Infection and Drug Resistance

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ORIGINAL RESEARCH

Longitudinal Study of the Drug Resistance in *Klebsiella pneumoniae* of a Tertiary Hospital, China: Phenotypic Epidemiology Analysis (2013–2018)

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Purpose: Multi-drug resistant *Klebsiella pneumoniae* (MDR KP) is spreading worldwide and has posed a huge medical burden to public health. However, studies on drug resistance surveillance of KP, especially MDR KP, with a large longitudinal sample size in a tertiary hospital are rare. This study aims to investigate phenotypic epidemiology characteristics of 4128 KP isolates in a Chinese tertiary hospital covering a period of 5 years.

Methods: All the KP clinical isolates were retrospectively collected from a tertiary hospital in Hunan province of China from Jan 5, 2013 to Jul 24, 2018. All the isolates were identified by MALDI-TOF MS analysis. Twenty-four antimicrobial agents were tested by antimicrobial susceptibility testing. Fisher exact test and logistic regression were used to analyze the association between clinical factors and antimicrobial non-susceptibility for seven second-choice antimicrobials.

Results: A total of 4128 KP isolates were collected in our study. The non-susceptible rates (NSRs) to ertapenem, imipenem and tigecycline increased considerably from 2013 to 2018 (13.6% to 28.6%, 10.1% to 28.9%, 10.8% to 46.5%, respectively). Amikacin presents the lowest NSR among 3 aminoglycosides (3.8–22.8%). The multi-drug NSRs among KP isolates to second-choice antimicrobials (88.6–100%) were higher than to all drugs (68.0%). The NSRs varied significantly among departments and sample sources. Higher ETP/IPM/AK NSRs (39.8/39.7/30.6%) were observed in Intensive Care Unit, and ETP/IPM non-susceptible isolates tended to distribute in cerebrospinal fluid. From 2015 to 2017, the NSRs of ETP, IPM, and AK showed an opposite trend of seasonal fluctuations to SXT.

Conclusion: Higher multi-drug resistance (MDR) rates were observed in KP isolates to second-choice antimicrobials than to others, among which MDR rates to carbapenems or AK are the highest. A unique pattern of MIC and time distributions of MDR were observed. Clinical factors including gender were correlated with MDR rates of KP. Isolates in ICU and CSF showed higher NSRs in carbapenems which should be paid more attention to, and temporal distribution of NSRs was observed.

Keywords: *Klebsiella pneumoniae*, drug resistance, NSR, phenotypic epidemiology

Introduction

Klebsiella pneumoniae, a member of the *Enterobacteriaceae* family, is one of the main pathogenic bacteria that causes hospital and community acquired infections. *K. pneumoniae* commonly infects the urinary tract, respiratory tract, surgical sites, as well as the bloodstream and can cause severe diseases such as pneumonia and sepsis.[1] According to the safety, efficacy, resistance, and price of antibiotics, antibiotics can be classified into non-restricted use (first-choice antimicrobials), restricted use

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(second-choice antimicrobials), and special use antimicrobials. First-choice antimicrobials have good efficacy and can be selected by doctors at all levels according to their needs. However, second-choice antimicrobials are usually allowed for use by the attending physician when patients are allergic or resistant to the first-choice antimicrobials. In recent years, the emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) and extended-spectrum β-lactamase (ESBL) poses a global healthcare challenge. At the same time, due to the increasing use of other second-choice antimicrobials against carbapenem-resistant *K. pneumoniae* (CRKP) and extended-spectrum β-lactam producing *K. pneumoniae* (ESBL-KP), the corresponding drug resistance rates have also increased.[2,3] Infections caused by these so-called superbugs are associated with high mortality because therapeutic options are limited.[4–9]

Carbapenems are especially effective in the treatment of *Enterobacteriaceae* bacterial infections that produce ultra-broad-spectrum beta-lactamase and cephalosporinase, and plays an important role in the treatment of multi-drug-resistant bacterial infections.[10] However, with the widespread and even clinician unreasonable use of carbapenems in recent years, the detection rate of CRKP increased year by year, posing a challenge to the clinical treatment. In recent decades, sporadic CRKP events and outbreaks have been reported in many countries and regions, including China.[11–13]

Other commonly selected drugs for the treatment of MDR KP include tigecycline (TGC), colistin, sulfamethoxazole-trimethoprim (SXT) and aminoglycosides such as amikacin (AK), gentamicin (GEN) and tobramycin (TOB). These antimicrobials generally have a lower resistance rate than first-choice antimicrobials and carbapenems in MDR KP infection. Tigecycline is a novel broad-spectrum antimicrobial approved by FDA in 2005, with excellent activity against all Gram-positive and nearly all Gram-negative bacterium.[14–17] Discovered in the 20th century, aminoglycosides are potent against various Gram-positive and negative bacterium, especially against *Enterobacteriaceae*. Aminoglycosides were substituted by cephalosporins, fluoroquinolones, and carbapenems for their toxicity and side effects since the 1980s, but received a renewed interest in recent years for the potential to treat multi-drug resistance (MDR) bacterial infections.[18]

The Chinese government pays great attention to the usage and resistance of carbapenems. According to the monitoring results of 192 tertiary hospitals in China, the consumption of carbapenems constantly increased from 1.83 defined daily doses (DDDs) per 100 bed days in 2011 to 3.38 DDDs per 100 bed days in 2017.[19] China Antimicrobial Resistance Surveillance System (CARSS) reported that the detection rate of CRKP in China increased from 4.9% in 2013 to 10.7% in 2018.[20] However, little is known about the department and temporal characteristics of the infection of other second-choice antimicrobials resistant KP in recent years in China. Longitudinal large-scale study can be useful for monitoring the drug resistance and controlling thenosocomial infections. In our study, we collected *K. pneumoniae* isolates during 2013–2018 from a tertiary hospital with 3500 beds and 76 wards in Hunan, China, investigating the multi-drug and second-line antimicrobial non-susceptible rates (NSRs) of isolates from various departments, sample types, and seasonal distributions, in order to provide a comprehensive landscape of the department-temporal characteristic of resistant KP infections in a typical tertiary hospital in China.

Methods

Bacterial Isolates and Identification

4128 *Klebsiella pneumoniae* isolates were recovered from the clinic laboratory of a tertiary hospital in Hunan province of China, covering all clinical samples tested positive for KP from January 5, 2013 to July 24, 2018. These isolates were continuously and unbiasedly collected, covering all patients who tested positive for KP in the hospital during this period. To avoid duplication and phenotypic changes, only the first strain, isolated from various samples of the same patient, was included. All isolates were identified by MALDI-TOF MS analysis. The isolates mainly came from ten departments in the hospital. Samples were taken from a variety of locations, including respiratory secretion, blood, drainage and puncture fluid, urine, wound secretion, digestive system secretion, abscess, tractus genitalis secretion, CSF, stool, and other sites. The patient information is managed through the hospital information management system. All patients will be given an ID number when they are admitted to the hospital, through which age and sex of the patients, date of sample submission, sample type, hospitalization, and department were collected.

Antimicrobial Susceptibility Test

All isolates were tested experimentally for their susceptibility to 24 common antimicrobial agents. The susceptibility to 20 antimicrobial agents was tested by the Vitek 2 system (bio Mérieux, Marcy l'Étoile, France) to determine the minimum inhibitory concentration (MIC).

