Microbiol. Biotechnol. Lett. (2022), 50(4), 584–591
http://dx.doi.org/10.48022/mbl.2208.08011
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

# Improving the Rapidity and Accuracy of Carbapenem-Resistant Enterobacteriaceae Detection by Shortening the Enrichment Duration

Miyoung Lee*

*Department of Biomedical Laboratory Science, Daejeon Institute of Science and Technology, Daejeon 35408, Republic of Korea*

Received: August 16, 2022 / Revised: October 9, 2022 / Accepted: October 28, 2022

**Identifying carbapenem-resistant Enterobacteriaceae (CRE) is necessary to prevent nosocomial CRE infection outbreaks. Here, a rapid identification method with reduced enrichment time was developed without compromising accuracy. A total of 49 rectal swabs requested for CRE screening at the Department of Diagnostic Medicine at Hospital B in Busan, Korea, were included in this study. Specimens were inoculated on MacConkey and CHROMID Carba media either directly or following enrichment for 3, 6, and 24 h in 100 µl trypticase soy broth containing an ertapenem disk. The enriched cultures were further inoculated on CHROMID Carba or MacConkey media containing an ertapenem disk. In total, 19 CRE and 5 carbapenem-intermediate Enterobacteriaceae isolates were obtained from the 49 swabs. Among the 19 CRE isolates, carbapenemase-producing Enterobacteriaceae constituted 13 strains. Moreover, of the 19 CRE isolates, 16 (81.25%) and 17 (88.24%) were identified from the direct cultures on MacConkey and CHROMID Carba media, respectively. After 3 h of enrichment, the proportions of the CRE identified in the media were: MacConkey medium, 16/19 (81.25%); CHROMID Carba medium, 17/19 (88.24%); and MacConkey medium containing an ertapenem disk, 17/19 (88.24%). The detection rates after 6 h of enrichment were the same for all three media (19/19 strains, 100%), whereas those after 24 h of enrichment were 21, 22, and 24 strains, respectively, but included false positives. These findings suggest that a 6-h enrichment before inoculation on the CHROMID Carba medium is optimal for the rapid and accurate detection of CRE in clinical samples.**

**Keywords:** **Carbapenem, resistance, Enterobacteriaceae, blakpc, CHROMID Carba medium**

## Introduction

Carbapenems are a class of antibiotics with extensive activity against gram-negative bacteria and some gram-positive bacteria; they are especially effective in the treatment of bacterial species that produce extended-spectrum β-lactamases and are commonly used for this indication [1]. Carbapenem antibiotics include imipenem (IMP), meropenem, doripenem, and ertapenem (ETP). Resistance to carbapenems is defined as tolerance to one or more of the antibiotics in this class [2]. Carbapenem-resistant Enterobacteriaceae (CRE) first emerged in the 1990s owing to misuse of carbapenems and have since become a serious public health threat worldwide, as they result in increased hospitalization periods, costs, and patient mortality rates [1, 3, 4]. In addition, according to the Antibiotic Resistance Threat Report by the Centers for Disease Control and Prevention (CDC), the prevalence of CRE infections in the US is 1.3 million cases per year, causing 1,100 deaths and costing approximately $130 million in medical expenses [4].

CRE can be divided into two groups based on their antibiotic resistance mechanisms: carbapenemase-producing CRE (CP-CRE) and non-carbapenemase-

***Corresponding author**
Phone: +82-42-580-6291, Fax: +82-42-580-6119
E-mail: hope@dst.ac.kr

producing CRE (non-CP-CRE) [5]. The primary mechanism of antibiotic resistance in CP-CRE is the production of carbapenem-degrading enzymes, whereas the mechanisms in non-CP-CRE include changes in the outer membrane proteins, overproduction of antibiotic-degrading enzymes (such as AmpC) and extended-spectrum β-lactamases, and production of run-off pumps [6–8].

In Korea, the first case of CP-CRE was reported in December 2010 [9, 10]. In 2017, the KCDC designated CRE infections as class III infectious diseases following the 2015–2016 epidemic. These were subsequently revised to class II infectious disease agents with a relatively high proliferation rate, and as of January 2020, patients with these infections are required to undergo isolation [11, 12]. To effectively manage CRE propagation, active surveillance is recommended to actively identify carriers at home and abroad [13, 14].

Therefore, screening tests are conducted to minimize CRE propagation through early identification of CRE carriers in all patients at admission, regardless of prior contact with a CRE-infected person [13, 14]. During a previous study, only 16.5% of CRE carriers developed an infection whereas the majority remained asymptomatic [15]. Active monitoring of CRE has been reported to reduce in-patient infections and mortality by enabling early initiation of antibiotic treatment [16].

