

Spread of Carbapenem-Resistant Enterobacteriaceae in a Region, China: How to Control?

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Abstract

Background

The increasing number of carbapenem-resistant Enterobacteriaceae (CRE) has become a serious problem globally. This study aimed to elucidate their geographically epidemiological characteristics and explore evidence-based infection control measures.

Results

A total of 930 phenotypically confirmed CRE isolates collected from 19 hospitals were genotypically characterised. *K.pneumoniae* (KP) and *E.coli* isolates were 787 (85.17%) and 96 (10.39%) among 924 carbapenemase-producing Enterobacteriaceae (CPE) isolates. Two major carbapenemase genes KPC-2 and NDM in CPE isolates accounted for 84.63% ($n = 782$) and 13.74% ($n = 127$). ST11 comprised 86.32% (631/731) of KPC-2 KP isolates. Wzi typing could discriminate ST11 KP clones and precisely track their transmission. Conjugation assays demonstrated that Some KPC-2- and NDM-bearing plasmids could be conjugatively transferred. The transferability was influenced by different STs and different wzis. CRE patients were becoming increasingly younger due to nosocomial CRE acquisition. The average length of hospitalization of these patients showed a downward trend mainly due to significant increases in voluntarily discharged rates and mortality rates. No associations between the rates of antibiotics consumption and CRE prevalence were observed. Evidence-based measures could effectively reduce the prevalence of ST11-wzi209 clone but failed to control the dissemination of ST11-wzi141 KP clone.

Conclusions

Continued vigilance for the importations should be maintained. Coordinated regional interventions are urgently needed to reduce CRE threat.

Background

Owing to the consequences of unreasonably excessive and frequently unnecessary use of antibiotics in humans and animals, bacterial strains that harbored novel and transmissible antibiotic resistance genes were continually emerging [1]. Of these, carbapenemase-producing Enterobacteriaceae (CPE) strains were of particular concern as they possessed an extremely high potential for transmission and had been associated with increased mortality, longer hospital stays and higher hospital costs [2]. This scenario had resulted in the introduction of tigecycline, colistin and ceftazidime-avibactam as the novel therapeutic agents for these infections. However, the extensive use of these antibiotics in clinical settings was rapidly followed by the emergence of new resistance to these drugs [3–5], leaving treatment options even fewer or non-existent. Given the paucity of novel or clinically proven effective antibiotics, infection control and prevention must be given an urgent priority.

In response, numerous international, national and regional guidelines and strategies developed to address the escalating threat of carbapenem-resistant Enterobacteriaceae (CRE) continued to increase worldwide [6, 7]. At present, there was still no consensus on the optimally effective interventions or the best combination of interventions to curtail the spread of CRE. Interventions in healthcare settings most often had involved bundled control measures, which varied by institution and effect [8]. These discrepancies might reflect differences in regional epidemiology that required distinct prevention strategies.

In China, since the first *Klebsiella pneumoniae* (KP) producing KPC-2 was reported in 2007 [9], the prevalence of carbapenem-resistant KP (CRKP), especially the KPC-2 ST11 clones, had increased. Recent data from the CHINET surveillance system showed that imipenem-resistance rate of KP increased from 3.0% in 2005 to 25% in 2018, with resistance rate reaching over 45% in some hospitals [10]. The annual upward trend was also obtained from the China Antimicrobial Resistance Surveillance System (<http://www.carss.cn/>), which further showed that the annual isolation rate of CRKP in 2014–2019 was for geriatric patients over the age of 65 years 7.4/8.7/9.9/10.2/11.3/12.2 and for adult patients 5.3/6.3/7.4/7.8/8.9/9.7 (%), respectively. However, very few detailed epidemiological data are available to uncover the underlying mechanisms for these increases.

In this context, we sought to determine the genotypic characterization of CRE in a region by performing a series of integrated analysis of clinical, epidemiologic, microbiologic and molecular data; and to assess the effectiveness of control measures by describing the course and management of CPE-associated outbreaks.

Results

Genotypic characterization of CRE isolates

A total of 930 phenotypically confirmed CRE isolates (643 from various clinical diagnostic specimens and 103 from stool specimens in our hospital as well as 184 from other 18 hospitals) during the period from 2013 through 2018 were screened for carbapenemase genes, 924 isolates were detected positive including 782 KPC-2 isolates, 127 NDM isolates and 7 IMP-4 isolates (Table 1). MLST and wzi assigned 787 carbapenemase-producing KP (CPKP) isolates to 27 STs and 32 wzi alleles (wzis), respectively. Among them, KPC-2 KP accounted for the largest proportion (92.88%, 731/787) with ST11-wzi209 being the most prevalent type (60.47%, 442/731), followed by ST11-wzi141 (21.61%, 158/731) (Table 2). The 96 carbapenemase-positive *E.coli* isolates contained 36 KPC-2-producers belonging to 9 distinct STs with a predominance of ST43 (55.56%, 20/36) and 60 NDM-producers belonging to 20 unique STs (13 isolates unidentified) dominated by ST167 (36.17%, 17/47).

