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ORIGINAL RESEARCH

Clinical and Microbiological Characteristics of Community-Onset Carbapenem-Resistant Enterobacteriaceae Isolates

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Objective: The aim of this study was to investigate the clinical and microbiological features of community-onset CRE (CO-CRE) obtained from outpatients at a tertiary hospital in China.

Patients and Methods: We isolated 64 CRE strains from outpatients and divided them into three groups: 36 hospital-acquired CRE (HA-CRE), 28 CO-CRE including 15 community-acquired CRE (CA-CRE) and 13 healthcare-associated CRE (HCA-CRE). Clinical information was collected. The antibiotic susceptibilities of the 28 CO-CRE strains were tested. Whole-genome sequencing (WGS) was conducted, and then drug resistance gene analysis was performed. CgMLST and SNP comparisons were used to analyze the genomic relationship with *E. coli* and *K. pneumoniae* strains, respectively.

Results: In this study, the 28 CO-CRE isolates included *K. pneumoniae* (53.6%), *E. coli* (28.6%), *E. cloacae* (7.1%), *C. freundii* (7.1%) and *E. asburiae* (3.6%). The CO-CRE isolates were mainly isolated from urine samples (75%). The ceftazidime/avibactam resistance rate of community-onset *E. coli* was significantly higher than that of community-onset *K. pneumoniae*, while the aztreonam, ciprofloxacin, levofloxacin, and chloramphenicol resistance rates were significantly lower ($P<0.05$). Thirteen of the 15 *K. pneumoniae* strains belonged to ST11 containing bla_{KPC-2}. Correspondingly, 8 *E. coli* strains belonged to 7 STs, and they all were NDM producers. *K. pneumoniae* belonged to two major clusters, while *E. coli* was sporadic. The number of SNPs separating ST11 *K. pneumoniae* isolates ranged from 7 to 2154.

Conclusion: Community-onset CRE is not rare, and the dissemination of *E. coli* was sporadic while *K. pneumoniae* was clonal spread with similar STs as HA-CRE. Active surveillance of CRE in the community setting is in demand.

Keywords: community-acquired CRE, healthcare-associated CRE , *E. coli*, *K. pneumoniae*, MLST, cgMLST

Introduction

Carbapenem-resistant Enterobacteriaceae (CRE), especially *Escherichia coli* and *Klebsiella pneumoniae*, are currently the main causes of nosocomial infections. The possible therapeutic options for the treatment of CRE infection are narrow (only polymyxins, aminoglycosides, and tigecycline work).[1] The mortality rate of bloodstream CRE infections is close to 70%.[2] Being so severe with a low cure rate, high medical consumption, and high mortality, the US Centers for Disease Control and Prevention (CDC) listed CRE as an urgent threat.[3]

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To date, the research on CRE in China has mainly focused on nosocomial infections, and only a few studies on community-onset infections are available.[4] Several studies established that the probability of CRE spreading from the medical environment to the community was great, owing to the highly transferable nature of plasmid-borne carbapenemases.[5] The latest review in 2017 concluded that the incidences of CRE occurring in the community range from 0.0% to 29.5% in the world,[6] and great attention should be paid to it. However, there was no accepted uniform standard of community-acquired CRE (CA-CRE) in those studies, and the clinical and microbiological features of CRE have not been thoroughly elucidated.

This study screened 64 CRE isolates from outpatients in the First Affiliated Hospital, College of Medicine, Zhejiang University, during 2015–2018, aiming to investigate the clinical characteristics of the patients and further analyze the microbiological features to provide evidence for the clinical control of CRE.

Methods

Study Design

A total of 64 CRE isolates were screened from outpatients in the First Affiliated Hospital, College of Medicine, Zhejiang University, during the period from 2015 to 2018. The United States Centers for Disease Control and Prevention (CDC) defined Enterobacteriaceae as CRE that test resistant to at least one carbapenem antibiotic (ertapenem, meropenem, doripenem, or imipenem) or produce a carbapenemase. Except for some Enterobacteriaceae (Proteus spp., Morganella spp., Providencia spp.), resistance to imipenem should not be used to class CRE because of the intrinsic resistance of Enterobacteriaceae to imipenem.[7] We consulted the Friedman criteria described previously[8] to classify CRE infections into three categories:

1. Hospital-acquired CRE (HA-CRE): Patients who had been hospitalized in the two weeks before admission or transferred from other hospitals were defined as having nosocomial infections.[5]
2. Healthcare-associated CRE (HCA-CRE): A positive culture taken ≤ 48 hours of admission could be classified as HCA-CRE if any of the following criteria were present: 1) hospitalization for ≥ 48 hours in the previous 90 days; 2) receipt of recent invasive operations such as catheter, bone marrow aspiration, mechanical ventilation, or peritoneal/pelvic drainage tube within 90 days; 3) receipt of intravenous mediation within 30 days; 4) receipt of hemodialysis or peritoneal dialysis; 5) residing in a long-term care facility or nursing home.
3. Community-acquired CRE (CA-CRE): A positive culture that did not meet the criteria above was considered to be a strictly community-acquired infection.[9,10]

HCA-CRE and CA-CRE are collectively called community-onset CRE (CO-CRE).

According to the definition above, the 64 CRE isolates in this study were divided into 36 HA-CRE and 28 CO-CRE, including 15 CA-CRE, and 13 HCA-CRE. Clinical information was collected by referring consulting the hospital's medical records database and giving a telephone follow-up (the study design flow chart is shown in Figure 1).

