

# Antimicrobial resistance trends among important clinical pathogens reported from the ARINCQ surveillance of bacterial resistance, 2012-2018: multi-center retrospective surveillance study in Chongqing

**Shan Sun**

the first affiliated hospital of Chongqing Medical University

**Xiaolang Tian**

University of the Chinese Academy of Sciences

**Li Yan**

Chongqing Medical University First Affiliated Hospital

**Jide Sun**

Chongqing Medical University First Affiliated Hospital

**Chuanming Zhang**

Chongqing Medical University First Affiliated Hospital

**Xiuyu Xu**

Chongqing Medical University First Affiliated Hospital

**Chunmei Jing**

Chongqing Medical University Affiliated Children's Hospital

**Yunying Wang**

Chongqing Medical University Second Affiliated Hospital

**Wei Chen**

Three Gorges Hospital affiliated to Chongqing University

**Zhongzheng Xiong**

Dianjiang People's Hospital of Chongqing

**Xin Yao**

Banan people's hospital

**Minjun Zhang**

The people's Hospital of Jiulongpo District, Chongqing

**Wen Qi**

The people's Hospital of Kaizhou District, Chongqing

**Renyan Wang**

Chongqing Jiangbei district people's hospital

**Shulin You**

The people's hospital of Nanchuan district, Chongqing

**Yong Wu**

Fengdu People's hospital of Chongqing

**Mei Zhang**

The people's hospital of Qijiang District, Chongqing

**Xiaofeng Yu**

Traditional Chinese Medicine Hospital of Dianjiang, Chongqing

**Guangde Pei**

The people's hospital of Shapingba District, Chongqing

**Juan Fang**

The people's hospital of Yubei district, Chongqing

**Zhongxiu Huang**

Qianjiang central hospital of Chongqing

**Ji Zhang**

The people's hospital of Changchou district, Chongqing

**Yuan Gao**

Jiangjin center hospital of Chongqing

**Gang Fu**

Chongqing University cancer hospital

**Niqi Xie**

The Dazu district people's hospital of Chongqing

**Zhen Ruan**

The ninth people's hospital of Chongqing

**Wenzhang Long**

Yongchuan Hospital of Chongqing Medical University

**Yuan Xiong**

Chongqing traditional Chinese Medicine Hospital

**Lijuan Li**

Chongqing University central hospital

**Lei Chen**

The people's hospital of Tongliang district, Chongqing

**Wenping Deng**

Fuling center hospital of Chongqing

**Xiaoli Ye**

The fifth people's hospital of Chongqing

**Chengfeng Shu**

Bishan hospital of Chongqing

**Yan Shen**

Chongqing Health Center for Women and Children

**Siqiang Niu**

Chongqing Medical University First Affiliated Hospital

**Shifeng Huang (✉ [sfhuang@hospital.cqmu.edu.cn](mailto:sfhuang@hospital.cqmu.edu.cn))**

Chongqing Medical University First Affiliated Hospital

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**Research**

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## Abstract

**Background:** Data on the Antimicrobial resistance (AMR) trends of the important clinical isolates in southwestern China is lacking. This study was conducted to monitor the temporal AMR trends of the most common pathogens contributing to the majority of human infections.

**Methods:** A multi-center retrospective surveillance study was conducted from 2012 to 2018. Antimicrobial susceptibility testing was carried out according to unified protocols using the Kirby-Bauer method or automated systems. Results were analyzed according to Clinical and Laboratory Standards Institute (CLSI) 2018 definitions. Data from the China Antimicrobial Resistance Surveillance System (CARSS) in Chongqing (ARINCQ) was analyzed by WHONET 5.6 software.

**Results:** The total number of bacterial isolates was between 49,636 and 128,460 annually. While the isolation rate of *Acinetobacter baumannii* (*A. baumannii*) presented decreasing trend, that of *Staphylococcus aureus* (*S. aureus*) showed increasing tendency. *Escherichia coli* (*E. coli*) showed decreasing susceptibility trends to most antibiotics except carbapenems. Resistance rates of *Klebsiella pneumoniae* (*K. pneumoniae*) to ceftazidime (CAZ), ceftriaxone (CRO), and cefepime (FEP) are decreasing over time. *Pseudomonas aeruginosa* (*P. aeruginosa*) and *S. aureus* demonstrated obvious declining resistance trends to most of the antibiotics tested. Resistance of *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) to high-level gentamycin are decreasing continuously over time. The resistance rate of *A. baumannii* to meropenem increased from 48.3% to 57.7%. The isolation rate of carbapenem-resistant *K. pneumoniae* (CRKPN) increased annually from 2.6% in 2012 to 6.1% in 2018. For CRKPN, consistent increasing trends in isolation rates were witnessed in both the children and the elderly groups, with its isolation rate in children being obviously higher than that in the elderly group from 2015 to 2018.

**Conclusions:** The prevalence of CRKPN has been significantly increased in both the children and the elderly groups ever since 2012, which calls for continuous resistance surveillance, colonization screening and clearance, environmental monitoring, and effective antimicrobial strategies. While carbapenems are still active against *K. pneumoniae* and *E. coli*, vancomycin or linezolid is still effective against *S. aureus* and *Enterococcus spp.* Notably, this is the first report of an CRKPN isolate co-harboring triple carbapenemase genes including *bla*<sub>KPC-2</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>IMP-4</sub> worldwide.

**Keywords:** antimicrobial resistance, surveillance, multi-center, carbapenems, Southwestern China, carbapenem-resistant *K. pneumoniae*

## Background

The China Antimicrobial Resistance Surveillance System (CARSS) (<http://www.carss.cn/>) covers all the provinces and autonomous regions in China. As Chongqing is the largest autonomous region in southwestern China and its demographic structure of urban-rural dualization is similar to that of the whole China, making the data of the antimicrobial resistance (AMR) in Chongqing representative in China. With the aim of gathering temporal trends on bacterial epidemiology and resistance from multiple laboratories in Chongqing, the Antimicrobial Resistance Surveillance Network in Chongqing (ARINCQ), a branch of CARSS, was organized in 2011, when there were only seven teaching hospitals. To prevent geographical bias, we successively enrolled hospitals in a geographical representative manner, and 59 hospitals have been enrolled in ARINCQ, almost covering all the regions and districts of Chongqing.