Molecular detection of carbapenemase-producing genes using polymerase chain reaction (PCR), or DNA sequencing is highly sensitive and specific; however, the need for substantial expertise and expensive equipment as well as high diagnostic costs limit their widespread use in laboratories [17]. Therefore, this study aimed to shorten the enrichment time required by standard methods to reduce the overall cost of testing and encourage hospitals to implement regular screening.

## Materials and Methods

### Ethics

This study was performed in accordance with the ethical guidelines presented in the Declaration of Helsinki, and the research protocol was approved by the Institutional Review Board of the BHS Hanseo Hospital, Busan, Korea (IRB Number: CTS-19-005) prior to the start of the study. The requirement for informed consent was waived by the review board as all experimental data were obtained through routine patient care activities and all samples were anonymized, meaning there was no extra risk to patients or personal data required for participation.

### Specimen collection

CRE screening among high-risk patients, regardless of symptoms, was carried out in the Department of Diagnostic Medicine at Hospital B located in Busan, Korea, between mid-October and November 2017. Two rectal swabs were collected from each patient, transferred to the laboratory in transport medium (Asan, Korea), and cultures performed immediately thereafter.

### Culture method for clinical specimens

**Direct inoculation.** Of the two swabs placed in a single transport medium tube, one was used to directly inoculate both MacConkey (Asanpharm, Korea) and CHROMID Carba medium (bioMérieux, France) and the other was retained and preserved. The inoculated plates were incubated overnight at 35 ± 2℃, in ambient air (Fig. 1A).

**Enrichment culture.** The same sample swab that was used for direct inoculation was used to inoculate a culture tube containing trypticase soy broth. Rectal swabs were used for direct inoculation into a culture tube containing a trypticase soy broth (TSB; Difco Laboratories, USA) and a 10 μg ETP disk (BBL, USA) and were allowed enrichment times of 3, 6, and 24 h at 35 ± 2℃ (Fig. 1B).

The enriched cultures were adjusted to 0.5 McFarland according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and then diluted in isotonic sodium chloride solution to a final ratio of 1:10 [18]. The samples were inoculated using three different media conditions, MacConkey agar (with and without an ETP disk) and CHROMID Carba medium, and were cultured for 24 h at 35 ± 2℃ (Fig. 1C).

For isolation on McConkey medium with an ETP disk, 100 μl of the diluted bacterial suspension was thinly spread onto MacConkey medium using a cotton swab. After 3–5 min of drying, a 10 μg ETP disk was planted onto the inoculated media, followed by culture for 24 h (Fig. 1D). Among the colonies growing within a 21 mm radius around the ETP disk, single colonies that were suspected to be Enterobacteriaceae were selected for

