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Surveillance Culture of Carbapenemase-Producing *Enterobacteriaceae* in a Tertiary-Care Hospital

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Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly being reported throughout the world, which is a significant problem for patient treatment and infection control. Carbapenem-resistance in *Enterobacteriaceae* is mainly due to carbapenem-hydrolyzing β-lactamase, which tends to spread through genetic mobile elements. Therefore, the detection of carbapenemase-producing *Enterobacteriaceae* (CPE) carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this study, we performed surveillance cultures for CPE in patients admitted to the hospital and evaluated the prevalence of CPE.
Methods: Stool cultures were obtained from a total of 228 patients at our tertiary-care hospital between March and May 2017. Stool specimens were inoculated on ChromID CARBA agar (bioMérieux, France) and incubated for 18-24 hours. Suspicious colonies with pink or bluish-green color were screened for CPE by the modified Hodge test (MHT) and carbapenemase inhibition test (CIT). We performed PCR to detect five carbapenemase genes, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA-48}.
Results: Among 228 isolates, seven were suspicious for CPE: four *Klebsiella pneumoniae*, one *Escherichia coli*, one *Enterobacter aerogenes*, and one *Serratia marcescens*. Two *K. pneumoniae* isolates showed positive reactions in both the modified Hodge test and inhibition test with phenylboronic acid. By PCR, bla_{KPC} was identified in these two *K. pneumoniae* isolates.
Conclusion: Our results showed a very low prevalence (2/228, 0.9%) of CPE in our tertiary-care hospital based on surveillance culture in a recent three month period. **(Ann Clin Microbiol 2018;21:8-11)**

Key Words: Carbapenemase-producing *Enterobacteriaceae*, Healthcare-associated infection, Infection control

INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly being reported in throughout the world, which is a significant problem for patient treatment and infection control [1,2]. CRE are also resistant to β-lactam, fluoroquinolone, aminoglycoside and trimethoprim/sulfamethoxazole, and the severe infections caused by CRE are very likely to cause high mortality and morbidity [2]. The risk factors for CRE include intensive care unit (ICU) hospitalization, tracheotomy, mechanical ventilation and antibiotic usage [3,4]. Carbapenem-resistance in *Enterobacteriaceae* is mainly due to carbapenem-hydrolyzing β-lactamase, which tends to spread through genetic mobile elements [5]. Therefore, the detection of carbapenemase-producing *Enterobacteriaceae* (CPE) carriers is particularly important for the prevention and epidemiological monitoring of these infections. Ambler classification class A β-carbapenemases such as *Klebsiella pneumoniae* carbapenemase (KPC); class B metallo-β-lactamases such as imipenemase (IMP), Verona integron-encoded metallo-β-lactamase (VIM), and New Delhi metallo-β-lactamase (NDM); and class D such as oxacillinase (OXA-48) are important carbapenemases [6,7]. The Korea Centers for Disease Control and Prevention (KCDC) reported 174 CPE isolates in hospitals with over 300 beds in Korea in 2014 [8]. In the KCDC report, the most common isolate was *K. pneumoniae* (59.8%), while OXA-232 was the most common carbapenemase gene (27.6%) based on the results of active surveillance of a hospital outbreak. The second most frequently de-

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tected gene was the KPC type (23.6%).

Although there have been several hospitals reporting CPE, there have not been cases of CPE acquired from a hospital-associated environment or outbreak at our institute until now. However, we might have missed the presence of colonized CPE in stool specimens because we did not perform surveillance cultures for CPE. Therefore, in this study, we performed surveillance cultures for CPE in patients admitted to our institute and evaluated the prevalence of CPE.

MATERIALS AND METHODS

Stool cultures were obtained from a total of 228 patients at a tertiary-care hospital from March to May 2017. There were 119 male and 109 female patients with mean age of 67 years (range 20-96). We carried out CPE culture using the remaining stool specimens from patients, which were requested for routine bacterial culture, *Clostridium difficile* culture, or vancomycin-resistant *Enterococcus* culture at the clinical microbiology laboratory. During this period, the isolates were consecutively recovered as one isolate per patient. Stool specimens were inoculated on ChromID CARBA agar (bioMérieux, Marcy l'Etoile, France) and incubated for 18-24 hours. Each isolate was identified by MicroScan Walkaway (Beckman Coulter, Brea, CA, USA) or Bruker Biotyper (Bruker Daltonics, Bremen, Germany) matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry systems. Suspicious colonies with pink or bluish-green color on ChromID CARBA agar (bioMérieux) were screened for CPE by the modified Hodge test (MHT) and carbapenemase inhibition test (CIT). CIT were performed using phenylboronic acid (PBA, Sigma, Korea) and ethylenediaminetetraacetic acid (EDTA, Sigma, Korea) to detect class A and class B carbapenemases, respectively. In addition, we performed PCR to detect the five carbapenemase genes bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and bla_{OXA-48} as previously described [9].