Susceptibility to other four antimicrobial agents, including cefuroxime (CXM), cefoperazone/sulbactam (SCF), tigecycline (TGC) and cefazolin (CFZ), was determined by the size of inhibition zone in Kirby-Bauer disk diffusion test. Bacteria were cultured on 5% sheep blood agar (BA) (Oxoid, UK) overnight at 37°C. At least 4 colonies were picked and emulsified in 0.8% saline to achieve a turbidity of 0.5 McFarland. The suspension was streaked onto the surface of Mueller-Hinton agar using a sterile cotton swab. Discs supplemented with antibiotics were placed onto the surface of agar plate using an automatic disc dispenser. The culture was then incubated at 37°C for 16 to18 hours before zone diameters were read. Antibiotic agents were classified into three categories as resistant, intermediate, or susceptible according to the CLSI guideline 2018. The MICs and inhibitory zone diameter (IZD) of different antimicrobials were interpreted using the CLSI 2018 breakpoints standards. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as controls.

Data Process and Statistical Analysis

Some patients were sampled multiple times for clinical purposes. As for the patients with multiple samples, the isolates with identical antibiotic phenotypes were considered to be duplicates, among which the isolate owns the most complete antimicrobial susceptibility test (AST) information was retained, and others were excluded from the dataset. If all isolates among the duplicates have equally complete AST information, the earliest isolate collected would be retained.

The category of antimicrobial agents (Supplementary Table 1) and the judgment of MDR KP were based on an international expert proposal for interim standard definitions for acquired resistance.[21] Univariate analysis of clinical risk factors of non-susceptibility was finished by Monte Carlo simulation fisher exact test (repeated 200,000 times). Multivariate analysis was managed by logistic regression in order to avoid false correlations between the analyzed clinical factors. All P-values were two-tailed, and a statistical significance was considered when $P < 0.05$.

Results

Clinical Characteristics of *Klebsiella pneumoniae* Isolates

Of the 4128 KP isolates, 509 isolates were regarded as duplicates according to their antimicrobial susceptibility results. 3619 non-redundant isolates were left after removing the duplicates (Supplementary Figure 1). These isolates were continuously and unbiasedly collected, covering all patients who tested positive for KP in the hospital from January 5, 2013, to July 24, 2018. The sample sizes of each year are shown in Table 1. Most samples (76.26%) were collected from 2015 to 2017 and isolates were only collected for half a year in 2018.

Among the 3619 isolates, the largest number of isolates were from the patients in intensive care unit (ICU) (n = 998, 27.6%), followed by surgery (n = 865, 23.9%), medicine (n = 625, 17.3%), and pediatrics (n = 404, 11.2%). Obstetrics and gynecology have the least number of isolates (n = 45.1.2%), five isolates had no department information (Table 1). Sample type distribution was also investigated. The majority of isolates came from respiratory tract (n = 2020, 55.8%), a reported main site of KP infection, followed by blood samples (n = 484, 13.4%), puncture/drainage fluid (n = 351, 9.7%), urine (n = 278, 7.7%), and stool had the lowest number of isolates among all sample types (n = 12, 0.3%).

The patients who were sampled had a wide range of age distribution (0–98 years old), with a median of 52. Among them, 2478 (68.5%) of the patients were male, while 1141 (31.5%) were female. For patients with admission information (n = 3335), the hospitalization rate was 60.8% (Table 1).

The Characteristics of Antimicrobial Resistance and MDR KP During 2013 to 2018

The resistance/intermediary/sensitive rates for 24 antimicrobial agents were calculated from 2013 to 2018, covering 14 antimicrobial categories (Table 2). The resistance rates toward ampicillin (AMP) were close to 100% in each year, which may be led by the intrinsic resistance of *Klebsiella pneumoniae*.[22] Not considering the AMP, Nitrofurantoin showed the highest NSR in all categories (83.1% in total), followed by 1st and 2nd generation cephalosporins (81.9% for CFZ, 75.8% for CXM, in total) and Penicillins + β-lactamase inhibitors (60.2% for AMC, 64.9% for SAM, in total). NSRs to 3rd and 4th generation cephalosporins were lower than 50% during 2013–2018, except CRO (58.6% in total).

Without regard to the intrinsic AMP resistance, 3355 (92.7%) samples were non-susceptible to at least one antimicrobial category, and 2462 (68.0%) samples had multi-drug resistance (non-susceptible to ≥1 agent in ≥3

Table 1 Clinical Characteristics of 3619 Isolates

Characteristics	Value[a]
Department	
ICU	998 (27.6)
Surgery	865 (23.9)
Medicine	625 (17.3)
Pediatrics	404 (11.2)
Outpatient	219 (6.1)
ITC & WM[b]	190 (5.3)
Rehabilitation	149 (4.1)
Infectious diseases	66 (1.8)
Oncology	53 (1.5)
Obstetrics & Gynecology	45 (1.2)
ND[c]	5 (0.1)
Sample type	
Respiratory secretion	2020 (55.8)
Blood	484 (13.4)
Drainage & Puncture fluid	351 (9.7)
Urine	278 (7.7)
Wound secretion	157 (4.3)
Digestive system secretion	87 (2.4)
Abscess	70 (1.9)
Others	70 (1.9)
Tractus genitalis secretion	48 (1.3)
CSF	42 (1.2)
Stool	12 (0.3)
Collecting year	
2013	239 (6.6)
2014	325 (9)
2015	892 (24.6)
2016	996 (27.5)
2017	872 (24.1)
2018	295 (8.2)
Age of patients, median years (range)	52 (0–98)
Sex of patients	
Male	2478 (68.5)
Female	1141 (31.5)
Hospitalization of patients[d]	
1	2029 (56.1)
0	1306 (36.1)
ND	284 (7.8)

Notes: [a]Values are reported as number (%) of isolates unless otherwise indicated. [b]ITC & WM: Integrated traditional Chinese & Western Medicine. [c]ND: data not available. [d]Hospitalization of patients: 1: inpatient 0: outpatient. All the data is calculated based on the sample size.

antimicrobial categories) (Figure 1A). Among all antimicrobial agents with antimicrobial susceptibility testing results, seven commonly used antimicrobials for KP treatment were selected for in-depth analysis, namely, 2 carbapenems (including ETP and IPM), 3 aminoglycosides (including AK, GEN, and TOB), SXT, and TGC. Isolates non-susceptible to the second-choice antimicrobials overall showed higher multidrug-resistant rates (88.6–100%), among which the isolates non-susceptible to carbapenems or AK showed stronger multidrug-resistance than those to other second-choice antimicrobials, with over 98% isolates being non-susceptible to >5 antimicrobial categories and over 30% non-susceptible to >10 categories (Figure 1B).

MICs results of six second-choice antimicrobials are shown in Figure 2. We discovered that the MIC values tended to distribute in low range or high range, rather than in the middle range. 876 (24.5%) isolates presented ETP MIC of ≥8 μg/mL, 763 (21.3%) isolates presented IPM MIC of ≥16 μg/mL. Unlike other five second-choice antimicrobials, 599 isolates presented TOB MIC of 8 μg/mL, leading to 17.1% intermediary rate.

By comparing the NSRs to the seven second-choice antimicrobials by year, we found that the NSRs to three aminoglycosides (AK, GEN, TOB) increased from 2013 to 2015, and then followed with a decreased trend, among which the NSR to AK was the lowest (Figure 3B). While the NSR to SXT dropped slowly from 40.2% to 35.3% (Figure 3C), a considerable increase towards two carbapenems (ETP, IPM) and TGC, which was commonly used to treat carbapenems and fluoroquinolone resistant KP, was shown (Figure 3A and D). We can also observe that the intermediary rate of TGC increased from 2014 to 2016 in Table 2.

