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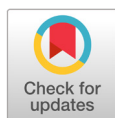
Environmental culture for carbapenemase-producing *Enterobacterales*

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Abstract

We conducted environmental cultures for carbapenemase-producing *Enterobacterales* (CPE) to evaluate the environmental contamination around patients with CPE. CPE was detected in the environmental cultures of four of the nine intensive care unit (ICU) inpatients with CPE. All four isolates were collected from sink surfaces in isolation rooms within the ICU. CPE isolates from the environment differed from those isolated from patients and had different carbapenemases. Even though CPE isolates from the environment of the ICU were not associated with CPE isolates from patients, the repeated isolation of CPE from sinks over several months is alarming.

Keywords: Carbapenem-resistant *Enterobacteriaceae* (*Enterobacterales*), Carbapenemase, Environment, Intensive care units



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The global prevalence of carbapenemase-producing *Enterobacterales* (CPE) has been increasing recently, posing a substantial threat to infection control [1,2]. In particular, the importance of environmental factors in CPE transmission has been recognized, and the potential for transmission through the environment has been demonstrated in outbreak cases [3]. Consequently, environmental management is important in CPE infection control.

Environmental culture involves collecting and culturing specimens from air, water, and environmental surfaces. When conducted properly, environmental culture can support epidemiological investigations and aid in determining the effectiveness of infection control measures; however, if conducted improperly, it can lead to wastage of clinical microbiology laboratory and infection control resources, and misleading data can lead to incorrect infection control measures. The purpose of environmental cultures is twofold: to monitor compliance with hygienic standards and detect the presence or absence of specific healthcare-associated infectious pathogens. The second is generally undertaken during outbreak investigations [4]. In cases involving carbapenem-resistant *Enterobacterales* (CRE), the Korean infection control guidelines recommend environmental culture testing in the event of an outbreak [5].

However, few studies have investigated environmental cultures for CPE in Korea [6,7]. In the present study, we conducted environmental cultures for CPE to evaluate the environmental contamination around patients with CPE.

Nine intensive care unit (ICU) inpatients with CPE isolated from clinical specimens referred for culture

between March 2017 and October 2017 were included. Clinical information such as sex, age, ward, medical department, comorbidities, hospitalization history, and clinical microbiology results were collected by reviewing electronic records. The specimens were collected from bed railings/controls, headboards, intravenous poles, call buttons, telephones, bedside tables, chairs, sinks, light switches, door handles, bathroom door handles, bathroom light switches, bathroom-assisted handles, toilet handles, bathroom sinks, and toilet seats. Additionally, specimens were collected from infusion pumps, monitor controls, monitor control touch screens, monitor cables, and ventilator controls. Culture for CPE detection was performed by incubating 10 µg meropenem disks in trypticase soy broth overnight followed by overnight incubation in MacConkey agar in accordance with the Centers for Disease Control and Prevention laboratory guidelines [8]. Identification of isolates and antimicrobial susceptibility testing were performed using the VITEK2 system (bioMérieux, Durham, NC, USA). RAPIDEC CARBA NP (bioMérieux, Marcy-l'Étoile, France) and Xpert Carba-R (Cepheid, Sunnyvale, CA, USA) tests were conducted to detect and genotype the carbapenemases.

CPE was detected in the environmental cultures of four of the nine cases (Table 1). All four isolates were collected from sink surfaces in isolation rooms within the ICU (Fig. 1). Environmental isolates were identified as *Serratia marcescens* (n = 2), *Enterobacter cloacae* (n = 1), and *Enterobacter aerogenes* (n = 1). All four isolates harbored *bla*_{NDM}. CPE isolates from the environment differed from those isolated from patients and had different carbapenemases. In all cases, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were detected at various environmental sites (data not shown). In Case 2, environmental cultures were conducted again after the source was disinfected, and CPE was not isolated.

Associations between water environments in hospitals and healthcare-associated infections have been reported [3,9]. Healthcare water environments, including potable water, faucets, sink surfaces, and wastewater drainage systems such as drains, sink/shower traps, toilets, and drainage pipes, can serve as reservoirs for nosocomial pathogens such as drug-resistant *Enterobacterales*, *Pseudomonas* spp., and *A. baumannii* [3]. This study found that sink surfaces were a major source of contamination by multidrug-resistant organisms, including CPE and *P. aeruginosa*. Similarly, previous reports have shown that sinks are a reservoir for CPE, are associated with CPE in patients, and are a transmission source [6,10,11].

Reports indicate that CPEs in the environment are associated with CPEs isolated from patients and may even cause outbreaks [6,12,13]. However, in this study, the carbapenemase genes of CPEs isolated from the environment differed from those of CPEs isolated from patients (the environmental isolates were all *bla*_{NDM}, and the patient isolates were *bla*_{KPC}), suggesting that the environmental and patient isolates were unrelated. Environmental cultures were performed in most cases shortly after isolation was initiated. Considering the timing of these procedures, environmental isolates were presumed to be present in the environment, regardless of the occurrence of CPE carriers.

This study has several limitations. The study included a small number of cases. In addition, the original study plan was to perform environmental cultures where the patient was staying when the CPE was isolated; however, this was possible only in one case. In other cases, the environmental culture was performed at the location where the patient was isolated after CPE isolation (isolation room in the ICU). This was due to the time required to identify the target cases, as it typically took more than 3 days to report CPE during the

study period, and we were unable to delay patient isolation and disinfection for research purposes because of the priority of patient care and infection control. Further studies are needed to demonstrate the horizontal transmission of *bla*_{NDM} among environmental isolates.

In the present study, we detected CPEs carrying *bla*_{NDM} in sinks in the ICU, which were not associated with the isolation of CPEs from patients. However, the repeated isolation of CPE from sinks over several months is alarming. More aggressive disinfection and environmental surveillance are needed to prevent contamination by CPE and other multidrug-resistant organisms in ICUs.

Table 1. Cases of carbapenemase-producing *Enterobacterales* isolated from environmental cultures in ICU

	Case 5	Case 6	Case 2	Case 9
Environmental isolate				
Identification	<i>Serratia marcescens</i>	<i>Serratia marcescens</i>	<i>Enterobacter cloacae</i>	<i>Enterobacter aerogenes</i>
Carbapenemase	NDM	NDM	NDM	NDM
Site	Sink surface	Sink surface	Sink surface	Sink surface
Date	2017-07-31	2017-08-25	2017-06-15	2017-10-11
Clinical isolate				
Identification	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
Carbapenemase	KPC	KPC	KPC	KPC
Specimen	Rectal swab	Rectal swab	Rectal swab	Rectal swab
Date	2017-07-28	2017-08-22	2017-06-12	2017-10-09
Clinical information				
Sex/Age (yr)	M/56	F/75	M/64	M/82
Diagnosis	Periampullary cancer	Sepsis, pneumonia	Aspiration pneumonia	Lymphoma, pneumonia
Department	General surgery	Nephrology	Pulmonology	Hematology
Admission	2017-07-24	2017-08-21	2017-05-10	2017-09-11

Abbreviations: ICU, intensive care unit; M, male; F, female.



Fig. 1. Overall view of the isolation room within the intensive care unit (A) and the sink (B) (arrow points to the location of the sink).

Ethics statement

This study was approved by the Institutional Review Board of the Ewha Womans University Mokdong Hospital (IRB No. EUMC 2016-11-026). The informed consent was waived for the nature of this study.

Conflicts of interest

Hae-Sun Chung has been an editor-in-chief of the *Annals of Clinical Microbiology* since January 2022. However, she was not involved in the review process of this article. No other potential conflict of interest relevant to this article was reported.

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