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ORIGINAL RESEARCH

Molecular Analysis and Antimicrobial Resistance Pattern of Tigecycline-Non-Susceptible *K. pneumoniae* Isolated from a Tertiary Care Hospital of East Asia

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Introduction: Tigecycline is one of the last resorts for carbapenem-resistant *K. pneumoniae* (CRKP) infections. Indeed, tigecycline-non-susceptible *K. pneumoniae* (TNSKP) strains are increasingly treated with the use of tigecycline. In this study, we attempted to better understand their epidemiological trends and characteristics. *K. pneumoniae* were collected from 2017 to 2020 at the First Affiliated Hospital of Nanchang University.

Methods: Thirty-four TNSKP strains were selected during the study period, all of which were analyzed using antimicrobial susceptibility testing, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE). PCR and DNA sequencing were performed for the detection of β-lactamase genes and carbapenemase genes, and the mutation analysis of *tet*(*A*), *tet*(*X*), *tet*(*L*), *tet*(*M*), *rpsJ, ramR*, and *oqxR*, which are related to tigecycline resistance. Virulence gene and capsular genotype testing were conducted to identify whether the TNSKP strains were hypervirulent *Klebsiella pneumoniae*.

Results: An epidemiology analysis showed that *Klebsiella pneumoniae* carbapenemase-2 (*KPC-2*) was the predominant carbapenemase in tigecycline non-susceptible carbapenem-resistant *K. pneumoniae* (TNSCRKP) (96.7%), and the dominant clone type was ST11-K14K64 (82.4%). Among them, 55.9% (19/34) of strains were from each department of ICU, particularly EICU and neurosurgery ICU. In order to further understand the molecular mechanisms of the TNSKP, a polymerase chain reaction of the resistant determinants was carried out. The results detected many tigecycline-resistant genes, such as *tet*(*A*) (97.1%), *tet*(*X*) (17.6%), *rpsJ* (97.1%), and *ramR* (8.8%).

Conclusion: As the results of this study reveal, we should take effective measures to control the increase in TNSKP.

Keywords: carbapenem-resistant, tigecycline-non-susceptible, *Klebsiella pneumoniae*

Introduction

Klebsiella pneumoniae belongs to the Enterobacteriaceae family and is an opportunistic pathogen that can transfer to multi-drug resistant strains and can cause various infections, including bacteremia, pneumonia, liver abscess, and urinary tract infection.[1,2] At present, the spread of multi-drug resistant *K. pneumoniae* strains is a worldwide problem, and especially the carbapenem-resistant *K. pneumoniae* (CRKP); their infections are usually associated with high mortality.[3]

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Tigecycline is a novel type of broad-spectrum glycyltetracycline antibiotics administered after minocycline development. It has a very wide range of activities against most Gram-positive bacteria, Gram-negative bacteria, and anaerobic bacteria, especially for multi-drug resistant strains.[4,5] Tigecycline is one of the few available options for the treatment of carbapenem-resistant bacterial infections.[6] Thus, tigecycline is considered to be the last line of defense for the treatment of this kind of bacterial infection. However, tigecycline-insensitive strains of *K. pneumoniae* are increasingly being reported, with some reports showing emergence in Taiwan,[7] Northern China,[8] Korea,[9] Austria,[10] etc. According to the China Antibiotic Resistance Surveillance System for antibiotic resistance, the tigecycline-non-susceptible *K. pneumoniae* (TNSKP) was up to 8.5% in 2019 (http://www.carss.cn/); thus, we should pay more attention to them in clinical infections.

In order to explore whether tigecycline-non-susceptible *K. pneumoniae* are also present in the First Affiliated Hospital of Nanchang University, and then to better understand their epidemiological trends and characteristics, we analyzed the *K. pneumoniae* in the hospital from 2017 to 2020, and screened for tigecycline-non-susceptible *K. pneumoniae* in order to perform further analysis. We aimed to provide more advice that may be useful for controlling the infection rates in the hospital.

Materials and Methods

Bacterial Strains and Data Collection

In this study, we collected 5729 *K. pneumoniae* non-duplicate strains from the First Affiliated Hospital of Nanchang University from 2017 to 2020. Cases of infection with tigecycline-non-susceptible *K. pneumoniae* strains were detected at different wards, and strains were isolated from different types of clinical specimens. Detailed clinical data were obtained from electronic medical records and informed consent was obtained from all patients included in the study. A concise flow chart is shown in Figure 1. All clinical isolates were identified using MALDI-TOF MS (Bruker, Sancordon Inc., Bremen, Germany). *K. pneumoniae* ATCC 13883 was used as a quality control strain for antimicrobial susceptibility testing. The *Salmonella enterica* serotype Braenderup strain (H9812) was used as a reference standard of pulsed-field gel electrophoresis (PFGE).

