High prevalence of CTX-M β-lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand

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Objectives: To determine the prevalence of CTX-M β-lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in a rural area of Thailand.

Methods: Bacteria in stool specimens were screened for extended-spectrum β-lactamase (ESBL) production on McConkey agar with cefotaxime and confirmed by the double-disc synergy test. Genetic detection and genotyping of CTX-M-type ESBL was performed by PCR with bacterial DNA extracted from isolates.

Results: A markedly high number (82 of 141, 58.2%) of the specimens showed the presence of CTX-M β-lactamase-producing Enterobacteriaceae, as confirmed by both phenotypic and genetic examinations. The majority of the CTX-M β-lactamase-producing bacteria were Escherichia coli (85.1%).

Conclusions: The study revealed the wide dissemination of CTX-M β-lactamase-producing Enterobacteriaceae in the healthy population.

Keywords: genotypes, faecal carriage, rural areas, ESBLs

Introduction

Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs), which are plasmid-encoded enzymes capable of hydrolysing extended-spectrum cephalosporins, are a growing problem in the treatment of nosocomial as well as community-acquired infections. Most ESBLs can be divided into three groups: TEM, SHV and CTX-M types. During the last 5 years, the CTX-M ESBL has become the most prevalent ESBL type.1

Bacteria that produce CTX-M enzymes not only cause nosocomial infections but also have the potential to spread beyond the hospital environment; in addition, interspecies plasmid transfer is observed in these bacteria,2 which further exacerbates public health concerns.3 Two studies conducted in the 2000s reported that CTX-M ESBL-producing Escherichia coli is an important cause of community-onset bloodstream infections.3,4 Even though many studies have been conducted for the detection and typing of ESBL-producing bacteria isolated from patients with infections, the prevalence of ESBL-producing bacteria in the healthy population is unclear. Therefore, in order to clarify the prevalence of ESBL-producing bacteria within community settings, particularly in healthy individuals, stool specimens of healthy individuals were examined for the presence of CTX-M ESBL-producing Enterobacteriaceae.

Materials and methods

From October to November 2008, stool specimens of healthy asymptomatic volunteers in a rural area of Kanchanaburi, Thailand, were collected. All the participants were screened for age (>20 years) and medical history. Exclusion criteria included any antibiotic treatment in the 3 months prior to specimen collection and confirmed diagnosis of digestive tract diseases. All of the participants gave their informed consent. A total of 160 stool specimens, one specimen each, from volunteers (age range, 25–86 years; average age ± standard deviation, 56.0 ± 9.8 years) were examined in this study. The phenotypic detection of ESBL-producing bacteria in the stool specimens was performed using McConkey agar with 2 mg/L cefotaxime (CTX-McConkey). The stool specimens were directly inoculated on the agar plates and incubated at 37°C for 24 h. Isolates were identified using conventional biochemical tests. The presence of ESBLs was confirmed by the double-disc synergy test using cefazidime, cefepime, cefotaxime, ceftidoxime, ceftriaxone, aztreonam and amoxicillin/clavulanic acid, as previously described.5,6

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Genetic detection and genotyping of CTX-M was performed using PCR with bacterial DNA, which was extracted from the isolates by boiling the bacterial suspensions. A solution with an extracted DNA concentration of 0.1 ng/µL was used as a template for PCR analysis. The PCR was performed with the universal blaCTX-M primer set as previously described. A DNA solution of a reference E. coli blaCTX-M-positive strain was used as a positive control for PCR analysis. In the case of genotyping of CTX-M genes, four primer sets that amplify group-specific CTX-M genes were utilized, as described previously. Group I, including CTX-M-1, -3, -10 to -12, -15, -22, -23, -28, -29 and -30; group II, including CTX-M-2, -4 to -7 and -20 and Toho-1; group III, including CTX-M-8; and group IV, including CTX-M-9, -13, -14, -16 to -19, -21 and -27 and Toho-2. The PCR products were visualized by 2% agarose gel electrophoresis and staining with ethidium bromide. The specificity of the PCR was confirmed by sequencing the PCR products.