Clinical Features

By consulting the hospital's medical records database and giving a telephone follow-up, clinical information was collected on age, gender, source of specimen, prior use of antimicrobial agents within 90 days, underlying diseases or comorbidity conditions (including malignancy, diabetes mellitus, chronic kidney disease, surgery history, and obstructive urinary tract disease), and invasive operations within 90 days (including catheter, bone marrow aspiration, abdominal or pelvic drainage tube, invasive blood pressure monitoring, lumbar puncture, and cystoscope). Patients ≥ 65 years old were classified as elderly patients.

Collection and Identification of Isolates

Sixty-four CRE isolates were collected from outpatients in the First Affiliated Hospital, College of Medicine, Zhejiang University, from January 2015 to December 2018 in this study. After obtaining single colonies, all isolates were identified and reidentified using an automated Vitek 2 system (bioMérieux, France). ATCC25922 (*Escherichia coli*) was used as a quality control isolate.

Antibiotic Susceptibility Testing

We conducted antibiotic susceptibility testing on the 28 community-onset CRE strains with 20 antimicrobial agents using agar dilution and broth dilution methods.

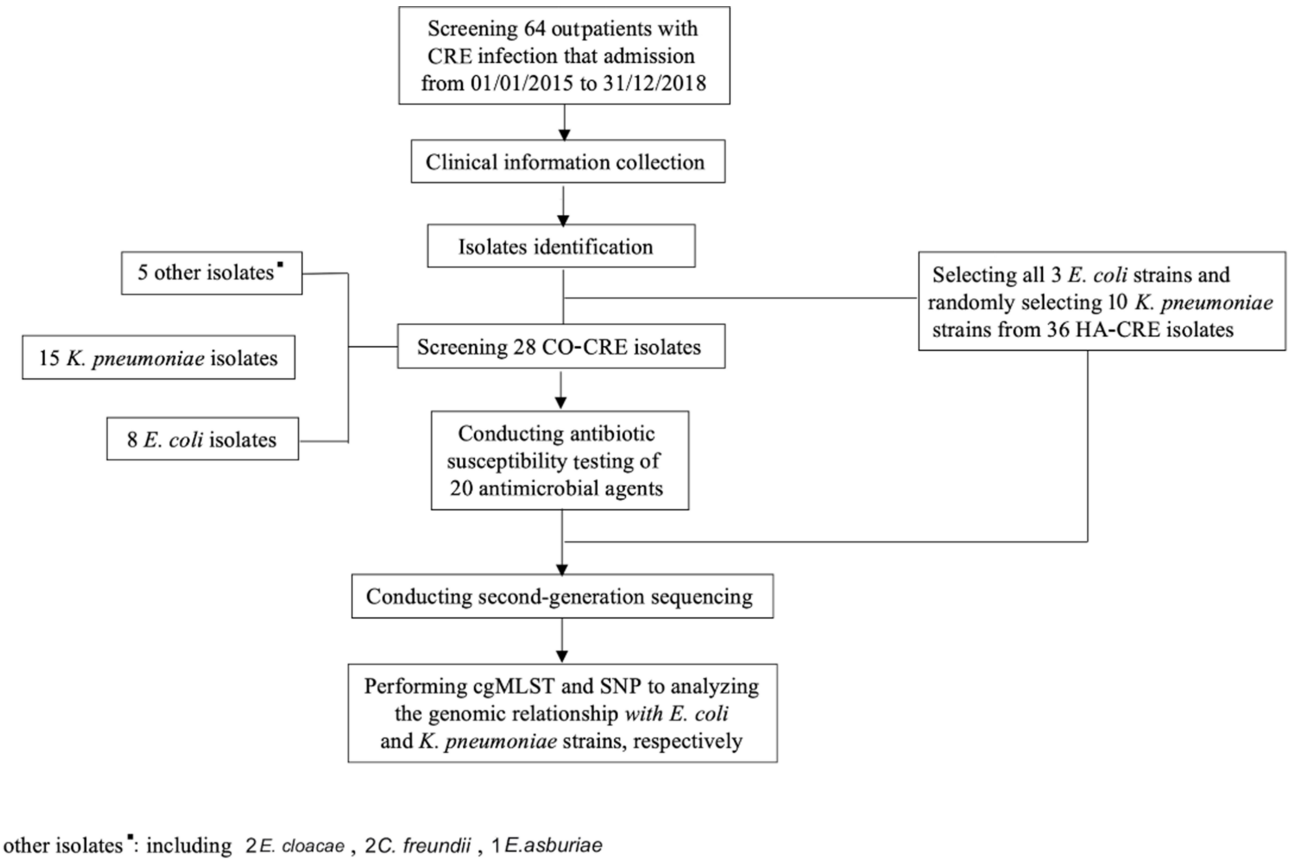

Figure 1 The study design flow chart.