Worldwide AMR changing tendencies were reported one after another [1,2,3,4], and the comprehensive and overall data of temporal AMR shifts of important clinical isolates in China was only reported by CHINET, which just included seventeen teaching hospitals [1,5]. We searched pubmed with the terms “antimicrobial resistance of nosocomial pathogenes”, and the retrieval results consisted of larger national surveillance studies, reviews, or single case studies, with no results of the studies including comprehensive Chongqing coverage. In addition, the longitudinal province-level AMR surveillance in China was limited, and was mostly about the AMR changing trends of isolates from bloodstream and intra-abdominal infections [6, 7, 8, 9, 10]. The overall annual AMR changing trends over long time periods are still lacking in southwestern China. The present study was initiated to provide clear and comprehensive AMR changing trends for the important nosocomial pathogens from 2012 to 2018 in southwestern China, and meanwhile to deeply explore into the cause of the key problems discovered.

# Methods

## Data enrollment criteria

All bacterial data including patients' information, bacterial identification and antimicrobial susceptibility from 59 microbiological laboratories in Chongqing, covering secondary and tertiary hospitals with a ratio of 1.5:1, were acquired from CARSS database in Chongqing (ARINCQ) between 2012 and 2018. Children were defined as patients no older than 14-year-old, adults were defined as patients between 15 and 65 years old, while the elderly were defined as patients older than 65 years old.

Only the first isolate from each individual patient was included in this study.

## Bacterial identification and antimicrobial susceptibility test

All the isolates were identified at the species level by semi- or automated systems, such as the VITEK2 compact (bioMérieux, Inc., Durham, NC) system, or the VITEK MS (bioMérieux, Hazelwood, MO, United States) automated system, and so on. Routine antimicrobial susceptibility testings were performed by using either the API system, the BD Phoenix System (Becton Dickinson, America), or the VITEK2 compact (bioMérieux, Inc., Durham, NC) system. According to the breakpoint recommendations by the Clinical and Laboratory Standards Institute, 2018 (CLSI-2018), *K. pneumoniae* or *E. coli* isolates which were resistant to at least one of the carbapenems, with the criteria of minimum inhibitory concentration (MIC) of  $\geq 2\mu\text{g/mL}$  for ertapenem,  $\geq 4\mu\text{g/mL}$  for imipenem, or  $\geq 4\mu\text{g/mL}$  for meropenem, were defined as carbapenem-resistant *Enterobacteriaceae* (CRE); while carbapenem-resistant *A. baumannii* (CRABA) or *P. aeruginosa* (CRPAE) strains were defined as MIC  $\geq 8\mu\text{g/mL}$  for imipenem or meropenem.

## Detection of Carbapenemase genes

Polymerase chain reaction was used to detect the potential presence of carbapenemase genes including *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>GES</sub>, *bla*<sub>OXA-48-like</sub>, and *bla*<sub>OXA-181-like</sub>, and all the variants of these carbapenemase genes were confirmed by sequencing. Moreover, the Carba NP test and sCIM were performed on all isolates to determine whether any bacteria produced carbapenemases by phenotypic methods but were negative by genotypic methods, or vice versa [11].

## Statistical analyses

Raw data was firstly processed by WHONET 5.6 software (version 5.6, <http://www.whonet.org/software.html>) and then analyzed on SPSS v.23.0 (SPSS, Chicago, IL, USA) software. The changing trends in AMR of each species and in the isolation rates of the main resistance phenotypes over the study period were determined using the linear trend analysis method. A statistically significant trend was established if the *P* value was  $<0.05$ . The *P* value was provided whenever there was a significant trend in the resistance. Moreover, the regression coefficient was calculated and indicated the change in the percentage of resistant isolates (on the y-axis) over time (on the x-axis) [12, 13]. A negative slope (-) indicated a decrease in resistance over time, whereas a positive slope (+) presented an increase in resistance over time. The difference in AMR between children and non-children was further assessed by Pearson Chi-square test. Statistical significance was confirmed if a two-tailed *P* value was no more than 0.05.

# Results

## Changing trends of the isolation percentages of the main clinical pathogen species

Between 2012 and 2018, the number of non-repetitive bacterial isolates ranged between 49,636 and 128,460 annually. The ratio of specimen types during the study period did not change. The percentages of the seven investigated species (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. aureus*, *E. faecium*, and *E. faecalis*) among total isolates were shown in Table 1. While the annual total percentage of the seven pathogen species among the total isolates didn't change much, accounting from 60.2 to 63.4%, the percentage of *A. baumannii* showed a slowly decreasing trend, whereas the ratio of *S. aureus* presented a slowly increasing tendency (Table 1).

**Table 1 Percentages of the main clinical pathogens among total clinical isolates**

<i>E.coli</i>		<i>K.pneumoniae</i>		<i>P.aeruginosa</i>		<i>A.baumannii</i>		<i>S.aureus</i>		<i>E.faecalis</i>		<i>E.faecium</i>		Total percentage among all
n	%	n	%	n	%	n	%	n	%	n	%	n	%	
9806	19.8	6520	13.1	4326	8.7	3650	7.4	4039	8.1	1054	2.1	886	1.8	61.0
15931	19.2	12513	15.1	7264	8.7	6348	7.6	6727	8.1	1539	1.9	1451	1.7	62.3
18784	19.7	13566	14.2	7374	7.7	6122	6.4	8608	9	1546	1.6	1546	1.6	60.2
19714	20.9	13753	14.6	7650	8.1	5523	5.8	8815	9.3	1811	1.9	1601	1.7	62.3
20408	21.7	13107	13.9	7752	8.2	5145	5.5	9456	10.1	1866	2.0	1846	2.0	63.4
21996	21.0	14464	13.8	8549	8.2	5665	5.4	10862	10.4	2176	2.1	2133	2.0	62.9
26436	20.6	16882	13.1	10381	8.1	6703	5.2	12977	10.1	2569	2.0	2614	2.0	61.1

