Faecal carriage of extended-spectrum β-lactamase-producing bacteria in the community

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حَمْل الجراثيم المنتجة لبيتا لاكتاماز الواسعة الطيف في البراز في المجتمع

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الخلاصة: حدد الباحثان حَمْل الجراثيم المنتجة لإنزيم بيتا لاكتاماز الواسعة الطيف في البراز في المجتمع في المملكة العربية السعودية. وقد أجريا تحريات على 716 عينة برازية (جمعاها من 505 أشخاص أصحاء و211 مريضاً ممّن يراجعون العيادات الخارجية في المجتمع)، بحثاً عن الجراثيم المنتجة لإنزيم بيتا لاكتاماز الواسعة الطيف، واستخدم الباحثون اختبار التآزر المزدوج على القرص، والتأكيد بطريقة القرص الإضافي لمعهد المعايير السريرية والمختبرية. ومن بين مجمل العينات وجد أن 91 مستفردة (12.7٪) منتجة لإنزيم بيتا لاكتاماز، ومن بين هؤلاء كان 87 مستفردة (95.6٪) من الإيشريكيات القولونية، و4 مستفردات (4.4٪) من الكلبسيلات الرئوية. كما تبين معدل مماثل لحَمْل الجراثيم المنتجة لإنزيم بيتا لاكتاماز الواسعة الطيف في البراز لدى 29 من المرضى الخارجيين في المجتمع (13.7٪)، ولدى 62 من الأشخاص الأصحاء (12.3٪). وقد استنتج الباحثون أن بإمكان المجتمع أن يكون مستودعاً للجراثيم المنتجة لإنزيم بيتا لاكتاماز الواسعة الطيف وللإنزيمات.

ABSTRACT We determined the faecal carriage of extended-spectrum β-lactamase-(ESBL)-producing bacteria in the community in Saudi Arabia. A total of 716 faecal specimens (from 505 healthy individuals and 211 community outpatients) were screened for ESBL using the double-disk synergy test and confirmed by the Clinical Laboratory Standards Institute combined disk method. We found 91 (12.7%) isolates were ESBL-producers. Of these, 87 (95.6%) were *Escherichia coli* and 4 (4.4%) *Klebsiella pneumoniae*. A similar rate of faecal carriage of ESBL-producers was demonstrated in community outpatients and healthy individuals: 62 (12.3%) healthy persons and 29 (13.7%) outpatients. We conclude that the community could be a reservoir of these ESBL-producing bacteria and enzymes.

Portage fécal de bactéries productrices de ß-lactamase à spectre élargi dans la communauté
RÉSUMÉ Nous avons déterminé le portage fécal de bactéries productrices de ß-lactamase à spectre élargi (BLSE) dans les structures de proximité en Arabie saoudite. Au total, 716 échantillons fécaux (provenant de 505 sujets sains et de 211 malades ambulatoires traités dans la communauté) ont fait l'objet d'un dépistage du BLSE à l'aide du test de la synergie entre deux disques et les cas ont été confirmés par la méthode des disques combinés du *Clinical Laboratory Standards Institute*. Sur l'ensemble, 91 isolats (12,7 %) étaient producteurs de BLSE, 87 d'entre eux (95,6 %) étant des isolats d'*Escherichia coli* et 4 (4,4 %) de *Klebsiella pneumoniae*. Un taux analogue de portage fécal de bactéries productrices de BLSE a été mis en évidence chez les 62 sujets sains (12,3 %) et chez les 29 patients ambulatoires traités dans la communauté(13,7 %). Nous en concluons que les structures de proximité pourraient être un réservoir de bactéries productrices de BLSE et de ces enzymes.

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Introduction

Multidrug resistance is increasingly seen in many Gram-negative bacteria as a result of widespread use of various antibiotics [*1,2*]. Extended-spectrum β-lactamases (ESBLs) are enzymes that commonly mediate resistance to β-lactam antimicrobial drugs in Gram-negative bacteria [*3*] and are most commonly found in *Escherichia coli* and *Klebsiella pneumoniae* [*2*]. ESBLs have the ability to hydrolyse penicillins, 3rd generation cephalosporins and monobactams [*4*]. They are highly susceptible *in vitro* to β-lactamase inhibitors such as clavulanic acid, but not cephamycins or carbapenems [*2*]. ESBL enzymes are encoded by transferable conjugative plasmids, which often code resistance determinants to other classes of antimicrobial agents and are also responsible for the dissemination of resistance to other Gram-negative bacteria in the community and in hospitals [*5*].