Ampicillin/sulbactam, piperacillin/tazobactam, ceftazidime/avibactam, cefepime, cefotaxime, cefoxitin, cefuroxime, ceftazidime, aztreonam, gentamicin, ciprofloxacin, levofloxacin, sulfamethoxazole, chloramphenicol, amikacin, ertapenem, imipenem and meropenem results were interpreted according to the Clinical and Laboratory Standards (CLSI 2019),[11] while tigecycline and polymyxin results were interpreted under the European Committee on Antimicrobial Susceptibility Testing (EUCAST2019)[12] The Carba NP test was also performed on the 28 community-onset CRE strains for the detection of carbapenemases according to the Clinical and Laboratory Standards (CLSI 2019).[11]

Genomic DNA Extraction and Analysis

The genomic DNA of 28 CO-CRE strains and 13 randomly selected HA-CRE strains was extracted using a QIAamp DNA MiniKit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Sequencing libraries were prepared using an Illumina-HiSeq™ 2000 (Illumina Inc., San Diego, USA) platform with 2 × 100 bp paired-end reads, and the resulting sequences were assembled into contigs using CLC Genomics Workbench 8.0 (CLC Bio, Denmark). Next, the Rapid Annotation using Subsystems Technology (RAST) annotation website server was used to annotate the genomes (http://rast.nmpdr.org/rast/cgi). Acquired resistance genes and sequence types (STs) were detected using the ResFinder 3.2 tool on the CGE server (https://cge.cbs.dtu.dk/services/ResFinder/).

Comparison of CgMLST and SNP

Core genome multilocus sequence typing (cgMLST) was used to analyze the genomic relationships of 11 carbapenem-resistant *E. coli* (CREC) and 25 carbapenem-resistant *K. pneumoniae* (CRKP) strains. Genome assemblies of these strains were imported into SeqSphere+ (version 4.1.9; Ridom) as FASTA files for cgMLST analysis. Phylogenetic analyses were performed based on validated *E. coli* cgMLST version 1.0 (2,336 target genes) and *K. pneumoniae* cgMLST (2,237 target genes). In addition, the single nucleotide polymorphisms (SNPs) of 13 *K. pneumoniae* strains belonging to the most representative ST were analyzed with Snippy v.4.4.5 (https://github.

com/tseemann/snippy) and snp-dists v0.6.3 (https://github.com/tseemann/snp-dists) with default parameters.[13]

Statistical Analysis

All clinical databases, divided into three independent groups, were analyzed using SPSS version 25.0. Mean ±SD values were reported for the normal distribution of continuous variables, while numbers and percentages were calculated for categorical variables. The homogeneity variance and analysis of variance were compared among the groups, and the chi-square test was used under an equal condition of normal distributed continuous variables. Categorical variables were compared using Fisher's exact test. A P value<0.05 was considered to be statistically significant.

Results

Differences Among Patients with CA-CRE, HCA-CRE and HA-CRE Infections Based on Clinical Data

The clinical characteristics of the 28 community-onset CRE (CO-CRE) collected from patients with infections are listed in Table 1 together with the gene characteristics of these isolates. As shown in Table 1, the most common pathogen of the 28 CO-CRE isolates was *K. pneumoniae* (n=15, 53.6%), followed by *E. coli* (n=8, 28.6%), other pathogens including *E. cloacae* (n=2, 7.1%), *C. freundii* (n=2, 7.1%) and *E. asburiae* (n=1, 3.6%). Twenty-one (75%) strains were from urine, 3 from sputum, 2 from secretion, 1 from blood and 1 from swab samples. The mean age of the 28 patients was 60.96±19.63 years with a range of 23–92 years. Half of the patients were men, and half were women. Fifteen (53.6%) patients were elderly patients. The comparisons of the CA-CRE, HCA-CRE, and HA-CRE isolates are summarized in Table 2. The rate of *K. pneumoniae* infection was highest in the HA-CRE group (P<0.05). The patients in the HCA-CRE group had the highest cephalosporin-use history and catheter history (P<0.05). No other significant differences in age, gender, underlying conditions, other invasive operations, or antibiotic-use history were found among the three groups.

Susceptibility Results of 28 CO-CRE Isolates to 20 Antimicrobial Agents

The MICs of 20 antimicrobial agents against 28 CO-CRE isolates were determined. All pathogens were not susceptible to ampicillin/sulbactam, cefotaxime, cefoxitin, cefuroxime, or ertapenem. Pathogens also showed high resistance to antibiotic agents, including piperacillin/tazobactam, cefepime, ceftazidime, aztreonam, imipenem, meropenem, azithromycin, ciprofloxacin, levofloxacin, sulfamethoxazole, and chloramphenicol (Supplementary Table 1). However, ceftazidime/avibactam, tigecycline, amikacin, and polymyxin showed good activity against the CRE isolates. Further comparisons according to species are shown in Figure 2. No significant difference was found in the antibiotic resistance rate within CRKP or CREC (Figure 2A and B). The ceftazidime/avibactam resistance rate of community-onset CREC was significantly higher than that of community-onset CRKP, while the resistance rates of CO-CREC to aztreonam, ciprofloxacin, levofloxacin, and chloramphenicol were significantly lower than those of CO-CRKP (P<0.05) (Figure 2C).

MLST and Resistance Genes of the CO-CRE Isolates

The gene characteristics of 28 community-onset CRE isolates are summarized on the right side of Table 1. According to the analysis, 15 CRKP strains belonged to 3 different sequence types (STs), including 13 belonging to ST11, 1 belonging to ST15, and 1 belonging to ST147. Correspondingly, 8 CREC strains belonged to 7 different clones. Having obtained the whole-genome data, we analyzed the carbapenem resistance genes. The CRKP strains belonging to ST11 mainly contained bla_{KPC-2}, except one strain carried no carbapenem genes. The ST15 and ST147 strains were found to carry NDM-1 and NDM-5, respectively. All CREC strains were NDM producers: 5 strains carried bla_{NDM-5}, 2 strains carried bla_{NDM-1}, and 1 strain carried both bla_{NDM-5} and bla_{KPC-2} (HCA18). Although no carbapenem genes were found in the 3 *E. cloacae* and *E. asburiae* strains (HCA24, HCA25, and HCA26), these strains were found to carry the AmpC beta-lactamase gene. Additional beta-lactam genes found were listed in the table. The Carba NP tests were negative for 4 strains (1 *K. pneumoniae*, HCA09; 2 *E. cloacae*, HCA24 and HCA25; and 1 *E. asburiae*, HCA26) but positive for all carbapenem gene producers. The confirmatory test results were consistent with the WGS results.