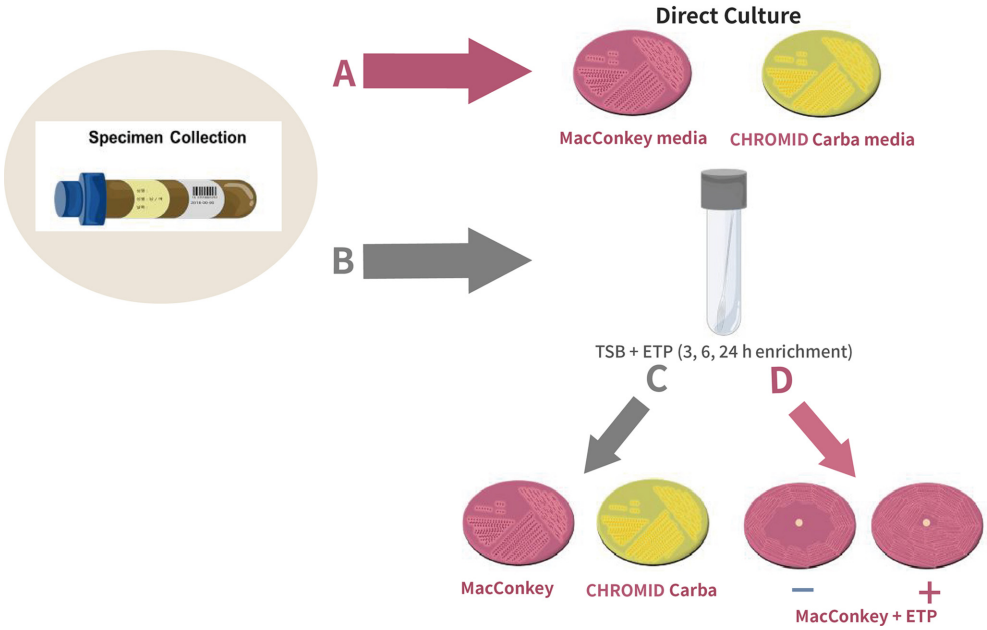


**Fig. 1. Schematic of the overall experimental procedure.** Rectal swab specimens were enriched in trypticase soy broth for 3, 6, and 24 h at 35 ± 2℃. The enriched cultures were then inoculated onto MacConkey medium, CHROMID Carba medium, and MacConkey medium containing an ETP disk. (A) Rectal swabs were used for direct inoculation on MacConkey and CHROMID Carba media. (B) Another batch of rectal swabs were placed in trypticase soy broth containing a 10 μg ETP disk and enriched for 3, 6, and 24 h at 35 ± 2℃. (C) The samples were then inoculated on MacConkey and CHROMID Carba media and cultured for 24 h at 35 ± 2℃. (D) For isolation on McConkey medium with an ETP disk, 100 μl of the diluted bacterial suspension was thinly spread onto MacConkey medium using a cotton swab. After 3-5 min of drying, 10 μg ETP disk was planted onto the inoculated media, followed by culture for 24 h.

species identification and antimicrobial susceptibility testing [19].

### Bacterial isolates and antimicrobial susceptibility testing

Clinical isolates were identified by using standard microbiological procedures and VITEK-2 (bioMérieux). The identification of all isolates was confirmed by 16S rDNA sequencing [20].

Susceptibilities to the following antimicrobials were determined using VITEK 2 AST N224 cards (Vitek; bioMérieux): amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), ampicillin (AMP), cefazolin (CFZ), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), amikacin (AMK), gentamicin (GEN), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), tigecycline (TGC), colistin (CST), ETP, and IMP. In the case of IMP, ETP, and meropenem resistance, zone diameter was confirmed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Becton Dickinson, USA). Strains that demonstrated resistance or sensitivity to any one of the three carbapenem antibiotics tested were classified as resistant and sensitive strains, respectively. The antibiotic susceptibility tests and minimum inhibitory concentration (MIC) breakpoint interpretations were performed according to CLSI guidelines [18]. Colistin susceptibility was determined by the broth microdilution method with freshly prepared cation-adjusted Mueller-Hinton broth, following the CLSI guidelines [18].

### Carbapenemase differential test

The carbapenem inactivation tests were performed according to the methods of Tsai *et al.*, with some modifications [21]. Briefly, an inoculation loop was used to resuspend isolated CRE colonies in two culture tubes containing 2 ml of TSB and either a 10 μg meropenem disk (BBL), only (mCIM), or supplemented with 5 mM EDTA (eCIM). Both tubes were incubated at 35℃ for 4 h ± 15 min. EDTA addition to the eCIM test results in

the chelation of metal ions necessary for metallo-β-lactamase function. Strains that grow in mCIM produce carbapenemases which allow the growth of the reference strain. mCIM positive isolates were then tested using eCIM. Growth of the reference strain in both tests indicated the production of serine-based carbapenemases; reference strain growth in mCIM, but not in eCIM indicated the production of metallo-based carbapenemases. The carbapenemases differential test was carried out as follows. The meropenem disks were transferred to Mueller-Hinton agar inoculated with a carbapenem-susceptible *Escherichia coli* ATCC 25922 strain and the plates were incubated at 35℃ for 16 to 20 h [21]. Following overnight incubation, the zones of inhibition were measured to determine whether meropenem had been hydrolyzed (growth of the indicator organism close to the disk) or was still active (a large zone of inhibition around the disk) [22].

Inhibition zone diameters were measured and interpreted according to CLSI guidelines [20]. Briefly, for mCIM, a 6–15 mm zone diameter or pinpoint colonies within 16–18 mm of the disk indicated the presence of active carbapenemases. The eCIM results, in contrast, were interpreted relative to a positive mCIM result for the respective isolate. The presence of metallo-β-lactamases was indicated if a zone diameter increase of ≥ 5 mm for eCIM was observed compared with that for mCIM.

