Prevalence and acquisition pathway identification of carbapenemase-producing *Enterobacterales* colonization

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Prevalence and acquisition pathway identification of carbapenemase-producing *Enterobacterales* colonization

Directed by Professor Young Goo Song

Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medical Science.

Kyoung Hwa Lee

December 2022

This certifies that the Doctoral Dissertation
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December 2022

ACKNOWLEDGEMENTS

I would like to thank my dissertation advisor, Professor Young Goo Song, who has been a valuable advisor and mentor to me. His support and encouragement as conceptualization of study have been immeasurable support for me during this process. I would also like to thank Professor Seok Hoon Jeong for his critical advice and feedback. His perspective on this research has helped me strengthen my work. Professor Dokyun Kim, Doctor Jun Sung Hong, and Soon Young Park supported the experiments for a long time, and this study could not have been completed without their support. I would also like to express my gratitude to them.

Furthermore, I would like to thank Professor Sang Hoon Han for always supporting my work and giving me insights into the research. In addition, I would like to thank the thesis committee members, Professor Young Hwa Choi, Professor Jun Yong Choi, and Professor Su Jin Jeong, for their critical opinions and valuable advice. Their review of the study has improved the research.

Finally, I thank all my family members. In particular, I am grateful to my two sons, Dong Youn, Sung Youn, and my husband, Tae Hoon Kim, for their sincere support.

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ABSTRACT

Prevalence and acquisition pathway identification of carbapenemase-producing *Enterobacterales* colonization

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Carbapenemase-producing *Enterobacterales* (CPE) are global concerns in treatment and infection control. In addition, the number of CPE outbreaks in hospitals is increasing despite the strengthening of contact precautions. Since 2018, active surveillance in the Emergency Room (ER) and universal surveillance in the Intensive Care Unit (ICU) using stool specimens have been implemented in our hospital. This study aimed to confirm the prevalence and transition rate of CPE infection from stool surveillance culture and to identify the acquisition pathway of CPE by sequencing analysis.

This is a longitudinal review of patients with stool surveillance cultures at a tertiary center in Seoul, South Korea, from July 2018 to June 2020. Pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and whole genome sequencing (WGS) were performed for carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* strains.

Among 1,620 patients who had undergone stool CPE surveillance cultures, only

7.11% of active surveillance at the ER and 4.46% of universal surveillance in the ICU were stool CPE positive. The transition rates from stool carriers to clinical CPE infections were 29.41% in the ER and 31.37% in the ICU. However, it was significantly high (55.0%) in the initial stool CPE-negative ICU patients.

Among the initial stool CPE-positive patients, hypertension (61% *vs.* 92.3%, P = 0.004), malignancy (28.8% *vs.* 53.8%, P = 0.027), and mechanical ventilation (25.4% *vs.* 53.8%, P = 0.011) were significant risk factors for clinical CPE infection. In the multivariate analysis, underlying hypertension (odds ratio = 5.18 [95% confidence interval, 1.93 – 8.43], P = 0.009) and malignancy (OR = 2.94 [95% CI, 1.55 - 7.96], P = 0.038) were found to be significant risk factors for clinical CPE infection from stool carriers. Molecular typing revealed that sequence type (ST) 307 and ST 395 were dominant in *K. pneumoniae*, and ST 410 was dominant in *E. coli* isolates. In addition, this study showed a high prevalence of *K. pneumoniae* $bla_{\text{KPC-2}}$ ST 307 of stool CPE.

In conclusion, active surveillance showed a higher detection rate than universal stool CPE screening. One-third of stool carriers ultimately developed clinical CPE infection. In addition, ST 307 and ST 395 were dominant in carbapenemase-producing *K. pneumoniae*. Thus, even if some genotypes are transmitted from outside the hospital, it can be confirmed that a particular strain is continuously dominant in the hospital. Therefore, in-hospital ICU surveillance, as well as active surveillance to block acquisition from outside, should be performed for the early detection of stool carriers and for early intervention in severe patients. Thus, close monitoring is needed to prevent propagation of CPE infection.

Keywords: Carbapenemase-producing *Enterobacterales*; active surveillance culture; risk factor; pulsed-field gel electrophoresis; whole genome sequencing

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I. INTRODUCTION

Since the discovery of penicillin in 1928, numerous antibiotics have been developed, and many bacterial pathogens have continued to acquire resistance with advances in the development of antimicrobial agents. Thus, in recent decades, a few novel antimicrobial agents have been developed, and antimicrobial resistance is increasing in community and hospital transmission [1,2]. These patterns demonstrate concerns, with optimal treatment becoming difficult and finally resulting in poor clinical outcomes.

Carbapenem-resistant *Enterobacterales* (CRE), a representative example of multi-drug resistant (MDR) gram-negative bacteria, are global health concerns and have been increasing rapidly in recent years, which is a major concern in treatment and infection control [3-6]. The prevalence of CRE in South Korea is reported to be less than 1%;

however, it has been growing exponentially every year since 2015, and the number of carbapenemase-producing *Enterobacterales* (CPE) outbreaks has also increased rapidly. Therefore, as a countermeasure against the exponentially increasing CPE infection, control techniques, such as antibiotic stewardship, as well as standard precautions, including hand hygiene, and contact precautions are necessary [3,7,8].

In addition to these actions, the importance of screening through risk assessment is emphasized. Early detection and subsequent isolation of CPE carriers are essential for preventing nosocomial transmission [9-12]. The Centers for Disease Control and Prevention recommends perirectal screening and isolation for patients colonized or infected with CPE. These strategies, combined with contact precautions, were found to be effective in reducing CPE transmission [13].

Furthermore, many studies have analyzed the risk factors for CPE infection or colonization, but the analysis of risk factors for progression to clinical CPE infection from stool carriers is limited [14-17]. Among many stool CPE colonizers, we wanted to determine which patients developed clinical CPE infection and the proportion of stool colonizers that progressed consecutively into developing clinical infection during surveillance cultures.

Therefore, this study aimed to confirm the prevalence of CPE colonization from stool screening cultures, assess the transition rate of clinical CPE infection from stool carriers, and identify the acquisition pathway of CPE. The epidemiological and clinical characteristics of stool CPE colonizers were analyzed to identify the risk factors for clinical CPE infection transition., The results of this study is expected to play an important role in treatment and infection control of CPE by blocking potential CPE propagation or outbreak.

II. MATERIALS AND METHODS

1. Stool surveillance culture and collection of clinical CPE strains

Patients with stool CPE surveillance culture at the Gangnam Severance Hospital, a tertiary center in Seoul, South Korea, from July 2018 to June 2020, were enrolled in this study. During this period, 1,620 patients over the age of 18 years underwent stool CPE cultures, and 85 patients were initially identified as CPE positive for stool culture.

