Detection of Extended Spectrum Beta-Lactamases in Escherichia Coli Isolated from the Community in Gaza Strip, Palestine

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الملخص

تهدف هذه الدراسة إلى كشف مدى إمكانية بكتيريا الاشيريشيا كولاي لإفراز إنـزيم بيتالاكتاميز الواسع المدى. ولقد تم تحديد نسبة إفراز هذا الإنزيم بين (300) ميكروباً من الاشيريشيا كولاي. ولقد بينت النتائج أن (11) ميكروباً (3.7%) من العدد الكلي يفرز هذا الإنزيم. وأظهرت معظم الميكروبات التي تفرز هذا الإنزيم مقاومة عالية للأموكسيسلين والسيفالكسين والسفيروكسيم والسيفوتكسيم حيث وصلت نسبة مقاومتهم إلـى (100.0%) والجنتاميسين (81.8%). ومن الواضح بعد تحليل البيانات أن كل الميكروبات المفـرزة لإنزيم بيتالاكتاميز الواسع المدى كانت مقاومة لأربعة مضادات حيوية أو أكثر. كمـا أن الغالبية (36.4%) كانت مقاومة لتسعة من المضادات الحيويـة. وقـد تبـين بالتحليـل الإحصائي وجود علاقة معنوية قوية بين الميكروبات التي تفرز هذا الإنزيم وبين كلٍ من الاستخدام السابق للمضادات الحيوية ودخول المستشفى والالتهابـات الـسابقة للمـسالك البولية.

Abstract

This study was designed in order to detect the production of extended spectrum beta-lactamase (ESBL) in the isolated *Escherichia coli*. The prevalence of ESBL production was determined among 300 isolates of *E. coli.* Eleven (3.7%) isolates proved to be ESBL. High resistance especially, to amoxycillin, cephalexin, cefuroxime, cefotaxime (100.0%) and gentamicin (81.8%) was observed. It was also observed that, all ESBL-producers displayed multiple resistance to four or more antimicrobial agents and the majority of the

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isolates (36.4%) were resistant to nine agents. Moreover, a highly significant association has been found between infection with ESBL-producers and the patients' prior antimicrobials use, previous hospitalization and preceding UTI.

Introduction:

Extended-spectrum beta-lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum cephalosporins and monobactams. These enzymes are produced mainly by *Klebsiella pneumoniae* and *Escherichia coli* (Yates, 1999 and Luzzaro *et al.*, 2001). Although, they have been detected in other organisms including *Salmonella* species, *Pseudomonas aeruginosa* and other enterobacteriaceae (Kaye *et al.*, 2000).

The first bacterial isolate resistant to the so-called extended-spectrum cephalosporins was identified in Germany in 1983 (Stobberingh *et al.*, 1999). And soon after, in several European countries such as France, Italy, Greece and Austria. ESBLs have also been detected in the USA and in Western Australia (Stobberingh *et al.*, 1999). Subsequently, much of the dramatic increase in bacterial resistance to beta-lactam antibiotics has been associated with the spread of ESBLs worldwide (Canadian External Quality Assessment, 1998).

It is known that ESBL producing strains can survive in the hospital environment (Hobson *et al.*, 1996). Outbreaks due to the dissemination of various ESBL-producing enterobacteriaceae species in hospitals and other health care facilities has been reported (Cormican *et al.*, 1996).

Boundaries between community and hospital environments are, however, becoming more blurred and this trend will continue due to the shift towards shorter hospital stays, the provision of more treatment at home (even of patients with severe and complicated illnesses) and the use of more short stay surgical interventions. This may have consequences for the development of resistance to antimicrobial drugs and spread of resistant strains in the community (Goossens & Sprenger, 1998).

Overcrowding, the increasing number of immunocompromized patients, increased travelling, the widespread use of broad-spectrum antibiotics, the sale of antibiotics over the counter and the inappropriate use of antibiotics are other contributing factors to the emergence of bacterial resistance in the community.

The aim of this study was to detect the production of ESBL in *E. coli* isolated from the community in the Gaza Strip, evaluate the susceptibility of ESBL-producers to various antimicrobial agents and to investigate some of the risk factors contributing to infection with ESBL strains.