CgMLST and SNP Analysis

Minimum spanning trees of the core genome sequences of *K. pneumoniae* and *E. coli* were generated (Figures. 3A and B, respectively). With the cluster distance threshold of

Table 1 Overview of Clinical Characters and Antimicrobial Resistance Genes of 28 Patients with Community-Onset CRE Infections

Patients' Clinical Characters									Gene Characters of Isolates				
		Specimen	Age/ Gender (M/F)	Department	Underlying Conditions	Surgery		Prior Antibiotic Use Within 90 Days	Carba NP Test	ST	AmpC	Carbapenems Genes	Other Beta-Lactam Genes
						Within 90days	Beyond 90days						
K. pneumoniae	CA02	urine	71/F	Urology Surgery	Malignancy	N	Y	N	+	11	N	KPC-2	CTX-M-65, SHV-155, SHV-172, SHV-31, TEM-1B
	CA03	sputum	65/M	Emergency Department	N	N	N	N	+	11	N	KPC-2	CTX-M-65, SHV-12, TEM-1B
	CA04	urine	86/M	Urology Surgery	N	N	N	N	+	11	N	KPC-2	CTX-M-65, SHV-182
	CA05	urine	82/F	Urology Surgery	Malignancy	N	Y	Fosfomycin	+	11	N	KPC-2	CTX-M-65, SHV-12, SHV-129, SHV-13, SHV-155, SHV-172, SHV-31, TEM-1B
	CA07	urine	73/M	Urology Surgery	Obstructive urinary tract disease	N	N	N	+	11	N	KPC-2	CTX-M-3, SHV-182, TEM-1B,
	CA08	blood	77/M	Emergency Department	N	N	N	N	+	11	N	KPC-2	CTX-M-14b, SHV-182, TEM-1B
	CA11	swab	45/F	Nephrology Center	Acute myeloid leukemia	N	N	N	+	11	N	KPC-2	CTX-M-65, SHV-182, TEM-1B
	CA12	urine	87/F	Urology Surgery	N	N	Y	N	+	11	N	KPC-2	CTX-M-65, SHV-11
	CA15	urine	75/M	Urology Surgery	N	N	Y	Macrolides	+	147	N	NDM-5	SHV-11, CTX-M-3, SHV-11, SHV-67, TEM-1B

(*Continued*)

Table 1 (Continued).

Patients' Clinical Characters									Gene Characters of Isolates				
		Specimen	Age/ Gender (M/F)	Department	Underlying Conditions	Surgery		Prior Antibiotic Use Within 90 Days	Carba NP Test	ST	AmpC	Carbapenems Genes	Other Beta-Lactam Genes
						Within 90days	Beyond 90days						
K. pneumoniae	HCA01	sputum	23/M	Nephrology Center	Acute myeloid leukemia	N	N	Cefoperazone/ Sulbactam, moxifloxacin, linezolid, imipenem, faropenem, cefuroxime, azithromycin	+	11	N	KPC-2	SHV-12, SHV-129
	HCA06	urine	32/F	Emergency Department	N	Y	N	Piperacillin/tazobactam, levofloxacin, fosfomycin	+	11	N	KPC-2	CTX-M-65, SHV-182, TEM-1B
	HCA09	sputum	19/M	Nephrology Center	Acute myeloid leukemia	N	N	N	−	11	N	N	CTX-M-65, SHV-182, TEM-1B
	HCA10	secretions	70/M	Anorectal Surgery	Malignancy	Y	N	Cefoperazone/ Sulbactam	+	11	N	KPC-2	SHV-182
	HCA13	secretions	45/F	Anorectal Surgery	N	Y	N	Cefoperazone/ Sulbactam	+	11	N	KPC-2	CTX-M-65, SHV-12, SHV-129, TEM-1B
	HCA14	urine	53/M	Nephrology Center	Chronic kidney disease, cerebral infarction	Y	Y	Moxifloxacin, amikacin	+	15	N	NDM-1	CTX-M-15, OXA-1, SHV-106, SHV-28, TEM-1B
E. coli	CA17	urine	59/F	Nephrology Center	N	N	Y	N	+	2705	N	NDM-1	CTX-M-55, CTX-M-14b, TEM-176
	CA19	urine	47/F	Nephrology Center	Obstructive urinary tract disease	N	N	N	+	354	N	NDM-5	CTX-M-14, NDM-5, TEM-1B
	CA20	urine	35/F	Urology Surgery	Renal abscess	N	N	N	+	410	N	NDM-5	CTX-M-65, TEM-1B
	CA21	urine	35/F	Nephrology Center	Chronic kidney disease	N	Y	Aztreonam	+	617	N	NDM-1	CTX-M-55, TEM-176

