Extended spectrum beta-lactamases in *Eschericia coli* isolated from community-acquired urinary tract infections in the Gaza Strip, Palestine

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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*,[1,2] although they have been detected in other organisms, including Salmonella species, Pseudomonas aeruginosa and other Enterobacteriaceae.[3]

ESBL-producing strains can survive in the hospital environment.[4] Outbreaks due to the dissemination of various ESBL-producing Enterobacteriaceae species in hospitals and other health care facilities have been reported. Boundaries between community and hospital environments are becoming more blurred, however, 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 the spread of resistant strains in the community.[6]

The aim of this prospective study was to detect the production of ESBL in *E. coli* isolated from the community in the Gaza Strip, and to evaluate the susceptibility of ESBL-producers to various antimicrobial agents.

Materials and Methods

Isolates were collected from outpatient adult females with clinical evidence of community-acquired urinary tract infections (UTI) during the period January to June 2001, in the Gaza Strip. UTIs were defined as the culture of a single organism from a midstream urine specimen at 10^5 colony forming units per milliliter.[7] Only one specimen per patient was processed. Identification and confirmation of *E. coli* isolates were done according to standard procedures.[7-9]

The susceptibility of the isolates to twelve antimicrobial agents was determined by the Kirby-Bauer disk diffusion technique according to the NCCLS recommendations.[10] 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 IU), amikacin (30 µg), gentamicin (30 µg) and nitrofurantoin (300 µg). We measured resistance to cefpodoxime (10 µg) by disk diffusion, which has been studied as a screening method for ESBL production using a sensitivity break point of 21 mm.[3]

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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. A ratio of 8 or greater for the ceftazidime to ceftazidime-clavulanic acid minimal inhibitory concentrations (MICs) and the cefotaxime to cefotaxime-clavulanic acid MICs was considered ESBL-positive.[11] The E-test was also used for determining the MIC values of the antimicrobial agents used in this study.

The quality control check was performed once weekly, using the reference strains E. coli ATCC 25 922 and a beta-lactamase-producing E. coli ATCC 35 218 to check the results of disks that contain a combination of beta-lactam antibiotics and a beta-lactamase inhibitor.

Results

Three hundred *E. coli* isolates were collected from outpatient females with a mean age of 41.5 years (range 18 to 65 years). Forty-eight (16.0%) of the 300 E. coli isolates were sensitive to all the antimicrobial agents tested. The remaining 252 (84.0%) isolates showed different resistance patterns. Putative ESBL-production was observed in 11 (3.3%) of the 300 E. coli isolates. All putative ESBL-producers were found to be resistant to cefpodoxime according to the disk diffusion test.

The ratio of MICs for ceftazidime/ ceftazidime-clavulanic acid and cefotaxime/cefotaxime-clavulanic acid was greater than 8 for 11 isolates, which were considered ESBL-producers (Table 1). Most of the isolates showed high resistance, especially to amoxycillin (11/11), cephalosporins (11/11) and gentamicin (9/11) (Table 2). All the isolates, however, were completely sensitive to amoxycillin-clavulanic acid and nitrofurantoin. Resistance of ESBL-positive isolates was confirmed by the E-test (data not shown). It was evident from the data that all ESBL-producers were resistant to four or more antimicrobial

Table 1. Minimum inhibitory concentration (MIC) and MIC ratio of ESBL-producing *E.coli* isolates

Isolate serial number	CTX	CTX-CLA	CTX/CTX-CLA ratio	CAZ	CAZ-CLA	CAZ/CAZ-CLA ratio
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

ESBL- extended-spectrum beta-lactamase, CTX- cefotaxime, CTX-CLA- cefotaxime-clavulanic acid, CAZ- ceftazidime, CAZ-CLA- ceftazidime-clavulanic acid

Table 2. Antimicrobial resistance of ESBL-producing pathogens.

	Number of resistant isolates (%)
Amoxycillin	11 (100)
Cephalexin	11 (100)
Cefuroxime	11 (100)
Cefotaxime	11 (100)
Cotrimoxazole	9 (81.8)
Gentamicin	9 (81.8)
Nalidixic acid	7 (63.6)
Ciprofloxacin	6 (54.5)
Tetracycline	5 (45.5)
Amikacin	1 (9.1)
Amoxycillin-clavulanate	0
Nitrofurantoin	0

ESBL - extended-spectrum beta-lactamase

agents and that 4 of the 11 isolates (36.4%) were resistant to nine agents.

Discussion

In our study, 11 (3.3%) of 300 E. coli isolates were ESBL-producers. Greater and fewer percentages of ESBL production have been reported worldwide by other investigators.[3,11-15] Similarities and differences on antimicrobial resistance may be due to different periods of data collection. Also, the populations investigated may differ in various socio-demographical, socioeconomical, socio-epidemiological, and clinical parameters.

Most of the isolates showed co-resistance to non-beta-lactam antimicrobials, especially to cotrimoxazole, gentamicin, nalidixic acid and ciprofloxacin. However, they were completely sensitive to amoxycillin-clavulanic acid and nitrofurantoin. Therefore, we conclude that amoxycillin-clavulanic acid, nitrofurantoin and amikacin remain effective treatment options for E. coli.

This study showed that all ESBL-producing isolates were resistant to four or more antimicrobial agents and that 4 of them (36.4%) were resistant to nine agents. The considerably high MIC values of amoxycillin, cotrimoxazole, tetracycline, nalidixic acid, cephalexin and cefuroxime observed in this study reflect the extent of the treatment problem for ESBL-producing isolates. Jett et al.[16] observed that 65% of ESBL-producing isolates were resistant to non-beta-lactam antimicrobials (cotrimoxazole, ciprofloxacin or gentamicin). In another finding, Livermore and Yuan [17] found that 4 of their 6 ESBL-positive isolates were resistant to most aminoglycosides.

Our study clearly shows that infection with these highly resistant isolates, which is usually confined to the hospital, is now being acquired in the community. Thus, we recommend continuous surveillance for ESBL-producing isolates in both hospitals and the community. This will be necessary for controlling the spread of these isolates, which complicate the treatment of community acquired UTIs.

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