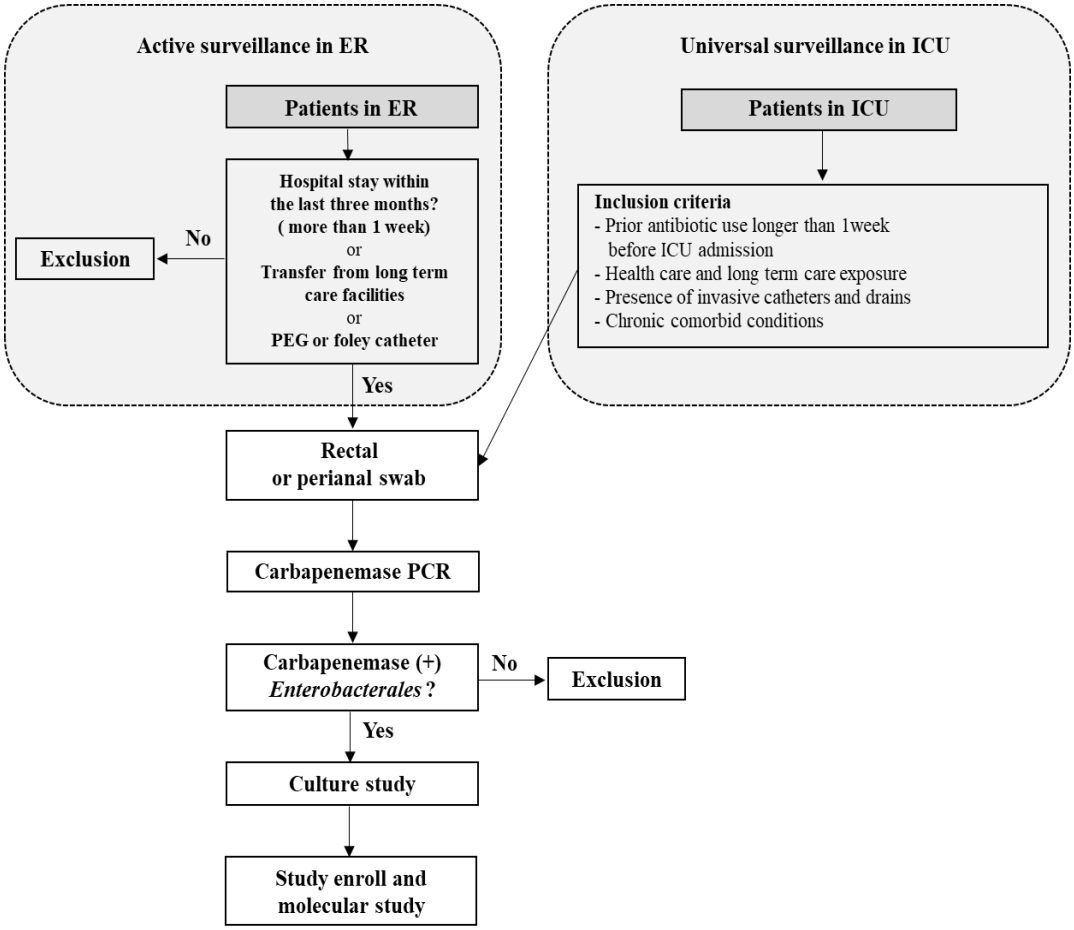


Figure 1. Flow chart of surveillance culture for carbapenemase-producing *Enterobacterales*

Initially, 1,535 patients were stool CPE negative, but eventually 42 patients showed CPE positive results on subsequent stool cultures. This confirmed that stool CPE-positive patients were enrolled in the research analysis. Stool CPE surveillance cultures were performed using two different routes. First, when patients needed to be admitted to an intensive care unit (ICU) for critical ill disease progression, all patients were required to undergo universal screening of stool CPE, according to the policy of our hospital.

Second, active surveillance cultures are implemented for patients who are admitted to the emergency room (ER) with CRE risk factors (prior use of carbapenem antibiotics, presence of invasive catheters, such as central venous catheters, and transfer from long-term care facilities).

Even if the screening is negative at initial surveillance, patients who reported CPE in clinical specimens, such as blood, sputum, urine, or bile, should be screened for stool colonization by stool culture. In addition to stool samples, rectal or perianal swab samples were also used for CPE culture screening (Figure 1).

2. Clinical variables of study populations

Demographic and clinical data were extracted from the electronic medical records using a clinical data warehouse system, including age, sex, comorbidities, and admission history from other medical institutions, such as general hospitals or long-term care facilities. The status of having an invasive catheter, such as a central venous catheter, dialysis catheter, chemo port, mechanical ventilator, and percutaneous drainage catheter, was identified at the time of the clinical CPE-positive report date.

Clinical CPE infections are defined as acute infectious diseases caused by CPE

pathogens. Clinical CPE bacteremia was defined when the same CPE strain as stool grew in blood culture, and CPE-induced pneumonia was defined as the CPE pathogen cultured by bronchoalveolar lavage, bronchial washing, or sputum (Group 4or 5 of Murray-Washington grading system). In case of urinary tract infection or intra-abdominal infection were defined as cases in which the same CPE was positive in related specimen culture, and there was evidence of medical image such as computed tomography or magnetic resonance imaging.

3. Ethics approval and informed consent

The protocol for this prospective study was reviewed and approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University College of Medicine in Seoul, South Korea (Reg. No. 6-2018-0165). All procedures were performed in accordance with the Declaration of Helsinki guidelines. Informed consent was obtained from all the participants prior to specimen collection.

4. Identification of acquisition pathway using molecular typing

Molecular typing was performed to differentiate between isolates of the same bacterial species. This method can be used to identify relatedness between different bacterial strains. Pulsed-field gel electrophoresis (PFGE) is based on the gel electrophoresis of restriction-digested genomic deoxyribonucleic acid. Traditional gel electrophoresis has a constant current in one direction; therefore, only small fragments can enter the gel and be separated. In PFGE, the direction of the current changes regularly (pulsed); thus, large fragments twist and move slowly through the gel. The band patterns determined the relatedness of the

isolates.

Multilocus sequence typing (MLST) was performed for carbapenemase-producing *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) strains. The polymerase chain reaction (PCR) amplification with seven housekeeping genes (http://pubmlst.org/databases) from genomic Deoxyribonucleic acid (DNA) was performed, and the internal fragments of the genes were compared. Each recorded sequence of a gene was assigned a number. Isolates with related sequence types were grouped into clonal complexes.

5. Whole Genome Sequencing and In Silico Analysis

Bacterial DNA was extracted using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. DNA concentrations were measured using a Qubit double-stranded DNA high-sensitivity assay kit (Invitrogen, Waltham, MA, USA) on a Qubit 3.0 fluorometer (ThermoFisher, waltham, MA). Genomic DNA was fragmented using g-TUBE (Covaris, Inc., Moburn, MA, USA).

Libraries were constructed using the SMRTbell DNA template with a fragment size of >10 kb and selected using a Blue Pippin system. Library quality was analyzed using a Qubit and Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA). The whole genome was sequenced using the PacBio Sequel II platform (PacBio, Menlo Park, CA, USA). Microbial assembly of the reads was performed using SMRT Link v11.1 (PacBio) with the default parameters. The assembled genomic contigs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

MLST and core genome MLST were performed using a Ridom SeqSphere+(Ridom, Münster, Germany). The plasmid replicon was identified using PlasmidFinder 1.3

(https://cge.cbs.dtu.dk//services/PlasmidFinder/), and resistance genes were analyzed using ResFinder 3.1 (https://cge.cbs.dtu.dk//services/ResFinder/) from the Center for Genomic Epidemiology server. Plasmid structures of the sequenced isolates were compared and visualized using the Proksee server (https://proksee.ca/).

Whole genome sequencing (WGS) was performed with nine selective *K. pneumoniae* and two *E. coli* strains to reveal the resistance genes and acquisition pathways. These strains mainly consisted of CPE bacteremia strains.

6. Statistical Analysis

Categorical variables were compared using the chi-square test and expressed as numbers (percentages). Continuous variables were expressed as mean ± standard deviation or median with interquartile range (IQR) according to normal distribution. A parametric independent t-test was used to compare continuous variables with normal distribution between the two groups, and analysis of variance was used for comparisons among the four different groups. Continuous variables without a normal distribution between the two groups were compared using the non-parametric Mann–Whitney U test, and the analysis among the four different groups was compared using the Kruskal–Wallis test. All two-tailed *P*-values or adjusted *P*-values of ≤ 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Version 23 (IBM Corp., Armonk, NY, USA).

