Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Bahrain

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Summary
Objectives: To determine the occurrence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in Bahrain.
Methods: Retrospective analysis of records (January 2005—December 2006) at the Microbiology Laboratory of the Salmaniya Medical Complex, Bahrain which is the major national diagnostic laboratory.
Results: Out of a total of 11,886 member of family of Enterobacteriaceae isolated, 2695 (22.6\%) were ESBL producers. Majority of ESBL isolates were from inpatients (\(n=2363; 87.7\%\)). \textit{Escherichia coli} (52.2\%) and \textit{Klebsiella pneumoniae} (24.3\%) were predominant and distributed comparatively in the hospital wards while \textit{Proteus} spp. (17.6\%) was predominant in medical wards. Urine was the major source (52.2\%) with low occurrence in blood cultures. No carbapenem resistant isolates was identified but resistance to three classes of antibiotics was exhibited by >25\% of the isolated ESBL strains. Nitrofurantoin resistance was identified in 38.2\% of urinary isolates.
Conclusion: This is the first report from Bahrain and it indicates that the prevalence of ESBL-producing isolates is high. Carbapenems were the most active drug against the ESBL-producing isolates. We recommend strict infection control to prevent trafficking into the community.

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Introduction

Globally there is a continuing upward trend in the prevalence of pathogens producing extended-spectrum beta-lactamases (ESBLs). As infections caused by these ESBL-producing organisms are associated with higher rates of mortality, morbidity as well as health care costs. This upward trend is of concern to health care providers. ESBL arises as a result of mutations in the TEM-1, TEM-2 or SHV-1 genes which are commonly found in the Enterobacteriaceae family [1]. Others enzymes, notably members of the ceftaxime resistance family (CTX-M), represent plasmid acquisition of broad-spectrum beta-lactamases originally determined by chromosomal genes. These mutations result in alterations in the amino acid configuration thus conferring on these enzymes the ability to hydrolyze a broader spectrum of beta-lactam antibiotics including penicillins, oximinocephalosporins and monobactams. However these plasmid-mediated enzymes have no detectable activity against cephamycins or the carbapenems (imipenem and meropenem). They are also generally susceptible to beta-lactamase inhibitors, such as clavulanate, sulbactam, and tazobactam. These ESBLs are most commonly identified in Gram-negative organisms, primarily in Klebsiella pneumoniae, Klebsiella oxytoca, and Escherichia coli but they have also been described in Acinetobacter, Burkholderia, Citrobacter, Enterobacter, Morganella, Proteus, Pseudomonas, Salmonella, Serratia, and Shigella spp. [2].

Since the 1983 report of the first outbreak involving ESBL-producing organisms in Germany, other reports from Europe, the USA and the Far East have confirmed the role of ESBL-producing organisms as important agents in nosocomial infections [1,3]. In recent years, variants of the original ESBL enzymes such as CTX-M beta-lactamases have become widespread with an endemic situation now prevailing in Asia, South America and parts of Europe [4–6]. As evidence indicates regional/national diversity in the prevalence of ESBL isolates there is a need for continued surveillance. Up to date, there have been no reports documenting the prevalence of ESBL isolates in the Kingdom of Bahrain. However data from other countries within the Arabian Gulf region suggest ESBL isolates constitute a major problem in nosocomial and community acquired infections with rates ranging from 7.5% to 31.7% reported in Kuwait to the highest of 41% which we have recently shown in data from United Arab Emirates [7–9]. In this report, we present the first data on ESBL-producing isolates identified in the Kingdom of Bahrain.

Materials and methods

Setting

The Kingdom of Bahrain is a group of small islands located in the Arabian Gulf region with a population of approximately 650,000. The Salmaniya Medical Complex (SMC) is a 1000-bed hospital that serves as a secondary and tertiary referral center for specialist care, laboratory diagnosis and admissions. The microbiology laboratory processes specimens for inpatients as well as outpatients seen at SMC clinics and primary health care facilities.