Table 1

Distribution of carbapenemase-producing Enterobacteriaceae isolates and their carbapenemase genes

Organisms	Carbapenemase genes	Number of adult clinical isolates in our hospital	Number of isolates from adult fecal specimens in our hospital	Number of clinical isolates in other hospitals
<i>Klebsiella pneumoniae</i>	KPC-2	519	90	122
	NDM-1	11		24
	NDM-5	6	2	2
	IMP-4	2		2
	KPC-2 and NDM-1	6		1
<i>Escherichia coli</i>	KPC-2	30	6	
	NDM-1	9	1	2
	NDM-5	23	1	20
	NDM-7	1		
	NDM-9	2		1
<i>Enterobacter cloacae</i>	NDM-1	7		2
<i>Klebsiella oxytoca</i>	KPC-2	3	1	
	NDM-1	4	1	
	IMP-4			3
Other species	KPC-2	10	1	
	NDM-1	5		3
	NDM-5 and KPC-2	1		

Table 2
Distribution of common STs and wzi alleles in carbapenemase-producing *Klebsiella pneumoniae*

Sequence types	Wzi alleles	Carbapenemase genes	Number of adult clinical isolates	Number of isolates from adult fecal specimens in our hospital	Number of clinical isolates in other hospitals
in our hospital					
ST11	wzi209	KPC-2	277	58	107
ST11	wzi141	KPC-2	149	8	1
ST11	wzi64	KPC-2	17		1
ST11	wzi89	KPC-2	3	1	1
ST11	wzi133	KPC-2			2
ST11	-a	KPC-2	2	2	2
ST15	wzi384	KPC-2	18	5	7
ST15	wzi173	KPC-2			2
ST15	wzi19	KPC-2	2		1
ST17	wzi141	NDM-5	3	2	
ST20	wzi84	NDM-1 or 5	1		10
ST23	wzi1	KPC-2 or NDM-1	13		
ST437	wzi109	KPC-2	21	6	
ST617	wzi162	KPC-2	6	6	
ST2068	wzi381	NDM-1	1		3
ST307	wzi173	IMP-4			2

a : No detected.

Among all 533 patients with CRE, 127 consecutive cases with clinical infections underwent stool screening, 96 of whom were positive (75.59%, 96/127). Eighty-six patients infected by CPE strains also intestinally colonized with the same organisms.

ST11-wzi209 KPC-2 KP was established in all hospitals and some of which could be traced back to patient transfer between hospitals. ST20-wzi84 NDM-1 KP and ST167 NDM-5 E.coli were detected in 5 hospitals. Some ST11 KPC-2 subclones (wzi89, wzi133, wzi64 and wzi141) and ST15 KPC-2 subclones (wzi173, wzi19) based on capsular typing were found in two hospitals. Some STs-wzis or species (ST15-

wzi384, ST2068-wzi381, IMP-4 K.oxytoca) were observed in three hospitals, and other relatively rare STs were only confined to their individual settings.

Clinical characteristics of CRE patients

The majority of CRE patients (78.5%) were male, but no statistical differences in clinical features between sexes were observed (Table 3). Further analysis showed that CRE patients were becoming increasingly younger, especially evident in 2016. This could be explained by the rapid increase in the number of patients under the age of 65 years (Table 3). One hundred and forty-seven (96.08%, 147/153) of these young cases were nosocomially acquired and the main reasons for primary admission were cerebral hemorrhage (41.45%), motor vehicle accident (11.18%), pulmonary infection (10.53%), fall injury (8.55%) and tumor (6.58%). More than half patients had a previous hospital admission within 1 month before the current admission. As shown in Table 3, the average length of hospitalization of CRE patients showed a downward trend mainly due to significant increases in voluntarily discharged rates and mortality rates. The number of imported CRE patients was 6 (5.94%, 101) in 2015 and increased to 32 (15.46%, 207) in 2018 ($p = 0.017$). There was a statistically significant increase in the number of exportations in CRE patients [1 (0.94%, 106) in 2016 vs 20 (9.66%, 207) in 2018 ($p = 0.004$)]. The number of intra-hospital transfer of CRE patients also increased gradually from 18 (17.82%, 101) in 2015 to 64 (30.92%, 207) in 2018 ($p = 0.015$).