Clinical Factors Affecting the KP Non-Susceptibility to the Second-Choice Antimicrobials

The department-temporal clinical factors associated with non-susceptibility to the seven second-choice antimicrobials were calculated (Table 3). Some previous studies have shown that long duration of hospital stay was considered as one of the risk factors for antimicrobial resistance,[23] in our study, however, NSR to tigecycline among the non-hospitalized group was significantly higher than that of the hospitalized group (40.60% VS 27.36%, $P < 0.001$). NSRs to other second-choice antimicrobials did not show significant difference between the two groups. For different genders, the NSRs to 2 carbapenems and 3 aminoglycosides among males were significantly higher than those among females ($P < 0.05$). For different age groups, the non-susceptibility to IPM, AK, GEN, TOB, SXT, and TGC showed

Table 2 Sample Distribution (R%/I%/S%)[a] of *Klebsiella pneumoniae* by Year

Antimicrobial Agent	2013	2014	2015	2016	2017	2018	Total
TZP	239 (10.0/9.2/80.8)	325 (16.6/6.5/76.9)	891 (26.0/3.8/70.1)	771 (34.1/5.8/60.1)	783 (33.6/4.6/61.8)	294 (28.2/3.4/68.4)	3303 (27.8/5.1/67.1)
AMC	45 (31.1/13.3/55.6)	56 (41.1/17.9/41.1)	121 (35.5/17.4/47.1)	208 (51.4/16.8/31.7)	5 (60.0/0.0/40.0)	0	435 (43.7/16.6/39.8)
SAM	195 (55.4/5.1/39.5)	269 (56.9/3.7/39.4)	770 (62.9/2.5/34.7)	788 (64.6/3.7/31.7)	867 (61.6/3.2/35.2)	294 (56.5/5.8/37.8)	3183 (61.4/3.6/35.1)
ETP	228 (12.3/1.3/86.4)	300 (9.3/0.0/90.7)	892 (25.4/0.6/74.0)	996 (31.3/0.9/67.8)	868 (28.6/0.3/71.1)	294 (28.2/0.3/71.4)	3578 (25.9/0.6/73.5)
IPM	238 (7.6/2.5/89.9)	325 (14.5/1.5/84.0)	892 (23.7/1.0/75.3)	996 (27.3/0.5/72.2)	870 (28.3/0.7/71.0)	294 (27.2/1.7/71.1)	3615 (24.2/1.0/74.8)
ATM	239 (46.0/0.0/54.0)	325 (42.5/0.3/57.2)	889 (45.6/0.8/53.7)	994 (50.5/0.2/49.3)	872 (48.3/0.2/51.5)	295 (43.7/0.0/56.3)	3614 (47.2/0.3/52.5)
AMP	239 (100.0/0.0/0.0)	325 (99.7/0.0/0.3)	886 (99.5/0.0/0.5)	996 (99.5/0.0/0.5)	871 (99.4/0.1/0.5)	295 (99.3/0.0/0.7)	3612 (99.5/0.0/0.4)
CFZ	138 (100.0/0.0/0.0)	184 (100.0/0.0/0.0)	484 (100.0/0.0/0.0)	823 (80.1/3.4/16.5)	843 (63.2/6.3/30.5)	291 (55.0/7.9/37.1)	2763 (78.1/3.8/18.1)
CXM	1 (100.0/0.0/0.0)	0	0	316 (61.1/18.7/20.3)	852 (58.9/16.7/24.4)	291 (54.0/18.2/27.8)	1460 (58.4/17.4/24.2)
CAZ	193 (32.6/3.1/64.2)	269 (33.5/1.1/65.4)	771 (40.9/3.2/55.9)	900 (43.1/3.2/53.7)	868 (41.7/3.8/54.5)	295 (40.0/1.7/58.3)	3296 (40.5/3.1/56.4)
CRO	238 (52.5/0.4/47.1)	325 (53.8/0.0/46.2)	892 (59.1/0.4/40.5)	995 (63.3/0.2/36.5)	872 (57.6/0.1/42.3)	295 (51.5/0.0/48.5)	3617 (58.4/0.2/41.4)
SCF	222 (26.6/12.2/61.3)	322 (19.6/9.0/71.4)	881 (24.4/5.3/70.3)	936 (29.3/8.4/62.3)	856 (31.1/11.0/57.9)	292 (32.5/8.6/58.9)	3509 (27.7/8.6/63.7)
FEP	239 (29.3/5.0/65.7)	325 (28.9/6.8/64.3)	892 (35.7/3.4/61.0)	995 (38.5/5.6/55.9)	871 (37.0/4.0/59.0)	294 (37.1/2.4/60.5)	3616 (35.8/4.5/59.7)
CTT	194 (10.8/0.5/88.7)	269 (12.3/2.6/85.1)	771 (24.1/1.3/74.6)	788 (27.7/1.3/71.1)	865 (26.0/1.3/72.7)	294 (27.6/1.4/71.1)	3181 (24.0/1.4/74.6)
FOX	46 (26.1/2.2/71.7)	56 (23.2/0.0/76.8)	121 (31.4/4.1/64.5)	97 (45.4/2.1/52.6)	7 (42.9/0.0/57.1)	0	327 (33.6/2.4/63.9)
AK	239 (3.8/0.0/96.2)	325 (12.6/0.6/86.8)	891 (20.9/0.1/79.0)	996 (22.6/0.2/77.2)	872 (19.7/0.2/80.0)	294 (15.6/0.0/84.4)	3617 (18.8/0.2/81.0)
GEN	239 (33.9/0.0/66.1)	324 (33.6/1.5/64.8)	892 (40.9/0.7/58.4)	994 (37.9/1.3/60.8)	872 (36.5/2.2/61.4)	294 (29.9/0.7/69.4)	3615 (37.0/1.2/61.7)
TOB	239 (12.1/22.2/65.7)	325 (18.5/19.4/62.2)	892 (28.6/15.5/55.9)	884 (27.4/16.6/56.0)	872 (26.0/18.0/56.0)	294 (19.0/13.9/67.0)	3506 (24.8/17.1/58.1)
CIP	239 (25.1/3.8/71.1)	325 (29.5/6.5/64.0)	891 (37.7/2.6/59.7)	996 (41.5/3.7/54.8)	871 (40.2/4.5/55.3)	294 (42.5/5.1/52.4)	3616 (38.2/4.0/57.9)
LVX	239 (17.6/5.0/77.4)	325 (23.7/3.4/72.9)	891 (31.2/3.4/65.4)	996 (36.3/3.2/60.4)	872 (34.4/3.0/62.6)	294 (38.4/4.1/57.5)	3617 (32.4/3.4/64.2)
MIN	1 (0.0/0.0/100.0)	0	38 (7.9/13.2/78.9)	139 (25.2/15.1/59.7)	0	0	178 (21.3/14.6/64.0)
NIT	220 (33.2/55.0/11.8)	325 (41.2/47.1/11.7)	890 (42.2/43.3/14.5)	994 (44.1/35.5/20.4)	868 (41.1/38.9/19.9)	292 (47.9/38.7/13.4)	3589 (42.3/40.8/16.9)
SXT	239 (40.2/0.0/59.8)	325 (40.3/0.0/59.7)	892 (34.9/0.0/65.1)	990 (33.8/0.0/66.2)	861 (36.0/0.1/63.9)	292 (35.3/0.0/64.7)	3599 (35.7/0.0/64.2)
TGC	37 (8.1/2.7/89.2)	52 (3.8/0.0/96.2)	148 (10.8/6.8/82.4)	537 (6.7/35.9/57.4)	852 (4.8/33.9/61.3)	282 (6.4/40.1/53.5)	1908 (6.1/31.8/62.2)

Notes: [a]R, resistant; I, intermediate; S, susceptible.