III. RESULTS

1. Prevalence of stool CPE colonization and clinical CPE infection

Among 1,620 patients who had undergone stool CPE surveillance cultures, majority (n=1,142) underwent stool culture at ICU admission. Among the 478 active surveillance cultures at the ER, 34 patients (7.11%) were initially stool CPE positive. Among 1,142 universal surveillance cases at ICU admission, 51 patients (4.46%) were initially stool CPE positive. The transition rates from stool carriers to clinical CPE infections were 29.41% in the ER and 31.37% in the ICU.

As a result of subsequent stool culture, 22 patients who initially had negative stool screening results during active surveillance transformed into CPE colonizers (n=15) or developed clinical CPE infections (n=7). Twenty patients who initially had negative stool results at ICU admission transformed into CPE colonizers (n=9) or developed clinical CPE infections (n=11). The transition from stool carriers to clinical CPE infection was 31.81% in patients admitted via the ER, which was similar to other transition rates. However, 55.0% of the patients who initially had a negative result at ICU admission, acquired clinical CPE infection from the stool colonizers (Table 1).

2. Baseline Characteristics of stool CPE surveillance culture-positive patients

Among the enrolled 1,620 patients, 85 patients were initially stool CPE positive in the overall surveillance culture, and 42 patients were initially stool CPE negative in surveillance culture, but eventually they were found to be stool CPE positive during the admission period. Of the initial stool CPE-positive patients, 59 patients were stool colonizers, but 26 patients had developed clinical CPE infection.

Table 1. Number of stool CPE positive and clinical CPE infection by surveillance culture

Surveillance culture	**Active surveillance at ER (n=478)**					**Universal surveillance at ICU (n=1,142)**				
Stool CPE	**Initial Stool (+) (n=34)**		**Initial Stool (-) (n=444)**			**Initial Stool (+) (n=51)**		**Initial Stool (-) (n=1,091)**		
Progress	**Carrier**	**Infection**	**f/u Stool (+)**		**f/u Stool (-)**	**Carrier**	**Infection**	**f/u Stool (+)**		**f/u Stool (-)**
			Carrier	**Infection**				**Carrier**	**Infection**	
Number of patients	24	10	15	7	422	35	16	9	11	1,071
Stool detection rate	34/478 (7.11%)		22/478 (4.60%)		422/478 (88.28%)	51/1,142 (4.46%)		20/1,142 (1.75%)		1,071/ 1,142 (93.78%)
Transition rate*	-	10/34 (29.41%)		7/22 (31.81%)			16/51 (31.37%)		11†/20 (55.0%)	

Data are expressed as number (%). *Transition rate from stool carrier to clinical CPE infection. †Pneumonia (n=8), bacteremia with pneumonia (n=1), bacteremia with intra-abdominal infection (n=1), and bacteremia with wound infection (n=1).

Abbreviations, CPE, Carbapenemase-producing *Enterobacterales*; ER, emergency room; ICU, intensive care unit.

The baseline patient characteristics are presented in Table 2. The median age was 68.0 [25–75% IQR, 61.0–79.0] years in stool CPE carrier group, and 70.0 [59.7–77.0] years in clinical CPE group. There were no significant differences in the age and sex variables. Comorbidities showed prominence with underlying hypertension (61.0% *vs.* 92.3%, $P = 0.004$) and malignancy (28.8% *vs.* 53.8%, $P = 0.027$) in the clinical CPE group than those in the stool CPE carrier group. In the subgroup analysis of malignancy type, gastrointestinal cancer did not show a significant difference compared to other malignancies. Among invasive catheters, the application of mechanical ventilation (25.4% *vs.* 53.8%, $P = 0.011$) was significantly higher in the clinical CPE group than that in the stool CPE carrier group.

Table 2. Baseline characteristics of stool surveillance culture-positive patients

Characteristics	**Initial Stool CPE (+) at surveillance**		***p*-value**
	Stool CPE carrier (n = 59)	**Clinical CPE infection (n = 26)**	
Age, year	68.0 [61.0-79.0]	70.0 [59.7-77.0]	0.962
Gender, male	41 (48.2)	17 (20.0)	0.708
Comorbidity			
Hypertension	36 (61.0)	24 (92.3)	0.004
Diabetes	17 (28.8)	8 (30.8)	0.855
Hepatitis	10 (16.9)	4 (15.4)	0.858
ESRD	9 (15.3)	5 (19.2)	0.649
Cardiovascular diseases	17 (28.8)	9 (34.6)	0.593
Malignancy	17 (28.8)	14 (53.8)	0.027
Gastrointestinal cancer	12 (20.3)	8 (30.8)	0.061

Other cancer*	5 (8.5)	6 (23.1)	
Invasive catheters			
Central venous catheter	37 (62.7)	17 (65.4)	0.814
Foley catheter	46 (78.0)	22 (84.6)	0.480
Dialysis catheter	6 (10.2)	3 (11.5)	0.850
Chemoport	2 (3.4)	1 (3.8)	0.916
Mechanical ventilation	15 (25.4)	14 (53.8)	0.011
Percutaneous drainage	16 (27.1)	10 (38.5)	0.296

Data are expressed as number (%) and median [25–75% IQR]. *Thyroid cancer (n=5), lung cancer (n=4), prostate cancer (n=1), and diffuse large B-cell lymphoma (n=1).

Abbreviations, CPE, Carbapenemase producing *Enterobacterales*; ESRD, end-stage renal diseases

3. Clinical Characteristics of Stool CPE surveillance culture-positive patients

The proportion of surveillance culture sites and universal screening at ICU admission was higher than that of active surveillance culture at the ER (40.7% vs. 59.3% in stool CPE carrier group, 38.5% vs. 61.5% in clinical CPE-positive group) in both groups, but there was no significant difference between the two groups ($P = 0.848$). In addition, most patients had a history of admission to another medical institution or readmission within 90 days (71.2% *vs.* 76.9%, $P = 0.583$). The use of carbapenem antibiotics within 90 days was 28.8% in the carrier group and 34.6% in the clinical CPE infection group ($P = 0.593$). The in-hospital mortality rate was not significantly different between the two groups (27.1% *vs.* 34.6%, $P = 0.485$). ICU care was more prominent in the clinical CPE group than that in the carrier group (67.8% *vs.* 76.9%, $P = 0.395$), and the median duration of ICU stay was significantly longer in the clinical CPE group than that in the carrier group (1.0 days *vs.* 6.5

days, $P = 0.017$) (Table 3).

Table 3. Clinical characteristics of stool surveillance culture-positive patients

Characteristics	Initial Stool CPE (+) at surveillance		*p*-value
	Stool CPE carrier (n = 59)	Clinical CPE infection (n = 26)	
Surveillance site			
Active at ER	24 (40.7)	10 (38.5)	0.848
Universal screening at ICU	35 (59.3)	16 (61.5)	
History of admission*	42 (71.2)	20 (76.9)	0.583
General hospital	25 (42.4)	12 (46.2)	0.860
Long-term care facility	17 (28.8)	8 (30.8)	
Direct transfer	28 (47.5)	11 (42.3)	0.661
Previous use of carbapenem*	17 (28.8)	9 (34.6)	0.593
In-hospital mortality	16 (27.1)	9 (34.6)	0.485
Total Hospital stay, days	23.0 [15.0-48.0]	41.5 [22.2-55.7]	0.185
ICU care	40 (67.8)	20 (76.9)	0.395
ICU duration, days	1.0 [0-8.0]	6.5 [1.0-27.0]	0.017
Time interval, days			
Admission to CPE positive patients	2.0 [0.0-12.0]	5.0 [0.0-22.0]	0.584
CPE positive to discharge	15.0 [10.0-30.0]	22.0 [8.7-42.7]	0.256

Data are expressed as number (%) and median [25–75% IQR]. *Within 90 days of admission.