**Detection of resistance genes.** PCR was performed for the detection of the carbapenemase genes ($bla_{KPC}$, $bla_{KPC-2}$, $bla_{IMP-1}$, $bla_{VIM-2}$, $bla_{NDM}$, $bla_{GES}$, and $bla_{OXA-48}$-like) in *Klebsiella pneumoniae* strains as previously described [23]. The primer sequences and amplicon sizes are indicated in Table 1. Briefly, for each reaction, 5 μl DNA extract was added to a reaction mixture comprising 0.7 U of Taq DNA polymerase (Doctor protein INC, Korea), 10× Taq buffer (2.5 μl) (Doctor protein INC), 10 mM dNTP mix (0.5 μl), 10 pmol of each primer, and distilled water added to obtain a 25 μl reaction volume. The reaction cycling conditions were as follows: 95℃ for 5 min, followed by 35 cycles of 95℃ for 30 s, 45–58℃ for 30 s, and 72℃ for 90 s (Table 1). A final extension step of 72℃ for 10 min was then carried out. The amplicons were visualized by performing 1% agarose gel electrophoresis and were then purified using a standard amplicon purification kit (Thermo Scientific, USA). The purified products were sequenced using a 3730xl DNA analyzer (Applied Biosystems, Weiterstadt, Germany), and the nucleotide sequences were analyzed using the

**Table 1. β-lactamase primers for PCR identification of resistance determinants.**

| Gene | Primer | Sequence (5′-3′) | Amplicon size (bp) | Annealing temperature (℃) |
|---|---|---|---|---|
| VIM | VIM_F | ATTGGTCTATTTGACCGCGTC | 780 | 58 |
| | VIM_R | TGCTACTCAACGACTGAGCG | | |
| IMPase | IMP_F | CATGGTTTGGTGGTTCTTGT | 488 | 45 |
| | IMP_R | ATAATTTGGCGGACTTTGGC | | |
| NDM | NDM_F | TGGAATTGCCCAATATTATGC | 813 | 45 |
| | NDM_R | TCAGCGCAGCTTGTCGGCCATGC | | |
| OXA-48 | OXA-48_F | TTGGTGGCATCGATTATCGG | 743 | 45 |
| | OXA-48_R | GAGCACTTCTTTTGTGATGGC | | |
| GES | GES_F | ATGCGCTTCATTCACGCAC | 863 | 56 |
| | GES_R | CTATTTGTCCGTGCTCAGGA | | |
| KPC-2 | KPC-2_F | ATGTCACTGTATCGCCGTCT | 893 | 52 |
| | KPC-2_R | TTTTCAGAGCCTTACTGCCC | | |
| KPC | Kpc_F | CTGTCTTGTCTCTCATGGCC | 795 | 52 |
| | Kpc_R | CCTCGCTGTGCTTGTCATC | | |

VIM, Verona integron-encoded metallo-β-lactamases; IMPase, imipenemase; NDM, New Delhi metallo-β-lactamase; OXA-48, oxacillinase; GES, Guiana extended-spectrum; KPC, *Klebsiella pneumoniae* carbapenemase.

27. Jeong S, Kim JO, Jeong SH, Bae IK, Song W. 2015. Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in gram-negative clinical isolates. *J. Microbiol. Methods.* **113**: 4-9.

Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Statistical analysis.** Receiver-Operating Characteristic (ROC) curve analysis was used for determining the sensitivity and specificity of carbapenem-resistant Enterobacteriaceae (CRE) detection from different samples.

## Results

### Specimen characteristics

Twenty-four isolates were obtained from the forty-nine rectal swabs through the methods of inoculation following enrichment and direct inoculation. Of these, 19 were CRE strains and 5 were carbapenem-intermediate Enterobacteriaceae (CIE). Out of the 19 CRE strains isolated, 13 were CP-CRE. The identified CRE strains included sixteen (84.2%) isolates of *K. pneumoniae*, two of (10.5%) *E. coli*, and one of (5.3%) *Klebsiella aerogenes* isolate (Fig. 2A–C). The CRE-infected patients had a mean age of 78.5 years, and 11 out of 19 were male.