Abbreviations: TZP, piperacillin/tazobactam; AMC, amoxillin/clavulanic acid; SAM, ampicillin/sulbactam; ETP, ertapenem; IPM, imipenem; ATM, aztreonam; AMP, ampicillin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; CTT, cefotetan; FOX, cefoxitin; AK, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LVX, levofloxacin; MIN, minocycline; NIT, nitrofurantoin; SXT, trimethoprim-sulfamethoxazole; CXM, cefuroxime; SCF, cefoperazone/sulbactam; TGC, tigecycline; CFZ, cefazolin.

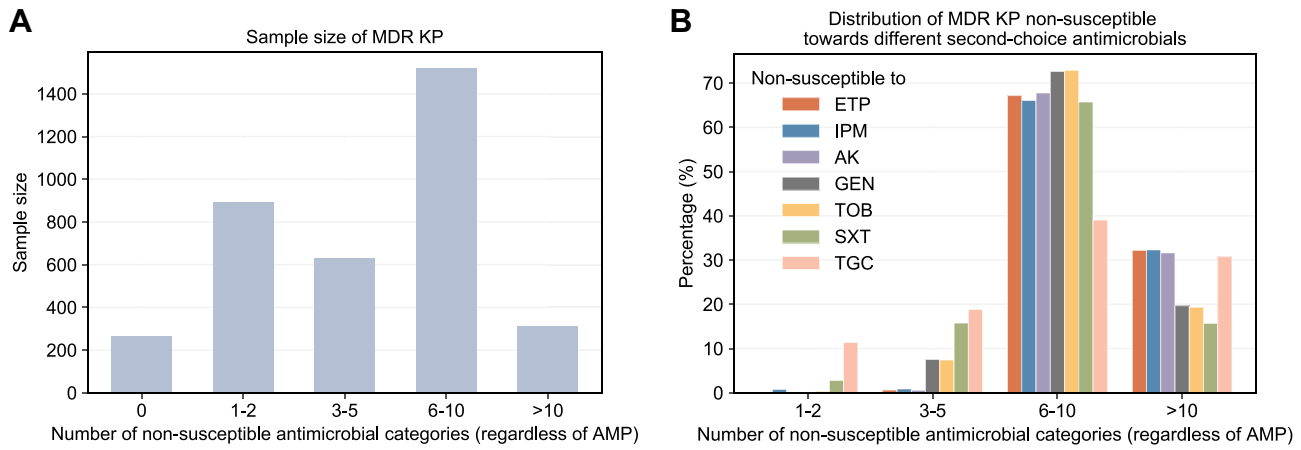

Figure 1 Distribution of multidrug-resistance patterns among KP isolates. The sample size distribution of KP in 24 common antimicrobial agents among all 3619 analyzed isolates (**A**) and percentage of isolates non-susceptible to seven second-choice antimicrobials in different amount antimicrobial categories (**B**). MDR KP was defined as the isolates non-susceptible to three or more than three categories. As an intrinsic resistance of KP, the resistance towards AMP was excluded from this analysis.
Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

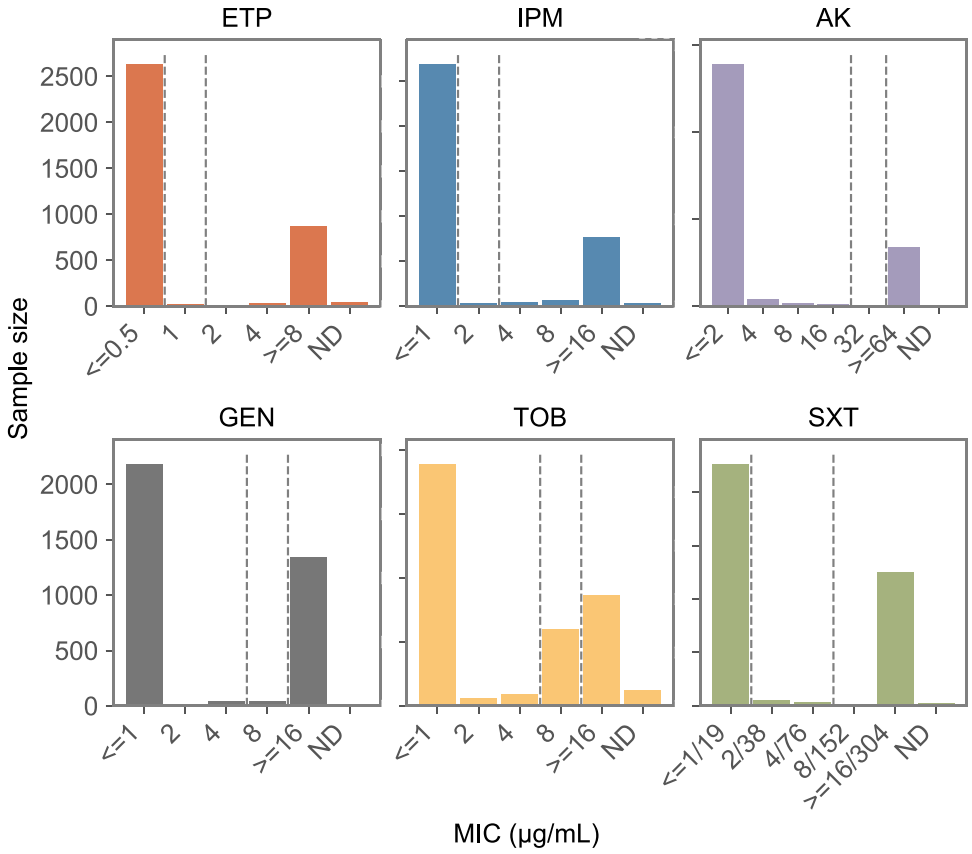

Figure 2 Distribution of MIC for six second-choice antimicrobials. MIC distributions among isolates for six drugs: ETP, IPM, AK, GEN, TOB, and SXT. ND: data not available. CLSI 2018 breakpoints were presented as grey dashed lines in each subplot, dividing isolates into susceptible (isolates to the left of the first line), intermediate (isolates between the two lines) and resistant (isolates to the right of the second line except "ND") types. The resistance to TGC was tested by Kirby-Bauer disk diffusion method, so there is no information on TCG MIC.
Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole.

significant bias ($P < 0.001$), with the lowest NSRs among newborns at the age of 0 to 1 (14.7%, 0.6%, 17.7%, 23.5%, 32.7%, 25.5%, respectively). Significant bias of non-susceptibility was also present in different sampling types/time. We subdivided the sampling time into quarterly units (Q1: Jan–Mar, Q2: Apr–Jun, Q3:

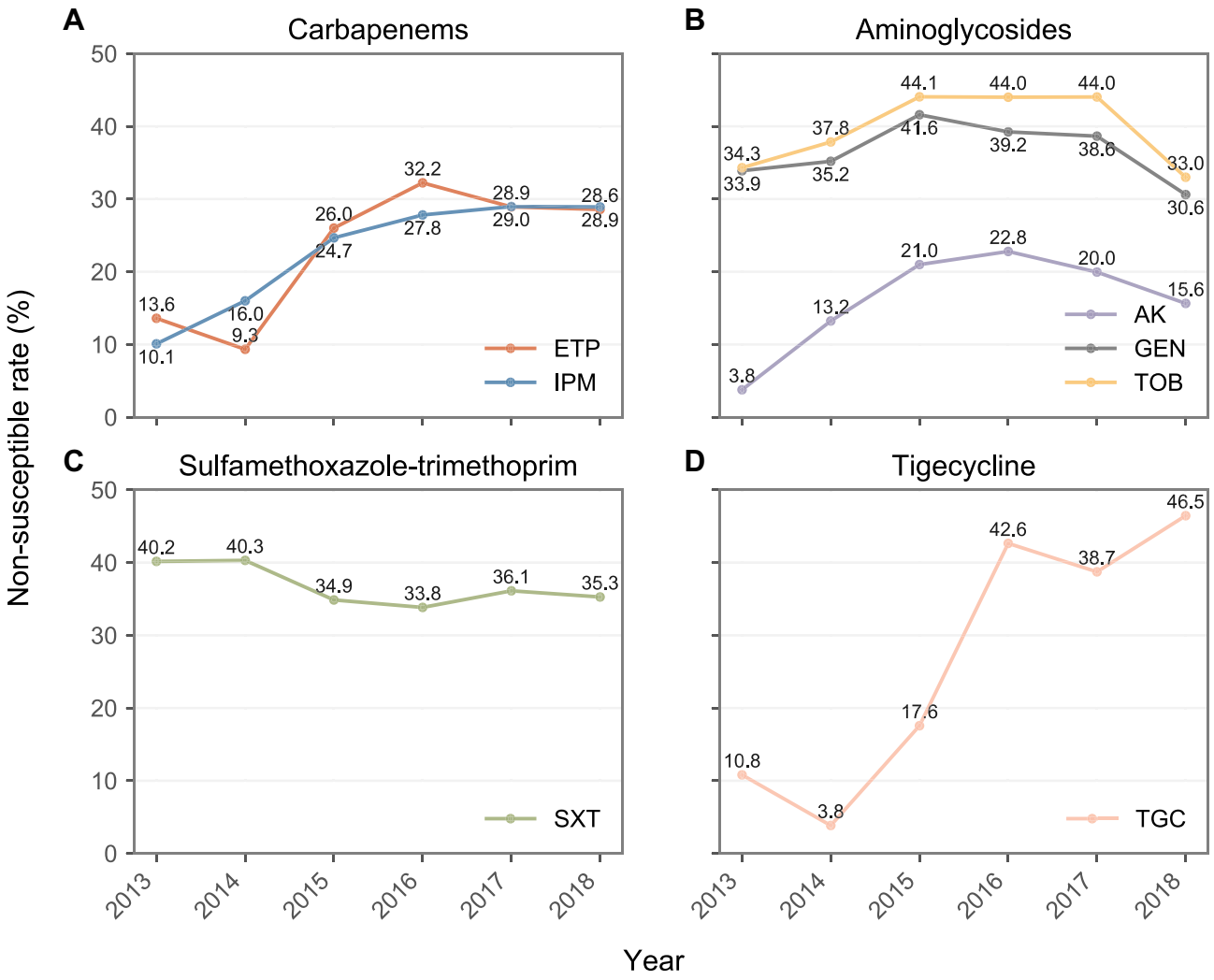

Figure 3 Dynamics in NSRs to second-choice antimicrobials from 2013 to 2018. The yearly change of NSRs to 2 carbapenems (ETP, IPM) (**A**), 3 aminoglycosides (AK, GEN, TOB) (**B**), sulfamethoxazole-trimethoprim (SXT) (**C**) and tigecycline (TGC) (**D**), between 2013 and 2018.
Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

Jul–Sep, Q4: Oct–Dec), and discovered the non-susceptibility towards ETP, IPM, AK, SXT, and TGC varied from quarter to quarter. All second-choice antimicrobials presented significantly different NSRs in different departments, while all second-choice antimicrobials (except TGC) presented significantly different non-susceptibility in various sample types.

Multivariate analysis by logistic regression was also managed (Table 4). We found that hospitalization showed a negative correlation with non-susceptibility to ETP, IPM, TOB, and TGC. Males were associated with non-susceptibility to ETP and IPM. Age was not correlated with any NSRs when it is regarded as a continuous variable. These results showed similar characteristics of antimicrobial non-susceptibility discovered in univariate analysis, except that no significant correlation of the non-susceptibility to AK with sample types was identified.

Department Distribution of Non-Susceptibility in KP

In the present study, we found that KP showed different department bias for non-susceptibility to different second-choice antimicrobials. For the seven second-choice antimicrobials, the NSRs to ETP, IPM, and AK were highest in ICU (39.8%, 39.7%, and 30.6%, respectively), while that to GEN, TOB, SXT, and TGC were higher in wards of integrated traditional Chinese and Western medicine (ITC & WM) and rehabilitation than in other departments (higher than 51.6%, 58.6%, 47.6%, and 42.1%, respectively). The resistance bias to TGC is not as obvious as that to other drugs, but is still significant (P = 0.037). The

Table 3 Univariate Analysis of Clinical Factors for Non-Susceptibility to Second-Choice Antimicrobials

		ETP		IPM		AK		GEN		TOB		SXT		TGC	
		NS[a]	S[b]	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S
Hospitalized	**P**	**0.418**		**0.078**		**1**		**0.942**		**0.246**		**0.11**		**<0.001***	
	0	353	949	346	958	251	1055	509	796	572	731	485	810	445	651
	1	514	1479	483	1545	391	1637	788	1239	803	1117	702	1321	148	393
Gender	**P**	**<0.001***		**<0.001***		**0.012***		**0.006***		**0.043***		**1**		**0.47**	
	M	710	1742	680	1796	497	1979	984	1491	1030	1364	880	1582	515	827
	F	237	889	230	909	189	952	399	741	438	674	407	730	207	359
Age	**P**	**0.689**		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**0.001***	
	≤1	79	253	50	289	2	337	60	279	75	244	111	228	40	117
	2–44	225	606	222	616	174	665	365	475	393	421	360	475	188	256
	45–59	296	827	298	837	229	906	430	703	464	641	373	754	212	376
	≥60	347	945	340	963	281	1023	528	775	536	732	443	855	282	437
Quarter[c]	**P**	**<0.001***		**<0.001***		**<0.001***		**0.354**		**0.254**		**<0.001***		**0.041***	
	A	246	527	229	558	184	603	305	482	308	455	225	561	133	227
	B	274	676	277	679	230	725	386	570	404	505	314	633	200	391
	C	230	796	220	816	140	897	385	651	419	578	412	618	191	296
	D	197	632	184	652	132	706	307	529	337	500	336	500	198	272
Infection site	**P**	**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**0.396**	
	Abscess	11	59	10	60	11	59	17	53	18	52	11	57	16	30
	Blood	128	348	118	366	73	411	157	326	161	303	169	312	87	160
	CSF	15	24	18	24	10	32	16	26	16	23	19	23	6	18
	Digestive system secretion	16	71	18	69	12	75	27	60	27	56	23	62	19	29
	Drainage & Puncture fluid	112	237	108	243	87	264	139	212	130	206	95	256	72	112
	Respiratory secretion	517	1480	506	1510	379	1640	783	1234	853	1116	731	1277	412	681
	Stool	11	1	3	9	1	11	3	9	8	3	2	10	0	3
	Tractus genitalis secretion	1	47	1	47	0	48	8	40	11	36	18	30	6	10
	Urine	71	205	65	213	61	216	121	157	134	136	134	143	69	76
	Wound secretion	44	113	43	114	39	118	91	66	91	59	69	88	23	46
	Others	21	46	20	50	13	57	21	49	19	48	16	54	12	21
Department	**P**	**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**<0.001***		**0.037***	
14	ICU	390	589	395	601	305	693	478	517	486	484	347	645	238	343
	Infectious diseases	14	52	14	52	13	53	18	48	22	43	17	49	15	22
	ITC & WM[d]	47	143	40	150	40	150	110	80	128	55	102	88	40	55
	Medicine	94	525	88	535	80	544	201	423	209	402	189	431	123	202
	Obstetrics & Gynecology	1	44	1	44	2	43	8	37	7	38	10	35	5	
	Oncology	3	50	3	50	1	52	9	44	10	42	12	40	6	15
	Outpatient	37	182	38	181	29	190	67	152	69	144	84	134	38	64
	Pediatrics	94	304	63	341	8	396	80	324	105	278	149	255	50	142
	Rehabilitation	42	106	42	107	33	115	77	72	85	60	71	78	30	39
	Surgery	224	632	225	640	174	691	334	531	346	488	305	553	176	287

