Journal of Microbiology, Immunology and Infection (2016) 49, 692–700

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp. isolates in Mongolia

Cheng-Yen Kao [a], Uuganbayar Udval [b], Yi-Ting Huang [a], Hsiu-Mei Wu [a], Ay-Huey Huang [a], Enkhbaatar Bolormaa [b], Jing-Jou Yan [c], Zorig Urangoo [b], Gunchin Batbaatar [b], Tulgaa Khosbayar [b,e,], Jiunn-Jong Wu [a,d,e,*]**

[a] *Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[b] *Department of Cellular Biology and Biochemistry, Mongolian National University of Medical Sciences, Mongolia*
[c] *Department of Pathology, National Cheng Kung University Hospital, National Cheng Kung University, Tainan, Taiwan*
[d] *Center of Infectious Disease and Signaling Research, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

Received 29 August 2014; received in revised form 30 April 2015; accepted 21 May 2015
Available online 27 June 2015

KEYWORDS
antibiotic resistance;
CTX-M;
Enterobacteriaceae;
Extended-spectrum β-lactamase;
PFGE

Abstract *Background/purpose:* The aim of this study was to determine the molecular characteristics of β-lactamase genes in extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae isolates from Mongolia.
Methods: Fifty-six ESBL-producing Enterobacteriaceae isolates were collected, of which 46 were *Escherichia coli*, seven were *Klebsiella pneumoniae*, and three were *K. oxytoca*. Minimum inhibitory concentrations for selected antibiotics were tested using the agar dilution method, and the β-lactamase genes were determined using polymerase chain reaction combined with sequencing. Pulsed-field gel electrophoresis (PFGE) was used for genotyping all isolates, and phylogenetic grouping was performed on ESBL-producing *E. coli* isolates.

* Corresponding author. Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Number 1, University Road, Tainan 70101, Taiwan.
** Corresponding author. Department of Cellular Biology and Biochemistry, School of Pharmacy and BioMedicine, Mongolian National University of Medical Sciences, Mongolia. P. O. Box 48/111, Ulaanbaatar 210648, Mongolia. Tel.: +976 99254526; fax: +976 11 321249.
E-mail addresses: khosbayar.t@mnums.edu.mn (T. Khosbayar), jjwu@mail.ncku.edu.tw (J.-J. Wu).
[e] These authors contributed equally to this article.

http://dx.doi.org/10.1016/j.jmii.2015.05.009

Conjugation tests combined with plasmid digestion assays were used to determine whether there was a horizontal spread in Mongolia.
Results: Among the 56 ESBL-producing isolates, 43 isolates (76.8%) were resistant to fluoroquinolones, but all isolates were susceptible to carbapenems and amikacin. The polymerase chain reaction sequencing results showed that the dominant CTX-M genotype was CTX-M-15 (19/46, 41.3%) in the ESBL-producing *E. coli* isolates. By contrast, CTX-M-14 and CTX-M-3 were the major genotypes found in *Klebsiella* spp. Phylogenetic analysis revealed that 21 ESBL-producing *E. coli* isolates belonged to group D (21/46, 45.6%), followed by group A (13/46, 28.3%), group B2 (11/46, 23.9%), and group B1 (1/46, 2.2%). Only four *E. coli* isolates (4/46, 8.7%) belonged to the ST131 clone. PFGE showed that the ESBL-producing Enterobacteriaceae were genetically unrelated. The conjugation assay showed that two plasmids harboring CTX-M-15 in *E. coli* isolates were genetic unrelated, whereas seven plasmids harboring CTX-M-14 (5/7 and 2/7) and four plasmids harboring CTX-M-55 (4/4) showed genetic relatedness, indicating the dissemination of resistance plasmids in this area.

Introduction

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (EPE) are increasing rapidly all over the world. At present, EPE are a growing threat to public health, leading to serious infections and raising key therapeutic problems.[1–5] ESBLs are among the Ambler Class A, resistant to β-lactam antibiotics except cephamycins and carbapenems, and are inhibited by clavulanic acid.[1] In addition, ESBLs are often located on plasmids harboring resistance genes to other antimicrobial classes, resulting in multidrug-resistant isolates and thus also facilitating their transfer to different hosts.[6,7]

The first ESBLs have evolved from native β-lactamases TEM and SHV by genetic mutation.[8,9] However, since the mid 2000s, a novel type of ESBL called CTX-M, from environmental *Kluyvera* spp., has emerged worldwide.[10] There are currently > 150 different CTX-M-type ESBLs recognized (http://www.lahey.org/Studies/) and grouped into six major subgroups based on their amino acid identities, named the CTX-M-1, -2, -8, -9, -25, and KLUC.[11]

CTX-M has been identified in several members of the Enterobacteriaceae family, and especially in *Escherichia coli*, which is the principal ESBL-producing member of the Enterobacteriaceae.[12,13] At present, CTX-M-15, derived from CTX-M-3 by a substitution of Asp-240-Gly, which increases its catalytic efficiency against ceftazidime,[14] is recognized as the most widely distributed CTX-M enzyme.[15] In addition, the current pandemic spread of ESBL-producing *E. coli* has been greatly facilitated by high-risk clones, mainly the clonal group O25b:H4-B2-ST131.[16,17] ST131 contains the CTX-M-15 enzyme, with high potential virulence, and represents a major public health problem.[16,17]

Many reports have documented the emergence of EPE.[18–20] In our previous study, 18.3% of the clinical *E. coli* isolated from patients in Mongolia are ESBL producers.[21] However, the distribution of ESBL genotypes and plasmid characteristics in EPE in Mongolia is still unclear. The aim of this study was to investigate the molecular epidemiology and genetic characteristics of clinical EPE isolates obtained from two Mongolian hospitals.

Materials and methods

Sampling and isolation of Enterobacteriaceae

The study protocols were approved by the National Ethics Committee of Mongolia (20120925/6). Fifty-six nonconsecutive, nonduplicate clinical isolates were obtained from inpatients hospitalized at National Central Hospital or National Center for Maternal and Child Health in Mongolia. These isolates were collected over a period of 5 months, between February 2013 and June 2013. Presumptive Enterobacteriaceae isolates were identified using standard microbiological methods. The isolates were stored at −80°C in Luria–Bertani (LB) broth containing 20% glycerol (v/v) until used.

Antimicrobial susceptibility testing and ESBL confirmation

Antimicrobial susceptibilities were determined by the disk diffusion method on Mueller–Hinton agar (Bio-Rad, Marne la Coquette, France) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. ESBL-producing isolates were screened using the double-disk synergy test in accordance with CLSI guidelines.[22] All ESBL-producing isolates were tested for minimum inhibitory concentration (MIC) of selected antimicrobial agents (from Sigma-Aldrich, St. Louis, MO, USA; amikacin, cefepime, cefotaxime, ceftazidime, ciprofloxacin, ertapenem, levofloxacin; from USP Standards, Rockville, MD, USA: cefoxitin, imipenem, meropenem) using the agar dilution method in accordance with CLSI guidelines. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains.

Molecular characterization of β-lactamases

The primers used in this study are described in Table 1. Strains with a positive double-disk synergy test were further studied using specific polymerase chain reaction (PCR) amplification and sequencing of ESBL genes. DNA was