Abbreviations: CPE, carbapenemase-producing *Enterobacterales*; ER, emergency room;

ICU, intensive care unit.

4. Microbiological characteristics of stool CPE surveillance culture-positive patients

Among the clinical CPE infection groups, pneumonia was predominant (50%), and bacteremia accounted for 26.9% (Table 4). The most common pathogen was *K. pneumoniae* in both groups, followed by *E. coli*. The most common genotype of CPE strains was identified as *Klebsiella pneumoniae* carbapenemase (KPC) type (76.3% *vs.* 65.4%, P = 0.524).

Table 4. Microbiological characteristics of stool surveillance culture-positive patients

Characteristics	**Initial Stool CPE (+) at surveillance**		***p*-value**
	Stool CPE carrier (n = 59)	**Clinical CPE infection (n = 26)**	
Clinical CPE infection			
Bacteremia	-	7 (26.9)	
Pneumonia	-	13 (50.0)	
Urinary tract infection	-	3 (11.5)	
Intra-abdominal infection	-	7 (26.9)	
Wound and joint infection	-	1 (3.8)	
Pathogen			
K. pneumoniae	48 (81.4)	18 (69.2)	
E. coli	10 (16.9)	8 (30.8)	0.300
E. cloacae	1 (1.7)	0 (0)	

Genotype			
KPC	45 (76.3)	17 (65.4)	
NDM & OXA-48	3 (5.1)	3 (11.5)	
OXA-48	6 (10.2)	5 (19.2)	0.524
NDM	4 (6.8)	1 (3.8)	
GES	1 (1.7)	0 (0)	

Data are expressed as number (%) and median [25–75% IQR].

Abbreviations: CPE, carbapenemase-producing *Enterobacterales*; KPC, *Klebsiella pneumoniae* Carbapenemase, NDM, New Delhi metallo-β-lactamase; OXA-48, Oxacillinase-48; GES, Guiana extended-spectrum beta-lactamase

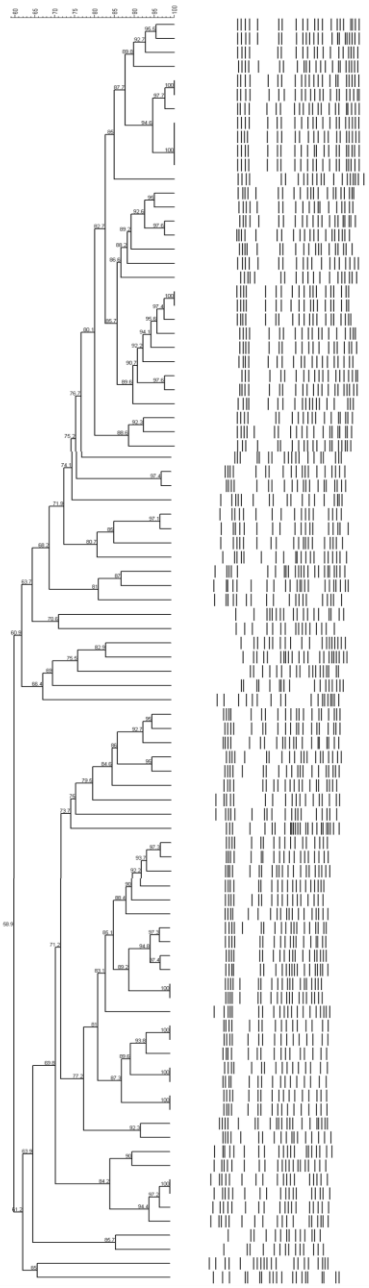
5. Risk factor comparison of stool carriers and clinical CPE infections

Multivariate analysis was performed to identify the risk factors for progression to clinical CPE infection from stool CPE carriers. Comorbidities with hypertension (odds ratio, OR 5.18 [95% CI, 1.93 - 8.43], $P = 0.009$) and malignancy (OR 2.94 [95% CI, 1.55- 7.96], $P = 0.038$) were found to be significant risk factors for the progression to clinical CPE infection from stool carriers. Mechanical ventilation and the length of ICU stay were not statistically significant (Table 5).

Table 5. Multivariate analysis of the risk factors of clinical CPE infection among stool colonizers

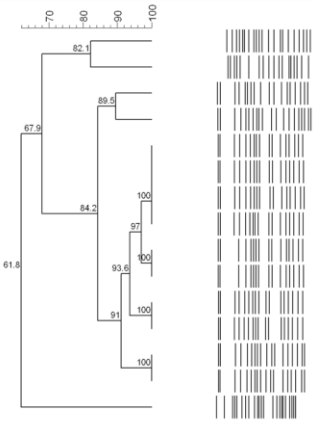
Variables	**OR (95% CI)**	***p*-value**
Hypertension	5.18 (1.93–8.43)	0.009
Malignancy	2.94 (1.55–7.96)	0.038
Mechanical ventilation	2.21 (0.71–6.79)	0.168
Duration of ICU stay, days	1.04 (0.99–1.09)	0.077

Abbreviations: CI, Confidence interval; ICU, intensive care unit; OR, odds ratio.