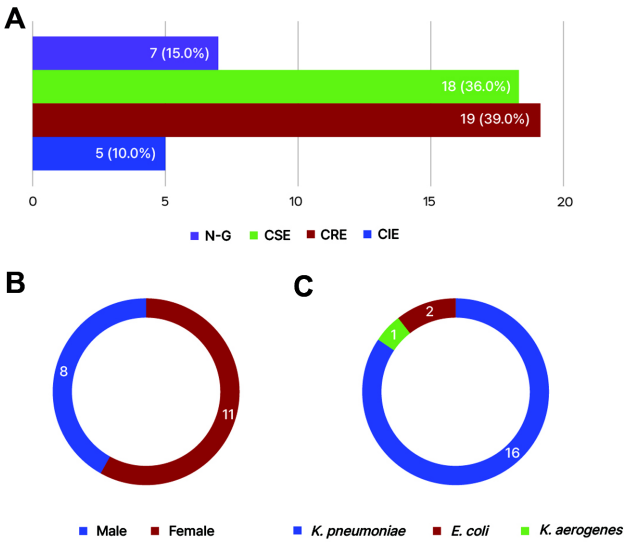


**Fig. 2. Patient characteristics. Results are based on the direct inoculation method.** (A) Bacterial strains were identified according to their antimicrobial susceptibilities: 19 carbapenem-resistant Enterobacteriaceae (CRE) strains, 18 carbapenem-sensitive Enterobacteriaceae (CSE) strains, 5 carbapenem-intermediate Enterobacteriaceae (CIE) strains, and 7 no growth (N-G) strains. (B) Distribution of all strains according to patient gender. (C) Distribution of the genotypes of the CRE isolates (n = 19).

### CRE detection rate

Following direct culture, 16/19 (81.25%) and 17/19 (88.24%) CRE strains were isolated from MacConkey and CHROMID Carba media, respectively. Similarly, after 3 h of enrichment, 16/19 (81.25%) and 17/19 (88.24%) CRE strains were isolated on MacConkey and CHROMID Carba media, respectively, whereas on the MacConkey medium containing an ETP disk, 17/19 strains (88.24%) were isolated. After 6 h of enrichment, the detection rates were the same for all three media (19/19 strains, 100%). However, after 24 h of enrichment, 21, 22, and 24 strains were isolated on MacConkey medium, CHROMID Carba medium, and MacConkey medium containing an ETP disk, respectively.

The number of positive cultures obtained following 24 h of enrichment in MacConkey medium (n = 21), CHROMID Carba medium (n = 22), and MacConkey medium containing an ETP disk (n = 24) was higher than that obtained following 6-h enrichment (n = 19, 19, and 19, respectively). The sensitivity and specificity of CRE identification were higher after 6 h of enrichment than with direct culture or enrichment for 3 or 24 h (Fig. 3). Therefore, the additional strains that were isolated after 24 h of enrichment were regarded as false positives and they were removed with carbapenemases treatment. It is possible that the concentration of antibiotics in the medium had been reduced over time, which would have allowed the growth of non-CRE.

Among the 24 strains, 19 were identified as CRE, and the false positive rate of 24 h enrichment culture was 9.52% (MacConkey agar), 13.64% (CHROMID Carba), and 20.83% (MacConkey medium containing an ETP disk). On CHROMID Carba agar, the specificity was 1.07 after direct inoculation, and 1.06, 1.00, and 0.90 when inoculated following 3 h, 6 h, and 24 h of enrichment cultures, respectively (Fig. 3). On MacConkey medium containing an ETP disk, the specificity was 1.07 after 3 h inoculation, and 1.00 and 0.90 when inoculated following 6 h and 24 h enrichment cultures, respectively.

### Antimicrobial susceptibility testing

MIC results for all the isolated strains showed that 19 strains were resistant to IMP and ETP, whereas five strains were ETP-sensitive or -intermediate. In IMP-resistant strains, the MIC was 4 μg/ml.