## BACTERIAL RESISTANCE AND TRENDS IN GRAM-NEGATIVE BACILLI

### *E. coli* and *K. pneumoniae*

Statistically significant decreases in AMRs of *E. coli* to nine (75%) out of the 12 tested antibiotics were observed between 2012 and 2018, which included SAM (from 51.2% to 40.8%,  $P<0.001$ ), SCF (13.0% to 4.8%,  $P<0.001$ ), TZP (4.8% to 2.9%,  $P<0.001$ ), CAZ (29.7% to 19.6%,  $P<0.001$ ), CRO (65.3% to 49.1%,  $P<0.001$ ), FEP (33.4% to 22.6%,  $P<0.001$ ), AMK (3.4% to 1.6%,  $P<0.001$ ), LVX (47.1% to 40.3%,  $P<0.001$ ), and SXT (62.8% to 52.7%,  $P<0.001$ ). Its resistance rates to IPM and MEM increased from 0.7% to 1.9% and 0.6% to 1.4% from 2012 to 2016, but then decreased to 1.0% and 0.9% in 2018, respectively. However, the overall resistance rates to IPM and MEM showed significant statistical increases over the study period ( $P<0.001$  and  $P<0.001$ ). The FOX resistance rates fluctuated around 12.0% ( $P=0.164$ ) (Table 2).

**Table 2. Resistance rates (%) of *E. coli* to commonly used antibiotics**

Antibiotics	2012 (n=9806)	2013 (n=15931)	2014 (n=18784)	2015 (n=19714)	2016 (n=20408)	2017 (n=21996)	2018 (n=26436)	<i>P</i> value	RC
Ampicillin/Sulbactam	51.2	46	44.5	44.4	45.1	43	40.8	<0.001	-0.881
Piperacillin/tazobactam	4.8	3	3.3	3.1	3.6	2.9	2.9	<0.001	-0.638
Cefoperazone/sulbactam	13.0	6.2	5.3	4.5	5.8	5.5	4.8	<0.001	-0.667
Ceftazidime	29.7	26.6	23	22.7	22.0	19.6	19.6	<0.001	-0.949
Ceftriaxone	65.3	62	55	54.1	52.2	51	49.1	<0.001	-0.951
Cefepime	33.4	28.5	29.1	27.8	21	18.2	22.6	<0.001	-0.883
Cefoxitin	13.3	9.2	9	12.5	11.9	11.2	10.7	0.164	-0.043
Imipenem	0.7	0.7	0.7	1.2	1.9	1.1	1.0	<0.001	+0.519
Meropenem	0.6	0.6	0.6	1.4	1.4	0.7	0.9	<0.001	+0.399
Amikacin	3.4	2.6	3.2	2.1	1.8	1.6	1.6	<0.001	-0.905
Levofloxacin	47.1	44.6	40.1	38.3	38.0	39.9	40.3	<0.001	-0.727
Sulfamethoxazole/trimethoprim	62.8	61.5	57.4	56.5	56.0	53.8	52.7	<0.001	-0.973

The values in parentheses indicate the total isolated numbers of *E. coli* in each year; RC, regression coefficient.

The overall resistance rates to all the antibiotics showed statistically significant changing trends over the study period. These antibiotics with increasing AMRs included IMP (from 1.8% to 5.5%,  $P<0.001$ ), MEM (2.3% to 5.0%,  $P<0.001$ ), SCF (from 6.7% to 11.8%,  $P<0.001$ ), FOX (from 14.1% to 18.2%,  $P<0.001$ ), AMK (from 2.9% to 5.2%,  $P<0.001$ ) and LVX (from 7.9% to 10.9%,  $P<0.001$ ). While the antibiotics with decreasing AMRs contained SAM (from 37.3% to 27.9%,  $P<0.001$ ), TZP (from 12.3% to 8.8%,  $P=0.003$ ), CAZ (from 24.2% to 15.7%,  $P<0.001$ ), CRO (33.7% to 26.4%,  $P<0.001$ ), FEP (from 19.6% to 15%,  $P<0.001$ ) and SXT (from 31.9% to 22.1%,  $P<0.001$ ) (Table 3).

**Table 3. Resistance rates (%) of *K. pneumoniae* to commonly used antibiotics**

Antibiotics	2012 (n=6520)	2013 (n=12513)	2014 (n=13566)	2015 (n=13753)	2016 (n=13107)	2017 (n=14464)	2018 (n=16882)	<i>P</i> value	RC
Ampicillin/Sulbactam	37.3	29.7	27.9	26	29.3	29.4	27.9	<0.001	-0.586
Piperacillin/tazobactam	12.3	6	5.9	5.8	6.9	8.1	8.8	0.003	-0.175
Cefoperazone/sulbactam	6.7	3.1	4.4	5.2	8.2	12.8	11.8	<0.001	+0.804
Ceftazidime	24.2	18.5	15.2	15.2	15.9	15.5	15.7	<0.001	-0.719
Ceftriaxone	33.7	33	24.2	23.8	26	27.7	26.4	<0.001	-0.592
Cefepime	19.6	14.6	14.6	13.9	12.7	12.4	15	<0.001	-0.651
Cefoxitin	14.1	8.8	10.2	10.9	12.6	16.3	18.2	<0.001	+0.675
Imipenem	1.8	1.5	1.6	2.3	4.6	5.5	5.5	<0.001	+0.917
Meropenem	2.3	1.5	1.4	2.8	3.9	4.5	5.0	<0.001	+0.892
Amikacin	2.9	2.4	3.4	1.6	3.3	4.7	5.2	<0.001	+0.702
Levofloxacin	7.9	7.2	6.9	6.9	8.4	9.6	10.9	<0.001	+0.78
Sulfamethoxazole/trimethoprim	31.9	26.3	23.9	22.9	24.1	22.8	22.1	<0.001	-0.825

The values in parentheses indicate the total isolated numbers of *K. pneumoniae* in each year; RC, regression coefficient.

### ***P. aeruginosa* and *baumannii* complex**

The resistance rates of *P. aeruginosa* to all the eleven investigated antibiotics were declining with statistical significance. The AMR rates of *P. aeruginosa* to all the antibiotics tested were less than 26% during the study period and all were below 19% in 2018 (Table 4).