Strain No.	Pulsotype	Sequence type	Isolation Date	Surveillance	Acquisition Site	CPE infection
J173	1	ST307	2019-09-26	ICU (-)	Ward	-
R39	1	ST307	2020-03-05	ICU (-)	Ward	Pneumonia
P329/#1	1	ST307	2020-06-01	ICU (-)	Ward	-
J123	1	ST307	2019-11-16	ER (-)	Ward	-
P120	1	ST307	2020-02-18	ICU (-)	Ward	Pneumonia, wound
R439	1	ST307	2020-02-20	ICU (-)	Ward	Pneumonia
T50	1	ST307	2020-02-17	ER (-)	Ward	-
B943	1	ST307	2019-06-14	ER (-)	ICU	Bacteremia, Pneumonia, IAI
R12	1	ST307	2019-10-11	ER (-)	Ward	Pneumonia
R16	1	ST307	2019-10-17	ER (-)	Ward	-
R941	1	ST307	2019-07-25	ICU (-)	Ward	Pneumonia
U75	1	ST307	2019-11-06	ICU (-)	Ward	-
J271	1	ST307	2019-12-28	ICU (+)	ICU	-
J99	1	ST307	2019-06-25	ER (+)	ER	Bacteremia
J122	1	ST307	2019-11-16	ICU (+)	ICU	-
U529	1	ST307	2019-11-18	ER (-)	Ward	-
R534	1	ST307	2019-08-11	ICU (+)	ICU	Bacteremia
U352	1	ST307	2019-03-16	ER (-)	Ward	-
J81	1	ST307	2019-10-30	ER (-)	Ward	-
J162-1	1	ST307	2019-04-26	ICU (+)	ICU	-
J163	1	ST307	2018-12-29	ER (+)	ER	-
J245	1	ST307	2019-11-27	ICU (+)	ICU	Bacteremia, Pneumonia
J68	1	ST307	2019-07-17	ICU (+)	ICU	-
J269	1	ST307	2019-11-30	ICU (+)	ICU	-
J19	1	ST307	2019-03-07	ICU (+)	ICU	-
J104/#1	1	ST307	2019-11-23	ICU (-)	Ward	Pneumonia
J261	1	ST307	2019-11-29	ICU (+)	ICU	Pneumonia
J168/#1	1	ST307	2018-10-28	ICU (+)	ICU	Pneumonia
J144	1	ST307	2019-11-18	ICU (+)	ICU	-
J96	1	ST307	2019-11-13	ICU (+)	ICU	-
J7	1	ST307	2019-03-03	ICU (+)	ICU	-
J74/#1	2	ST307	2018-12-13	ER (+)	ER	-
J197	3	ST307	2019-12-20	ICU (+)	ICU	-
J75	3	ST307	2019-11-11	ER (-)	Ward	-
J110-2	4	ST307	2019-08-21	ICU (+)	ICU	-
R1251/#1	5	ST395	2019-05-10	ICU (-)	Ward	
U578	5	ST395	2020-01-17	ER (-)	Ward	UTI
J162-2	5	ST395	2018-12-29	ER (+)	ER	-
J147/#1	5	ST395	2019-07-04	ICU (-)	Ward	Pneumonia
J114	6	ST11	2019-07-25	ER (+)	ER	-
U1402	6	ST11	2019-11-07	ICU (-)	Ward	Pneumonia
R127	6	ST307	2019-07-26	ER (+)	ER	-
J61	7	ST735	2020-01-08	ICU (+)	ICU	-
KPN ATCC	-	-	-	-	-	-
J12	8	ST307	2020-02-02	ICU (+)	ICU	-
J235	8	ST307	2020-01-30	ICU (+)	ICU	Bacteremia, Pneumonia
J72	9	ST307	2019-10-12	ER (-)	Ward	-
J161	10	ST307	2018-12-29	ER (+)	ER	-
U868	11	ST307	2020-02-25	ER (-)	Ward	-
J73	12	ST307	2019-10-12	ER (+)	ER	-
T211	12	ST307	2019-09-24	ER (+)	ER	-
K190	12	ST307	2019-09-28	ER (-)	Ward	-
C176	12	ST307	2019-09-26	ICU (+)	ICU	-
P274	12	ST307	2019-08-26	ER (-)	Ward	-
J102	12	ST307	2019-04-18	ICU (+)	ICU	-
J32	13	ST395	2019-06-07	ER (+)	ER	Pneumonia
U1379	14	ST11	2019-08-06	ER (+)	ER	-
J34	15	ST307	2019-05-10	ER (-)	Ward	-
J56	16	ST307	2019-05-13	ER (+)	ER	-
P105	16	ST307	2019-07-01	ER (+)	ER	IAI
P58	16	ST307	2020-02-11	ER (-)	Ward	-
P329	16	ST307	2019-10-24	ICU (-)	Ward	Bacteremia, UTI, IAI
R538	16	ST307	2019-10-21	ER (-)	Ward	Pneumonia
R322	16	ST307	2020-02-19	ICU (-)	Ward	Pneumonia
J117-J	16	ST307	2019-11-15	ER (+)	ER	-
R118	16	ST307	2019-11-07	ER (-)	Ward	-
J184	16	ST307	2019-10-30	ICU (-)	Ward	Pneumonia
U38	16	ST307	2019-08-06	ICU (-)	Ward	UTI
R1037	16	ST307	2019-09-28	ER (-)	Ward	Pneumonia, UTI
R767	16	ST307	2019-09-19	ER (+)	ER	-
U428	16	ST307	2019-11-13	ICU (-)	Ward	-
J89	16	ST307	2018-10-14	ICU (+)	ICU	-
U326	16	ST307	2018-11-11	ER (+)	ER	-
J80	16	ST307	2018-10-12	ER (+)	ER	Pneumonia
C207	16	ST307	2018-12-20	ER (+)	ER	-
J104	16	ST307	2018-09-23	ICU (+)	ICU	-
J168	16	ST307	2018-10-28	ICU (+)	ICU	Pneumonia
R1207	16	ST307	2018-10-29	ICU (-)	Ward	Bacteremia, Pneumonia
J60-J	17	ST307	2018-11-11	ICU (+)	ICU	-
K132	17	ST307	2018-12-10	ER (-)	Ward	Pneumonia, UTI
J147	18	ST395	2018-10-24	ICU (+)	ICU	-
R1236	18	ST395	2019-12-21	ER (-)	Ward	-
J117-K	18	ST395	2018-12-22	ER (+)	ER	-
J79	18	ST395	2018-09-19	ICU (+)	ICU	Pneumonia, UTI
J29	18	ST395	2018-09-13	ICU (+)	ICU	-
J115	18	ST395	2019-01-22	ICU (+)	ICU	-
J128	19	ST147	2018-11-20	ER (+)	ER	IAI
R128	19	ST307	2019-09-27	ICU (+)	ICU	Pneumonia
J241	20	ST307	2019-12-25	ICU (+)	ICU	-
R1380	21	ST307	2019-06-11	ER (+)	ER	Wound/Joint

Figure 2. PFGE dendrogram with the corresponding MLST sequence types of the carbapenemase-producing *K. pneumoniae*



Strain No.	Pulsotype	Sequence type	Isolation Date	Surveillance	Acquisition Site	CPE infection
E. coli ATCC	-	-	-	-	-	-
J40	1	ST1193	2019-07-10	ICU (+)	ICU	-
J104	2	ST410	2018-09-23	ICU (+)	ICU	-
R718	2	ST410	2019-12-04	ICU (-)	Ward	Pneumonia
J21	2	ST410	2019-05-06	ICU (-)	Ward	-
J29	2	ST410	2019-05-08	ER (+)	ER	-
J37	2	ST410	2019-10-06	ER (+)	ER	-
R681	2	ST410	2018-10-16	ICU (+)	ICU	Pneumonia
J126	2	ST410	2018-12-24	ER (+)	ER	-
J129	2	ST410	2018-12-25	ICU (+)	ICU	-
J108	2	ST410	2019-01-19	ER (+)	ER	Bacteremia
R135	2	ST410	2019-01-08	ICU (+)	ICU	Pneumonia
J136	2	ST410	2020-01-17	ER (-)	Ward	-
J142	2	ST410	2019-11-18	ICU (+)	ICU	IAI
J149	3	ST648	2019-10-23	ER (+)	ER	UTI

Figure 3. PFGE dendrogram with the corresponding MLST sequence types of the carbapenemase-producing *E. coli*

6. The molecular epidemiologic analysis

The PFGE profiles showed two large clustered groups in *K. pneumoniae* isolates (Figure 2) and *E. coli* isolates (Figure 3), respectively. By the MLST analysis matching, sequence type (ST) 307 and ST 395 was dominant in *K. pneumoniae*, and ST 410 was prominent in *E. coli* isolates group. By matching the CPE isolation date, type of CPE surveillance culture, isolation location of CPE strain one by one, Figure 2 and 3 showed correlation among the CPE strains. The seven housekeeping genes for MLST analysis used in this study were shown in Table 6.

Among the 88 carbapenemase-producing *K. pneumoniae* strains, nine strains were selected for WGS analysis. All carbapenemase-producing *K. pneumoniae* strains are pathogens that cause clinical CPE infections. Of these, eight caused CPE bacteremia (P329, R1207, J 245, J99, P120, B943, J235, and R534 strain) and only one strain (J80) caused pneumonia.