RESULTS AND DISCUSSION

Among 228 isolates, seven were suspicious for CPE: four *K. pneumoniae*, one *Escherichia coli*, one *Enterobacter* aerogenes and one *Serratia marcesens* (Table 1). The *E. coli* colonies had a pink color, while the other colonies were green. Two *K. pneumoniae* isolates (nos. 3 and 4) showed positive reactions for both the MHT and CIT with PBA. As a result, bla_{KPC} was identified in these two *K. pneumoniae* isolates. One *K. pneumoniae* isolate (no. 1) showed a negative MHT reaction but positive CIT with both PBA and EDTA. However, none of the five carbapenemase genes were detected by PCR. This isolate may hyperproduce AmpC β-lactamase with porin loss and reveal carbapenem resistance as previous studies [9,10]. *E. coli*, *E. aerogenes* and *S. marcesens* isolates were negative in the MHT and CIT, and PCR did not detect any carbapenemase genes in these isolates.

Two KPC-producing *K. pneumoniae* (nos. 3 and 4) isolates were obtained from stool specimens from an 82-year-old female with septic shock and a 37-year-old male with cerebral infarction, respectively. These patients required prolonged hospitalization in ICU for 28 days and 25 days, respectively. The two KPC-producing isolates were resistant to ampicillin/sulbactam, cefotaxime, cefepime, imipenem, meropenem, ertapenem, levofloxacin, and trimethrprim/sulfamethoxazole, but were suscep-

Table 1. Isolates suspicious for carbapenemase-producing *Enterobacteriaceae* on chromogenic agar

No. of isolate	Sex/age	Species	Colony	Modified Hodge test	Inhibition test (zone diameter, mm)			CPE gene
					MEM	PBA	EDTA	
1	M/71	*K. pneumoniae*	Green	−	13	22 (+)	17 (+)	ND
2	F/73	*K. pneumoniae*	Green	−	21	24 (w+)*	22 (−)	ND
3	M/37	*K. pneumoniae*	Green	+	**14**	**22 (+)**	16 (−)	***bla*$_{KPC}$**
4	F/80	*K. pneumoniae*	Green	+	**14**	**22 (+)**	14 (−)	***bla*$_{KPC}$**
5	M/84	*E. coli*	Pink	−	26	27 (−)	27 (−)	ND
6	M/68	*E. aerogenes*	Green	−	21	23 (−)	22 (−)	ND
7	M/74	*S. marcesens*	Green	−	27	28 (−)	28 (−)	ND

*Indicates a positive result when the size difference was 4 mm or more and weakly positive at 3 mm.
Abbreviations: MEM, meropenem; PBA, phenylboronic acid; EDTA, ethylenediaminetetraacetic acid; CPE, carbapenemase-producing *Enterobacteriaceae*; M, male; ND, not detected; F, female.

tible to colistin and tigecycline.

Jeong et al. [11] suggested that the prevalence and predominant genotypes of CPE in Korea showed hospital-specific differences such as epidemic presence, sporadic presence, and absence. Therefore, they suggested that CPE dissemination is at an early stage in Korea. There are two limitations in this study: the short period of hospital surveillance and the limited number of patients referred to the laboratory for stool culture. The limitation of this study was that the surveillance culture for evaluation the prevalence was not performed for all patients admitted in hospital or intensive-care unit.

In this study, our institute may be a sporadic presence hospital in this time. Our results indicated a very low prevalence (2/228, 0.9%) of CPE in a tertiary-care hospital based on surveillance culture. However, continuous monitoring and infection control for CPE should be performed to prevent transmission of this superbug.

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=국문초록=

카바페넴분해효소 생성 장내세균에 대한 감시배양

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배경: 카바페넴 내성 장내세균(Carbapenem-resistant *Enterobacteriaceae*, CRE)은 전세계적으로 점차 증가하고 있으며, 환자의 치료 및 감염관리에 매우 중요하다. CRE의 카바페넴 내성은 주로 이동성을 가진 카바페넴분해 효소 때문인 것으로 알려져 있다. 따라서, 카바페넴분해효소를 분비하는 장내세균(carbapenemase-producing *Enterobacteriaceae*, CPE)의 보균자 검출은 원내 감염의 예방 및 감시에 중요하다. 본 연구에서는 국내 1개 3차병원에서 CPE 감시배양을 시행하여 발생률을 조사하였다.

방법: 2017년 3월부터 5월까지 1개 3차병원에 내원한 총 228명 환자의 대변배양을 시행하였다. 대변은 ChromID CARBA agar (bioMérieux, France)에 접종하고, 18-24시간 배양하였다. CPE가 의심되는 집락에 대해서 modified Hodge test (MHT)와 carbapenemase inhibition test (CIT)을 시행하였고, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM} 및 bla_{OXA-48} 유전자에 대해서 PCR 및 염기서열을 분석하였다.

결과: CPE가 의심되는 균주는 228균주 중에서 7주로, *Klebsiella pneumoniae* 4주, *Escherichia coli* 1주, one *Enterobacter aeroginosa* 1주, *Serratia marcescens* 1주였다. *K. pneumoniae* 2주가 MHT와 CIT에 양성이었고, KPC 유전자가 검출되었다. 본 기관의 최근 3개월간 CPE 발생률은 0.9% (2/228)임을 알 수 있었다.

결론: CPE 감시 배양을 통해서 낮은 발생률(2/228, 0.9%)을 확인하였으며, 지속적인 감시배양이 필요할 것으로 사료된다.

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