Molecular Characterization and Epidemiology of Extended-Spectrum Beta-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae Isolates in the United Arab Emirates

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Abstract
Objectives: The aim of this prospective study was to assess the extent of dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae at the hospital level in the United Arab Emirates (UAE). Materials and Methods: A total of 662 Escherichia coli and Klebsiella pneumoniae samples were collected from three UAE hospitals between January and December 2008. ESBL screening and confirmatory test for ESBL phenotype were conducted using the VITEK system. Molecular typing was performed using specific primers and then sequencing. Results: A total of 240 (36%) samples were identified as ESBL producers, including both E. coli (n = 150) and K. pneumoniae (n = 90). All of these isolates were resistant to cefazoline and cefotaxime, but remained susceptible to imipenem. Molecular analysis revealed that, of the 240 ESBL producers, 228 carried the ESBL bl a genes. A majority of the strains 199 (87%) expressed the CTX-M-15 gene. The SHV-28 gene was detected in 29 (13%) of the strains. Conclusion: The present study highlighted the emergence and dissemination of CTX-M-15-producing E. coli and K. pneumoniae in the UAE. This is the first report of SHV-28-producing Enterobacteriaceae in the country.

Introduction
The dynamic spread of Gram-negative bacteria resistant to newer β-lactam antibiotics has alerted researchers to the epidemiological problem of β-lactam resistance in recent years. In a large part, this resistance has resulted from the production of several kinds of β-lactamases encoded by mobile genes. Among these, class A extended-spectrum β-lactamases (ESBLs) predominate in the family of Enterobacteriaceae. Class A ESBLs hydrolyze penicillins, cephalosporins (except cephemycins), and monobactams are inhibited by β-lactam inhibitors [1, 2].

There is no consensus for the precise definition of an ESBL. A commonly used working definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance by hydrolysis of the following antibiotics: the penicillins, first-, second- and third-generation cephalosporins, and aztreonam (but not the cephemycins or car-
bapenems) that are suppressed by β-lactamase inhibitors, like clavulanic acid [3].

In addition to the classical TEM and SHV enzymes, several ESBLs of predominantly Ambler molecular class A phenotypes have recently been reported, including the CTX-M enzymes [4]. From the late 1980s, ESBLs have disseminated dangerously across wide geographic regions [1]. This proliferation may be the consequence of plasmid transmission among nonrelated Enterobacteriaceae, as observed in French hospitals [5]. It may also be mediated by the clonal dissemination of enzyme-producing microorganisms [6]. Sometimes transmission of ESBL-producing strains between hospitals may occur, followed by their clonal expansion and/or the horizontal transfer of plasmids carrying the ESBL gene [7]. Although ESBL-producing strains have been reported in several countries, very little resistance data representing the Middle East have been published [8, 9]. Infections caused by ESBL-producing strains are increasing in frequency in the United Arab Emirates (UAE), an Arabian Gulf country in the Middle East with a dynamic and ethnically diverse population of 4 million. However, no epidemiological survey of ESBL-producing strains has previously been performed in this country. Hence, the aim of this study was to investigate the extent of carriage of ESBL-producing Enterobacteriaceae and characterize the types of ESBLs produced at the hospital level in the UAE.

### Materials and Methods

Six hundred and sixty-two samples of Enterobacteriaceae sp. were isolated from in-patients (5–55 years, mean age 36 years) who had been admitted to the medical care units of three medical centers in the UAE: Zayed Military Hospital, a 260-bed, tertiary-care governmental hospital, Alain Medical Center, and Alfalah Medical Center, both secondary-care hospitals. Of the 662 samples, 450 were from male patients. All strains were isolated between 1 January and 31 December, 2008. The majority of strains isolated were from urine specimens (400 specimens); others were from blood (66 specimens), tracheal/bronchial aspirates (15 specimens), wound swabs (58 specimens), sputum (108 specimens), and ear discharges (15 specimens). The study was carried out in the Microbiology Laboratory at Zayed Military Hospital.

**Bacterial Strains and Susceptibility Testing**

An automated identification and microdilution system (VITEK, bioMérieux, Inc., France) and an overnight ESBL panel were used to identify the bacterial species and determine the minimum inhibitory concentrations of the broad-spectrum cephalosporins, including cefotaxime, ceftazidime, and cefepime; the monobactam antibiotic aztreonam, and the ureidopenicillin piperacillin. The results were recorded and interpreted according to the National Committee on Clinical Laboratory Standards guidelines [10].

**Determination of ESBL-Producing Phenotype**

The ESBL-producing phenotypes of the 662 isolates were determined with an AST-GN27 VITEK ESBL card (VITEK, bioMérieux) according to the manufacturer’s instructions. The E. coli ATCC 25922 and K. pneumoniae ATCC 700603 strains were used as negative and positive controls for ESBL production, respectively.