Table 3 Characteristics of adult patients with carbapenem-resistant Enterobacteriaceae according to the years

	2014-18 Male (n = 360) Female (n = 170)	2014 (n = 25)	2015 (n = 101)	2016 (n = 106)	2017 (n = 91)	2018 (n = 207)
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Age (years)	72.14 ± 16.68	71.92 ± 15.37	80.36 ± 10.70	76.94 ± 13.60	70.99 ± 17.58	69.41 ± 17.03
P value	0.514			0.213	0.037	0.423
Patients under 65 years (No.)	104 (28.89)	49 (28.82)	2 (8)	13 (12.87)	39 (36.79)	35 (38.46)
P value	0.960			0.843	< 0.001	0.809
Previous hospitalization within last one month	211 (58.61)	90 (52.94)	19 (76)	71 (70.3)	67 (63.21)	49 (53.85)
P value	0.219			0.572	0.279	0.183
Hospital length of stay (days)	41.95 ± 37.31	42.04 ± 40.81	53.96 ± 37.69	47.88 ± 31.38	41.60 ± 33.60	42.86 ± 33.34
P value	0.951			0.748	0.069	0.967
Outcome						
Improvement	191 (56.85)	93 (57.41)	8 (33.33)	52 (54.74)	68 (64.76)	59 (67.82)
Voluntary discharge	62 (18.45)	37 (22.84)	3 (12.5)	17 (17.89)	16 (15.24)	15 (17.24)
Death	83 (24.70)	32 (19.75)	13 (54.17)	26 (27.37)	21 (20)	13 (14.94)
P value	0.329			0.051	0.333	0.646
Patient importation	52 (14.44)	17 (10)	6 (24)	6 (5.94)	12 (11.32)	13 (14.28)
P value	0.156			0.018	0.170	0.533
Patient transfer between wards	106 (29.44)	37 (21.76)	8 (32)	18 (17.82)	27 (25.47)	26 (28.57)
P value	0.063			0.117	0.182	0.625
Patient exportation	24 (6.67)	8 (4.71)	1 (4)	6 (5.94)	1 (0.94)	4 (4.40)
						20 (9.66)

P value	0.376	0.124
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P values were comparisons between sexes or comparisons between adjacent two years. *: *P* value was the comparison between 2018 and 2015. Patient importation: CRE patients were recruited from the community ($n = 17$), tertiary care hospitals ($n = 29$), secondary care hospitals ($n = 16$), community care hospitals ($n = 5$), and other regional hospitals ($n = 2$). Patient exportation: CRE patients were transferred to secondary care hospitals ($n = 19$), community care hospitals ($n = 5$), and other regional hospitals ($n = 8$).

Description of ST11 clones outbreaks and infection control measures

ST11-wzi209 clone was introduced into ICU in June 2014 and the numbers subsequently increased, causing an outbreak involving 12 cases during March 15-April 2015. Environmental screenings for room surfaces, equipment, and staff hands did not yield any CRE isolates except some carbapenem-resistant *Acinetobacter baumannii* isolates. But one isolate of ST11-wzi209 was recovered from the bed sheet after ultraviolet light disinfection, suggesting an important mode of transmission via the hands of healthcare workers from contaminated bed linens to new patients and the inadequate disinfection. Additional control measures were then developed and implemented: patient wiping with chlorhexidine once per day, disinfection of bed linens with an ozone sterilizer twice a week, reinforcement of patients' environmental disinfection with chlorine-based compound twice-daily, and regular training with special emphasis on hand hygiene and contact precautions. Afterward, a substantial decrease was observed in the number of new cases (Fig. 1). However, the strains were still detected in various wards, mainly due to the continuous introductions from other hospitals and the community as well as the transfer of CRE patients between wards.

ST11-wzi141 clone initially appeared in neurosurgery ward in April 2017 and spread rapidly to different wards, causing several outbreaks in the ICU and neurosurgery wards largely due to frequent patient transfers between both wards. Successive attempts failed to identify sources or reservoirs of the epidemic clone during the surveillance of the affected wards' environment. Despite the above measures implemented, the widespread transmissions of the clone to various wards were not contained, and it had evolved into an endemic situation in the hospital (Fig. 1).