**Table 4. Resistance rates (%) of *P. aeruginosa* to commonly used antibiotics**

Antibiotics	2012 (n=4326)	2013 (n=7264)	2014 (n=7374)	2015 (n=7650)	2016 (n=7752)	2017 (n=8549)	2018 (n=10381)	P value	RC
Piperacillin	24.4	22.7	18.2	15.7	15.8	13.4	13.4	<0.001	-0.953
Ticarcillin/Clavulanic acid	24.9	25.3	22.4	19.8	21.2	21.5	16.4	<0.001	-0.869
Piperacillin/tazobactam	13.3	14.6	10.9	9.5	10.6	9.3	9.9	<0.001	-0.804
Ceftazidime	15.4	17.8	15.7	13.2	13.5	12.2	12.7	<0.001	-0.826
Cefepime	12.5	14.3	12.7	11	10.2	9.5	11	<0.001	-0.775
Aztreonam	19.1	18.7	16.8	15.8	16	15.7	18.4	0.020	-0.465
Imipenem	20.2	15.7	15.1	13.7	13.9	12.5	12.3	<0.001	-0.896
Meropenem	18.2	14.4	12.7	11.7	11.4	9.7	10	<0.001	-0.925
Ciprofloxacin	18.4	14.7	13.1	11.2	10.1	9.1	10.5	<0.001	-0.902
Levofloxacin	16.4	13.3	11.5	10.2	9.3	9	10.6	<0.001	-0.834
Amikacin	10.5	9.5	7.8	4.9	4.5	3.1	3.3	<0.001	-0.965

The values in parentheses indicate the total isolated numbers of *P.aeruginosa* in each year; RC, regression coefficient.

For *A. baumannii* complex, while its resistance rates to MEM (from 48.3% to 57.7%,  $P<0.001$ ), IMP (from 52.1% to 55.4%,  $P<0.001$ ), TZP (from 48.0% to 56.5%,  $P<0.001$ ) and LVX (from 36.9% to 47.2%,  $P<0.001$ ) annually increased from 2012 to 2018, its resistance rates to CAZ, AMK and MNO decreased from 64.1% to 57% ( $P<0.001$ ), 55.7% to 46.5% ( $P<0.001$ ) and 19.5% to 14.1% ( $P<0.001$ ), respectively. There were no continuous changing trends for FEP and PIP, with their respective resistance rates fluctuating between 55.5% and 59.2% ( $P=0.490$ ), and 52.5% and 70.6% ( $P=0.952$ ) (Table 5).

**Table 5. Resistance rates (%) of *A. baumannii* to commonly used antibiotics**

	2012	2013	2014	2015	2016	2017	2018		
Antibiotics	(n=3650)	(n=6348)	(n=6122)	(n=5523)	(n=5145)	(n=5665)	(n=6703)	<i>P</i> value	RC
Piperacillin	52.5	64.9	61.9	64.8	70.6	58	58.6	0.952	+0.173
Piperacillin/tazobactam	48	54.1	49.2	53.5	58.6	54.9	56.5	<0.001	+0.744
Ceftazidime	64.1	61.6	56.9	59.9	60.3	56.5	57	<0.001	-0.764
Cefepime	56.8	58	55.5	57.9	59.2	55.7	56.6	0.490	-0.087
Imipenem	52.1	52.7	48.2	53.2	51.7	51.3	55.4	<0.001	+0.375
Meropenem	48.3	53.3	51.5	57.4	62.4	55.2	57.7	<0.001	+0.717
Levofloxacin	36.9	34.6	33.6	44.6	44.6	43.1	47.2	<0.001	+0.829
Amikacin	55.7	48.2	44.2	44.7	50.9	46.2	46.5	<0.001	-0.474
Minocycline	19.5	25.2	12.3	29.2	22.4	12.5	14.1	<0.001	-0.366

The values in parentheses indicate the total isolated numbers of *A. baumannii* in each year; RC, regression coefficient.

## BACTERIAL RESISTANCE AND TRENDS IN GRAM-POSITIVE COCCI

### *S. aureus*

Evident annual decreasing AMR trends were observed for *S. aureus* to 10 of 13 (76.9%) investigated antibiotics comprising GEN (from 28.9% to 11.2%), RIF (from 10.3% to 2%), LVX (from 28.6% to 9.5%), SXT (from 29.5% to 13.9%), ERY (from 64.1% to 57.6%), CLI (from 43% to 37.4%), TET (from 33.3% to 22%), PEN (from 95.9% to 93.7%) and OXA (from 26.9% to 24.6%). No LNZ-, VAN- or TEC-resistant *S. aureus* isolates were observed during the study period (Table 6).



**Table 6. Resistance rates (%) of *S. aureus* to commonly used antibiotics**

Antibiotics	2012 (n=4039)	2013 (n=6727)	2014 (n=8608)	2015 (n=8815)	2016 (n=9456)	2017 (n=10862)	2018 (n=12977)	<i>P</i> value	RC
Penicillin G	95.9	95.6	96.3	95.9	94.8	93.6	93.7	<0.001	-0.848
Oxacilli	26.9	27.2	29.4	28.2	26.2	25.9	24.6	<0.001	-0.624
Gentamicin	28.9	24.5	22.8	19.8	13.2	12.1	11.2	<0.001	-0.979
Rifampin	10.3	8.3	6.7	5	2.4	2.2	2	<0.001	-0.971
Levofloxacin	28.6	23.6	19	15.5	11.6	10.3	9.5	<0.001	-0.973
Sulfamethoxazole/trimethoprim	29.5	26.8	23.5	22	19.8	15.4	13.9	<0.001	-0.994
Azithromycin	65.8	65.4	66.7	64.6	62.1	65.5	63.3	<0.001	-0.580
Clindamycin	43	43.9	43.1	44.8	41.3	36.7	37.4	<0.001	-0.796
Erythromycin	64.1	63.2	63.2	61.2	59.6	58.9	57.6	<0.001	-0.983
Tetracycline	33.3	29.2	27.4	25.2	24.9	22.3	22	<0.001	-0.967
Linezolid	0	0	0	0	0	0	0	-	-
Vancomycin	0	0	0	0	0	0	0	-	-
Teicoplanin	0	0	0	0	0	0	0	-	-

The values in parentheses indicate the total isolated numbers of *S. aureus* in each year; The horizontal line indicates no statistical analysis; RC, regression coefficient.