**DNA Analyses**

Bacterial DNA extraction was performed with the QIAamp DNA Mini kit (QIAGEN). DNA extracts that were positive for the 16S rRNA gene in PCR assays were screened for the presence of the blaTEM, blaSHV, and blaCTX-M genes with consensus PCR primers in a ROTER GENE 3000 PCR system (QIAGEN). The PCR assays were performed with QIAGEN standard PCR mixtures and the HOTSTART Taq master mix kit (QIAGEN), as described previously [11]. The PCR specifications are shown in Table 1. All primers were synthesized by Eurofins MWG, Germany. Bidirectional sequencing was performed with a BigDye v. 3.1 cycle sequencing kit and a model 3100 genetic analyzer (Applied Biosystems). Editing and alignment of DNA sequences were performed with the Geneious 4.6 software package (Biomatters Ltd.).

| Table 1. PCR primers and specifications used in this study [11] |
|-----------------|-----------------|-----------------|-----------------|
| Amplicon       | Primer sequence (5’ to 3’) | Size of amplicon, bp | Annealing temperature °C |
| 16S rRNA gene  | AGA GTT TGA TCM TGG CTC AG | ~1,500           | 55              |
|                 | ACG GHT ACC TTG TTA CGA CTT |                 |                 |
| blaTEM         | ATG AGT ATT CAA CAT TTC CG  | 858              | 50              |
|                 | CCA ATG CTT AAT CAG TGA GG  |                 |                 |
| blaSHV         | ATG CGT TAT ATT CGG CTG TG  | 862              | 58              |
|                 | AGC GTT GCC AGT GCT CGA TC  |                 |                 |
| blaCTX-M       | SCS ATG TGC AGY ACC AGT AA  | 585              | 58              |
|                 | ACC AGA AYV AGC GGC GAC     |                 |                 |

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Results

Isolate Selection and Antibiotic Susceptibility

Of the 662 samples, 240 (36%) were identified as ESBL-producer phenotypes, of which 150 (62%) were E. coli and 90 (38%) K. pneumoniae. All ESBL-producing strains were resistant to the extended-spectrum cephalosporins, cefazolin and cefotaxime (95% of the minimum inhibitory concentrations were >64 mg/l), but remained susceptible to imipenem. Half (50%) of the ESBL-producing strains were resistant to gentamicin, and the remaining 50% were resistant to trimethoprim-sulfamethoxazole.

Detection and Distribution of bla Genes

Of the 150 ESBL-positive E. coli isolates, 141 (94%) carried the bla CTX-M 15 gene only. The remaining 9 (6%) strains did not carry bla genes. Of the 90 ESBL-positive K. pneumoniae isolates, 29 (32.2%) carried the bla SHV28 gene, 58 (64.4%) the bla CTX-M15 gene and the remaining 3 (3.3%) did not carry bla genes. The TEM gene was not detected in any of the samples in this study.

Discussion

In this study, we identified more ESBL-producing E. coli strains (62%) than ESBL-producing K. pneumoniae (38%), consistent with Canadian and Lebanese epidemiological studies [12, 13], but contrary to previous studies performed in Korea [14] and the UK [15]. Twelve isolates (nine E. coli and three K. pneumoniae) were phenotypically ESBL-positive but did not carry the CTX-M, TEM, or SHV genes. These isolates may carry other genes that encode a β-lactamase that confers bacterial resistance to the penicillins, the first-, second-, and third-generation cephalosporins and aztreonam. For example, other β-lactamases include PER, VEB-1, PES-1, Oxa, and other ESBLs. Further studies will be conducted for these isolates in the future.

Molecular sequencing revealed that CTX-M-15 was the prevalent ESBL produced. Unlike most CTX-Ms that preferentially hydrolyze cefotaxime, CTX-M-15, an Asp-240-Gly variant of CTX-M-3, showed increased catalytic efficiency in the hydrolysis of ceftazidime [16]. All ESBL-producing isolates were resistant to cefazoline and cefotaxime, but less resistant to ceftazidime. For this reason, a double test with cefazoline and cefotaxime may be a reliable method for detecting ESBL production in UAE hospitals.

The rapid emergence of the CTX-Ms as the predominant ESBL type is not an isolated UAE phenomenon. A recent report on ESBL types in the Enterobacteriaceae in Argentinian public hospitals found that CTX-Ms accounted for roughly 70% of all ESBLs [17]; similar findings were reported in studies conducted in Japan [18], China [19], the United Kingdom [15], Lebanon [13], and Spain [20]. CTX-M-15 has also been identified in several other countries, including Taiwan [21], Turkey [22], France [23], and the United Kingdom [24].

In 29 isolates, the SHV-28 gene was detected (13%), but only in the K. pneumoniae isolates. Among the SHV-type β-lactamases, SHV-28 and related enzymes appear to be the most prevalent ESBLs worldwide; they were responsible for outbreaks of nosocomial infections in several countries [25–27]. In the present study, SHV-28 was found only in the K. pneumoniae samples. TEM-derived ESBL enzymes were not detected in the present study.

Conclusion

The present study highlighted the emergence and dissemination of CTX-M-15-producing E. coli and K. pneumoniae in the UAE. This is the first report of SHV-28-producing Enterobacteriaceae in the country. Further studies are required for the other types and variants of ESBLs in the UAE.

References


Mulvey MR, Bryce E, Boyd D, Ofner-Agosti.