Data collection

Retrospective analysis of laboratory records at SMC over a 2-year period from January 2005 to December 2006 was carried out to identify ESBL-producing isolates detected during the study period. Only one positive culture per patient was included hence repeated positive cultures from the same patient were excluded from the analysis. Data on patient demographics, specimen source (inpatient or outpatient), specimen type as well as the antibiotic susceptibility profile of the isolates were recorded.

Detection of ESBL isolates

During the study period, bacterial identification, screening for ESBL and antimicrobial susceptibility testing were performed with the BD Phoenix™ (Becton Dickinson Diagnostic Systems, MD, USA) Automated Microbiology system which incorporates the BD Xpert system. Specifically for ESBL detection, the Phoenix™ ESBL test used fixed concentrations of the following drugs or drug combinations: cefpodoxime, ceftazidime, ceftazidime plus clavulanic acid (CA), cefotaxime plus CA, and ceftriaxone plus CA. The isolates were subcultured on MacConkey agar to obtain a pure culture from which a 0.5 McFarland suspension was obtained and tested according to the manufacturer provided protocol. Confirmation of the ESBL phenotype was carried out using the double disc diffusion method. This was carried out using antibiotic discs containing a combination of cephaplorin plus clavulanic acid in conjunction with a corresponding cephalosporin disc alone and interpreted according to CLSI guidelines [10]. The following antibiotic discs were used: ceftazidime (CAZ 30 μg), ceftazidime plus clavulanic acid (CAZ/CA 30/10 μg), cefotaxime (CTX 30 μg) and cefotaxime plus clavulanic acid (CTX/CA 30/10 μg) and were all obtained from Becton Dickinson, USA. Briefly, isolates were subcultured on...
MacConkey agar and 0.5 McFarland standard prepared from the pure colonies. These were then inoculated on Mueller Hinton agar plates, antibiotic discs were applied and the plates were incubated in ambient air at 35 °C for 16–18 h. The diameter of the zones of inhibition were measured and ≥5 mm increase in the zone of inhibition for the CAZ/CLA and CTX/CLA containing disc vs. the corresponding CAZ or CTX disc was considered positive for ESBL.

Using the BD Phoenix™ system, we assessed the susceptibility patterns of the ESBL-producing isolates to a panel of antibiotics including amoxicillin/clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, imipenem, meropenem and piperacillin/tazobactam, according to manufacturer recommended protocol. ESBL K. pneumoniae ATCC 700603, E. coli ATCC 25922 were used as a positive control and negative control, respectively. In addition, antibiotic sensitivity to imipenem and meropenem using the disc diffusion susceptibility testing method was also carried out. The antibiotic discs used were imipenem 10 μg and meropenem 10 μg and results were interpreted according to manufacturer provided guidelines. All urinary isolates were tested for susceptibility to nitrofurantoin. Data analysis (chi-squared test) was done using Sigma Stat ver 3.5 (Systat Software Inc., San Jose, California, USA) and p < 0.05 was considered statistically significant.

Results

Out of a total of 11,886 member of family of Enterobacteriaceae isolated, 2695 (22.6%) were ESBL producers. The mean age of the patients was 52.6 ± 24.8 years with a female preponderance (55.7% vs. 44.3% males). Majority of isolates (2363; 87.7%) were obtained from inpatients at SMC. E. coli (1414) and K. pneumoniae (654) were the predominant Enterobacteriaceae. Table 1 shows the distribution of the different bacterial isolates. Urine was the major source of ESBL isolates (1406; 52.2%) and 26.9% (725) were from wound swabs/pus while 13.2% (357) were identified in respiratory specimens including sputum, deep tracheal aspirate and bronchioalveolar lavage. The occurrence of ESBL producers in bacteriaemia was low as only 7.7% (207) were from blood cultures. Providentia spp. were isolated mainly from respiratory specimens (21/25) while Morganella morganii was isolated from wound (n = 2) and urine (n = 1). The distribution of the remaining isolates according to specimen type showed that a significantly higher proportion of urinary isolates were E. coli (68.3%); p < 0.05 and for respiratory specimens both K. pneumoniae (40.2%) and Proteus spp. were predominant (37.5%) (Fig. 1). With the exception of Citrobacter and Enterobacter, the distribution of the other bacteria in wound/pus and blood specimens was not statistically significant (Fig. 1). As E. coli, K. pneumoniae and Proteus spp. constitute 94.4% of