### *E. faecalis* and *E. faecium*

The resistance rates of *E. faecalis* to VAN fluctuated around 0.3% ( $P=0.163$ ). While the resistance rates to LNZ showed decreasing trend over time ( $P=0.009$ ). Quinolone resistance rates only showed a slight change with no statistical significance. Its resistance rates to GEH and ERY respectively declined from 49.8% to 38.6% ( $P<0.001$ ) and from 74.4% to 66.9% ( $P<0.001$ ). Its resistance rate to AMP maintained below 7.4% during the surveillance periods (Table 7). On the contrary, the resistance rates of *E. faecium* to AMP remained higher above 82% during the study period with no obvious change ( $P=0.871$ ). Obvious continuous decrease of resistance rate to GEH and ERY was observed from 69.8% to 50.7% ( $P<0.001$ ) and 89.1% to 85.6% ( $P=0.001$ ), respectively. *E. faecium* was highly resistant to quinolones with resistance rate of higher than 79% during the study period. While its resistance rates to both LNZ and VAN showed declining trend from 0.8% to 0.4% ( $P=0.019$ ) and 1.2% to 0.8% ( $P<0.001$ ) between 2012 and 2018, respectively (Table 8).

**Table 7. Resistance rates (%) of *E. faecalis* to commonly used antibiotics**

	2012	2013	2014	2015	2016	2017	2018		
Antibiotics	(n=1054)	(n=1539)	(n=1546)	(n=1811)	(n=1866)	(n=2176)	(n=2569)	<i>P</i> value	RC
Ampicillin	5.2	6.6	7.4	6.4	4	5.2	4.6	0.001	-0.511
Gentamicin-High	49.8	42.2	45.3	43	43.4	40.8	38.6	<0.001	-0.831
Levofloxacin	24.7	23.5	23.5	23.3	20.7	22.1	23.6	0.238	-0.534
Ciprofloxacin	25.4	27.3	27.9	26.6	23.3	26.7	27.5	0.869	+0.024
Moxifloxacin	21	22.3	21.4	20.7	17.9	17.4	22.9	0.362	-0.279
Erythromycin	74.4	73.4	72.2	69.6	68.7	69.5	66.9	<0.001	-0.960
Tetracycline	82.5	77.2	77.5	78.8	79.6	80.7	78.9	0.730	-0.071
Linezolid	1.7	3.4	3.5	2.5	2.1	1.4	2.2	0.009	-0.376
Vancomycin	0.3	0.3	0.3	0.5	0.3	0.1	0.2	0.163	-0.445

The values in parentheses indicate the total isolated numbers of *E. faecalis* in each year; RC, regression coefficient.

**Table 8. Resistance rates (%) of *E. faecium* to commonly used antibiotics**

	2012	2013	2014	2015	2016	2017	2018		
Antibiotics	(n=886)	(n=1451)	(n=1546)	(n=1601)	(n=1846)	(n=2133)	(n=2614)	<i>P</i> value	RC
Ampicillin	88.1	82.8	82.3	83.3	84.2	84.1	84.3	0.871	-0.281
Gentamicin-High	69.8	64.2	60.2	58.2	57.1	52.6	50.7	<0.001	-0.981
Levofloxacin	82.3	79.1	81.1	83	84.2	81.8	80.9	0.518	+0.203
Ciprofloxacin	84.7	85.8	86.6	86.8	86.5	85.1	83.4	0.032	-0.337
Moxifloxacin	90.2	88.8	87.2	87.1	90.5	88.7	87.2	0.243	-0.317
Erythromycin	89.1	87.4	88	86	86.9	85	85.6	0.001	-0.880
Tetracycline	59.4	56.2	56.7	58.5	64.1	60	60.4	0.003	+0.526
Linezolid	0.8	0.5	1	0.5	0.4	0.3	0.4	0.019	-0.677
Vancomycin	1.2	2.4	2.9	1.8	1.4	0.7	0.8	<0.001	-0.575

The values in parentheses indicate the total isolated numbers of *E. faecium* in each year; RC, regression coefficient.

## Changing trends of the main resistance phenotypes for the key pathogen species

### MRSA and VRE

The isolation rates of MRSA and VREFM were under 30% and 3%, respectively, with a respective increase of 2.5% and 1.7% from 2012 to 2014 firstly, and a decrease of 4.8% and 2.1% respectively from 2014 to 2018. And both the isolation rates of MRSA and VREFM showed declining trends over time ( $P<0.001$ ). VREFA isolation rates fluctuated around 0.3% ( $P=0.163$ ) (Figure 1).

### CRE, CRABA and CRPAE

CRKPN isolation rates increased annually from 2.6% in 2012 to 6.1% in 2018 ( $P<0.001$ ). Although both of the CRECO and CRABA isolation rates presented increasing trends from 2012 to 2018 ( $P=0.016$  and  $P<0.001$ , respectively), the isolation rates of CRABA maintained stable from 2013 to 2018 ( $P=0.673$ ). The isolation rate of CRECO also declined continuously for the recent two years, remaining at a low level of 1.3%. CRPAE isolation rates decreased annually from 23.8% in 2012 to 15.2% in 2018 ( $P<0.001$ ) (Figure2).

### Isolation rates of CRKPN and CRECO according to different age groups

As for CRKPN isolation rates, all the three groups witnessed statistically annual increasing trends over time ( $P<0.001$ ): from 2.8% (35/1252) in 2012 to 10.9% (236/2169) in 2018 in the children group ( $P<0.001$ ); from 2.8% (88/3135) in 2012 to 4.4% (303/6896) in 2018 in the adults group ( $P<0.001$ ); from 1.5% (33/2133) in 2012 to 6.3% (489/7817) in 2018 in the adults group ( $P<0.001$ ). The isolation rates of CRKPN in the children group presented a sharp increase and have been obviously higher than those in the non-children groups ever since 2015 (2015, 5.8% vs. 2.6%,  $P<0.001$ ; 2016, 8.0% vs. 5.0%,  $P<0.001$ ; 2017, 9.5% vs. 5.5%,  $P<0.001$ ; 2018, 10.9% vs. 5.4%,  $P<0.001$ ) (Figure 3).