Disk diffusion assay using IMP, ETP, and meropenem

http://dx.doi.org/10.48022/mbl.2208.08011

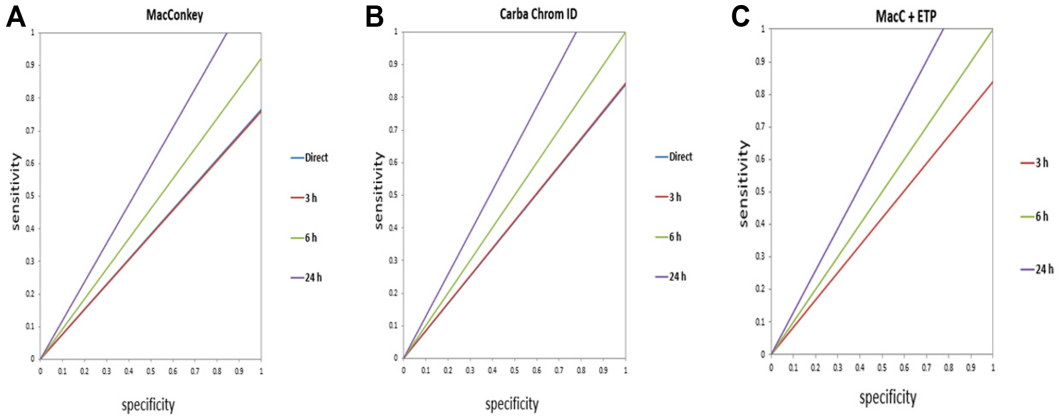


**Fig. 3. Receiver-Operating Characteristic (ROC) curve. ROC curve for determining the sensitivity and specificity of carbapenem-resistant Enterobacteriaceae (CRE) detection from different samples.** Inoculating with 6- and 24-h-enriched cultures resulted in higher specificity and sensitivity of CRE detection. (A) MacConkey medium. (B) CHROMID Carba medium. (C) MacConkey agar with an ertapenem disk. The Direct line of Fig. 3A and 3B overlaps the 3 h-enriched cultures line.

revealed resistance in 19 strains and sensitivity in five strains. Broth microdilution revealed that, based on the MIC values, 19 strains showed resistance, and five strains were susceptible to all carbapenem antibiotics tested.

Antimicrobial susceptibility testing showed that the 19 strains were resistant to penicillin, most cephalosporins, carbapenems, and monobactams. The tolerance rates to GEN, TGC, and SXT were 50%, 48%, and 17%, respectively. No strain was found to be resistant to AMK (Table 2).

### Carbapenemase (class A, B, D) differential test

Table 2 shows the results of carbapenemase differential testing. Of the 24 strains tested for eCIM and mCIM, 13 strains were mCIM-positive indicating carbapenemase production in the isolates.

### Detection of carbapenemase genes

Among the 19 CRE isolates, 13 showed positivity for the KPC-2 gene of which 12 were in. *K. pneumoniae* and one was in *E. coli*. However, no strain was confirmed to carry $bla_{IMP}$, $bla_{VIM}$, $bla_{NDM}$, $bla_{GES}$, and $bla_{OXA-48}$.

## Discussion

CRE infections are associated with high medical expenses and poor prognosis because their resistance to first-line antibiotic treatment limits therapeutic options [24]. Therefore, understanding the mechanism of antibiotic resistance in bacteria is extremely important for infection control and epidemiological studies. Therefore, a quick and effective detection method for CRE strains is essential as a countermeasure to avoid therapeutic failure as well as to prevent and control the spread of these multidrug-resistant bacteria. Here, the average age of the participants was 78.5 years which is in line with previous reports of CRE infections being more common among older populations [25]. The Korea Center for

**Table 2. Antimicrobial susceptibilities of carbapenem-resistant Enterobacteriaceae isolates and phenotypes.**

| Isolate (N) | Susceptibility n (%) of isolates | | | | | | Carbapenemase detection test n (%) | | Gene type n (%) |
|---|---|---|---|---|---|---|---|---|---|
| | FEP | AMK | GEN | TGC | SXT | CST | eCIM | mCIM | KPC-2 |
| CRE strains (19) | 0 (0) | 18 (94.7) | 7 (36.8) | 11 (57.9) | 3 (15.8) | 19 (100) | 0 | 13 (68.4) | 13 (68.4) |
| CIE strains (5) | 2 (40%) | 5 (100) | 2 (40) | 5 (100) | 5 (100) | 5 (100) | 0 | 0 | 0 |

CRE, carbapenem-resistant Enterobacteriaceae; CIE, carbapenem-intermediate Enterobacteriaceae; KPC-2, *Klebsiella pneumoniae* carbapenemase; FEP, cefepime; AMK, amikacin; GEN, gentamicin; TGC, tigecycline; SXT, trimethoprim-sulfamethoxazole; CST, colistin; eCIM, EDTA-modified carbapenem inactivation method; mCIM, modified carbapenem inactivation method.

Disease Control and Prevention (KCDC) reported that most CRE infections occur in general hospitals. This trend may also be indicative of a potential increase in CRE infection rates in convalescent hospitals [24].

Therefore, rapid and accurate identification of CRE in these settings is important to implement infection control measures, which include isolation of patients with CRE infections. The general methods of CRE detection used in the diagnosis of CRE infections include the disk diffusion test, broth microdilution method, and using automated systems. However, isolating CRE according to the CLSI guideline takes an average of 4 to 5 days and slow diagnosis increases the burden on patients, caregivers, and medical personnel. As a prompt infection control strategy, the rapid detection of multidrug-resistant bacteria by identifying asymptomatic carriers is expected to reduce the spread of multidrug-resistant bacteria.