Table 6. Oligonucleotide sequences of the primers used in the study

Strain	Locus	Primer name	Primer Sequence	PCR product size (bp)
K. pneumoniae	*gapA*	gapA_F	TGAAATATGACTCCACTCACGG	662
		gapA_R	CTTCAGAAGCGGCTTTGATGGCTT	
	infB	infB_F	CTCGCTGCTGGACTATATTCG	462
		infB_R	CGCTTTCAGCTCAAGAACTTC	
	mdh	mdh_F	CCCAACTCGCTTCAGGTTCAG	756
		mdh_R	CCGTTTTTCCCCAGCAGCAG	
	pgi	pgi_F	GAGAAAAACCTGCCTGTACTGCTGGC	718
		pgi_R	CGCGCCACGCTTTATAGCGGTTAAT	
	phoE	phoE_F	ACCTACCGCAACACCGACTTCTTCGG	602
		phoE_R	TGATCAGAACTGGTAGGTGAT	
	rpoB	rpoB_F	GGCGAAATGGCWGAGAACCA	1075
		rpoB_R	GAGTCTTCGAAGTTGTAACC	
	tonB	tonB_F	CTTTATACCTCGGTACATCAGGTT	539
		tonB_R	ATTCGCCGGCTGRGCRGAGAG	
E.coli	*adk*	adk_F	GCAATGCGTATCATTCTGCT	536
		adk_R	CAGATCAGCGCGAACTTCAG	
	fumC	FumC_F	CCACCTCACTGATTCATGCG	469
		FumC_R	CGGTGCACAGGTAATGACTG	
	gyrB	gyrB_F	CGGGTCACTGTAAAGAAATTAT	460
		gyrB_R	GTCCATGTAGGCGTTCAGGG	
	icd	icd_F	TACATTGAAGGTGATGGAATCG	518
		icd_R	GTCTTTAAACGCTCCTTCGG	
	mdh	mdh_F	TCTGAGCCATATCCCTACTG	452
		mdh_R	CGATAGATTTACGCTCTTCCA	
	purA	purA_F	CTGCTGTCTGAAGCATGTCC	510
		purA_R	CAGTTTAGTCAGGCAGAAGC	
	recA	recA_F	AGCGTGAAGGTAAAACCTGTG	478
		RecA_R	ACCTTTGTAGCTGTACCACG	

Abbreviations: *K. pneumoniae, Klebsiella pneumoniae; E. coli, Escherichia coli;* PCR, polymerase chain reaction; bp, base pair.

Table 7. Characteristics of sequenced genome with carbapenemase-producing *K. pneumoniae* in this study

Strain No.	Chromosome	Number of CDS	MLST ST	cgMLST complex type	Acquired resistance gene	Plasmid type	Plasmid size	Carbapenemase	Co-resistance gene
R534	5,512,581	5,489	307	2303	blaCTX-M-15 (2), blaSHV-28, oqxA, fosA	IncX3	49,829	KPC-2	SHV-11
B943	5,369,959	5,455	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,827	KPC-2	SHV-11
J80	5,403,293	5,509	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	ND	45,638	KPC-2	-
J99	5,246,359	5,283	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,830	KPC-2	SHV-11
J235	5,128,161	5,206	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,836	KPC-2	SHV-11
J245	5,357,970	5,452	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,836	KPC-2	SHV-11
P120	5,369,136	5,458	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,835	KPC-2	SHV-11
P329	5,583,828	5,701	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,839	KPC-2	SHV-11
R1207	5,400,042	5,499	307	2303	blaCTX-M-15, blaSHV-28, oqxA, fosA	IncX3	46,838	KPC-2	SHV-11

Abbreviations, *K. pneumonia*, *Klebsiella pneumoniae*; No, number; CDS, coding sequence; MLST, Multi-locus sequence typing; ST, sequence type; ND, non-detectable

Table 8. Characteristics of sequenced genome with carbapenemase-producing *E. coli* in this study

Strain No.	Chromosome	Number of CDS	MLST ST	cgMLST complex type	Acquired resistance gene	Plasmid type	Plasmid size	Carbapenemase	Co-resistance gene
J37	4,842,532	5,058	410	17569	blaCMY-2	IncFII/IncX3	123,898	OXA-181	aadA2, aac(6')-Ib-cr, sul1, dfrA12, qnrS1, tet(B), blaOXA-1, blaTEM-1, blaCTX-M-15
J136	4,847,726	5,806	410	17568	blaCMY-2	IncX3	51,476	OXA-181	-
R718	4,844,524	5,054	410	9289	blaCMY-2	IncX3	51,479	OXA-181	-

Abbreviations, *E. coli*, *Escherichia*; No, number; CDS, coding sequence; MLST, Multi-locus sequence typing; ST, sequence type;

ND, non-detectable

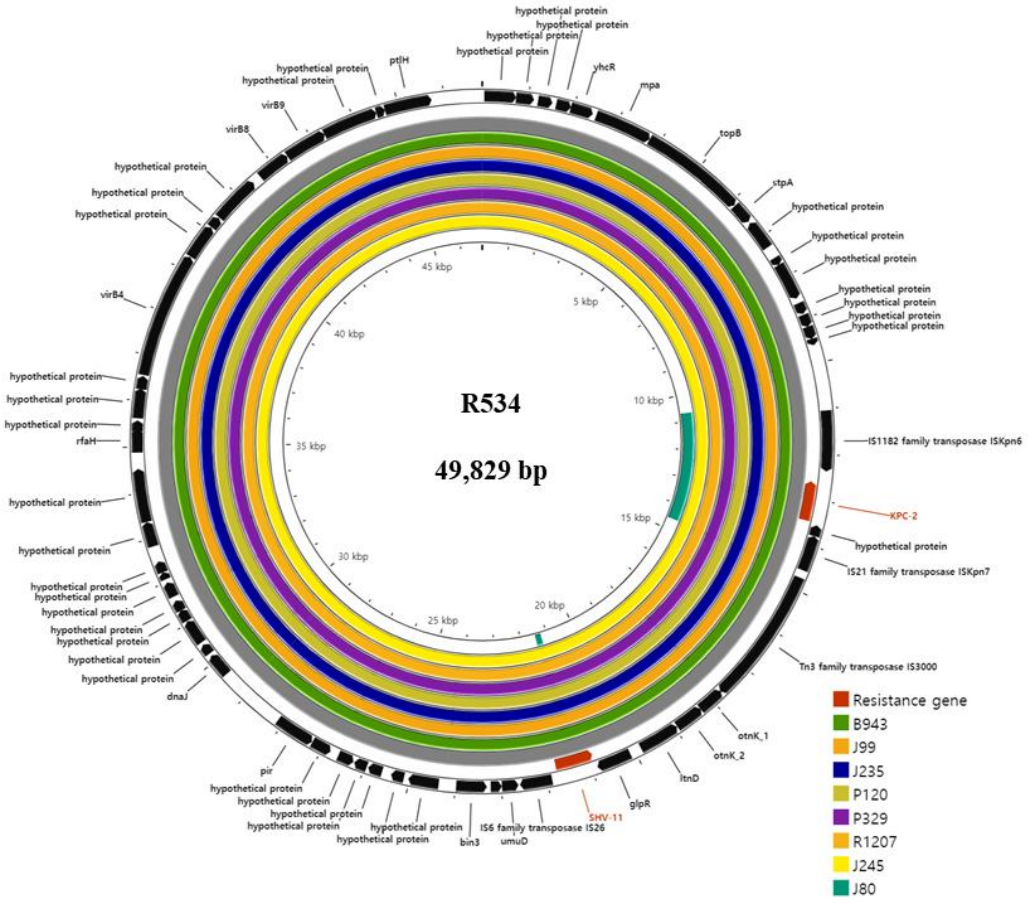


Figure 4. Whole genome sequencing of carbapenemase-producing *K. pneumoniae*

However, the patient did not progress to bacteremia. Among the 14 carbapenemase-producing *E. coli* strains, three strains were included for WGS analysis, and two of these strains (J37 and J 136) were only stool colonizers, and the R718 strain was the cause of CPE pneumonia.