As for CRECO isolation rates, while there was no consistent changing tendency in the elderly group ( $P=0.202$ ), there was a significant declining trend in the adults group ( $P<0.001$ ) during the study period. For the children group, however, there was a consistent statistically increasing trend from 0.6% in 2012 to 6.4% in 2016 ( $P<0.001$ ), and then it dropped surprisingly from 6.4% in 2016 to 4.2% in 2018. Furthermore, the CRECO isolation rates in children have been remarkably higher than those in the non-children groups ever since 2015 (2015, 2.7% vs.1.7%,  $P<0.001$ ; 2016, 6.4% vs.1.4%,  $P<0.001$ ; 2017, 4.4% vs. 0.8%,  $P<0.001$ ; 2018, 4.2% vs.1.0%,  $P<0.001$ ) (Figure 4).

### Distribution of carbapenemase genes from the CRKPN isolates

Carbapenemase-producing *Enterobacteriaceae* (CPE) is the most pervasive antibiotic resistance threat to health services worldwide. Few alternative antibiotics (eg, colistin, fosfomycin, and tigecycline) remain, and what's more, resistance can extend even to agents still in development or recently approved. Thus, public health efforts are beginning to emphasize containment of CPE in populations and health-care networks. This requires an understanding of the geographical distribution of CPE infections, their population reservoirs, and the risk factors for acquisition. However, there is little internationally comparable data.

In the present study, a total of 79 CRKPN isolates from 17 hospitals covering all the five geographical areas in Chongqing were collected during 2015-2016. 84.8% of the CRKPN isolates harbored carbapenemase genes, with  $bla_{KPC}$ ,  $bla_{NDM}$  and  $bla_{IMP}$  accounting for 51.9% (41/79), 39.2% (31/79) and 5% (4/79), respectively.  $bla_{VIM}$ ,  $bla_{GES}$ , and  $bla_{OXA}$  were not detected in this study. 11.4% (9/79) of the strains harbored two or three types of carbapenemase genes ( $bla_{KPC-2}$  and  $bla_{NDM-1}$ ,  $n=7$ ;  $bla_{KPC-2}$  and  $bla_{IMP-4}$ ,  $n=1$ ;  $bla_{KPC-2}$ ,  $bla_{NDM-1}$  and  $bla_{IMP-4}$ ,  $n=1$ ). The distribution map of carbapenemase genes in Chongqing was shown in Figure 5.

## Discussion

While a nationwide multi-center study in Korea showed increased AMRs to 3rd CEPs both for ECO and KPN from 2004 to 2012 [14], our study demonstrated gradual decreasing resistance tendencies for most antibiotics including third and fourth-generation cephalosporins (3<sup>rd</sup> and 4<sup>th</sup> CEPs), which was congruent with a previous report from CHINET in China which declared decreasing resistance trends to 3<sup>rd</sup> and 4<sup>th</sup> CEPs for ECO and KPN during 2012-2017 [1].

The emergence of CRE raises a global health-care threat. CRE infections are related to high mortality because therapeutic options are very limited [15, 16, 17, 18]. As to the isolation rates of carbapenem-resistant *Enterobacteriaceae*, that of CRKPN was much higher than that of CRECO according to Figure 2. Although the isolation rate of CRKPN in western China was lower compared with

that from CHINET [1], the increasing CRKPN isolation rates are still noteworthy, especially more attention should be paid to the CRE isolated from children and the elders.

To further clarify the epidemiology of CRKPN in different patient age cohorts, the annual changing trends of isolation rates of CRKPN with a comparison with those of CRECO stratified by age were shown in Figure 3 and 4. It could be concluded that the isolation rates of CRKPN in both the children and the elders increased consistently over time, which contributed significantly to continuous increasing annual CRKPN isolation rates. Notably, more attention should be paid to CRKPN in children its isolation rates in children were approximately twice higher than those in adults from 2015 to 2018.

Carbapenemase-producing *Enterobacteriaceae* (CPE) is the most pervasive antibiotic resistance threat to health services worldwide. The most globally widespread carbapenemase genes are typically carried on mobile genetic elements which can be freely exchanged between bacterial strains and species via horizontal gene transfer. Unfortunately, most of the antimicrobial surveillance systems only target specific strains or species and therefore are not well equipped for examining genes of drug resistance. To better understand the molecular carbapenem resistance mechanisms in CRKPN isolates in Chongqing, the carbapenemase genes in the 79 collected CRKPN isolates were detected. KPC-2 was found to be the most prevalent carbapenemase type in CRKPN (41/79, 51.9%), which was lower than that was reported by Qi Wang et al. (919/1201, 76.5%) who enrolled CRKPN isolates from 25 provinces across China [11]. NDM, including NDM-1 (27/79, 34.2%) and NDM-5 (4/79, 5.1%), was the secondary most predominant carbapenemase type, the percentage of which were much higher than that was reported by Qi Wang et al. (NDM-1, 9.3%; NDM-5, 1.7%) [11]. It was especially noticeable that one CRKPN strain isolated from a patient with tumor co-expressed 3 types of carbapenemase genes (*bla*<sub>KPC-2</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>IMP-4</sub>). To the best of our knowledge, there is only one study from Saudi Arabia that reported the first isolation of *K. pneumoniae* isolates co-harboring triple carbapenemase genes (including *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-48</sub>). Among the 21 carbapenem-resistant *K. pneumoniae* isolates tested from Saudi Arabia, 17 strains carried triple resistant genes including *bla*<sub>KPC</sub>, *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-48</sub>, while the other 4 carried double resistant genes (*bla*<sub>KPC</sub> and *bla*<sub>OXA-48</sub>) or (*bla*<sub>NDM-1</sub> and *bla*<sub>OXA-48</sub>) [19].

As to the geographical distribution of the carbapenemase genes, *bla*<sub>NDM</sub> was predominant in the middle of Chongqing. The differential compositions of carbapenemase gene types in different areas in Chongqing might due to nosocomial spread or irrational uses of antibiotics in different regions.