Correlation Between Antibiotic Consumption And Cre Prevalence

Yearly consumption rate of carbapenems decreased from 38.95 in 2016 to 27.22 DDDs/1000 PDs in 2018, while the usage of third-generation cephalosporins increased from 37.98 in 2016 to 68.39 DDDs/1000 PDs in 2018. No significant associations of annual CRE prevalence were found with yearly consumption rates of carbapenems ($r = -0.13$, $P = 0.806$), fluoroquinolones ($r = -0.301$, $P = 0.562$), first-

generation cephalosporins ($r = 0.732, P = 0.098$), second-generation cephalosporins ($r = -0.529, P = 0.280$), third-generation cephalosporins ($r = -0.1, P = 0.851$), and beta-lactam/beta-lactamase inhibitor combinations ($r = -0.485, P = 0.329$) (Fig. 2).

Transferability of carbapenemase genes via plasmid conjugation

A total of 211 clinical CRE isolates were tested for mobility of carbapenemase-bearing plasmids by conjugation with *E.coli* J53, 54 isolates were successfully transferred. High conjugation rates were found among NDM plasmids in both CPKP (54.84%, 17/31) and *E.coli* (46.67%, 14/30). The conjugation rate of the KPC-2 plasmids in *E.coli* was high (37.5%, 3/8), but relatively low (10.08%, 12/119) in CPKP. All ST15-wzi19 KPC-2-producing KP strains were readily transferable whereas all ST15-wzi384 KPC-2-producers were not conjugative. Among the ST11 KPC-2 clone group, isolates of ST11-wzi209 ($n = 53$, ST11-wzi64 ($n = 5$), ST11-wzi89 ($n = 3$) and ST11-wzi133 ($n = 1$) failed to transfer, while only one strain of the 7 ST11-wzi141 isolates was able to transfer its plasmid, suggesting that different STs, different wzis and the local environment of the host in which bacteria inhabited might influence conjugation..

Discussion

In this study, KPC-2 was the most frequently detected carbapenemase gene, and ST11 KPC-2 KP clones were the most prevalent isolates. The wzi typing method could further subdivide KP strains of the same STs into different subgroups and accurately track their transmission. CRE patients were becoming increasingly younger, largely due to nosocomial CRE acquisition. Although basic control measures had been strictly implemented throughout the study period, they were fundamentally inadequate to curb their transmission. The implementation of evidence-based measures had proven to be effective for some ST11 clones.

Molecular typing methods were especially essential in transmission surveillance and outbreak investigation and management. Pulsed field gel electrophoresis (PFGE) and enterobacterial repetitive intergenic consensus sequence-based PCR (ERIC-PCR) were the most important molecular typing tools for the analysis of bacterial strain clonality. However, these methods were associated with poor reproducibility and often tended to produce subjective biases, making direct comparison of data across different laboratories an immense challenge [11, 12]. Moreover, these approaches were not suitable for precisely tracking CRKP epidemics due to their high clonality [13]. In this study, we also found that ST11 KP clones were closely related and not accurately discriminated by ERIC-PCR (data not shown). So we tried to use the wzi typing method for typing the isolates of KP. Our findings showed that it was sufficient to distinguish isolates showing the same ST types. Furthermore, this method could identify the extent of the transmission, evaluate the trends and allow for direct comparisons among laboratories. So it should be applicable to the ST11 clone group as this group was the most predominant clone group and continued to rise in China.

Conjugation results showed that the carbapenemase genes could reside on transferable plasmids. As a result of frequent plasmid horizontal transfers, newly emerging clones of different STs and/or wzis, and

species diversity tended to increase over time. We found that the number of STs in CPKP were positively associated with the prevalence of CRE (Fig. 3), suggesting that horizontal transfer events would be rapidly increased if rigorous infection control measures were not implemented to disrupt their clonal spread. Factors such as temperature, substrate, plasmid content, and donor and recipient strain identity influenced conjugation rate [14]. In the study, conjugation efficiency was influenced by different STs and different wzis. Further exploring the underlying mechanisms influencing horizontal plasmid transfer might help design effective ways to block them.

Unnecessary and excessive use of antibiotics usually led to the emergence, colonization, clonal expansion and plasmid transmission of CRE strains [15, 16]. There existed a close correlation between the carbapenem consumption and the rate of carbapenem-resistant gram negative bacilli [17]. However, there were studies suggesting no effect of change in antimicrobial use in reducing CRKP infections [18, 19]. The current work did not find any association between antimicrobial consumption and CRE prevalence, the possible reason might be that the rapid spread of resistant bacteria and subsequent outbreaks masked these relationships.