	CA23	urine	78/F	Nephrology Center	N	N	N	N	+	69	N	NDM-5	N
E. coli	HCA16	urine	69/F	Respiratory Medicine	Malignancy/ diabetes mellitus	N	N	Cephalosporin, macrolides, meropenem	+	69	N	NDM-5	N
	HCA18	urine	92/M	Emergency Department	Chronic kidney disease, diabetes mellitus, heart failure	N	Y	Cephalosporin, macrolides	+	167	N	KPC-2, NDM-5	CTX-M-15, OXA-1, SHV-12, SHV-13, SHV-155, SHV-172, SHV-31
	HCA22	urine	69/F	Nephrology Center	Atherosclerosis	N	N	N	+	10*	N	NDM-5	TEM-1B
E. cloacae	HCA24	urine	66/M	Urology Surgery	Obstructive urinary tract disease	N	N	Cephalosporin, clarithromycin	—	93	Y	N	ACT-7, TBM-1B
	HCA25	urine	62/M	Infectious Disease	Obstructive urinary tract disease	N	N	Cephalosporin, fosfomycin	—	93	Y	N	ACT-7, TBM-1B
E. asburiae	HCA26	urine	77/F	Urology Surgery	Obstructive urinary tract disease, schistosomiasis hepatopathy	N	N	Cephalosporin, fosfomycin	—	1065	Y	N	ACT-6
C. freundii	HCA27	urine	59/M	Urology Surgery	Obstructive urinary tract disease, diabetes mellitus, chronic kidney disease	N	N	Biapenem, nitrofural, cephalosporin, macrolides	+	116	N	NDM-1	SHV
	CA28	urine	46/M	Nephrology Center	Chronic kidney disease	N	Y	N	+	118	N	NDM-1	TEM

Abbreviations: Y, yes; N, no; +, positive; —, negative.

Table 2 Comparisons of Clinical Characters Among CA-CRE, HCA-CRE, and HA-CRE[a] with All 64 CRE Cases

Variable	No.(%) of CA-CRE (n=15)	No.(%) of HCA-CRE (n=13)	No.(%) of HA-CRE (n=36)	P-value	P[b]	P[c]	P[d]
Age>65	7(46.7)	6(46.2)	14(38.9)	0.832			
Male	6(40.0)	8(61.9)	23(63.9)	0.320			
Pathogens							
K. pneumoniae	9(60.0)	6(46.2)	30(83.3)	0.028*	0.705	0.144	0.024*
E. coli	5(33.3)	3(23.1)	3(8.3)	0.068			
Other pathogens	1(6.7)	4(3.1)	3(8.3)	0.086			
Underlying conditions							
Malignancy	2(13.3)	2(15.4)	10(27.8)	0.549			
Diabetes mellitus	0	3(23.1)	4(11.1)	0.121			
Chronic kidney disease	2(13.3)	4(30.8)	2(5.6)	0.054			
Surgery history	6(40.0)	4(30.8)	23(63.9)	0.081			
Obstructive urinary tract disease	2(13.3)	4(30.8)	4(11.1)	0.286			
Invasive operation within 90 days							
Catheter	0	9(69.2)	10(27.8)	<0.001*	N/A	N/A	0.018*
Bone marrow aspiration	0	2(15.4)	3(8.3)	0.293			
Abdominal/Pelvic drainage tube	0	3(23.1)	7(19.4)	0.148			
Invasive blood pressure monitoring	0	1(7.7)	1(2.8)	0.420			
Lumbar puncture	0	1(7.7)	0	0.203			
PICC [d]	0	0	4(11.1)	0.388			
Mechanical ventilation	0	0	1(2.8)	1.000			
Cystoscope	0	1(7.7)	1(2.8)	0.420			
Prior antibiotic use within 90 days							
Carbapenems	0	3(23.1)	17(47.2)	0.001*	N/A	N/A	0.191
Cephalosporin	0	10(76.9)	11(30.6)	<0.001*		N/A	0.008*
Macrolides	1(6.7)	3(23.1)	0	<0.001*	0.311	N/A	N/A
Moxifloxacin	0	2(15.4)	8(22.2)	0.132			
Fosfomycin	1(6.7)	2(15.4)	1(2.8)	0.141			
Amikacin	0	1(7.7)	1(2.8)	0.420			
Linezolid	0	1(7.7)	1(2.8)	0.420			
Piperacillin/tazobactam	0	1(7.7)	4(11.1)	0.589			
Aztreonam	1(6.7)	3(23.1)	0	0.010*	0.311	N/A	N/A

Notes: [a]CA-CRE, HCA-CRE, and HA-CRE: abbreviations of "community-acquired CRE, healthcare-associated CRE, and hospital-acquired CRE"; [b]P: comparison between CA-CRE & HA-CRE; [c]P: comparison between HCA-CRE & HA-CRE; [d]P: comparison between CA-CRE & HCA-CRE; *P < 0.05.
Abbreviation: N/A, not applicable.

10 alleles in the core genome for CREC, no strains belonged to the same cluster. Two major CRKP clusters included all ST11 community-onset and hospital-acquired isolates, except isolates of ST147 and ST15. Additionally, the numbers of SNPs separating ST11 *K. pneumoniae* isolates in this study ranged from 7 to 2154 (Figure 4). Among the ST11 group, strains CA02 and HAZY60 were virtually identical with 7 SNP differences, suggesting that they originated from a single clone.

Discussion

To date, the available studies have indicated that CRE infection does great harm to the public, increasing medical resource consumption and leading to high mortality.