To our relief, the AMR of *P. aeruginosa* in Chongqing all showed decreasing tendencies, which was consistent with the results from CHINET [1]. Though significant decreases in resistance trends were also seen in *P. aeruginosa* to amikacin and gentamicin in a 10-year study from 2005 to 2014 in the third largest tertiary healthcare university hospital in Serbia, significant increasing trends were witnessed in resistance rates to imipenem and meropenem [20]. In order to comprehensively understand the antibiotic resistance situation in *P. aeruginosa*, more detailed hierarchical researches of *P. aeruginosa* should be executed.

While important temporal decreases in susceptibility rates among *A. baumannii* complex isolates were observed for all the antimicrobial agents tested from medical centers enrolled in the SENTRY Program [21], our study demonstrated an approximate 10 percent increase of the annual resistance rates of *A. baumannii* complex to meropenem, which was similar to that of a multi-center study report by K. Dafopoulou, et al. in Greece, from 2010-2015 [3]. Nevertheless, nearly a ten percentage decrease in amikacin resistance rate was observed for *A. baumannii* in our study.

For *S. aureus*, MRSA isolation rates presented a slow decrease and sustained at lower levels below 30%, which was different from the report of CHINET in China, which showed a significant decrease from 47.9% in 2012 to 35.3% in 2017 [1]. As for *Enterococcus* spp, the isolation rate of vancomycin resistant *Enterococcus* (VRE) maintained at a low level as compared with the result from CHINET [1].

Our study had several limitations. First, the AST results of tigecycline and polymyxin B were not included due to methodological defects employed. Second, the distribution map of the carbapenemase genes only cover fifteen districts out of the 38 districts in Chongqing due to economic burdens and strain preservation problems. Third, this was a retrospective observational study in Chongqing, and our results might not be applicable to other settings. In the future, more CRE isolates from other hospitals will be included so that we can understand the molecular mechanisms and their dynamic changes more comprehensively.

## Conclusion

In conclusion, our study provided a comprehensive provincial picture of the significant changes and trends in antimicrobial resistance of clinically important pathogens in Chongqing, which could be used to update the evidence-based guidelines on antimicrobial prescriptions in southwest, China. Although overall, many AMRs showed decreasing trends, further hierarchical researches should be executed according to different age groups, hospital wards, resistance degrees (MDR/XDR/PDR), and so on. To the best of our knowledge, this is the first report on a CRKPN isolate co-harboring triple carbapenemases including *bla*KPC-2, *bla*NDM-1 and *bla*IMP-4 worldwide. At a time when novel and effective antibiotic compounds have not become available, containment of CPE is bound to rely on stricter infection control measures in hospitals.

## Abbreviations

AMR: Antimicrobial resistance; BSIs: Bloodstream infections; CARSS: China Antimicrobial Resistance Surveillance System; CLSI: Clinical and Laboratory Standards Institute; CRABA: Carbapenem-resistant *Acinetobacter baumannii*; CRE: Carbapenem-resistant *Enterobacteriaceae*; CRECO: Carbapenem-resistant *Escherichia coli*; CRKPN: Carbapenem-resistant *Klebsiella pneumoniae*; CRPAE: Carbapenem-resistant *Pseudomonas aeruginosa*; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; VREFM: Vancomycin-resistant *Enterococcus faecium*; WHO: World health organization

### Antimicrobials

PEN: Penicillin G; AMP: Ampicillin; OXA: Oxacillin; PIP: Piperacillin; TZP: Piperacillin/Tazobactam; CZO: Cefazolin; CXM: Cefuroxime; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; ATM: Aztreonam; ETP: Ertapenem; IMP: Imipenem; MEM: Meropenem; CIP: Ciprofloxacin; LEV: Levofloxacin; MXF: Moxifloxacin; AMK: Amikacin; GEH: Gentamicin-High level; TOB: Tobramycin; ERY: Erythromycin; CLI: Clindamycin; CHL: Chloramphenicol; TCY: Tetracycline; LZD: Linezolid; VAN: Vancomycin

## Declarations

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### Availability of data and materials

All the data of this article is available from the corresponding author if reasonably requested.

### Authors' contributions

All the authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. SS, SQN and SFH designed this study, participated in statistic calculations and interpretation of data, and drafted this manuscript. LY, CMZ and JDS collected the raw data of ARINCQ by Whonet 5.6.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

## Competing interests

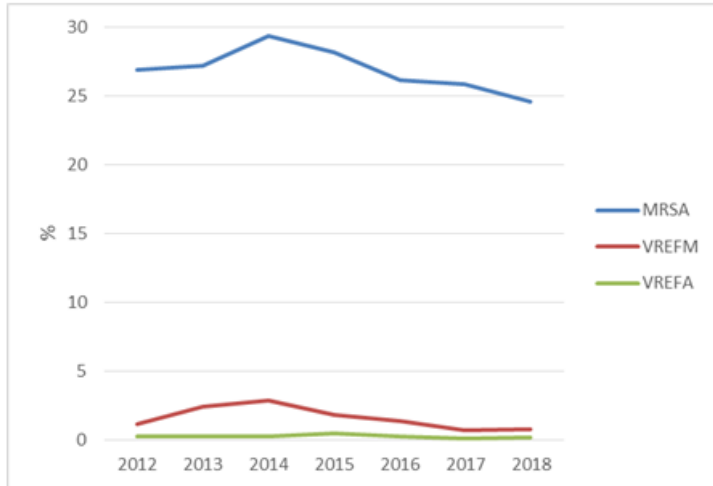
The authors declare that they have no competing interests.