The implementation of evidence-based control measures reduced the number of ST11-wzi209 clone during the study period, but insufficient to control the prevalence of ST11-wzi141 clone after its introduction to the hospital. This clone had spread to all wards and become the main clone, and it still continued to accrue despite these control measures taken, suggesting a higher transmission capacity or greater epidemic potential than ST11-wzi209 clone. The same Intervention(s) might have different effects on different bacteria due to different modes of transmission [20–22]. Our findings demonstrated that different clones had different transmission features and required different preventative strategies. The lack of success in the control of ST11-wzi141 clone indicated that other determinants that contributed to the sustained transmission were still needed to be explored in further studies. Most notably, some problems that might undermine control efforts were desperately needed to be addressed: the lack of routine CRE rectal surveillance program for high-risk patients, no obvious environmental sources despite repeated environmental samplings, lack of evidence of transmission, overcrowding and medical staff shortages.

The spread of CRE across hospitals due to the mobility of patients was increasingly recognized as an important mode of transmission [23–25]. Our findings indicated that the increased importation and exportation of CRE patients as well as frequent transfers of CRE patients between wards presented a rising trend, suggesting that CRE patients were expected to further increase in the future. Enterobacterial strains that producing KPC-2 or NDM-5 had already spread throughout the region and most hospitals would experience an increasing frequency of CRE introduction without any inter-hospital CRE status communication. In China, patients usually chose hospitals completely according to their individual preference without any restriction from regional or local health authorities. This situation might provide great opportunities for the movement of asymptomatic carriers to transmit CRE. Currently, almost all infection control efforts focused exclusively on individual institutions, while regional or national guidelines for controlling the dissemination of CRE across health care settings were still deficient. The

values of regional/national strategies had been demonstrated by experiences in Israel [26] and others [27, 28], where CRE prevalence could be effectively controlled through coordinated interventions. Our findings indicated that active rectal screening cultures, development of various adequate CRE molecular typing in clinical laboratories, especially wzi typing, timely communication of CRE status among institutions or wards upon patient transfer, and public education regarding CRE transmission routes and preventive measures would be essential control measures to address the future CRE challenge.

Conclusions

CRE were still evolving and spreading rapidly, and they would become a persistent problem in the future, mainly due to the continuing risk of importation pressures and deficits in regional strategies. Hence, continued vigilance and comprehensive epidemiology studies specific for institutions or regions were urgently needed to develop effective control measures to combat the ever-changing threat.

Methods

Study design and definition

This study was conducted in Hebei General Hospital, a 1830-bed tertiary care hospital located in Hebei, China. Seven secondary- and 11 other tertiary-care hospitals located in geographically separated areas in this province voluntarily participated in the study. These hospitals were requested to collect CRE isolates from clinical samples as well as the related basic clinical and epidemiological data between September 2015 and December 2017. For strains that caused outbreaks in these hospitals, only one representative strain was collected.

The demographic, clinical and epidemiological data were collected via electronic medical record review. CRE cases were defined by the isolation of CRE in any biological sample obtained from the patients. Imported cases were defined as patients with known CRE acquisition prior to admission or found to be positive in the first 48 h of admission. Exported cases were defined as known CRE patients discharged to other hospitals. Infection and /or colonization were defined according to the criteria established by the U.S. Centers for Disease Control and Prevention (CDC) [29].

Microbiological methods

Various clinically indicated samples, screening samples (stool or rectal swabs), and environmental samples were streaked onto blood agar plates and Eosin methylene blue agar (EMB) plates, and incubated aerobically for 48 h at 35°C. Bacterial isolates were identified to the species level by MALDI-TOF MS. The susceptibility of bacterial isolates to different antimicrobial agents were determined using the VITEK cards (bioMe'rieux) following the manufacturer's instructions and interpreted according to the criteria of the latest Clinical Laboratory Standards Institute (CLSI) document. Meropenem and imipenem susceptibilities were also carried out by the Kirby-Bauer disk diffusion method on EMB agar containing meropenem (1mg/L) for culture screening purposes or on Mueller-Hinton agar for confirmation if a

discrepancy in susceptibilities between carbapenems by Vitek 2 occurred. Enterobacterial isolates resistant to at least one of the carbapenems or positive for carbapenemase genes were regarded as CRE. *E. coli* ATCC 25922 was used as a quality control.

Molecular methods

Resistance genes of non-repetitive CRE isolates were tested by PCR using previously described primers and conditions [30–32]. Bacterial typing was performed by multilocus sequence typing (MLST) using primers listed in the online databases (<https://bigsdb.pasteur.fr/klebsiella/klebsiella.html> for KP and https://bigsdb.pasteur.fr/ecoli/primers_used.html for *E.coli*) and by wzi typing for KP isolates [33]. Amplicons were sequenced and compared with the reported sequences from GenBank by Blast.