Materials and Methods:

Data were collected via patient interviews, or the patient's family and analysis of 300 *E. coli* isolated from outpatient females with clinical evidence of community-acquired urinary tract infections (UTI) during the year 2001 in the Gaza Strip. Identification of organisms was based on colonial morphology, Gram stain reaction and biochemical characteristics (Burnett *et al.*, 1994 and Vandepitte *et al.*, 1996). For confirmation of *E. coli* strains, a commercial identification biochemical system (API 20 E bioMerieux) was used (Baron *et al.*, 1994).

E. coli isolates were then stored in Mueller Hinton broth supplemented with 15% glycerol at –85 °C until further analysis.

The susceptibility of the 300 isolates to twelve antimicrobial agents was determined by the Kirby-Bauer disk diffusion technique on Mueller Hinton agar plates according to the NCCLS recommendations (NCCLS, 2001).

The disks and concentrations of the twelve antimicrobial agents that are commonly used for the treatment of UTIs in our region were as follows: amoxycillin (25 μg), amoxycillin-clavulanic acid (30 μg), cephalexin (30 μg), cefuroxime (30 μg), cefotaxime (30 μg), cotrimoxazole (1.25-23.75 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), tetracycline (30 UI), amikacin (30 μg), gentamicin (30 μg) and nitrofurantoin (300 μg).

Resistance to cefpodoxime disk (10 μg) has been studied as a screening method for ESBL production by disk diffusion technique, using sensitivity break point of $\leq$ 21 mm (Kaye *et al.*, 2000).

The Epsilometer test (E-test) method was used for confirmation of ESBL production (AB Biodisk, Solna, Sweden). The strips were used according to the manufacturer's instructions. Briefly, an overnight culture of the microorganism diluted to a turbidity equal to that of 0.5 McFarland units was swabbed on Mueller Hinton agar plates. After drying for 15 min, the E-test strips were applied on the plates and incubated at 37°C for 18 to 24 hours. The MICs on both ends were read on the intersection of the inhibition ellipse and the E-strip edge. A ratio of MIC ceftazidime, ceftazidime-clavulanic acid and MIC cefotaxime, cefotaxime-clavulanic acid equal to 8 or greater was considered to be ESBL-positive (Stobberingh *et al.*, 1999).

The E-test was also used for determining the MIC values of the antimicrobial agents used in this study.

The quality of performance was controlled once weekly, using the reference *strains E. coli* ATCC 25 922 and a beta-lactamase producing *E.*

coli ATCC 35 218 to control results of disks that contain combination of beta-lactam antibiotics and a beta-lactamase inhibitor.

The Chi-square test was used for the statistical analysis of the data and P-values of ≤ 0.05 were considered significant.

Results:

The data analysis of the 300 *E. coli* isolates revealed that, a total of 48 (16.0%) of the isolates were sensitive to all the antimicrobial agents tested. The remaining 252 (84.0%) of the isolates showed different resistance patterns.

Putative ESBL-production was observed in 11 (3.7%) of the 300 *E. coli* isolates. All putative ESBL-producers were found to be resistant to cefpodoxime according to the disk diffusion test.

By calculating the ratio of MIC ceftazidime, ceftazidime-clavulanic acid and cefotaxime, cefotaxime-clavulanic acid, the results confirmed that the 11 isolates have ratios greater than 8 and were thus considered ESBL-producers (Table 1).

Table (2) represents the percentage of resistance of ESBL-producers to the twelve antimicrobials tested. Most of the isolates showed high resistance especially, to amoxycillin (100.0%), cephalosporins (100.0%) and gentamicin (81.8%). All the isolates, however, were completely sensitive to amoxycillin-clavulanic acid and nitrofurantoin.

Resistance of ESBL-positive isolates have been confirmed by the E-test. Table (3) demonstrates the distribution of the MICs resistance ranges for the different agents.

The MICs of amoxycillin as well as of cotrimoxazole and tetracycline were high and all reached the resistance breakpoints of 256, 32 and 256 µg/ml, respectively.

The MICs of nalidixic acid were clustered around the breakpoint of 256 µg/ml with a mean of 237.7µg/ml.

