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

Tadahiro Sasaki1, Itaru Hirai1,2, Marie Niki1, Tatsuya Nakamura3, Chalit Komalamisra4, Wanna Maipanich4, Teera Kusolsuk4, Surapol Sa-nguankiat4, Somchit Pubampen4 and Yoshimasa Yamamoto1,2*

1Department of Bioinformatics, Osaka University Graduate School of Medicine, Osaka, Japan; 2Kobe University Graduate School of Medicine, Kobe, Japan; 3Clinical Central Laboratory, Kansai Medical University Hospital, Osaka, Japan; 4Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

*Corresponding author. Department of Bioinformatics, Osaka University Graduate School of Medicine, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel: +81-6-6879-2580; Fax: +81-6-6879-2499; E-mail: yyamamot@sahs.med.osaka-u.ac.jp

Received 28 October 2009; returned 14 December 2009; revised 18 December 2009; accepted 4 January 2010

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 ceftazidime, cefepime, cefotaxime, cefpodoxime, ceftriaxone, aztreonam and amoxicillin/clavulanic acid, as previously described.3,5

© The Author 2010. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
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 PCR amplification primers 5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3' (sense) and 5'-TGG GTR AAR TAR GTS ACC AGA ARY AGC GG-3' (antisense), as described previously. 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; the remaining 5 isolates (5.7%) were negative for this gene. These CTX-M gene-negative isolates showed that one was both TEM and SHV type but other isolates were not. The genotyping of 82 CTX-M gene-positive isolates showed that 9 (11.0%), 0 (0%), 0 (0%) and 71 (86.6%) isolates belonged to groups I, II, III and IV, respectively. The remaining two isolates were not classified into these groups.

Of the 141 specimens that showed bacterial growth on CTX-McConkey agar, 54 (38.3%) were not ESBL-producing bacteria, as determined by the double-disc synergy test. 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. (5.7%) and Klebsiella spp. (5.7%).

Table 1. Detection of CTX-M<sup>a</sup>

<table>
<thead>
<tr>
<th>CTX-M gene&lt;sup&gt;c&lt;/sup&gt;</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL phenotype&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>82</td>
<td>5</td>
<td>87</td>
</tr>
<tr>
<td>-</td>
<td>3</td>
<td>51</td>
<td>54</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>56</td>
<td>141</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bacterial isolates grown on CTX-McConkey agar were assessed.

<sup>b</sup>ESBL phenotype was determined by the double-disc synergy test.

<sup>c</sup>CTX-M gene was determined by PCR.

Table 2. Enterobacteriaceae recovered from stool specimens<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>No. of isolates</th>
<th>ESBL phenotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ESBL phenotype with CTX-M gene&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>93</td>
<td>66.0%</td>
<td></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>

<sup>a</sup>Bacterial isolates grown on CTX-McConkey agar were identified.

<sup>b</sup>ESBL phenotype was determined by the double-disc synergy test.

<sup>c</sup>CTX-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 these 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.

Acknowledgements
The excellent technical assistance of Kozue Moriai and Maiko Akahane is acknowledged.

Funding
This work was supported in part by the Program of Funding Research Centers for Emerging and Reemerging Infectious Diseases, MEXT (Ministry of Education, Culture, Sports, Science and Technology), Japan.

Transparency declarations
None to declare.

References