Intervention measures

Evidence-based guidelines on prevention and control of CRE were unavailable in China. Infection control measures for CRE employed in all participating hospitals were based primarily on the guidelines for the control of multi-drug resistant (MDR) organisms and typically included hand hygiene, contact precautions and/or isolation precautions, healthcare staff education, environmental cleaning and disinfection, aseptic procedures and antimicrobial stewardship (AMS) policies.

In our institution, infection surveillance and control were conducted by the department of preventive medicine. Microbiology laboratory, clinical departments and preventive medicine department shared daily data about MDR organisms. The basic control measures for CRE prevention were as follows: hand hygiene with alcohol-based hand rubs, patients with CRE isolates placed on contact precautions, CRE status marked in the electronic medical record and environment disinfection with a chlorine-based compound. Monthly environmental surveillance cultures were performed for relevant wards, which included air sedimentation cultures, hand swab cultures and environmental swab cultures. Since the release of the National Action Plan to Contain Antimicrobial Resistance (2016–2020) in September 2016, antimicrobial stewardship had been reinforced. Carbapenems were added in the restricted antibiotic list, requiring preauthorization of prescriptions. Monthly multidisciplinary antibiotic meeting was undertaken in the whole hospital, with a particular emphasis on rational use of antibiotics.

When an outbreak occurred, an infection control task force was established immediately and multidisciplinary meetings were frequently held to explore additional effective control measures. Investigative findings on the basis of clinical observations, epidemiological surveys and molecular evidence were immediately fed back to guide development or refinement of control measures.

Antibiotic Consumption

Data on the rates of antibiotic consumption were obtained from hospital pharmacy database and expressed as the defined daily dose (DDD)/1000 patient-days (PDs). Four main classes of antibacterial

agents including carbapenems, fluoroquinolones, cephalosporins, and beta-lactam/beta-lactamase inhibitor combinations were analysed in this study.

Conjugation Experiments

To determine the transferability of the carbapenemase genes, a conjugation experiment was carried out in mixed broth cultures at 37°C [34]. Carbapenemase positive donor strains from various clinical samples were randomly selected according to the colonial morphotypes (size, color, viscosity, etc.). Azide-resistant *E. coli* J53 was used as recipient. Transconjugants were selected on EMB containing azide (200µg/ml) and meropenem (1 mg/L) and inspected by PCRs.

Statistical analysis

Data were reported as mean ± SD, number, percentage or frequency according to data distribution. Qualitative variables were compared by Student's t tests or Mann-Whitney U tests, while categorical variables were compared by Chi-squared or Fisher's exact tests. Pearson's or Spearman's correlation coefficient was used to determine the associations between variables. All tests were two-tailed and *P* values of < 0.05 were considered statistically significant. All analyses were conducted using SPSS software.

Abbreviations

CRE: Carbapenem-resistant Enterobacteriaceae; CPE:Carbapenemase-producing Enterobacteriaceae; KP:Klebsiella pneumoniae; CRKP:Carbapenem-resistant Klebsiella pneumoniae; MDR:Multi-drug resistant; wzis:wzi alleles; ST:sequence type

Declarations

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Authors' contributions

JSW and CF Y had made substantial contributions to the conception and design of the study, the acquisition, analysis and interpretation of data. JSW had been responsible for critically drafting and revising the manuscript for all critical intellectual content. WWY, YPLand PZ have made substantial contributions to sample collections, experiments, and data acquisition and interpretation. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Protocols for collection of samples as well as the experiment plan and all methods were performed in accordance with the guidelines and regulations of all participating hospitals and approved by the institutional ethical committees. Written informed consent was waived because all samples collected from patients were as part of routine management and surveillance.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