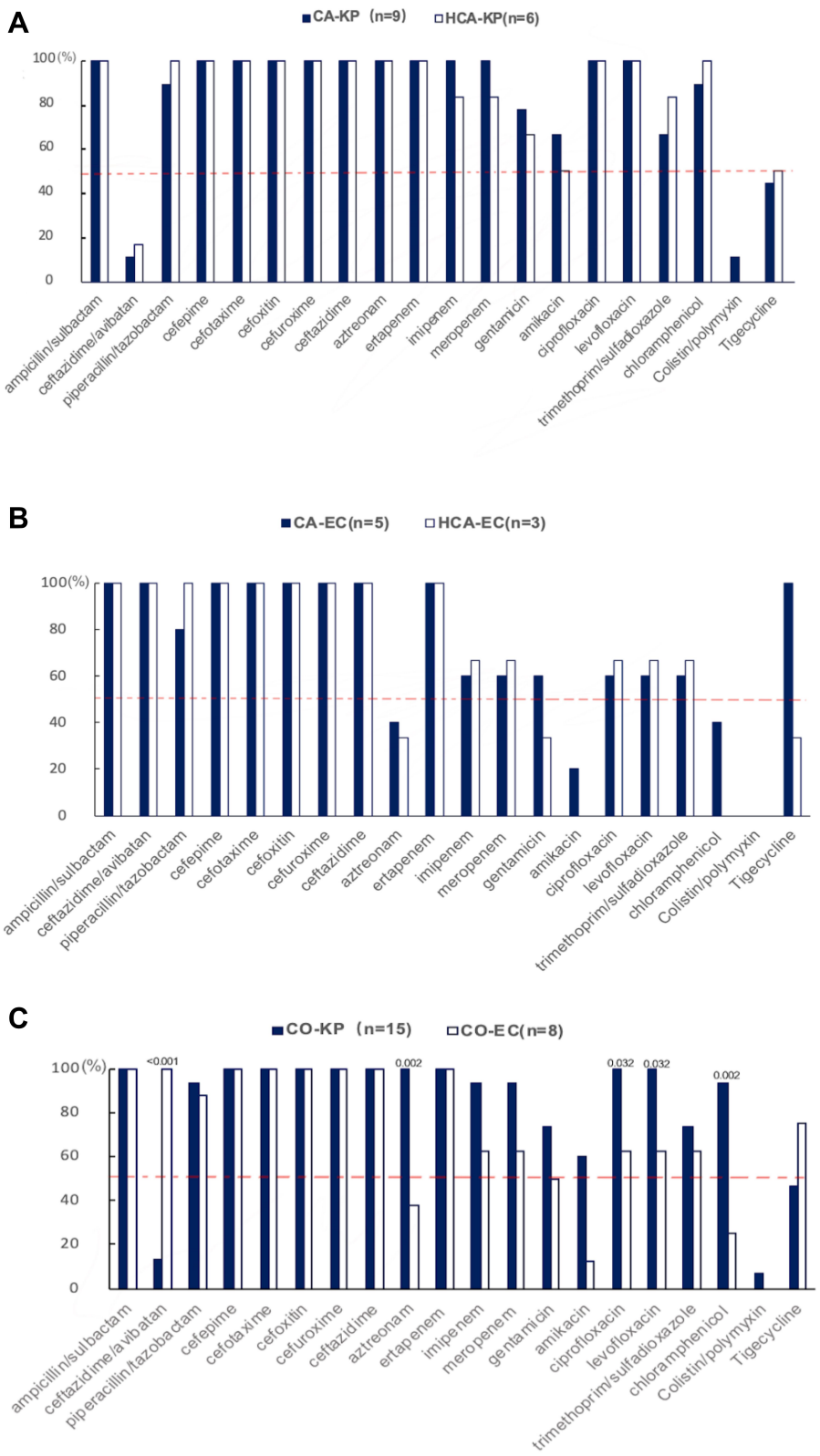

Figure 2 (**A**) Antibiotic-resistance rate comparisons of 9 community-acquired *K. pneumoniae* (CA-KP) and 6 healthcare-associated *K. pneumoniae* (HCA-KP) isolates to 20 common agents, with no significant difference (P>0.05). The red dotted line provides a reference with a rate of 50%. (**B**) Antibiotic-resistance rate comparisons of 5 community-acquired *E. coli* (CA-EC) and 6 healthcare-associated *E. coli* (HCA-EC) isolates to 20 common agents, with no significant difference (P>0.05). The red dotted line provides a reference with a rate of 50%. (**C**) Nonsusceptible rate comparisons of 15 community-onset *K. pneumoniae* (CO-KP) and 8 community-onset *E. coli* (CO-EC) isolates to 20 common agents. The significant P-value is marked above the bar chart (P<0.05). The red dotted line provides a reference with a rate of 50%.

