Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

High prevalence of bla_{CTX-M} in *Enterobacteriaceae* isolates from the Kingdom of Bahrain

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ARTICLE INFO

Article history:
Received 16 June 2011
Received in revised form 10 August 2011
Accepted 15 October 2011
Available online 20 December 2011

Keywords:
ESBL
CTX-M
Bahrain
Escherichia coli
Klebsiella pneumoniae

ABSTRACT

Objective: To determine the molecular epidemiology of extended-spectrum β-lactamase (ESBL) by testing a cohort of clinical ESBL-producing bacterial isolates that were isolated in the Kingdom of Bahrain. **Methods:** ESBL producing *Enterobacteriaceae* isolates (based on phenotypic tests) were collected from Microbiology Laboratory of the Salmaniya Medical Complex, Bahrain between January-June 2006. Antibiotic susceptibility to a panel of antibiotics was performed and bla_{CTX-M} genes were detected by multiplex PCR. **Results:** A total of 230 isolates (*Escherichia coli*, *n*=180; *Klebsiella pneumoniae*, *n*=50) were studied, 98% were CTX-M type. For *Escherichia coli* isolates, 65 (36.1%) harbored CTXM+TEM combination and 68 (37.8%) had CTX-M alone. In contrast, for *Klebsiella pneumoniae* isolates only 5 (10.0%) harbored the CTX-M combination, and none had CTX-M only. The bla_{CTX-M} gene was found predominantly in urine isolates (*n*=145/230; 63.0%). Sensitivity to imipenem and nitrofurantoin was 100% and 60%, respectively. CTX-M carriage was associated with the resistance to fluoroquinolones, trimethoprim-sulfamethoxazole and aminoglycosides. **Conclusions:** Our study documentes high prevalence of CTX-M ESBL type among *Escherichia coli* and *Klebsiella* from the Kingdom of Bahrain. The apparent dissemination of CTX-M producers could represent a substantial barrier in the treatment of community-acquired infections. The use of extended-spectrum cephalosporins, quinolones, and aminoglycosides is compromised, leaving carbapenems as the therapeutic option for severe infections caused by ESBL producers.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are β-lactamases capable of conferring bacterial resistance to the pencillins, first-, second-, and third-generation cephalosporins and aztreonam, but not to the cephamycins or carbapenems as demonstrated by hydrolysis of these antibiotics. ESBLs have been described in range of *Enterobacteriaceae* and *Pseudomonadaceae*, but they are most often identified in *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*)[1-3].

Many genera of gram-negative bacteria possess naturally occurring, chromosomally mediated β-lactamase. The first plasmid-mediated β-lactamase in gram negative bacteria, TEM-1, was described in the early 1960s. The TEM-1 enzyme was found in a single strain of *E. coli* that was isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM[4]. Being plasmid and transposon mediated has facilitated the spread of TEM-1 to other species of bacteria. Currently, TEM-1 is the most frequently encountered β-lactamase in Gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1. TEM-1 is also responsible for the ampicillin and penicillin resistance that is seen in *Haemophilus influenzae* and *Neisseria gonorrhea*. TEM-1 is able to hydrolyze penicillins and early cephalosporins, such as cephalothin and cephaloridine. TEM-2, the first derivative of TEM-1, had a single amino

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acid substitution yet, it did not change the substrate profile. TEM-3, first reported in 1989, was the first TEM-type β-lactamase that displayed ESBL phenotype. Now, over 120 TEM-type β-lactamases have been described of which the majority is ESBLs[4,5].

Another common plasmid-mediated β-lactamase found in *K. pneumoniae* and *E. coli* is SHV-1 (sulphydryl variable). The SHV-1 is most commonly found in *K. pneumoniae*. Unlike the TEM-type β-lactamases, there are few derivatives of SHV-1. Currently, the majority of SHV-type derivatives possess the ESBL phenotype.

In the recent years, a new family of plasmid-mediated ESBLs called CTX-M which preferentially hydrolyze cefotaxime, has emerged. CTX-M enzymes have mainly been found in strains of *Salmonella enterica* serovar Typhimurium and *E. coli*, but have also been described in other species of *Enterobacteriaceae*. CTX-M enzymes are not closely related to TEM or SHV β-lactamases, as they only show approximately 40% similarity in sequence[4,5].

According to a recent study, the prevalence of ESBL producing *Enterobacteriaceae* is 22.6% in the Kingdom of Bahrain[6]. Furthermore, the majority of ESBL isolates from inpatients (87.7%) comprised of *E. coli* (52.2%) and *Klebsiella pneumoniae* (24.3%) are predominant and distributed comparatively in the hospital wards, while *Proteus* spp (17.6%) is predominant in medical wards[6]. To date, no data on this type of circulating ESBL is available at the regional level.