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Figures

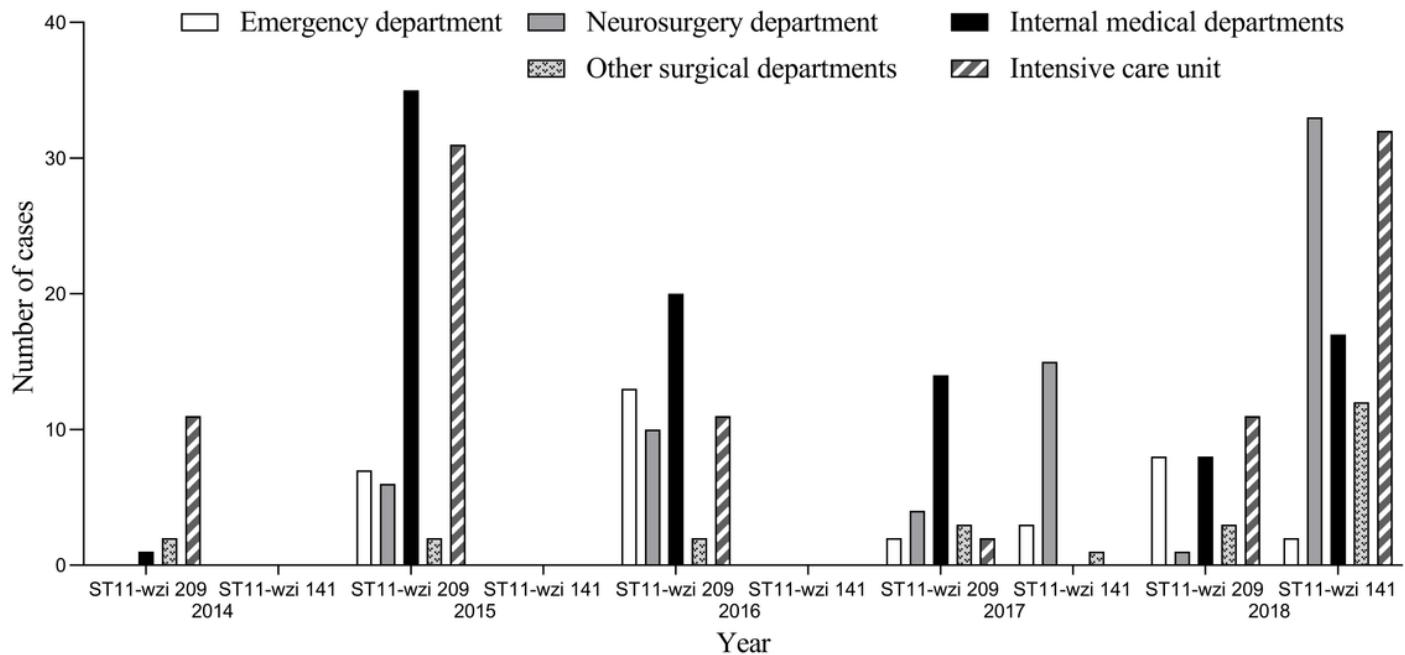


Fig. 1 Distribution of cases according to the years and departments

Figure 1

See image above for figure legend

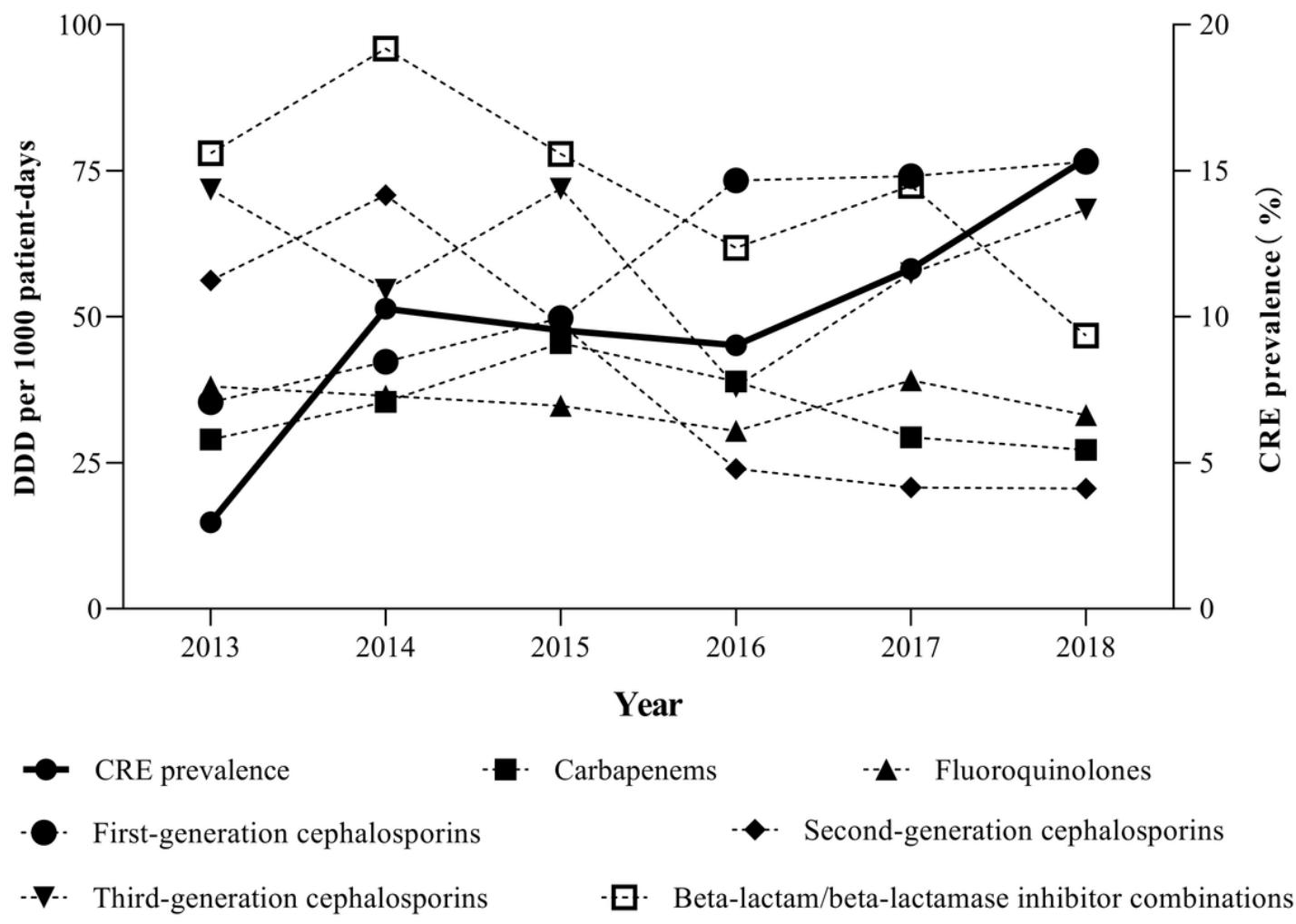


Figure 2