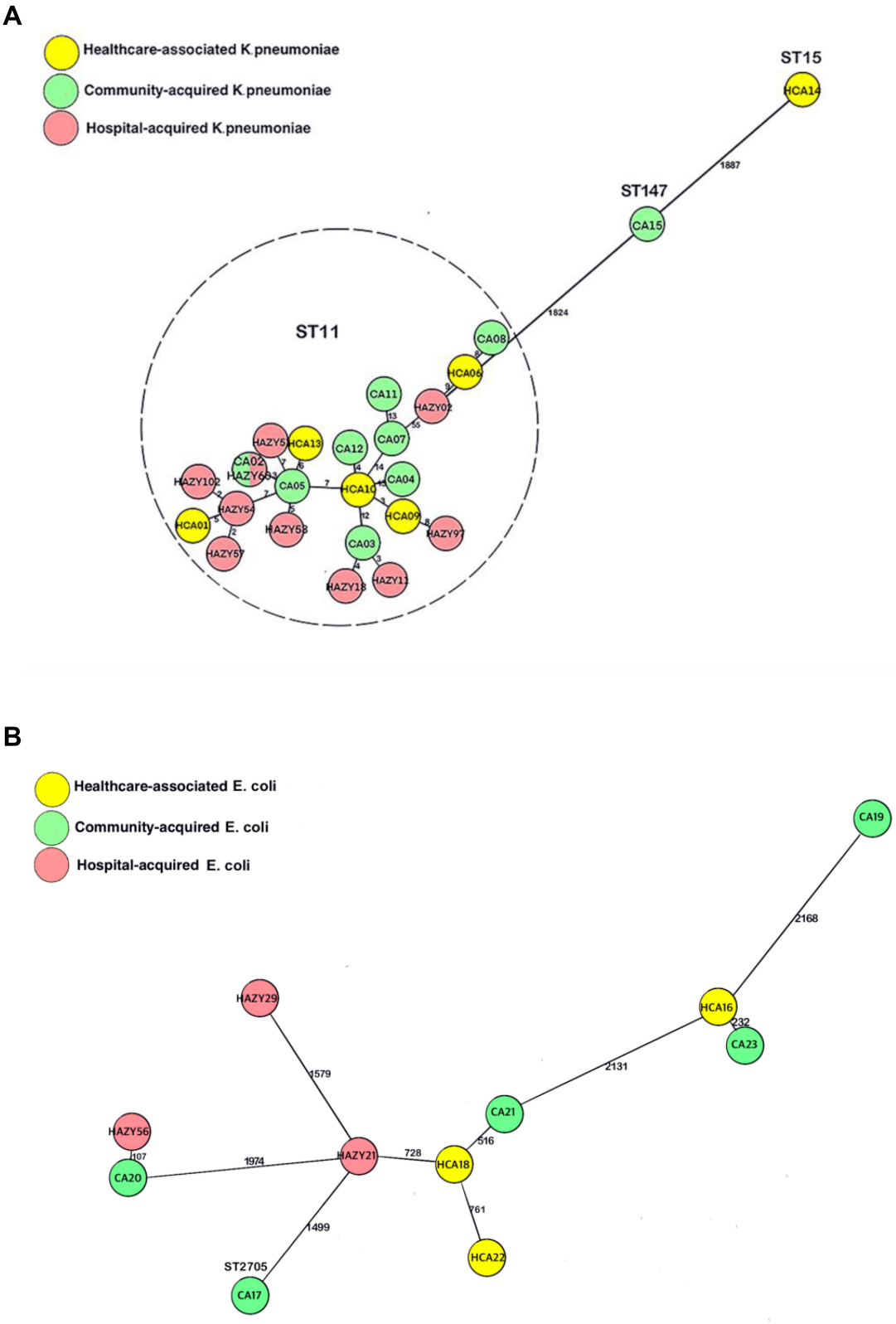

Figure 3 (**A**) Minimum spanning tree of core genome sequences of *K. pneumoniae* collected from 2015~2018 (n = 25, 15CO-KP and 10HA-KP). The cluster distance threshold is 15 alleles. Each circle (node) represents one or multiple identical sequences. The number between the nodes illustrates the number of target genes with different alleles. The text in each circle indicates the case identifier. The sequence types were labeled above the circles. (**B**) Minimum spanning tree of core genome sequences of *E. coli* collected from 2015~2018 (n = 11, 8 CO-EC and 3 HA-EC). The cluster distance threshold is 10 alleles. Each circle (node) represents one or multiple identical sequences. The number between the nodes illustrates the number of target genes with different alleles. The text in each circle indicates the case identifier.

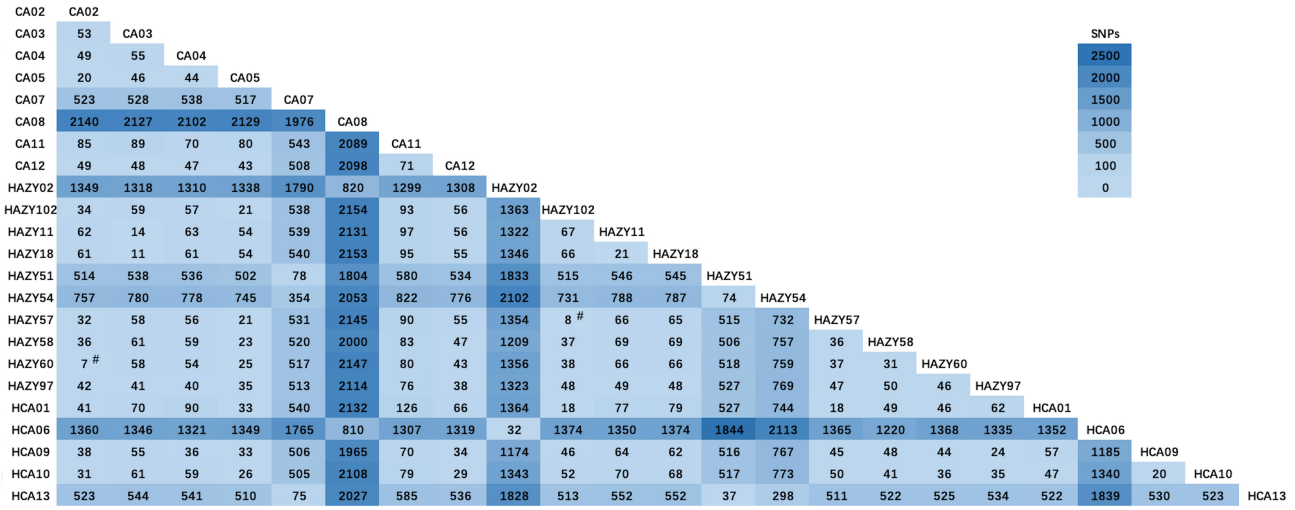

Figure 4 The number of single-nucleotide polymorphisms (SNPs) between each ST11 *K. pneumoniae* strain heatmap. The deeper the color is, the larger the SNP quantity scale. # indicates core genome SNP differences ≤10.