This study aims to describe the molecular epidemiology of ESBL by testing a sample of clinical isolates of ESBL-producers that were isolated in the Kingdom of Bahrain.

2. Materials and methods

2.1. Clinical isolates

This study was carried out at Salmaniya Medical Centre, which is a 1 000-bed tertiary hospital and receiving samples from primary health care centers. Over a six month period, from 1st January to 30th June 2006, 230 consecutive isolates were collected. Fifty *K. pneumoniae* and 180 *E. coli* isolates were phenotypically identified as ESBL producers by the double disc diffusion. The isolates were mainly from urine, blood, respiratory, or wound cultures. The quality control strains used for this study included *E. coli* ATCC 51446 and *K. pneumoniae* ATCC 700603 as positive controls. *E. coli* ATCC 25922 was used as negative control.

2.2. Determination of ESBL genotype

The detection of bla_{TEM}, bla_{SHV}, bla_{CTX-M} was performed with multiplex PCR using three set of specific primers (Thermo Fisher Scientific, Germany) as previously described[7].

A single reaction mixture (total volume = 50 μL) contained 25 μL of 2× ready PCR master mix (Qiagen, USA), 0.2 μM of each primer, and 5 μL crude plasmid DNA extract were used in all PCRs. A Perkin-Elmer 9600 apparatus (Perkin-Elmer, USA) was used and the reactions were run under the following conditions: 95 ℃ for two minutes followed by thirty cycles of 95 ℃ for 1 minute, 55 ℃ for 1 minute and 72 ℃ for 1 minute, with a final extension at 72 ℃ for 10 minutes. The resulting PCR products were run in 1.5% agarose gels (Sigma Chemical Co. USA). Deoxyribonucleic acid (DNA) fragments were separated by horizontal electrophoresis cell (Bio-Rad, USA) at 80 volts/cm for 60 minutes, gels were stained with 0.5 μg/mL ethidium bromide (Sigma chemical co., USA) and photographed under UV insight transilluminator (Bio-Rad, USA).

2.3. Antibiotic susceptibility testing

The antibiotic susceptibility pattern of ESBL producing isolates to a panel of antibiotics including amikacin, ciprofloxacin, imipenem, nitrofurantoin, amoxicillin sulbactam, pperacillin-tzobactam was recorded. The antimicrobial susceptibility testing was performed using disc diffusion method in accordance with CLSI interpretation standard.

2.4. Statistical analysis

Chi-squared test was performed on the Sigma Stat ver 3.5 software (Systat Software Inc, San Jose California, USA). $P<0.05$ was considered as statistically significant.

3. Results

3.1. Types of ESBLs

CTX-M was the most common ESBL in the ESBL producing *E. coli* isolates, as 170 of the 180 (94.4 isolates harbored CTX-M gene either alone or in combination with other ESBL gene. SHV was least common in ESBL producing *E. coli* isolates, as only 40 of the 180 isolates had SHV gene. TEM was found in 100 of the 180 ESBL producing *E. coli* isolates.

For the ESBL producing *K. pneumoniae* isolates, CTX-M was detected in 45 (90.0 isolates, SHV was detected in 40 of the 50 (80 isolates, and TEM was also detected in 40 of the 50 isolates indicating that the three types of ESBLs were equally prevalent in our *K. pneumoniae* isolates.

3.2. Analysis of isolates that harbored more than one ESBL gene

The multiplex PCR results showed that of the 180 ESBL-producing *E. coli* isolates 30 harbored CTX-M+TEM+SHV,

65 CTX-M+TEM, 10 CTX-M+SHV, 5 TEM alone, 68 CTX-M alone, and five isolates were negative. For the 50 ESBL-producing *K. pneumoniae* isolates, 35 harbored CTX-M+TEM+SHV, 5 CTX-M+TEM, 5 CTX-M+SHV, and two isolates were nontypeable. None of the *K. pneumoniae* isolates carried CTX-M alone or TEM alone. Figure 1 shows gel electrophoresis image of multiplex PCR products of bla_{CTX-M} genes.

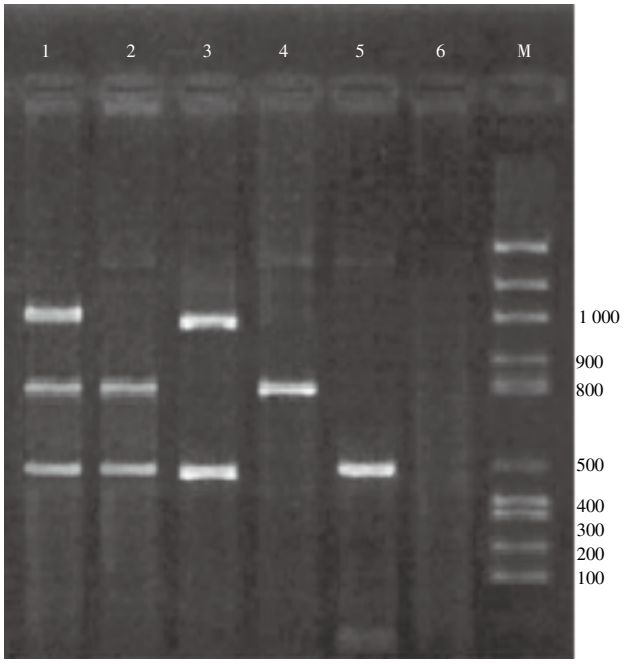

Figure 1. Gel electrophoresis image showing the multiplex PCR products of bla_{CTX-M} .