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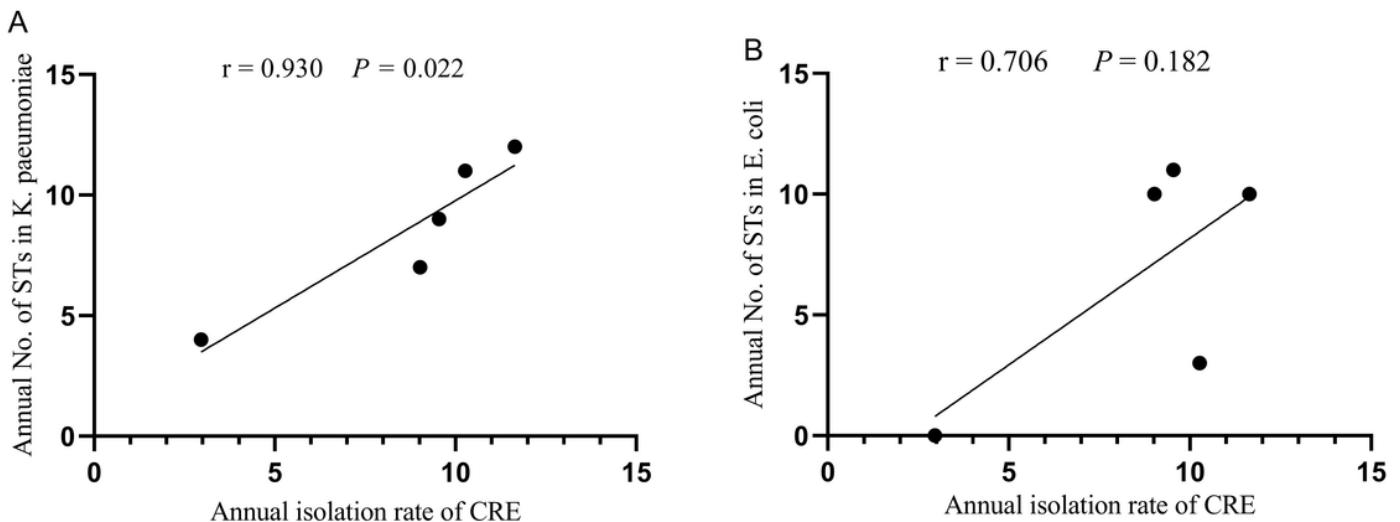


Fig.3 Correlation between the annual number of STs in *K.pneumoniae* (A) or *E.coli* (B) and the annual isolation of carbapenem resistant enterobacteriaceae (CRE)

Figure 3

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