**Results**

Of 160 specimens, 141 (88.1%) showed bacterial growth on CTX-McConkey agar. As shown in Table 1, of these 141 isolates, 87 (61.7%) were ESBL-producing bacteria as determined by the double-disc method. The genetic analysis of these isolates by PCR revealed that 82 of 87 (94.3%) were positive for the CTX-M gene. These CTX-M gene-negative isolates showed that one of the 85 ESBL-producing isolates (1.2%) had no CTX-M gene. The majority of these isolates (51 of 54, 94.4%) also did not have the CTX-M gene in their DNA. Only 3 of 54 isolates had the CTX-M genotype, but they did not exhibit the ESBL phenotype.

As shown in Table 2, the majority of CTX-M ESBL-producing bacteria were identified as E. coli (85.1%), followed by Citrobacter spp. (7.5%) and Klebsiella spp. (5.7%).

**Table 1. Detection of CTX-M**

<table>
<thead>
<tr>
<th>ESBL phenotype</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>82 (58.2%)</td>
<td>5 (3.5%)</td>
<td>87 (61.7%)</td>
</tr>
<tr>
<td></td>
<td>3 (2.1%)</td>
<td>51 (36.2%)</td>
<td>54 (38.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>85 (60.3%)</td>
<td>56 (39.7%)</td>
<td>141 (100%)</td>
</tr>
</tbody>
</table>

aBacterial isolates grown on CTX-McConkey agar were assessed. 
bESBL phenotype was determined by the double-disc synergy test. 
cCTX-M gene was determined by PCR.

**Table 2. Enterobacteriaceae recovered from stool specimens**

<table>
<thead>
<tr>
<th></th>
<th>No. of isolates</th>
<th>ESBL phenotype a</th>
<th>ESBL phenotype with CTX-M gene a</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>93</td>
<td>66.0%</td>
<td>74</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>9</td>
<td>6.4%</td>
<td>5</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>2</td>
<td>1.4%</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>5</td>
<td>3.5%</td>
<td>5</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>4</td>
<td>2.8%</td>
<td>0</td>
</tr>
<tr>
<td>Not Enterobacteriaceae</td>
<td>28</td>
<td>19.9%</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>100%</td>
<td>87</td>
</tr>
</tbody>
</table>

aBacterial isolates grown on CTX-McConkey agar were identified. 
bESBL phenotype was determined by the double-disc synergy test. 
cCTX-M gene was determined by PCR.

**Discussion**

The specificity of the PCR method utilized in this study with regard to the detection of CTX-M genes has been examined by using well-characterized reference strains of the ESBL phenotype; moreover, the ability of this PCR method to detect isolates with CTX-M ESBLs has also been studied. The PCR method utilized in this study also amplified the chromosomally located K-1 enzyme gene, which can be found in Klebsiella oxytoca. However, in this study, K. oxytoca was not detected in the specimens identified as ESBL-producing bacterial isolates.

A recently conducted review of the prevalence of ESBL-producing strains in Asia has reported that the faecal carriage rate of this species is probably ~10% in the Indian and Chinese populations. However, marked variation was observed in the incidence and genotype of ESBL-producing strains in hospitals located close to one another and certainly among countries. In Thailand, the incidence of the ESBL phenotype (26% Enterobacteriaceae) in isolates from patients was reported to be high. In Thailand, no studies have been published with regard to the asymptomatic carriage of ESBL-producing strains in stools. In other countries, only a few studies have been published on the prevalence of ESBL-producing Enterobacteriaceae in healthy subjects; these studies reported low detection rates (2.3%–13.1%).

In the present study, the reasons for the very high detection rate (58.2%) of CTX-M ESBL-producing Enterobacteriaceae,
particularly type IV, in stool specimens are unclear. A high incidence of ESBL-producing bacterial strains has been reported in various animals and food products in different countries. Therefore, one of the possible reasons for the high incidence of CTX-M ESBL-producing Enterobacteriaceae in humans might be that these bacteria are acquired through the food chain. Nevertheless, the countrywide dissemination of CTX-M ESBL-producing bacteria should be studied in further detail by conducting a nationwide study.

Thus, this study revealed the surprisingly high incidence of CTX-M ESBL-producing Enterobacteriaceae in asymptomatic healthy individuals. Therefore, tracking and monitoring the worldwide spread of Enterobacteriaceae that produce CTX-M ESBLs within community settings is essential from the viewpoint of public health.

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Transparency declarations
None to declare.

References