Notes: [a]NS: non-susceptible, [b]S: susceptible, [c]Quarter: Q1: Jan–Mar, Q2: Apr–Jun, Q3: Jul–Sep, Q4: Oct–Dec, [d]ITC & WM: Integrated traditional Chinese & Western Medicine. *With significant differences under the Monte Carlo simulation fisher exact test (B=200,000). 5 Isolates without department information and 284 isolates without hospitalization information were excluded in the analysis.

Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

Table 4 Multivariate Analysis of Risk Factors for Non-Susceptibility to Second-Choice Antimicrobials

	OR (95% CI)						
	ETP	**IPM**	**AK**	**GEN**	**TOB**	**SXT**	**TGC**
Hospitalized							
I	0.81 (0.74–0.89)*	0.77 (0.70–0.84) **			0.83 (0.76–0.90) *		0.56 (0.49–0.63) ***
ND[a]			0.58 (0.48–0.70) **	0.63 (0.55–0.74) **	0.59 (0.50–0.68) ***		1.55 (1.33–1.80) **
Gender							
M	1.31 (1.20–1.44) **	1.24 (1.13–1.36) *					
Quarter[b]							
C	0.67 (0.60–0.75) ***	0.69 (0.61–0.77) **	0.50 (0.44–0.56) ***			1.70 (1.53–1.88) ***	1.40 (1.20–1.63) *
D	0.65 (0.58–0.74) ***	0.64 (0.56–0.72) ***	0.56 (0.49–0.64) ***			1.63 (1.45–1.82) ***	
Infection site							
Blood	2.15 (1.50–3.06) *	2.30 (1.59–3.33) *				2.73 (1.93–3.87) **	
CSF	3.01 (1.86–4.85) *	4.43 (2.75–7.13) **				3.65 (2.31–5.77) **	
Drainage & Puncture fluid	2.26 (1.59–3.23) *	2.44 (1.69–3.53) *		1.88 (1.39–2.54) *			
Others	2.97 (1.92–4.59) *	2.85 (1.83–4.45) *					
Respiratory secretion					1.87 (1.40–2.48) *	2.68 (1.91–3.76) **	
Stool	56.28 (18.57–170.56) ***				14.59 (6.91–30.81) ***		
Tractus genitalis secretion						3.96 (2.43–6.44) **	
Urine	2.14 (1.48–3.09) *	2.12 (1.45–3.11) *		2.13 (1.56–2.91) *	2.46 (1.81–3.36) **	4.30 (3.01–6.14) ***	
Wound secretion	2.54 (1.73–3.72) *	2.83 (1.91–4.21) **		4.46 (3.21–6.19) ***	4.71 (3.39–6.53) ***	3.97 (2.74–5.75) ***	
Department							
Infectious diseases	0.38 (0.28–0.52) **	0.39 (0.28–0.53) **	0.51 (0.37–0.71) *	0.42 (0.32–0.56) **	0.55 (0.41–0.72) *		
ITC & WM[c]	0.53 (0.44–0.64) ***	0.44 (0.36–0.53) ***	0.60 (0.50–0.73) *	1.51 (1.28–1.77) *	2.26 (1.90–2.70) ***	2.04 (1.73–2.40) ***	
Medicine	0.27 (0.24–0.31) ***	0.26 (0.22–0.29) ***	0.33 (0.28–0.38) ***	0.48 (0.43–0.54) ***	0.48 (0.43–0.53) ***	0.74 (0.66–0.82) **	
Obstetrics & Gynecology	0.07 (0.02–0.19) **	0.07 (0.03–0.20) *		0.38 (0.24–0.60) *	0.23 (0.14–0.37) **		
Oncology	0.09 (0.05–0.16) ***	0.09 (0.05–0.16) ***	0.04 (0.02–0.12) **	0.22 (0.15–0.31) ***	0.23 (0.16–0.34) ***		
Outpatient	0.30 (0.24–0.36) ***	0.30 (0.25–0.37) ***	0.36 (0.29–0.45) ***	0.47 (0.40–0.55) ***	0.43 (0.36–0.51) ***		
Pediatrics	0.41 (0.34–0.50) ***	0.28 (0.23–0.34) ***	0.04 (0.03–0.07) ***	0.29 (0.24–0.34) ***	0.36 (0.30–0.43) ***		0.46 (0.36–0.59) **
Rehabilitation	0.57 (0.47–0.70) **	0.59 (0.49–0.73) *	0.61 (0.49–0.76) *				
Surgery	0.51 (0.45–0.57) ***	0.51 (0.46–0.57) ***	0.53 (0.47–0.60) ***	0.65 (0.59–0.72) ***	0.69 (0.62–0.76) ***		

Notes: [a]ND: data not available, [b]Quarter: Q3: Jul–Sep, Q4: Oct–Dec, [c]ITC & WM: Integrated traditional Chinese & Western Medicine. *0.01 ≤ P-value < 0.05, **0.001 ≤ P-value < 0.01, ***P-value < 0.001. Age was regarded as a continuous variable in the logistic regression. Only factors showed significance were listed in this table, the intercept of the fitting formula was not shown here.

Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

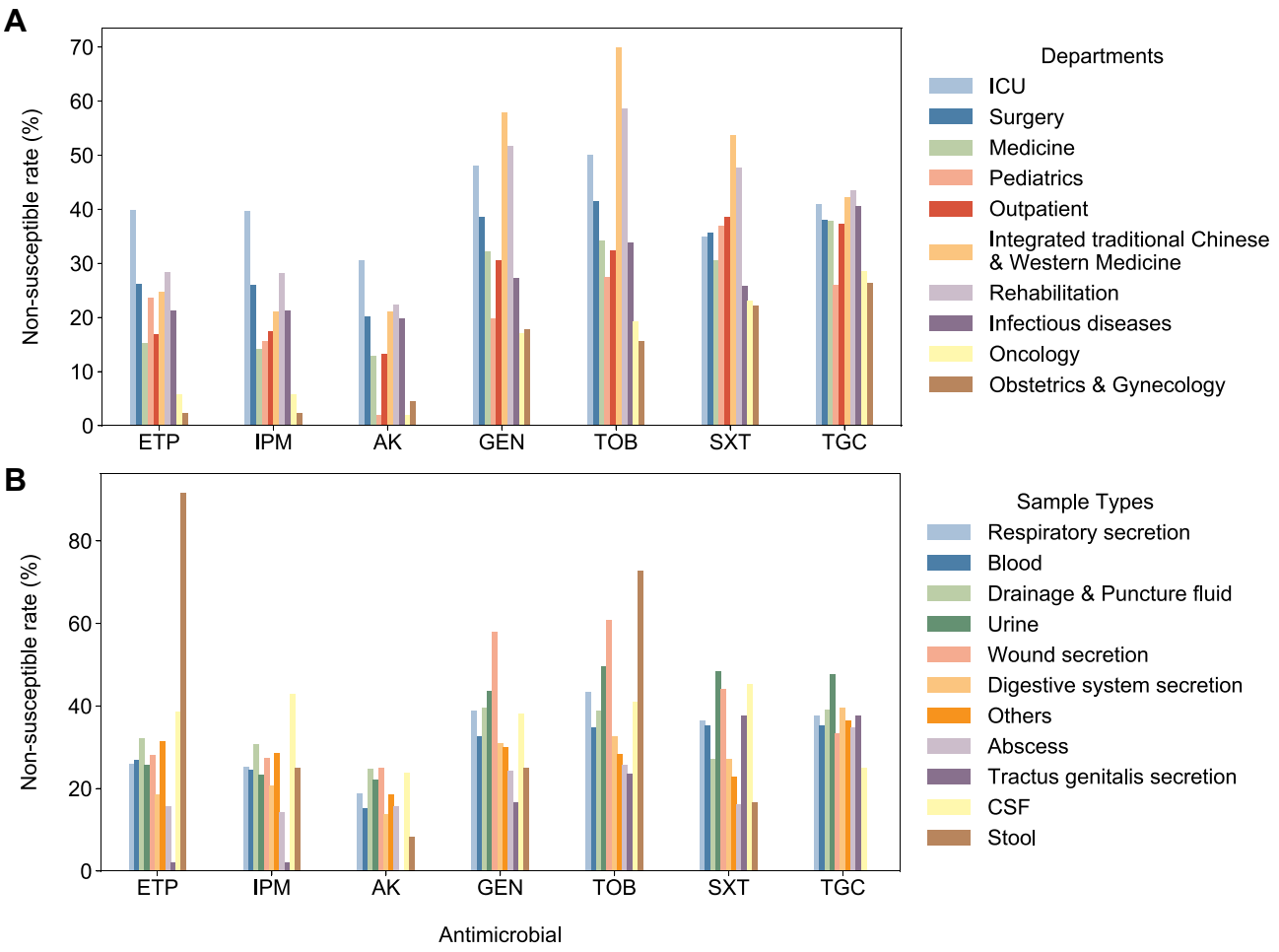

Figure 4 NSRs to seven second-choice antimicrobials in different departments and sample types. The Non-susceptible rates to seven second-choice antimicrobials of *K. pneumoniae* strains recovered from different departments (**A**) and different sample types (**B**) are presented in this figure.
Abbreviations: ETP, ertapenem; IPM, imipenem; AK, amikacin; GEN, gentamicin; TOB, tobramycin; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

NSRs to second-choice antimicrobials were always lower in the oncology ward and obstetrics and gynecology ward (Figure 4A).

As for different sample types, the NSRs to two carbapenems were higher in CSF (38.5% and 42.9%, respectively) and lower in genital secretion (2.1%), and three aminoglycosides (AK, GEN, and TOB) had higher NSRs in the wound secretion (24.8%, 58.0%, and 60.7%, respectively) (Figure 4B). The NSRs of KP from stool varied considerably, probably due to the small sample size.

Temporal Distribution of Non-Susceptibility in KP

We further analyzed the dynamics of NSRs with seasons for seven selected drugs. Interestingly, we found seasonal fluctuations in the non-susceptibility to four second-choice antimicrobials during 2015–2017, which constituted the majority (76.3%) of samples. During this period, ETP, IPM, and AK had higher NSRs in the first and second quarters of each year. However, the volatility of SXT NSR was the opposite of these three drugs, with the rate higher in the third and fourth quarters (Figure 5).

Discussion

Multidrug-resistant *Klebsiella pneumoniae* is a common problem exposed in various countries in the world. Some studies have found that plasmids carrying carbapenem resistant gene often carry other drug-resistant genes as well, and can be widely spread in KP population, leading to the increase of MDR CRKP.[24,25] This phenomenon is also reflected in Chinese data. According to the antimicrobial resistance report from CHINET in 2018, carbapenem-resistant *Klebsiella pneumoniae* were highly resistant to most commonly used antimicrobial agents.[26] Consistent with the previous reports, in this study we found that

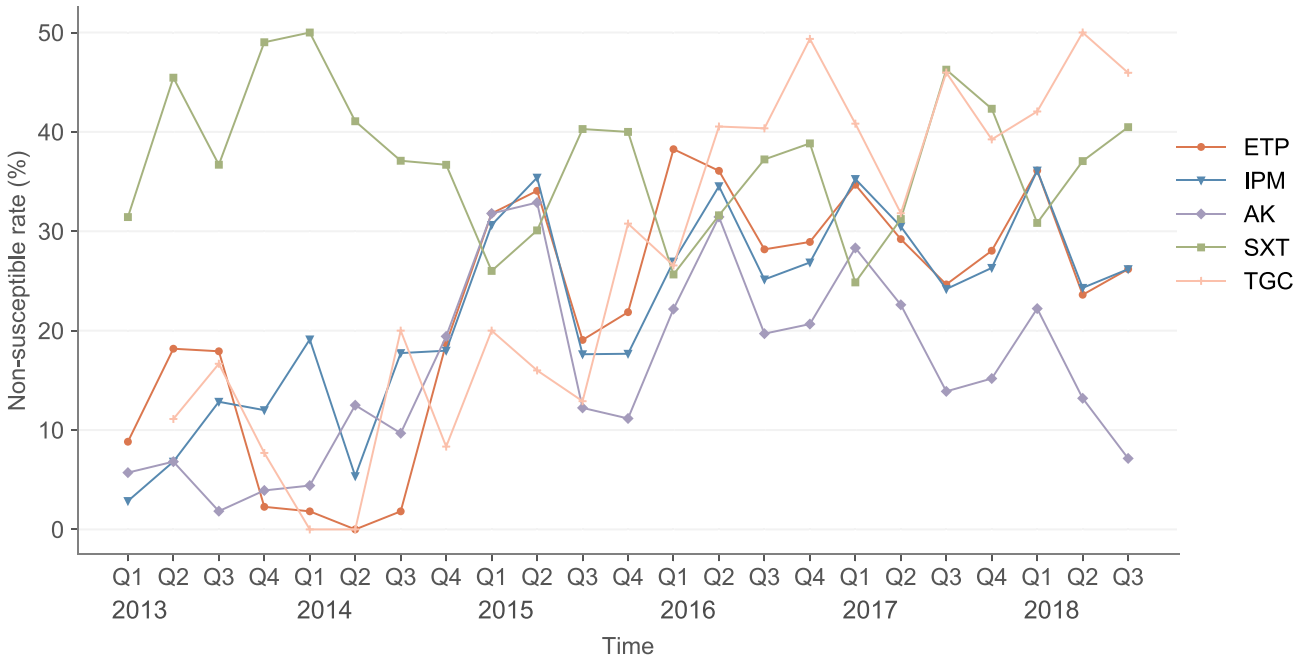

Figure 5 Seasonality of non-susceptibility to five second-choice antimicrobials which showed significance in univariable and multivariable test. The non-susceptible rates to five second-choice antimicrobials between 2013 and 2018, ETP, IPM, AK, SXT presented fluctuations on a yearly basis, and the seasonality of SXT non-susceptibility was opposite to that of ETP, IPM, and AK. Different antimicrobials were presented in different colors and line styles. Q1: Jan–Mar, Q2: Apr–Jun, Q3: Jul–Sep, Q4: Oct–Dec.

isolates non-susceptible to carbapenems and AK were associated with stronger multidrug-resistance (with over 98% isolates being non-susceptible to >5 antimicrobial categories and over 30% non-susceptible to >10 categories) than other isolates. In addition, second-choice antimicrobials were frequently used in the treatment of MDR KP, which may also promote the NSR of MDR KP to second-choice antimicrobials.