Rapid molecular methods, such as Xpert Carba-R (Cepheid, USA), can be directly implemented for detecting *K. pneumoniae* carbapenemases, New Delhi metallo-β-lactamase, Verona integron-encoded metallo-β-lactamase, imipenemase, and oxacillinase genes [26]. However, since PCR apparatuses are not readily available in convalescent and general hospitals, rapid and accurate bacterial detection through microorganism culture-based methods would be extremely useful if made widely available. Thus, this study aimed to investigate the benefits of a shortened enrichment time for CRE bacterial cultures compared with that of the direct culture, which is the current standard, for the accurate and rapid detection of CRE. This method was tested using 49 swabs and yielded 24 isolates (CRE:19, CIE:5) from a general hospital in Korea.

In this study, before bacterial identification, bacterial suspensions were first enriched in an enrichment medium containing an ETP disk for 3 h, 6 h and 24 h at 35 ± 2℃, followed by inoculation of the enriched bacterial suspension on MacConkey agar, CHROMID Carba media, on the MacConkey medium containing an ETP disk and incubation for 24 h at 35 ± 2℃.

Previous studies have shown that the number of CRE positive results increases when BHI Broth of higher concentrations is used [27]. However, as the growth of intestinal bacteria exponentially increases after 1 h, reducing the enrichment time might be useful to shorten the overall bacterial detection process. For this purpose, different enrichment times of 3, 6, and 24 h were tested.

This study revealed that 6 h of enrichment in TSB resulted in higher sensitivity and specificity of CRE detection compared with the 24 h of direct culture, which corresponds to the average duration of a bacterial growth cycle. Furthermore, 3 h of enrichment resulted in low sensitivity, whereas 24 h of enrichment led to low specificity. The results also showed that although direct culture in MacConkey or CHROMID Carba media may accelerate diagnosis, it reduced the accuracy of CRE detection.

We acknowledge that this study had several limitations. First, this experiment was conducted using only 49 rectal swab samples. Future studies with larger sample sizes should be conducted. strains carrying the KPC-2 gene were selected as representatives of the isolated carbapenemase-producing Enterobacteriaceae (CPE) strains; thus, the results need to be verified using strains carrying other CPE genes. Second, the identification of false positive CRE strains in 24-hour enrichment cultures seems to have been caused by a decreased titer of antibiotics over time. In this regard, antibiotic concentration tests should be accompanied.

Future studies should develop an effective infection management strategy following the identification of CRE.

In conclusion, in this study, an improved method to detect CRE with high rapidity and accuracy by shortening the enrichment time was successfully developed. Since CRE is of great concern in a clinical setting, this study provides valuable insight into the detection of CRE in rectal swab samples.

## Author Contributions

MYL performed the experiments, analyzed the data, and wrote the manuscript.

## Acknowledgments

This research was supported by the BHS Hanseo Hospital in 2019.

## Conflict of Interest

The authors have no financial conflicts of interest to declare.