The characteristics of the whole genome of the 12 CPE strains (nine strains of carbapenemase-producing *K. pneumoniae* and three strains of carbapenemase-producing *E.*

coli) are shown in Tables 7 and 8. Except for the J80 strain, the other eight carbapenemase-producing *K. pneumoniae* strains showed the same acquired resistance genes (blaCTX-M-15, blaSHV-28, oqxA, and fosA) on the chromosome and IncX3 at the plasmid level. In addition, they shared the co-resistance gene SHV-11 (Table 7). The Phylogenetic tree based on sequences of the core genome for the carbapenemase-producing *K. pneumoniae* complex detected in this study is shown in Figure 5.

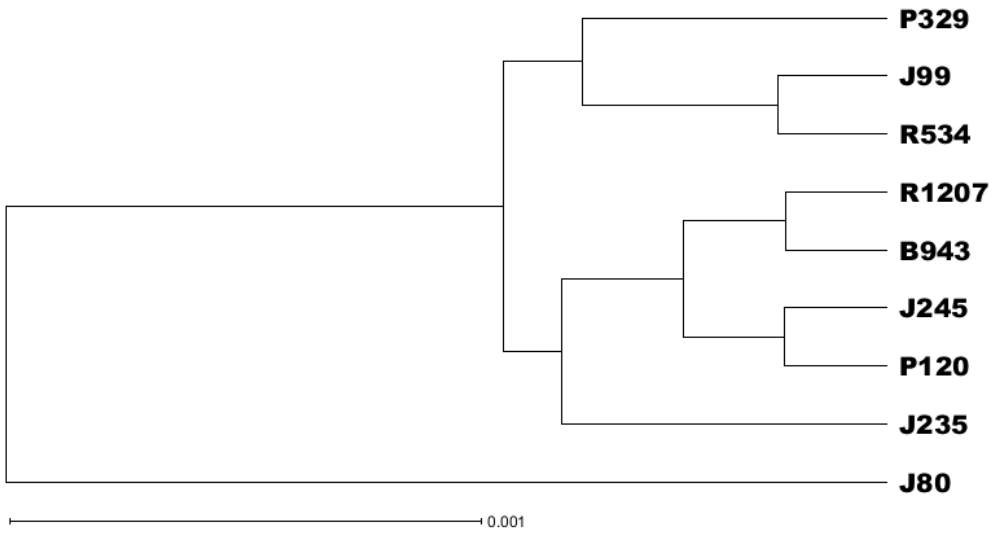


Figure 5. Phylogenetic tree based on sequences of core genome for carbapenemase-producing *K. pneumoniae* complex detected at single center hospital

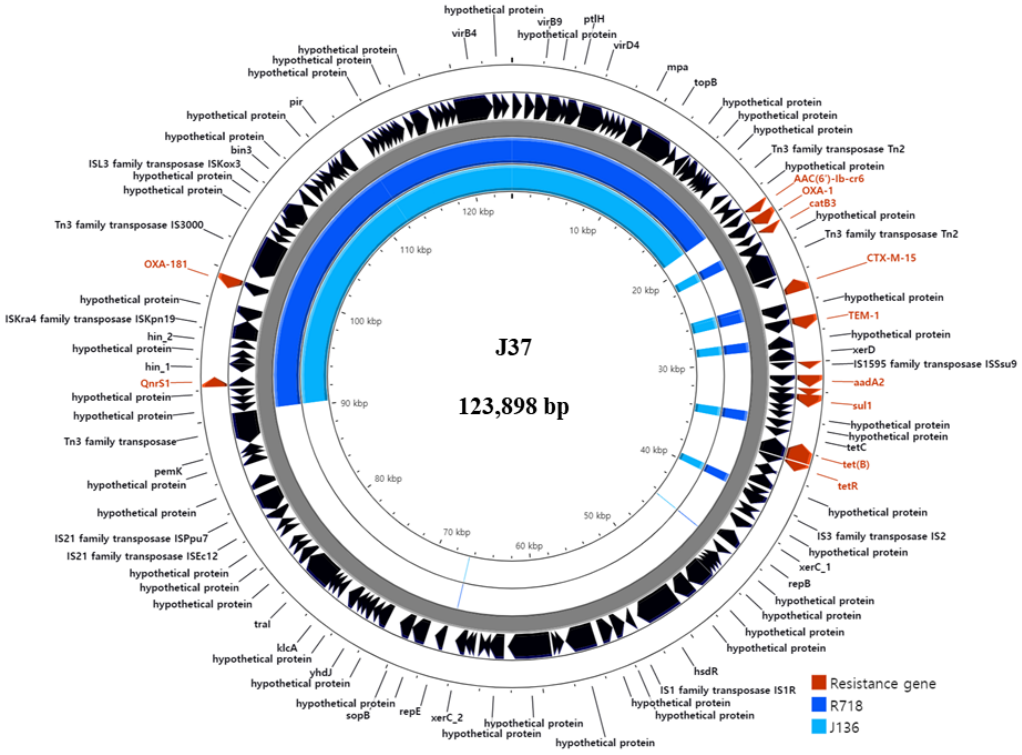


Figure 6. Whole genome sequencing of carbapenemase-producing *E. coli*

According to this dendrogram, most strains showed similarity, with the exception of J80. In the carbapenemase-producing *E. coli* sequencing, the J136 and R718 strains were similar to the blaCMY-2 resistance gene (Table 8). The circular diagram of whole genome sequencing for each strain and resistance gene is shown in Figures 4 and 6.

IV. DISCUSSION

In this study, we identified the positive rate of stool surveillance culture as 7.11% for active surveillance in the ER and 4.46% for universal surveillance in the ICU. The positive rate of active surveillance was higher than that of surveillance culture performed on ICU patients. In a single Korean hospital, the CPE acquisition rate was 3.2% among close contact patients who were defined as overlapping hospital stays in the same room or ICU [18]. In this study, the subsequent CPE positivity rate was 1.75–4.60%.

Among the stool colonizers, the transition rates of clinical CPE infection from stool carriers were similar between the initial surveillance negative of the two different groups (29.41% in the ER and 31.37% in the ICU). However, the transition rate was higher in the initially CPE-negative patients at ICU admission (55.0%) than that in the other patients. The ICU patients were critically ill and had multiple high-risk factors for CPE transmission. Among them, pneumonia (n = 8) accounted for the majority of clinical CPE infections, and six of them were treated with mechanical ventilator care. Critical illness and underlying medical conditions, such as pneumonia, are risk factors for CPE infection or colonization in the ICU [19].

The results of this study support not only active surveillance for the selective screening in high-risk groups but also large-scale screening in groups that can cause critical medical outcomes when an outbreak occurs, such as in the ICU. Of course, the progression to clinical CPE infection rather than stool colonization in these ICU patients is affected by the complexity of the overall patient's systemic condition, such as antibiotic use, ventilator care, and invasive catheter. The importance of performing universal surveillance culture in the ICU prior to ICU admission is also emphasized by the results of previous studies [8,20].

