which may be the reason in the transmission and spread of infection by these microbes. blaCTX-M was the most prevalent ESBL gene followed by blaTEM and finally, blaSHV, where the blaOXA gene was not detected in any of the isolated bacteria.

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