## References


1. European Centre for Disease Prevention and Control (ECDC). 2019. Carbapenem-resistant Enterobacteriaceae, second update - 26 September 2019, pp. 1-10. ECDC, Stockholm.
2. Centers for Disease Control and Prevention. 2015. FAQs about choosing and implementing a CRE definition. Atlanta: Centers for Disease Control and Prevention. Available from https://www.cdc.gov/hai/organisms/cre/definition.html. Accessed Aug. 28, 2019.
3. Marston HD, Dixon DM, Knisely JM, Palmore TN, Fauci AS. 2016. Antimicrobial resistance. *JAMA* **316**: 1193-1204.
4. Centers for Disease Control and Prevention (CDC). 2019. Antibiotic resistance threats in the United States, 2019, pp. 73. CDC. Atlanta (GA).
5. McCann E, Srinivasan A, DeRyke CA, DePestel DD, Murray J, Gupta V. 2018. Carbapenem-nonsusceptible gram-negative pathogens in ICU and non-ICU settings in US hospitals in 2017: A multicenter study. *Open Forum Infect. Dis.* **5**: ofy241.
6. Potter RF, D'Souza AW, Dantas G. 2016. The rapid spread of carbapenem-resistant Enterobacteriaceae. *Drug Resist. Updat.* **29**: 30-46.
7. Iovleva A, Doi Y. 2017. Carbapenem-resistant Enterobacteriaceae. *Clin. Lab. Med.* **37**: 303-315.
8. Lee HJ, Lee DG. 2018. Carbapenem-resistant Enterobacteriaceae: recent updates and treatment strategies. *J. Korean Med. Assoc.* **61**: 281-289.
9. Kim JO, Song SA, Yoon EJ, Shin JH, Lee H, Jeong SH, *et al.* 2017. Outbreak of KPC-2-producing Enterobacteriaceae caused by clonal dissemination of *Klebsiella pneumoniae* ST307 carrying an IncX3-type plasmid harboring a truncated Tn4401a. *Diagn. Microbiol. Infect. Dis.* **87**: 343-348.
10. Jeong SH, Kim HS, Kim JS, Shin DH, Kim HS, Park MJ, *et al.* 2016. Prevalence and molecular characteristics of carbapenemase-producing Enterobacteriaceae from five hospitals in Korea. *Ann. Lab. Med.* **36**: 529-535.
11. Korea Centers for Disease Control & Prevention (KCDC). 2019. Infectious diseases surveillance yearbook, 2019, pp. 38. Report No.: 11-1352159-000048-10. KCDC, Cheongju.
12. Korea Centers for Disease Control & Prevention (KCDC). Infectious disease portal. 2020. Available from: http://www.cdc.go.kr/nptbiz/npp/ist/simple/simplePdStatsMain.do. Accessed Mar. 7, 2020.
13. Korea Centers for Disease Control & Prevention (KCDC). 2018. Healthcare associated infection control guideline: VRSA/CRE. pp. 38-51. Report No.: 11-1352159-00832-10. KCDC, Cheongju.
14. Centers for Disease Control and Prevention (CDC). Facility guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). 2015. Available from: https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf. Accessed Mar. 7, 2020.
15. Tischendorf J, de Avila RA, Safdar N. 2016. Risk of infection following colonization with carbapenem-resistant Enterobacteriaceae: A systematic review. *Am. J. Infect. Control.* **44**: 539-543.
16. Ho KW, Ng WT, Ip M, You JH. 2016. Active surveillance of carbapenem-resistant Enterobacteriaceae in intensive care units: Is it cost-effective in a nonendemic region? *Am. J. Infect. Control.* **44**: 394-399. https://doi.org/10.1016/j.ajic.2015.10.026
17. Poirel L, Nordmann P. 2015. Rapidec Carba NP test for rapid detection of carbapenemase producers. *J. Clin. Microbiol.* **53**: 3003-3008.
18. Clinical and Laboratory Standards Institute. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Wayne, Pennsylvania.
19. Simner PJ, Martin I, Opene B, Tamma PD, Carroll KC, Milstone AM. 2016. Evaluation of multiple methods for detection of gastrointestinal colonization of carbapenem-resistant organisms from rectal swabs. *J. Clin. Microbiol.* **54**: 1664-1667.
20. Copur-Cicek A, Ozad Duzgun A, Saral A, Sandalli C. 2014. Determination of a novel integron-located variant (blaOXA-320) of Class D β-lactamase in *Proteus mirabilis*. *J. Basic Microbiol.* **54**: 1030-1035.
21. Tsai YM, Wang S, Chiu HC, Kao CY, Wen LL. 2020. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. *BMC Microbiol.* **20**: 315.
22. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. 2015. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One* **10**: e0123690.
23. Jeong S, Kim JO, Jeong SH, Bae IK, Song W. 2015. Evaluation of peptide nucleic acid-mediated multiplex real-time PCR kits for rapid detection of carbapenemase genes in gram-negative clinical isolates. *J. Microbiol. Methods* **113**: 4-9.
24. Korea Centers for Disease Control and Prevention. Distribution of carbapenem-resistant Enterobacteriaceae (CRE) in Korea, 2018-2020. Available from https://www.kdca.go.kr/upload_comm/syview/doc.html?fn=163238757067000.pdf&rs=/upload_comm/docu/0034/. Accessed Mar. 11, 2022.
25. Seo SM, Jeong IS. 2020. External validation of carbapenem-resistant Enterobacteriaceae acquisition risk prediction model in a medium sized hospital. *J. Korean Acad. Nurs.* **50**: 621-630.
26. Kim D-K, Kim HS, Pinto N, Jeon J, D'Souza R, Kim YS, *et al.* 2016. Xpert CARBA-R assay for the detection of carbapenemase-producing organisms in intensive care unit patients of a Korean tertiary care hospital. *Ann. Lab. Med.* **36**: 162-165.
27. Peretz A, Pastukh N, Isakovich N, Koifman A, Brodsky D, Mizrahi H, *et al.* 2016. Efficacy of an enrichment media for increasing threshold for carbapenem-resistant Enterobacteriaceae screening. *J. Clin. Lab. Anal.* **30**: 563-566.