In previous studies, medical invasive catheters, antibiotic exposure, mechanical

ventilation, and underlying chronic diseases were known risk factors for CPE colonization or infection [17,20,21]. Univariate analysis showed that the risk factors of clinical CPE infection transition among stool CPE carriers were underlying hypertension or malignancy, long ICU stay, and mechanical ventilation. Another study of CPE stool-colonized malignancy or hematopoietic stem cell transplant patients showed CPE blood stream infection risk as ICU hospitalization (OR 2.82, [95% CI 1.10–7.20], $P = 0.042$); however, solid tumor was a protective risk factor (OR 0.21 [95% CI 0.05–1.01], $P = 0.038$) [22]. In this study, there was a small study population with clinical CPE infection; thus, further analysis is required.

The PFGE dendrogram showed that the two large cluster groups continued to spread in this hospital during the study period of over 2 years. Although there were a few minor groups, two major clusters (ST 307 and ST395) were continuously prevalent in hospitals, and CTX-M-15 was the dominant *K. pneumoniae* type that was not different from the epidemic strains announced by Kor-GLASS [23]. However, ST 410 isolated from *E. coli* has not been previously reported; therefore, caution is needed.

This study had some limitations. First, regular or serial follow-up of stool culture was not performed in the initial study design; thus, the negative control group could not be compared with the study population. In addition, we could not analyze negative conversion. Second, molecular typing using PFGE and MLST did not include all CPE strains because of subculture failure. The acquisition pathway was estimated by the time of admission and date of culture reporting. Lastly, as this was a single-center study, only the characteristics of our hospital, surrounding areas, and property of patients could be reflected.

However, this study had several strengths. This comprehensive analytic data of stool CPE culture shows the prevalence of stool CPE colonization and the transition rate to clinical

CPE infection. In addition, this study identified the risk factors for clinical CPE infection from stool colonization, which can aid in infection control and prevention. Lastly, through WGS analysis of major CPEs identified in a single medical institution for a long period of 2 years, it was possible to identify resistance genes and confirm the major genotypes.

V. CONCLUSION

In conclusion, active surveillance showed a higher detection rate than universal stool CPE screening. If the result of the initial surveillance culture was positive or negative, one-third of the stool carriers ultimately developed clinical CPE infection. Interestingly, patients with severe disease who had tested negative prior to ICU admission showed a higher rate of progression to clinical CPE infection. This relates to the identification of significant risk factors, such as hypertension, malignancy, ventilator care, and prolonged ICU stay.

In addition, ST 307 and ST 395 were dominant in the case of carbapenemase-producing *K. pneumoniae* from CPE strains collected in a single center. Although the strains causing CPE bacteremia were identified at different times over 2 years, they were confirmed to have the same genotype. For carbapenemase-producing *E.coli*, ST 410 was found to be dominant in this hospital.

Thus, even if some genotypes are transmitted from outside the hospital, it can be confirmed that a particular strain is continuously dominant in the hospital. Therefore, in-hospital ICU surveillance, as well as active surveillance to block acquisition from outside, should be performed for the early detection of stool carriers and for early intervention in severe patients; thus, close monitoring is needed to prevent propagation of CPE infection.

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ABSTRACT(IN KOREAN)

카바페넴분해효소 생성 장내세균속균종 보균의 유병율과 획득 경로 확인

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이 경 화

Carbapenemase producing Enterobacterales (CPE)는 치료 및 감염 관리에 있어 전세계적으로 위협이되며 높은 관심을 갖는 균주임. 또한, 표준 주의, 접촉 주의을 강화함에도 불구하고 병원 내 CPE 발생이 증가하고 있음. 2018년부터 우리 병원에서는 대변 검체를 이용한 응급실 대변감시배양 및 중환자실 입실 전 전수 대변감시배양을 시행하고 있음. 본 연구는 대변감시배양에서 CPE 감염의 유병률과 대변 보균자에서 CPE 감염의 전이율을 확인하고, 분자생물학적 분석을 통해 원내 유행하는 CPE의 역학적 특성을 확인하고자 함.

2018년 7월부터 2020년 6월까지 3차 의료기관에서 대변감시배양을 받은 환자의 균주와 임상 정보를 수집하였음. Whole genome sequencing 은 Carbapenemase producing *Klebsiella pneumoniae*와 *Escherichia coli* 균주에 대해 시행되었음.

대변 CPE 감시 배양을 실시한 1,620명의 환자 중 응급실에서 시행한 감시

배양 환자의 7.11%와 중환자실에서 시행한 감시 배양 환자의 4.46% 만이 대변 CPE 양성으로 확인되었음. 대변 보균자에서 임상 CPE 감염으로의 전환율은 응급실 감시 배양 양성 환자에서 29.41%, 중환자실 감시 배양 양성 환자에서 31.37% 였으나, 초기 중환자실 감시배양에서 음성인 환자에서는 현저하게 높음을 확인하였음 (55.0%).

대변 CPE 보균자와 임상 CPE 감염환자 군의 비교에서 고혈압 (61% *vs.* 92.3%, $P = 0.004$), 악성종양 (28.8% vs. 53.8%, $P = 0.027$), 기계적 환기 (25.4% *vs.* 53.8%, $P = 0.011$) 및 중환자실 재실 기간 (6.5 days *vs.* 1.0 days, $P = 0.017$)은 임상 CPE 감염의 중요한 위험 요소였음. 다변량 분석에서는 기저 고혈압 (Odds ratio = 5.18 [95% Confidence interval, 1.93 – 8.43], $P = 0.009$)과 악성 종양 (OR = 2.94 [95% CI, 1.55 - 7.96], $P = 0.038$) 이 대변 보균자로부터의 임상적 CPE 감염의 중요한 위험 인자로서 통계적 유의성을 보였음.

동정된 균주 간 연관성 확인을 위해 시행한 Pulsed-field gel electrophoresis (PFGE)와 Multi-locus sequence typing (MLST) 분석 결과 sequence type 307과 ST 395는 *K. pneumoniae*에서, ST 410은 *E. coli* 분리주에서 우세함을 확인하였음. 또한 본 연구에서는 *K. pneumoniae* blaKPC ST 307의 원내 높은 유병율을 확인하였음. 이를 통해 병원 외부에서 전파되는 일부 유전자형이 있더라도 특정 유전형이 병원 내에서 지속적으로 우세함을 확인할 수 있었음.

결론적으로, 중환자실에서 시행하는 감시배양보다 응급실에서 시행하는 고위험군 대변 감시배양에서 더 높은 대변 CPE 검출률을 보였음. 그리고 대변 보균자의 1/3이 궁극적으로 임상적 CPE 감염으로 진행됨을 확인함. 또한,

carbapenemase producing *K. pneumonia* ST 307과 ST 395 가 원내 유행함을 확인하였음. 이를 통해 병원 외부에서 전파되는 일부 유전자형이 있더라도 특정 유전형이 병원 내에서 지속적으로 우세함을 확인할 수 있었음. 따라서 외부로부터의 유입을 차단하는 고위험군 감시 배양뿐만 아니라, 중환자실에서 지속적인 감시 배양을 통해 대변보균자를 조기에 발견하고 중증 환자에 대한 조기 개입을 시행하여 CPE 감염의 전파를 방지하기 위한 면밀한 모니터링이 필요함.

핵심되는 말: Carbapenemase producing *Enterobacterales*; active surveillance culture; risk factor; pulsed-field gel electrophoresis; whole genome sequencing