On the other hand, gentamicin resistant ESBL-producers showed MICs that ranged from 8 to 256 µg/ml with a mean of 38.2 µg/ml.

The MICs of cephalexin and cefuroxime were in the range of 32-256 µg/ml with a mean of 197.8 and 189.1µg/ml, respectively.

It is evident from the data that, all ESBL-producers were resistant to four or more antimicrobial agents and that the majority of the isolates (36.4%) were resistant to nine agents.

The 11 ESBL-producing isolates were analyzed with respect to the patients' prior antimicrobials use, previous hospitalization and preceding UTI. Table (4), shows that, all the ESBL-producers were isolated from patients who had used antimicrobials. In addition, 10 (90.9%) of the isolates were collected from patients with previous hospitalization. Whereas, 9 (81.8%) of the ESBL producers were isolated from those with previous UTI.

Chi-square analysis indicated that the association between the ESBL-producers and all the studied patients' characteristics is highly significant ($P<0.001$).

Discussion:

The results of this study confirmed that, out of 300 E. coli isolates, 11(3.7%) of the isolates were ESBL-producers. Lower ratios have been reported by many investigators, worldwide. For instance, Stobberingh *et al.* (1999) and De Champs *et al*.(2000) noted that, ESBL-producing E.coli ranged from 0.1 to 1.5%.However, other investigators have reported higher ratios. For example, in Greece, Vatopoulos *et al*.(1990),in Korea, Pai *et al.* (1999),in New York, Saurina *et al*.(2000) and in some USA hospitals Kaye *et al*.(2000) indicated that, ESBL-producing isolates ranged from 4.0 to 40.0%.

Similarities and differences in data concerning antimicrobial resistance may be due to the fact that they may have been collected on different periods. Also, the investigated target populations may have various sociodemographical, socioeconomical, socioepidemiological and clinical parameters.

Table (2) represents resistance rate of ESBL-producers to twelve antimicrobials. Most of the isolates showed co-resistance to non beta-lactam antimicrobials, especially to cotrimoxazole, gentamicin, nalidixic acid and ciprofloxacin. They, however, were completely sensitive to amoxycillin-clavulanic acid and nitrofurantoin. In this regard, we can conclude that, for E. coli isolates, amoxycillin-clavulanic acid, nitrofurantoin and amikacin remain effective treatment options.

This study showed that, all ESBL-producing isolates were resistant to four or more antimicrobial agents and the majority of them (36.4%) were resistant to nine agents.

The considerably high MIC values of amoxycillin, cotrimoxazole, tetracycline, nalidixic acid, cephalexin and cefuroxime (Table-3) reflect the extent of treatment problem for ESBL-producing isolates.

Jett *et al*. (1995), observed that, 65% of ESBL-producing isolates were resistant to non beta-lactam antimicrobials (cotrimoxazole,

ciprofloxacin or gentamicin). In another finding by Livermore & Yuan (1996), an association between ESBL production and aminoglycosides resistance has been reported where, four out of six ESBL-positive strains were resistant to most aminoglycosides.

Table (4) summarizes the relation between the ESBL-positive isolates and patients' prior antimicrobials use, previous hospitalization and preceding UTI. A highly statistical significance was found between the ESBL-producers and all studied patients' characteristics ($P<0.001$).

Similar finding was reported by Kim *et al.* (2002) who have shown that, the risk factors for infection with ESBL-producing organisms were prior hospitalization, prior use of oxyimino-cephalosporins and admission to an intensive care units.

This study clearly shows that, infection with these highly resistant isolates, which is usually confined to hospitals, is now being acquired in the community. Thus, we recommend continuos surveillance for ESBL-producing isolates in both hospitals and community in order to develop programs for controlling the spread of these isolates which will increase the problem of community acquired UTIs treatment.

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Table (1): The MICs ratio of the ESBL-producing isolates.