Infection caused by ESBL-producing bacteria is an emerging problem in the community setting in many parts of the world [*6*]. Several reports have addressed faecal carriage of these organisms during nosocomial outbreaks [*4,7*]. Although carriers of ESBL producers are expected to be present in general practice, their occurrence has rarely been reported and there are few studies conducted in the community or in hospital settings during nonoutbreak situations [*8,9*]. Recent studies have shown a significant increase in ESBL producers among community bacterial isolates [*10,11*].

In our hospital laboratory we do not routinely screen faecal isolates of the family Enterobacteriaceae for ESBL production. However, as we have detected ESBL-producers in 12.7% of the urinary isolates of outpatients in our hospital [*12*] and as part of community surveillance, we conducted the current study to determine faecal carriage of ESBL-producing organisms in a community setting.

Methods

The study was performed from June 2006 to February 2007 at Almana General Hospital in the Eastern province of Saudi Arabia. A total of 716 faecal samples were studied: 505 from healthy individuals attending a routine pre-employment clinic and 211 from community outpatients (143 Saudi and 68 non-Saudi). The healthy persons were non-Saudis of different ethnic background from the Asian continent. Around 96% of the outpatients were recorded as suffering from acute gastroenteritis; for the remaining patients the stool request forms did not have clinical details about the presenting complaint.

Stool samples were spread onto 2 MacConkey agar plates, one supplemented with 1 µg/mL of cefotaxime and another with 1 µg/mL of ceftazidime, and incubated in ambient air at 35 °C for a minimum of 24 h before initial examination [*13*]. Plates demonstrating no growth in a primary examination were incubated for another 24 h. Growing organisms were identified by standard techniques using the API system (BioMérieux, France) [*14*].

Antimicrobial susceptibility was determined by the disk diffusion test, using Clinical and Laboratory Standards Institute (CLSI) guidelines [*13*]. Samples yielding bacteria that grew on MacConkey agar were initially identified as suspicious for ESBL. Isolates were screened for ESBL production by the double-disk synergy test in which an amoxicillin–clavulanate (AMC) (20 µg/10 µg) disk was placed in the centre with ceftazidime (CAZ) (30µg) and cefotaxime (CTX) (30µg) disks at a 15 mm distance

from AMC. Strains producing ESBL were defined as those showing synergism between AMC and any one of CTX and CAZ [*15*]. The standard CLSI combined disk method involving CAZ and CTX with and without the inhibitor clavulanic acid (30 µg) (Mast Diagnostics, Merseyside, UK) was used to confirm the presence of ESBL [*13*]. ESBL production was indicated by an increase in zone size of more than 5 mm with and without clavulanic acid.

Quality control was done using *K. pneumoniae* ATCC 700603 (positive control) and *E. coli* ATCC 25922) (negative control).

Results

Bacterial isolates other than *E. coli* and *K. pneumoniae*, such as *Pseudomonas* spp., which grew on the MacConkey agar, and resistant strains showing no synergy between CAZ or CTX and AMC, were disregarded.

Of the 716 stool samples tested, isolates that were resistant to CTX and/or CAZ were cultured from 106 (14.8%) and ESBL producers from 91 (12.7%) (Table 1). Of the 91 ESBL-producing isolates, 87 (95.6%) were *E. coli* and 4 (4.4%) *K. pneumoniae.*

ESBL-producing strains were found in 62 (12.3%) of the isolates from 505 healthy individuals and 29 (13.7%) of the 212 isolates from community patients had ESBL-producing strains (20 from adults and 9 from children). EBSL producers were found in 18 (12.6%) isolates from the 143 community patients who were Saudi nationals and 11 (16.2%) from the 68 non-Saudi nationals.

The susceptibility data of the ESBL-producing *E. coli* and *K. pneumoniae* are summarized in Table 2. The antibiotics with the highest activity against the ESBL-producing isolates were carbapenems (imipenem/meropenem) and amikacin.

Discussion

This study demonstrates the presence of ESBL in faecal strains of *E. coli* and *K. pneumoniae* from both community patients and healthy individuals. More than 95% of isolates with this mechanism were *E. coli,* which is the most common pathogen among the Enterobacteriaceae. Despite normally living harmlessly in the gut, *E. coli* can cause various types of infections, especially urinary tract infection. In a study published from our hospital, 10.2% of the uropathogens isolated from outpatients were ESBL-producing *E. coli* [*16*]. In another study we showed that > 12% of the Gram-negative uropathogens isolated from community patients were ESBL-producers [*12*]. Some reports from Europe and Canada also suggest that infections caused by ESBL-producing organisms are emerging among community patients [*17,18*].