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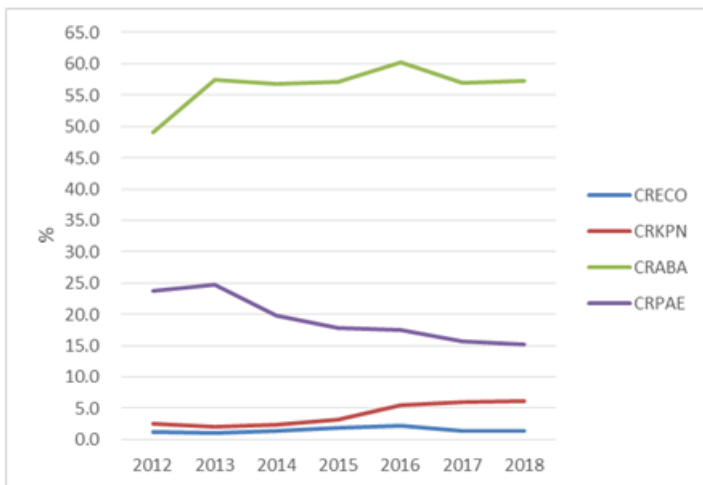
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## Figures



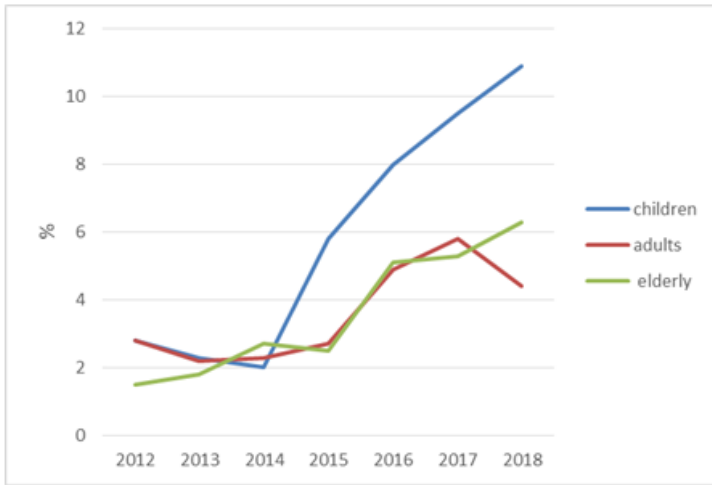
**Figure 1**

Isolation rates of MRSA, VREFM and VREFA from 2012-2018 MRSA, methicillin-resistant *Staphylococcus aureus*; VREFM, vancomycin-resistant *Enterococcus faecium*; VREFA, vancomycin-resistant *Enterococcus faecalis*



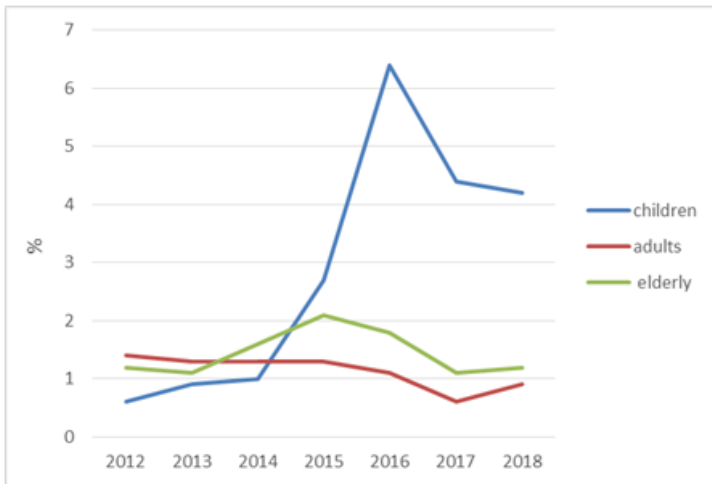
**Figure 2**

Isolation rates of CRECO, CRKPN, CRABA and CRPAE from 2012 to 2018 CRECO, carbapenem-resistant *Escherichia coli*; CRKPN, carbapenem-resistant *Klebsiella pneumoniae*; CRABA, carbapenem-resistant *Acinetobacter baumannii*; CRPAE, carbapenem-resistant *Pseudomonas aeruginosa*



**Figure 3**

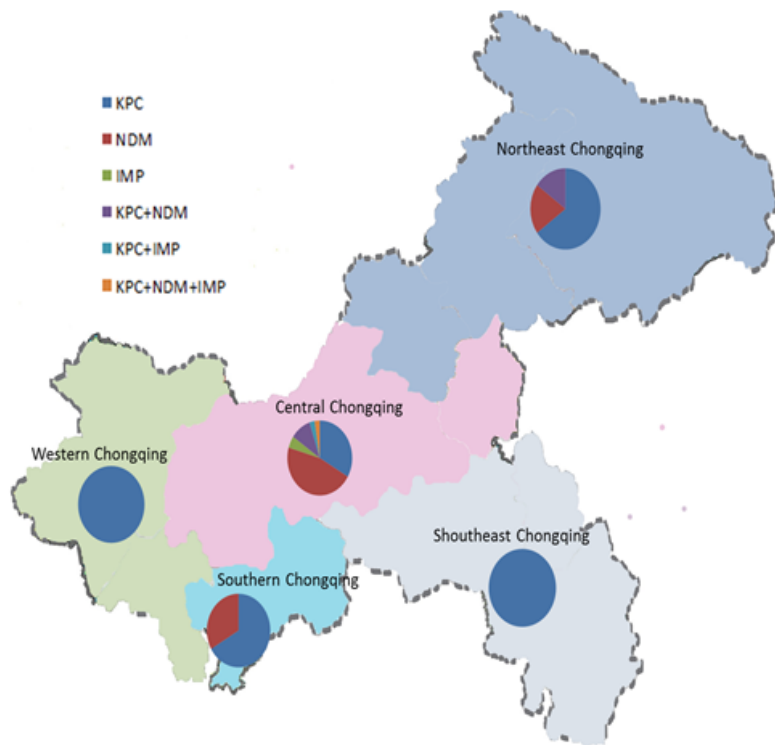
Isolation rates of CRKPN for different age groups from 2012 to 2018 CRKPN, carbapenem-resistant *Klebsiella pneumoniae*



**Figure 4**

Isolation rates of CRECO for different age groups from 2012 to 2018 CRECO, carbapenem-resistant *Escherichia coli*





**Figure 5**

Distribution map of the carbapenemase genes from the CRKPN isolates in Chongqing CRKPN, carbapenem-resistant *Klebsiella pneumoniae*. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.