Isolate Serial Number	MIC ratio for ESBL confirmation agents					
	CT	CTL	CT/CTL	TZ	TZL	TZ/TZL
93	> 16	0.047	340.4	> 32	0.19	168.4
111	> 16	0.25	64.0	> 32	0.5	64.0
113	> 16	0.032	500.0	3.0	0. 094	31.9
117	16	0.25	64.0	> 32	0.5	64.0
149	> 16	0.032	500.0	16.0	0. 125	128
185	> 16	0.064	250.0	1.0	0. 094	10.6
242	> 16	0.032	500.0	1.5	0. 094	15.9
280	> 16	0.19	84.2	2.0	0.19	10.5
281	> 16	0.38	42.1	> 32	0.38	84.2
285	> 16	0.047	340.4	> 32	0.19	168.4
288	> 16	0.25	64.0	8.0	0.5	16.0

CT, cefotaxime; CTL, cefotaxime-clavulanic acid; TZ, ceftazidime; TZL, ceftazidime-clavulanic acid; CT/CTL; cefotaxime to cefotaxime-clavulanic acid ratio; TZ/TZL, ceftazidime to ceftazidime-clavulanic acid ratio.

Table (2): Susceptibility of ESBL-producers to the selected antimicrobials.

Antimicrobial agent	Sensitive % (n)*	Resistant % (n)
AMX	0.0 (0)	100.0 (11)
CF	0.0 (0)	100.0 (11)
CMX	0.0 (0)	100.0 (11)
CXT	0.0 (0)	100.0 (11)
SXT	18.2 (2)	81.8 (9)
GM	18.2 (2)	81.8 (9)
NA	36.4 (4)	63.6 (7)
CIP	45.5 (5)	54.5 (6)
TE	54.5 (6)	45.5 (5)
AN	90.9 (10)	9.1 (1)
ACM	100.0 (11)	0.0 (0)
NTFN	100.0 (11)	0.0 (0)

*No.=number of isolates

AMX, Amoxycillin; SXT, Cotrimoxazole; TE, Tetracycline; NA, Nalidixic acid; CIP, Ciprofloxacin; GM, Gentamicin; CF, Cephalexin; CMX, Cefuroxime; CXT, Cefotaxime; ACM, Amoxycillin-clavulanic acid; NTFN, Nitrofurantoin; AN, Amikacin.

Table (3): The MIC values for drug resistant ESBL-producing isolates

Isolate number	MIC (µg/ml) for antimicrobials resistant isolates											
	AMX	SXT	TE	NA	CIP	GM	CF	CMX	CXT	ACM	NTFN	AN
	32-256	8-32	16-256	32-256	4-32	8-256	32-256	32-256	8-32	32-256	128-512	32-256
93	256	32	256	256	32	8	256	256	32	S	S	S
111	256	32	S	256	4	48	48	32	16	S	S	S
113	256	32	256	128	S	64	256	256	32	S	S	48
117	256	32	S	256	4	48	48	32	16	S	S	S
149	256	32	256	256	32	16	256	256	32	S	S	S
185	256	32	256	256	32	S	64	256	24	S	S	S
242	256	32	S	S	S	64	128	256	32	S	S	S
280	256	S	S	S	S	16	256	256	32	S	S	S
281	256	32	256	256	32	32	256	256	32	S	S	S
285	256	S	S	S	S	S	256	64	32	S	S	S
288	256	32	S	S	S	48	256	256	32	S	S	S
Mean MIC (µg/ml)	256	32	256	237.7	22.6	38.2	189.1	197.8	28.4	S	S	48

S=Sensitive (less than the lower value for each of the resistant ranges)

Table (4): The relation between ESBL-producers and patients' characteristics among resistant isolates.

Variable	ESBL-producer % (n)*	Non ESBL-producer % (n)
Prior antimicrobial use**		
Yes	100.0 (11)	83.8 (202)
No	0.0 (0)	16.2 (39)
Total	100.0 (11)	100.0 (241)
Previous hospitalization**		
Yes	90.9 (10)	41.1 (99)
No	9.1 (1)	58.9 (142)
Total	100.0 (11)	100.0 (241)
Preceding UTI**		
Yes	81.8 (9)	32.4 (78)
No	18.2 (2)	67.6 (163)
Total	100.0 (11)	100.0 (241)

*(n)=number of isolates

**=Significant at P-value of $\leq$0.05