Lane 1: PCR products of CTX-M, TEM and SHV (with the sizes of 550 bp, 840 bp and 1 040 bp respectively); Lane 2: PCR products of CTX-M and TEM; Lane 3: PCR products of CTX-M and SHV; Lane 4: PCR product of TEM only; Lane 5: PCR product of CTX-M only; Lane 6: Negative control; 100 bp DNA molecular marker.

3.3. Source of infection and antibiotics sensitivity

The majority of isolates (157/230; 68.3%) were obtained from inpatients. The presence of bla_{CTX-M} alone was significantly higher in isolates obtained from outpatient (n=52) as compared to those from inpatient (n=16) (P<0.05) (Table 1). However, the presence of CTX-M gene and/or the combination with other ESBL genotype was found predominantly in isolates obtained from urinary specimen (n=154/230; 67.0%). The distribution of the ESBL genotypes according to specimen type is shown in Table 2.

There was a high level of sensitivity to imipenem (100.0%) and amikacin (>95.0%) in all isolates. In contrast, the level of resistance to ciprofloxacin, trimethoprim and nitrofurantoin was 98.5%, 93.8% and 78.0%, respectively.

Table 1
Distribution of ESBL types among isolates from inpatients and outpatients.

ESBL types	Number of patients	
	Inpatient	Outpatient
CTX-M + TEM + SHV	61	4
CTX-M + TEM	58	12
CTX-M + SHV	10	5
CTX-M only	16	52
TEM only	5	0
Negative	7	0
Total	157	73

Table 2
Distribution of ESBL type isolates in different specimens.

ESBL types	Clinical specimens			
	Urine	Blood	Wound	Respiratory
CTX-M+TEM+SHV	35	2	8	20
CTX-M+TEM	65	0	0	5
CTX-M+SHV	5	1	5	4
CTX-M	40	0	8	20
TEM	4	1	0	0
Negative	5	0	0	2
Total	154	4	21	51

4. Discussion

The prevalence of ESBLs among *E. coli* and *Klebsiella* isolated from different body sites indicated that these β-lactamases have increased significantly in Bahrain in recent years[6]. This follow-up study aimed to identify the prevalent ESBL types and the strain diversity among the ESBL-producing organisms isolated. This was the first systematic study of ESBL types in Bahrain. All but 15 of the 230 ESBLs identified in this study were CTX-M. A similar predominance of CTX-M ESBLs has been reported in recent years in many countries throughout the world[8-20]. Data from the Arabian Peninsula showed a lower percentage of CTX-M carriage among isolates 34%[21]. However, in our study *E. coli* isolates harboring the CTX-M only showed the highest percentage 37.8% (68/180), of which 76.4% (52/68) were outpatients indicating the emergence of this type in the community.

Our results indicate that CTX-M-producing *E. coli* and *K. pneumoniae* are probably already established in the community. Although hospital isolates of ESBL remain in higher percentage as compared to community isolates, however, there is an increase in the community isolates since our last study in 2002[6]. These results indicate that the ESBL trafficking between hospital and community strains has occured. This occurrence of CTX-M enzymes in the community presents treatment problems.

Concerning antimicrobial susceptibility, all the CTX-M-producing isolates investigated in this study retained susceptibility to carbapenems and most of them also retained

susceptibility to amikacin and piperacillin-tazobactam. High resistance rates were observed with ciprofloxacin, and amoxicillin-clavulanate. The higher rates of resistance to ciprofloxacin observed with the CTX-M-producers could be due to genetic linkage of bla_{CTX-M} with other resistance determinants on the same genetic element and/or to the expansion of clones carrying these resistance determinants. This high percentage of CTX-M-producers in our setting represents a challenge for the treatment for urinary tract infection and other infections in both the community as well as the hospital, since all strains were not only resistant to cephalosporin but also to other antibiotics. The spread of CTX-M-positive bacteria considerably changes the way we think about treating community-acquired infections, and limits the oral antibiotics that may be administered.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors wish to thank the staff of Microbiology Section, Pathology Department, Salmaniya Medical Complex for collection and identification of the bacterial isolates.

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