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Original Article

Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in a primary care hospital in Japan, 2010–2013

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ABSTRACT

Recently, carbapenemase-producing Enterobacteriaceae (CPE) have been spreading worldwide and have become a threat in healthcare systems. We investigated the isolation frequency and molecular epidemiological characteristics of CPE isolated from clinical samples collected at a primary care hospital over the four years of 2010–2013 in Japan. CPE were detected in 17 (0.34%) of 4875 isolates by the broth microdilution method, sodium mercaptoacetate inhibition test, and modified Hodge test using meropenem disks. The frequency of CPE isolates was 0.09% in 2010, 0.17% in 2011, 0.16% in 2012 and 0.82% in 2013. Isolates positive for carbapenemase included *Klebsiella pneumoniae* (0.92%), *Escherichia coli* (0.12%), *Enterobacter cloacae* (0.80%), *Klebsiella oxytoca* (0.55%), *Enterobacter aerogenes* (0.81%) and *Proteus mirabilis* (0.08%). Antimicrobial susceptibility testing showed low MICs for piperacillin-tazobactam, amikacin, ciprofloxacin and levofloxacin, and only one multidrug-resistant strain. The carbapenemase genotype of all strains was IMP-6, and 94% of the strains were simultaneous CTX-M-2 producers. Two *K. pneumoniae* and 3 *E. coli* isolates showed the same pulsed-field gel electrophoresis group. Multilocus sequence typing detected no international high-risk clone types. Plasmid replicon typing detected IncN from all CPE strains, and IncF and IncFIB were simultaneously detected in 24% and 18%, respectively. All patients with detected CPE were inpatients, and many were elderly long-term hospitalized patients or had a history of prior vancomycin or levofloxacin antibiotic administration. The rapid spread of CPE is a concern in Japan. Preventive measures must be implemented against the spread of CPE after considering the epidemiological trend of CPE detection, antibiograms, and risk factors.

1. Introduction

Recently, carbapenemase-producing Enterobacteriaceae (CPE) have been spreading throughout the world and have now become a threat in healthcare systems [1]. The carbapenemase-encoding gene that is located on the R-plasmid of CPE is important in preventive measures against healthcare-acquired infection because it is frequently conjugally transferred to other bacterial species. In addition, carbapenemase-encoding genes simultaneously comprise several genes that tolerate antimicrobial agents other than beta-lactam antibiotics, such as fluoroquinolone or aminoglycoside, and often confer multidrug resistance. Therefore, CPE will often show multidrug resistance in antimicrobial susceptibility testing [2]. Consequently, the mortality rate from severe infections caused by CPE is very high [3]. The worldwide spread of CPE will be of great concern in the future.

The main genotype of carbapenemase contains KPC, GES, IMP, VIM, NDM and OXA-48 and is different in each country around the world [4]. For example, the main carbapenemase genotype in the United States has been KPC since this type was discovered in 1996, whereas in India, it has been NDM since it was discovered in 2008. Furthermore, the carbapenemase genotype of Europe is not limited to one type but is distributed among various genotypes, particularly, OXA-48, VIM and KPC [4,5]. The carbapenemase genotype of CPE isolated in Japan is different from that of other countries. It is IMP for most of the CPE genotypes isolated in Japan, and the

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isolation of other types is rare [6]. However, there are few reports on isolation frequency and long-term annual change in CPE in Japan. In addition, molecular epidemiological characteristics such as CPE to be isolated in Japan by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and plasmid replicon typing have not yet been revealed. Therefore, the purpose of this study was to clarify the isolation frequency and molecular epidemiological characteristics of the CPE, including all of the carbapenemase genotypes in Japan, of a primary care hospital over the four years of 2010–2013.

2. Materials and methods

2.1. Bacterial isolates

Specimens were collected from January 2010 to November 2013. A total of 4875 isolates of Enterobacteriaceae including *Escherichia coli* (3255 isolates), *Klebsiella pneumoniae* (870 isolates), *Klebsiella oxytoca* (179 isolates), *Enterobacter cloacae* (249 isolates), *Enterobacter aerogenes* (123 isolates) and *Proteus mirabilis* (199 isolates) were isolated from clinical specimens collected at Tenri Hospital (a 1001-bed primary care hospital in Nara, Japan). This study was approved by the ethics committee of Tenri Hospital (reference number 667), and informed consent was obtained from all patients.

2.2. Identification and antimicrobial susceptibility testing

All strains were identified with a MicroScan WalkAway 40 Plus System and the Neg Breakpoint Combo 6.23J measurement panel (both, Siemens Healthcare Diagnostics, Inc., Los Angeles, CA, USA). In addition, the strains identified as CPE were also identified by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry using MALDI Biotyper (Bruker Daltonik GmbH, Bremen, Germany). Antimicrobial susceptibility tests were performed with the broth microdilution method using a dry plate 'EIKEN' (Eiken Chemical Co., Ltd., Tokyo, Japan) that conforms to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Quality control was managed by using *E. coli* ATCC 25922.