| Table 1 ESBL-producing isolates identified 2005–2006 at SMC Bahrain. |
|------------------------|------------------------|------------------------|
| **ESBL isolate**       | **Number of isolates** | **%**                  |
| *Escherichia coli*     | 1414                   | 52.5                   |
| *Klebsiella pneumoniaea* | 654                 | 24.3                   |
| *Proteus spp.*         | 473                    | 17.6                   |
| *Citrobacter spp.*     | 64                     | 2.4                    |
| *Enterobacter spp.*    | 62                     | 2.3                    |
| *Providencia spp.*     | 25                     | 0.9                    |
| *Morganella morganii*  | 3                      | 0.1                    |

| Total                  | 2695                   | 100                    |

a No *Klebsiella oxytoca* identified.

b *Proteus mirabilis* and *Proteus vulgaris*.

![Figure 1](https://example.com/image.png) Distribution of ESBL producers according to type of clinical specimen.
Our ESBL isolates, further analysis was carried out only for these. The distribution of these isolates in the hospital units showed that both *E. coli* and *K. pneumoniae* were globally distributed being prevalent in comparable proportion in all the wards (Fig. 2). However, while the highest number of *E. coli* isolates were from the obstetric and gynaecology unit, *K. pneumoniae* was most prevalent in the paediatric unit. In contrast, *Proteus* spp. was mainly prevalent in the medical unit at a significantly higher number compared to the other units in the hospital and it was notable that no *Proteus* isolates were obtained from the paediatric unit (Fig. 2).

Over 25% of *E. coli*, *K. pneumoniae* and *Proteus* spp. isolates were resistant to the different classes of antibiotics tested (beta-lactams, quinolones and aminoglycosides) except for piperacillin/tazobactam where only 12.6% of *Proteus* spp. were resistant (Fig. 3). For the beta-lactam/beta-lactamase inhibitor combinations, there was a high degree of resistance to amoxicillin/clavulanate (>70% of isolates). However, compared to amoxicillin/clavulanate, the proportion of isolates showing resistance to piperacillin/tazobactam was significantly lower (*p* < 0.05) (Fig. 2); which was consistent with other studies [11]. The likely explanation for this observation is that the majority of our strains might be of CTX-M type which is better inhibited by tazobactam than by clavulanate. The highest degree of resistance to ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole was seen with *Proteus* spp. Susceptibility to nitrofurantoin was carried out for 1547 urinary isolates of which 591 (38.2%) showed resistance. All isolates were susceptible to both imipenem and meropenem using both the BD automated system and disc diffusion method.

**Discussion**

This is the first report on the prevalence and antibiotic-susceptibility pattern of ESBL-producing Gram-negative bacteria isolated in Bahrain, giving a first insight into the occurrence of these isolates in the country. The ESBL detection rate of...
22.8% is reflective of national prevalence as SMC is the major national tertiary hospital and the microbiology laboratory processes specimens obtained from inpatients at SMC, as well as outpatient clinics and primary care facilities across the region. Reported national data show that, in Europe, the prevalence of ESBL ranges from a low of 3% in Sweden to as high as 34% in Portugal [12], 30–60% in South America while in Asia surveys have indicated ESBL detection ranging from 5% to 8% in isolates from Korea, Japan, Malaysia and Singapore with higher rates of up to 24% for other Asian countries [13–15]. Within the Arabian Gulf region, the lowest ESBL prevalence was described in a report from Kuwait (7.5%) [7]. However in another study a higher percentage was reported (31.7%) [8]. In UAE the prevalence of ESBL was shown to be 41% [9]. However, in both cases the study population were inpatients only. ESBL detection among inpatients and outpatients in a maternity unit in the eastern region of Saudi Arabia was reported as 27.5% but only K. pneumoniae isolates were studied [16]. In 2002, Babay [17] reported ESBL production in 36% of Enterobacteriaceae from inpatients in a hospital in Riyadh. More recently, 15.8% and 8.9% ESBL prevalence were reported in blood cultures and urinary isolates, respectively [18,19]. Thus, compared to regional and international data, the ESBL detection rate described here tends to be towards the upper end of the spectrum and is therefore a cause for concern.