Several studies have depicted that there is a tendency of CRE to spread from the hospital to the community.[6] However, as no uniform standard of community-acquired CRE was accepted in those studies and the clinical and microbiological features have not been thoroughly elucidated, our study examined the clinical and microbiological characteristics of CA-CRE with HCA-CRE and HA-CRE, which might provide additional information.

In this four-year study, clinical isolates of CRE were collected from 64 outpatients, and several significant findings in the clinical features of these strains were observed. First, the most common pathogen in community-onset CRE was *K. pneumoniae*, followed by *E. coli, E. cloacae, C. freundii* and *E. asburiae*. This result was consistent with previous studies in the USA[14,15], Spain,[16] and Greece[17] but slightly different from previous studies in India,[18] and Australia,[19] where *E. coli was* the most common pathogen. The prevalence of CRE may vary worldwide. In this study, there were no other significant differences in other clinical characteristics among the three groups (CA-CRE, HCA-CRE, and HA-CRE), including age, sex, and underlying conditions, except that prior catheter and cephalosporin use history in the HCA-CRE group was significantly higher. No significant difference in the resistance to antibiotics was found between community-acquired and healthcare-associated CRE isolates. The options for therapy are limited; although the pathogens showed high resistance to most antibiotic agents, ceftazidime/avibactam, amikacin, tigecycline, and polymyxin showed good activity against CRE, indicating that they might be good drugs. We referred to previous studies for antibiotic resistance rates in hospitals,[20,21] and found similar pharmacological activity of those four agents, demonstrating that CO-CRE appears to mirror HA-CRE.

In this study, the predominant sequence type of community-onset *CRKP* was ST11, which carried the blaKPC−2 carbapenemase gene. This is consistent with a previous nationwide study in China.[21] Strain HCA09 had no carbapenemase resistance mechanism, which may have resulted from the loss of OmpK36 porins. All community-onset CREC strains were bla_{NDM} gene carriers, including NDM-1 and NDM-5. NDM was reported to be prevalent in most parts of Asia and thought to be the cause of sporadic outbreaks all over the world, including the USA, Japan, and the Netherlands.[22] A previous study suggested that NDM-1 was widely disseminated in the environment mainly via seepage and drinking water supplies,[23] which could explain the results in our study. Moreover, two healthcare-associated *E. cloacae* strains and one healthcare-associated *E. asburiae* strain were found to carry no carbapenemase gene (HCA24, HCA25, and HCA26), and the carbapenem resistance of these genes might have joint action with the AmpC beta-lactamase gene.[24]

As >100 alleles differed in all *E. coli* isolates in our study, the strains were classified as nonrelated. We can conclude that CREC dissemination in the community was sporadic. Correspondingly, we observed a close genetic relatedness among CRKP isolates for HA-CRE, CA-CRE, and HCA-CRE. Two major *K. pneumoniae* clusters included all ST11 community-onset and hospital-acquired isolates, except isolates of ST147 and ST15, which provided strong evidence that strains prevalent in

communities and hospitals had the same origin. *K. pneumoniae* strains CA02 and HAZY60 were virtually identical with 7 SNP differences, suggesting that they originated from a single clone. We performed further investigation by extending the hospitalization records to one year, including their admission time, admission department and any operation relationship, but no clinical epidemiological relationship was found. The results of this study suggested that CA-CRKP might be spread from hospitals, consistent with a previous study indicating that the mean time of patients carrying CRE with negative culture results was approximately one year.[25] To support our finding, further studies are needed to confirm the evidence that inpatients carried the bacteria out of the hospital unconsciously and transmitted it to healthy people through the environment.[26]

One limitation would be that the study analyzed the sensitivity against 20 antimicrobials in Enterobacteriaceae acquired in the community, but not in the hospital; however, it uses these hospital strains to compare clinical and phylogenetic data. Another limitation is that the study was carried out in a single center and is not representative of all countries.

In conclusion, community-acquired CRE is not rare, and *E. coli* dissemination was sporadic with weak ST relationships with HA-CREC, while *K. pneumoniae* was clonal spread with similar STs as HA-CRKP. Currently, the main treatment of infection is based on empiric antibiotic therapy, without first consideration of CRE, leading to treatment failure at times. Active surveillance of CRE in the community setting is in demand. To control the rapid spread of CRE and avoid cross-infection in the community, interventions should be implemented, including effective decolonization of CRE in patients who have been hospitalized and good hygiene.3

Ethics Approval

This study was approved by the Ethics Committees of the First Affiliated Hospital, College of Medicine, Zhejiang University (Approval No. IIT20200116A), and all participants gave informed consent. The study complied with the Declaration of Helsinki.

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Author Contributions

Name of All contributing authors: Hangbin Hu, Jinchao Mao, Yiyi Chen, Jie Wang, Piaopiao Zhang, Yan Jiang, Qing Yang, Yunsong Yu, Tingting Qu. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

All authors have no conflicts of interest in this work.

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