Original Article

Rapid Identification and Subtyping of *Enterobacter cloacae* Clinical Isolates Using Peptide Mass Fingerprinting*

WANG Yi Qian[1,¶], XIAO Di[1,¶], LI Juan[1], ZHANG Hui Fang[1], FU Bao Qing[2], WANG Xiao Ling[3], AI Xiao Man[4], XIONG Yan Wen[1], ZHANG Jian Zhong[1,#], and YE Chang Yun[1,#]

1. State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 2. Department of Clinical Laboratory, Daqing Oilfield General Hospital, Daqing 163411, Heilongjiang, China; 3. Department of Clinical Laboratory, Shanxi Province Chinese Medicine Hospital, Taiyuan 030012, Shanxi, China; 4. Department of Medical Laboratory, Beijing Hospital, Beijing 100730, China

Abstract

Objective To establish a domestic database of *Enterobacteria cloacae (E. cloacae)*, and improve the identification efficiency using peptide mass fingerprinting.

Methods Peptide mass fingerprinting was used for the identification and subtyping of *E. cloacae*. Eighty-seven strains, identified based on *hsp60* genotyping, were used to construct and evaluate a new reference database.

Results Compared with the original reference database, the identification efficiency and accuracy of the new reference database was greatly improved at the species level. The first super reference database for *E. cloacae* identification was also constructed and evaluated. Based on the super reference database and the main spectra projection dendrogram, *E. cloacae* strains were divided into two clades.

Conclusion Peptide mass fingerprinting is a powerful method to identify and subtype *E. cloacae*, and the use of this method will allow us to obtain more information to understand the heterogeneous organism *E. cloacae*.

Key words: *E. cloacae*; Identification; Peptide mass fingerprinting

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INTRODUCTION

Enterobacter cloacae (E. cloacae) is a facultatively anaerobic gram-negative bacillus belonging to the Enterobacteriaceae family. This microorganism can be widely found in nature and in saprophytic environments (e.g., soil and sewage); this microbe is also part of the commensal enteric flora of the human gastrointestinal tract. In recent decades, *E. cloacae* has emerged as a nosocomial pathogen with clinical significance in hospitals[1-6]. In previous work, sequencing of the heat shock protein 60 gene (*hsp60*) has been helpful for the phylogenetic analysis of *Enterobacter*. Using *hsp60* genotyping, *E. cloacae* was divided into 12 genetic clusters (cluster I-XII) and

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¶The authors contributed equally to this work.

#Correspondence should be addressed to YE Chang Yun, Professor, PhD, Tel/Fax: 86-10-58900747, E-mail: yechangyun@icdc.cn; ZHANG Jian Zhong, Professor, PhD, Tel/Fax: 86-10-58900754, E-mail: zhangjianzhong@icdc.cn

Biographical notes of the first authors: WANG Yi Qian, female, PhD, majoring in gastrointestinal diseases epidemiology; XIAO Di, female, PhD, majoring in application of proteomics in the diagnosis of infectious diseases.

an unstable sequence cluster (cluster XIII)[7]. It is reported that different genetic clusters of *E. cloacae* result in different pathological outcomes[8-13]. However, there is no fast and effective method to identify the genetic clusters of *E. cloacae* from clinical specimens. The BioMerieux biochemical identification system usually confuses *E. cloacae* with *Klebsiella* (*pneumoniae/oxytoca*) or other species from clinical environments. 16S rDNA sequencing could only identify *E. cloacae* at the species level and is very time consuming and expensive.

Peptide mass fingerprinting (PMF) based on Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is very useful for the identification of a variety of microorganisms[14-17]. Beginning with whole cells, cell lysates, or crude bacterial extracts, the acquired fingerprint spectrum (including signal intensity and mass-to-charge ratio) shows species-specific patterns in cultures grown for a wide range of times using a variety of growth conditions. Through comparison of the acquired spectra with a corresponding reference library, the bacteria can be identified within minutes by analysing the data using various algorithms. With accuracy and automation, high-throughput methods make PMF superior to conventional identification techniques that are based on genome-based identification schemes and biochemical methods[18]. This study aimed to assess the performance of PMF in the identification and subtyping of *E. cloacae*. PMF data from 86 defined strains were analysed using Biotyper 2.0 software. A new reference database (NRD) and a super reference database (SRD) (including the common characteristics of NRD) were constructed and evaluated. Based on the peptide-mass fingerprints, we created a main spectra projection (MSP) dendrogram and analysed the dendrogram for specific peaks that could be used for the identification and subtyping of *E. cloacae*.

MATERIALS AND METHODS

Bacterial Strains

Eighty-six isolates belonging to 10 genetic clusters of *E. cloacae,* which were isolated from patients in different hospitals, were used in the study (Table 1). Each genetic cluster contained 2 to 16 isolates. A reference strain (ATCC 13047) was also included. All isolates were characterized for clonality using pulsed-field gel electrophoresis and subtyped using *hsp60* genotyping[7,19,20]. Because no strain from clusters VII, X, and XII was isolated from the clinical samples, our study did not contain these three genetic clusters.

In this study, all strains were isolated from human patients for routine diagnostic purposes. All participants gave written informed consent. This study was approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, China CDC, according to the medical research regulations of the Ministry of Health, China [Approval No. ICDC-2014003].

MALDI-TOF MS Sample Preparation and Data Acquisition

Each strain was selected from brain-heart agar plates. After culturing at 37 °C for 12 h, two samples were prepared from each strain. The extraction method

Table 1. *Enterobacter cloacae* Strains Isolated from China

Cluster	No. of strains	Source	Province	Year
I	10	SP (9), Ur (1)	HB, HLJ	2011, 2012, 2013
II	7	SP (3), Ur (1), SC (1), BI (2)	HB, HLJ, SX	2010, 2011, 2012, 2013
III	14	SP (7), Ur (1), SC (5), BL (1)	HB, HLJ	2011, 2012
IV	5	SP (3), BI (1), OT (1)	HB, HLJ	2011, 2012, 2013
V	6	SP (3), SC (2), BI (1)	HB, HLJ	2011, 2012, 2013
VI	15	SP (8), Ur (2), SC (3), OT (2)	HB, HLJ, BJ	2011, 2012, 2013
VIII	16	SP (9), SC (4), BL (1), ST (2)	HB, HLJ	2011, 2012
IX	5	SP (4), BL (1)	HB, HLJ	2010, 2011, 2012
XI	2	SP (2)	HB	2011, 2012
XIII	6	SP (3), BI (1), BL (1), ST (1)	HB, HLJ, SX, GZ	2011, 2012, 2013
Total	86			

Note. Sources: SP (sputum), UR (urine), SC (secretion), BI (bile), BL (blood culture), ST (stool), OT (others) Regions: BJ (Beijing), GZ (Guizhou), HB (Hebei), HLJ (Heilongjiang), HN (Henan), QH (Qinghai), SX (Shanxi).