While ESBL isolates remain important in nosocomial infections, emerging data from parts of Europe, Asia and South America indicates that community acquired infections caused by ESBL-producing strains in particular those that harbour the CTX-M gene is now endemic in many countries and this trend has been accompanied by the recognition of E. coli as the major source of ESBLs in the community [20–22]. In contrast, majority (~88%) of ESBL producers in our setting were isolated from inpatients which indicates that infections associated with ESBL-producing pathogens in our population still remain largely nosocomial in nature.

A predominance of either K. pneumoniae or E. coli has often been reported among the ESBL isolates identified in different geographical regions. In Pakistan a prevalence of ESBL-producing K. pneumoniae vs. ESBL-producing E. coli (70% vs. 28.6%) has been reported [23] and in Italy a 2003 nationwide survey found that the most prevalent ESBL-positive species among hospitalized patients was E. coli a switch from the predominance of K. pneumoniae in 1999 [24]. The predominant ESBL producer in our setting is E. coli accounting for over half of the ESBL isolates identified. However, there was no significant difference in the distribution of E. coli and K. pneumoniae ESBL isolates in the wards within the hospital. Thus, given the global distribution of these isolates, particularly E. coli, unless strict infection control measures are enforced, there is the high likelihood of trafficking of these ESBL producers from the hospital into the community. This is particularly pertinent as the majority of ESBL isolates were from urine specimens and the detection of ESBL producers in urine has been described as an epidemiologic marker of colonization and potential for trafficking [25].

The antimicrobial susceptibility results show that both imipenem and meropenem remain the most effective class of drugs for ESBL isolates both showed full susceptibility with the two testing methods we used. However, there remains a need for continued surveillance and judicious use of these antibiotics as a recent report from our region has documented the occurrence of carbapenem resistant E. coli isolate [9]. As new findings indicate that the spread of CTX-M type ESBLs, especially in E. coli, may provide a favorable background for selection of carbapenem resistance [26] molecular characterization of the ESBL isolates in our setting is needed. For the other classes of antibiotics tested, >25% of the ESBL isolates showed resistance to the classes of antibiotics tested. This level of resistance perhaps represents the higher end of the spectrum relative to other reported data from the Arabian Gulf Region. This is of concern as clinically it indicates that there are limitations in the antibiotic choices available for the treatment of these infections. Studies have indicated a relationship between antibiotic usage and resistance and we speculate that similar scenario may be at play in our setting as recent reports have shown that there is a high level of antibiotic prescription and misuse occurring in Bahrain [27]. The use of beta-lactam/beta-lactamase inhibitor combinations for the treatment of infections caused by ESBL-producing organisms has been associated with treatment failures. The high level of resistance to amoxicillin-clavulanate indicates it limited use for treatment of ESBL infections in our setting. However, relative to ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole, our ESBL isolates show higher levels of susceptibility to piperacillin/tazobactam. The progressive increase of infections caused by ESBL-producing bacteria with their attendant cross-resistance to antibiotics of different classes has resulted in a re-evaluation of the potential use of old antimicrobials such as nitrofurantoin. Our data presented herein represents the first report on nitrofurantoin for urinary
ESBL-producing isolates in our region. The finding indicates that only about two-thirds of the isolates are sensitive to nitrofurantoin. This is much lower than other reports which have shown >88% susceptibility in surveys in USA, 89% in India and 98% in Hong Kong [28—30]. Although based on these data from other countries, it has been suggested that nitrofurantoin may be considered as an alternative therapeutic agent for urinary tract infections caused by ESBL producers, our findings indicate that responses may be less than satisfactory in our setting in view of the degree of resistance present.

In conclusion, these findings indicate although there is a high prevalence of ESBL-producing bacteria in our setting, it remains largely a nosocomial infection. However, in view of the global distribution of these isolates in the hospital the potential for trafficking remains a high possibility. Further work on the molecular characterization of ESBL producers isolated in Bahrain and an urgent need for control measures to reduce the spread of these pathogens is recommended.

Conflict of interest

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