The presence of ESBL-producing *E. coli* in the gut not only contributes to difficult-

Table 1 **Frequency of ceftazidime/cefotaxime resistance and extended-spectrum β-lactamase (ESBL) production in faecal isolates from outpatients and healthy persons**

Source of isolates	Ceftazidime/ cefotaxime resistant		ESBL positive		ESBL negative	
	No.	%	No.	%	No.	%
Outpatients (*n* = 211)	34	16.0	29	13.7	182	86.3
Healthy persons (*n* = 505)	72	14.3	62	12.3	443	87.7
Total (*n* = 716)	106	14.8	91	12.7	625	87.3

Table 2 **Antimicrobial susceptibility of the extended-spectrum β-lactamase-producing isolates of *Escherichia coli* and *Klebsiella pneumoniae***

Source	Cefepime		Ciprofloxacin		Gentamicin		Piperacillin/ tazobactam		Amikacin		Imipenem/ meropenem	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Outpatients (*n* = 29)	0	0.0	12	41.0	15	51.7	22	75.9	26	89.7	29	100.0
Healthy persons (*n* = 62)	3	4.8	23	37.0	37	59.7	60	96.8	62	100.0	62	100.0
Total (*n* = 91)	3	3.3	35	38.5	52	57.0	82	90.0	88	96.7	91	100.0

to-treat extraintestinal infections, but can also result in the transfer of antibiotic-resistance determinants to other strains of *E. coli* and other organisms within the gastrointestinal tract [*4*]. Their presence increases the risk of transmission to other individuals as a result of human-to-human transmission or through the environment [*19*]. In addition, the admission of carriers to hospitals increases the risk of infection for other hospitalized patients [*20*].

The spread of ESBL-producing organisms to the community could be related to previous hospital acquisition as some hospitalized patients continue to carry ESBL-producing bacteria over prolonged periods, which may contribute to their extrahospital propagation [*21*]. Their emergence in the community could also be caused by the overuse of antibiotics in community patients. Antibiotic use creates a selective pressure on host bacteria in the large bowel, leading to the emergence of antimicrobial-resistant organisms, which in turn causes an increase in the number of carriers harbouring resistant bacteria and enhances the opportunity for these bacteria to cause infections [*22*]. In our study, 13.7% of the faecal isolates from community patients were ESBL producers, and around 96% of these patients were suffering from acute gastroenteritis. The percentage of faecal ESBL producers among non-Saudi community patients (16.2%) was higher than that among Saudi nationals (12.6%). Although there was no history of any recent hospitalization or antibiotic consumption among the community patients, many are likely to have been exposed to multiple courses of antibiotics due to the unrestricted or over-the-counter availability of antibiotics in developing countries.

Several risk factors for colonization and infection with ESBL-producers outside hospitals have been identified, including prior treatment with β-lactams (e.g. penicillins, cephalosporins) and quinolones [*23*]. The widespread use of β-lactams in the community to treat infections could be associated with the selection of antibiotic resistance mechanisms in pathogenic and nonpathogenic isolates of *E. coli* [*20*]. Previous fluoroquinolone use has also been demonstrated to be a risk factor for the acquisition of ESBL-producing isolates [*10*]. In our study, the fluoroquinolone (ciprofloxacin) resistance rate among the ESBL-producing organisms was high (61.5%).

The rate of faecal carriers of ESBL producers among community patients (13.7%) was only slightly higher than among healthy individuals (12.3%). Community-acquired strains possessing ESBLs might be selected from the existing gastrointestinal flora, when they are exposed to broad-spectrum antimicrobial agents [*24*]. Although we could not find documentation confirming recent exposure of the healthy individuals

to antibiotics, the easy access to and unrestricted sale of antibiotics is likely to create a general pool of resistant organisms in the population. The existence of ESBL-producing organisms in the gut of healthy individuals has clinical implications as intestinal tract colonization is a prerequisite for infection by ESBL-producers [*25*].

Our findings demonstrate that even if laboratories do routine screening to detect ESBL-producing bacteria, the results are unlikely to represent the actual prevalence of ESBL producers in the community, and asymptomatic carriers may remain unnoticed for a long period of time.

Because of the significant public health implication, infectious disease physicians, microbiologists and community doctors or general practitioners need to be aware that ESBL-producing strains of bacteria are not only circulating in hospital environments but in the community as well, and they should deal with them accordingly. Confirmation of community-based transmission of ESBL requires further investigation, including molecular studies, to determine the reservoirs and vehicles for dissemination of ESBL within the community. Laboratory monitoring and detection of ESBL-producing bacteria are important steps in the appropriate treatment of patients and infection control efforts. It is also crucial in the tracking and monitoring of these resistant bacteria in community surveillance programmes.

Although asymptomatic Saudi individuals were not studied for faecal carriage of ESBL producers, the data from Saudi outpatients are likely to be close to that in asymptomatic people as *E. coli* and *K. pneumoniae* are part of the normal intestinal flora. Further study is required to verify this.

Finally, the scope of this study was limited to identifying ESBL producers by standard phenotypic methods.

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