According to the national drug resistance report of *Klebsiella pneumoniae* from China Antimicrobial Resistance Surveillance System (CARSS) (http://www.carss.cn/) in 2018, CRKP had a higher rate in neonatal and ICU populations (15.3% and 21.9%, respectively) as well as in CSF, and a lower rate in wound pus.[20] However, in this study, KP isolated from genital secretions had the lowest NSR towards carbapenem, followed by samples of various abscesses, and the proportion of CRKP in the neonatal population was not significantly higher than that of other groups. The preference of CRKP for ICU and CSF samples was consistent with the national data. In addition, the CRKP detection rate from the 2018 national data (10.1% nationwide, 11.4% in Hunan) was lower than the carbapenem resistance rate in this study (ETP 28.2%, IPM 27.2%). The discrepancy between our discovery and the nationwide surveillance data could be caused by several reasons. First, the nationwide surveillance sampled from numerous hospitals in the country, including both primary and secondary hospitals, while our study focuses on samples from one large tertiary hospital in China. Most patients admitted to our hospital are critically ill patients after a referral from primary and secondary hospitals where these patients have already undergone medication. This may represent a sampling difference. Second, the varieties in the method of Antimicrobial Susceptibility Test (AST) in the nationwide data could also cause a discrepancy in non-susceptible rates, comparing to our standard MIC test system. In addition, we only rechecked the TGC sensitivity from a few sterile parts by manual broth dilution method, which may be the main reason for the high TGC drug resistance. These differences in resistance characteristics indicate the need to analyze regional KP drug resistance.

Department of ITC & WM in the local hospital takes the patients with nerve function defect as its main therapeutic group, which is similar to those patients treated in the rehabilitation department. In the present study, isolates from ICU had the highest NSRs to ETP, IPM, and AK (39.8%, 39.7%, and 30.6%, respectively), while isolates from ITC & WM and rehabilitation had the highest NSRs to GEN, TOB, SXT, and TGC (higher than 51.6%, 58.6%, 47.6%, and 42.1%, respectively). We analyzed the antimicrobial consumption in ICU, ITC & WM, and

Table 5 Consumption of Four Second-Choice Antimicrobials in Hospital from 2013 to 2018

	Antimicrobial Prescription Numbers				Folds to ICU Prescription Numbers[a]			
	IPM	AK	GEN	TGC	IPM	AK	GEN	TGC
ICU	44,307	8171	1784	21,209	1	1	1	1
ITC & WM[b]	1278	4279	262	987	0.029	0.524	0.147	0.047
Rehabilitation	568	1064	571	72	0.013	0.13	0.32	0.003

Notes: [a]Folds to ICU prescription numbers were equal to dividing prescription numbers of target department by prescription numbers of ICU, [b]ITC & WM: Integrated traditional Chinese & Western Medicine.

rehabilitation (Table 5), and discovered that ICU consumed much more carbapenems than other departments of the hospital, this may be one of the reasons for the high resistance to carbapenems in ICU. However, the consumption of AK, GEN, and TGC in these three departments shows no correlations with their NSRs, underlying a more complicated mechanism.

The antimicrobial usage could also influence the resistance characteristics in different sample types. Some antibiotics that can cross the blood-brain barrier during inflammation,[27] IPM tend to reach higher concentrations than other second-choice antimicrobials in CSF. Though this unique mechanism may result in higher NSRs to ETP and IPM in isolates from CSF, drug penetration is also influenced by the nature and extent of the infection, more factors need to be investigated (Figure 4B).

A similar seasonal variation of drug resistance rates was also reported on *Escherichia coli* and *Streptococcus pneumoniae*.[28–30] In addition, some studies have shown that the abundance of drug-resistant genes in the environment varies with the season.[31–34] In our study, seasonal variation characters have also been found. The change of ETP, IPM, and AK in the period from 2015 to 2017 is basically consistent with the previous studies. The NSR reaches its peak in the first half of each year and drops to its trough in the second half of the year. However, the fluctuation pattern of SXT is contrary to the above three drugs, which is opposite to the previous report of *Escherichia coli*.[30]

In this research, 4128 isolates of KP were collected from a tertiary hospital in Hunan, China, covering a period of six years, and 3619 isolates were used in the analysis. The long-term, large sample-size retrospective study provides a fundamental picture of local KP infection. By analyzing the clinical information, we found that the NSRs to the second-choice antimicrobials varied significantly in different departments, sample types, and seasons. A full understanding of the department-temporal characteristics of the antimicrobial resistance provides reference for clinicians in KP prevention and treatment. Our research has several limitations. First, it is impossible to include all the influencing factors in the multivariate analysis so we can get more comprehensive information. This requires more investigation of clinical information in the later study. Second, the information of drug use has not been accurately counted, and antimicrobial consumption in the community was not taken into account with the analysis. All these suggest that we need to establish a more perfect and strict drug use supervision system in clinical practice. In addition, the special-temporal characteristics reported in the current study have regional limitations. In conclusion, our investigation of the NSRs in KP from a typical tertiary hospital revealed that MDR KP prevails and the carbapenem resistance rates of the strains in the ICU department and CSF source are higher. The non-susceptibility of ETP, IPM, and AK showed an opposite trend of seasonal fluctuations to non-susceptibility of SXT from 2015 to 2017. Our data provide a bias for the control and prevention work in the KP clinical treatment. More effective control measures should be taken to reduce the high resistance rates and curb the spread of MDR KP, and our study would be a good representation for the guidance of KP treatment in other clinical centers.

Highlights

- A total of 4128 *Klebsiella pneumoniae* isolates were collected in our study
- The non-susceptible rates to ertapenem, imipenem, and tigecycline increased considerably from 2013 to 2018
- Higher multi-drug resistance rates were observed in second-choice antimicrobials than all drugs of KP isolates
- Higher ETP/IPM/AK NSRs were observed in ICU than in other departments, and ETP/IPM non-susceptible isolates tended to distribute in CSF than in the other sample types

Ethical Approval and Consent to Participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Xiangya Hospital of Central South University (XHCSU), Changsha, China (No. 201,806,861). Written informed consent was obtained from individual or guardian participants.

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Author Contributions

JL, WL and NP conceived and designed the project, YZ, SW participated in sample collection, QL, ZJ, MZ, and JH performed the bacterial culturing and drug sensitivity testing, XC and TL performed the bioinformatic analyses, NP, QL, and XC interpreted the data. All authors participated in discussions, XC and NP wrote the manuscript. 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

The authors declare that they have no conflict of interest.

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