2.3. Screening for CPE

Among all target strains, for those which showed a MIC of ≥8 mg/L to any of the 3rd- or 4th-generation cephalosporins (ceftriaxone [CTRX], ceftazidime [CAZ] and cefepime [CFPM]) and a MIC of ≥2 mg/L to any of the carbapenems (imipenem [IPM], meropenem [MEPM], and doripenem [DRPM]) in the antimicrobial susceptibility testing, an inhibition assay using sodium mercaptoacetate and a modified Hodge test using meropenem disks were performed [7,8].

2.4. Multiplex PCR amplification and sequencing for carbapenemase and ESBL

Six carbapenemase-producing genotypes, comprising the IMP-1, IMP-2, VIM, KPC, GES and NDM groups, and five ESBL-producing genotypes, comprising the SHV, TEM, CTX-M-1, CTX-M-2 and CTX-M-9 groups, were searched for using multiplex PCR [4,9–15]. The PCR products were amplified using an Applied Biosystems Veriti 96 Thermal Cycler (Life Technologies, Tokyo, Japan). Moreover, PCR direct sequencing using an Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific Inc., MA, USA) was performed for strains that were positive for one of the genotypes in the multiplex PCR assay. For sequence of IMP-1 group, forward primer 5′-ATGAGCAAGTTATCTG-3′ and reverse primer 5′-TTAGTTGCTTGGTTTTGATGG-3′ were designed.

2.5. Molecular epidemiological analysis

The CPE identified as being of the same species were characterized by MLST, PFGE and plasmid replicon typing. MLST of *K. pneumoniae* was performed according to the MLST scheme of the Pasteur Institute (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). That of *E. coli* was performed according to the Achtman MLST scheme (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli), and that of *E. cloacae* was performed according to the pubMLST scheme (http://pubmlst.org/ecloacae/). PFGE analysis was performed using the restriction enzyme *Xba*I (Roche Diagnostics, Inc., Tokyo, Japan) on a CHEF-Dr II system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) [16]. The reaction conditions included the following running parameters: 6.0 V/cm, 5.3 s initial switching time, 49.9 s final switching time and 20 h of total running time at 14 °C. The dendrograms were obtained using Fingerprinting Plus Version 1.12 (Bio-Rad Laboratories, Inc.) and calculated using Dice coefficients and the unweighted pair group method using arithmetic averages [16]. Isolates were considered to belong to the same PFGE group if their Dice similarity index was ≥80%. PCR-based replicon typing was performed on 837 strains as described by Carattoli et al. [17]. Eighteen primer pairs targeting the FIA, FIB, FIC, HI1, HI2, I1-Ic, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FII replicons were used in separate PCR reactions.

2.6. Characteristics of patients with detected CPE

We searched the medical records of the patients in whom CPE was detected to extract data on inpatient background (sex, age, number of hospitalization days, underlying disease, antimicrobial dosage history, therapeutic drugs and outcome) and investigated the characteristics of these patients.

3. Results

3.1. Prevalence of CPE isolates

The frequencies of isolation of CPE over 2010–2013 are shown in Table 1. The frequency of CPE isolation was 0.1% (1/1015) in 2010, 0.2% (2/1168) in 2011, 0.2% (2/1232) in 2012 and 0.8% (12/1460) in

Table 1
Number of isolates of CPE in each term.

Organism	Number of isolates (%)				Total
	2010	2011	2012	2013	
Klebsiella pneumoniae					
Collected isolates	184	206	213	267	870
CPE	1 (0.54)	1 (0.48)	1 (0.46)	5 (1.87)	8 (0.92)
Escherichia coli					
Collected isolates	664	770	833	988	3255
CPE	0 (0)	0 (0)	0 (0)	4 (0.40)	4 (0.12)
Enterobacter cloacae					
Collected isolates	43	63	60	83	249
CPE	0 (0)	0 (0)	0 (0)	2 (2.40)	2 (0.80)
Klebsiella oxytoca					
Collected isolates	39	43	44	53	179
CPE	0 (0)	1 (2.32)	0 (0)	0 (0)	1 (0.55)
Enterobacter aerogenes					
Collected isolates	37	31	23	32	123
CPE	0 (0)	0 (0)	1 (4.34)	0 (0)	1 (0.81)
Proteus mirabilis					
Collected isolates	48	55	59	37	199
CPE	0 (0)	0 (0)	0 (0)	1 (2.70)	1 (0.50)
Total					
Collected isolates	1015	1168	1232	1460	4875
CPE	1 (0.09)	2 (0.17)	2 (0.16)	12 (0.82)	17 (0.34)

CPE carbapenemase-producing Enterobacteriaceae.