Antimicrobial Susceptibility Testing

The MICs of amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftazidime, ceftriaxone, ertapenem, imipenem, meropenem, amikacin, ciprofloxacin, and tigecycline were determined using the broth microdilution methodology. Except for tigecycline, the sensitivity of other antibacterial agents was determined with reference to the CLSI M100 guidelines (2020). The interpretive criteria for tigecycline was according to US Food and Drug Administration (FDA) standards.

Molecular Detection of Resistance Genes

We carried out polymerase chain reaction detection of β-lactamase genes, including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{LAP}, and carbapenemase genes, including bla_{VIM}, bla_{KPC}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48}. Potential tigecycline-resistance determinants containing *tet*(*A*), *tet*(*X*), *tet*(*L*), *tet*(*M*), *rpsJ, ramR*, and *oqxR*[11–13] were also amplified in the tigecycline-non-susceptible strains and sequenced in positive strains; then, *rpsJ, ramR*, and *oqxR* were compared with the wild-type reference strain *K. pneumoniae* MGH78578 (GenBank accession number CP000647) as previously described.[11] The primers used to amplify the coding sequence of each gene are listed in Table S1.

Molecular Detection of Virulence Genes and Capsular Genotype

It will be more difficult to treat clinically strains that express both virulence genes and are tigecycline non-susceptible. Thus, we detected virulence genes, including *rmpA, aero, uge, ironB, mrkD*, and *kpn*, and we used the *wzi* capsule gene to determine the capsule genotype (K-type) in the tigecycline non-susceptible strains.

Multilocus Sequence Typing (MLST) and Pulsed-Field Gel Electrophoresis (PFGE) for Tigecycline-Non-Susceptible Strains

According to the protocol described on the MLST *Klebsiella pneumoniae* website (https://bigsdb.pasteur.fr/klebsiella/primers_used.html), all tigecycline non-susceptible strains were tested with seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB*, and *tonB*[14]). The results of MLST were analyzed using the international *Klebsiella pneumoniae* MLST database established at the Pasteur Institute in France in 2005.[14] Allele sequence and sequence type (ST) are verified on https://bigsdb.pasteur.fr/klebsiella/. PFGE was performed as previously described,[15] the screened

tigecycline non-susceptible *K. pneumoniae* strains were picked from fresh pure colonies into the buffer, and each of them was placed into small plastic containers. The DNA was digested by the restriction enzyme XbaI (TakaRa, Japan) 10U for 4 h at 37 °C. Electrophoresis was conducted using 0.5 × TBE buffer at 14 °C for 18 h, and pulse time was 2–64 s. *Salmonella enterica* serotype Braenderup strain (H9812) was used as a classical molecular weight standard. We finally used Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium) to analyze homogeneity. Clinical isolates with more than 80% homology were defined as the same PFGE cluster.

Results

Clinical Characteristics of Patients Infected with TNSKP

In this study, a total of 5729 *K. pneumoniae* strains were collected, there were 1282, 1397, 1564, and 1486 strains in 2017–2020, respectively, and of them 1851 (32.3%, 1851/5729) strains were CRKP. The *K. pneumoniae* strains showed high susceptibility to TGC, but we also found 34 patients infected with TNSKP; the demographic and clinical characteristics of the 34 strains are shown in Figure 2. Among the 34 isolates, the number of specimens from sputum was the largest, accounting for 64.7% (22/34), and the number of specimens from urine 17.6% (6/34) ranked second, followed by blood 5.9% (2/34), ascites 5.9% (2/34), catheter tip 2.9% (1/34), and wound fluid 2.9% (1/34). The TNSKP strain was not found in 2017, but the TNSKP rates rose during the following years, by 0.1% (2/1397), 0.6% (9/1564), and 1.5% (23/1486) in 2018–2020, respectively. Analysis of the departmental distribution of the patients revealed that 55.9% (19/34) of the patients with non-susceptible strains were from the intensive care unit (ICU), particularly from EICU 14.7% (5/34) and neurosurgery ICU 14.7% (5/34). In 2018, both of the TNSKP strains were from neurosurgery ICU. In addition to this, common departments also had more TNSKP strains, such as the rehabilitation department 11.8% (4/34) and neurosurgery 11.8% (4/34).

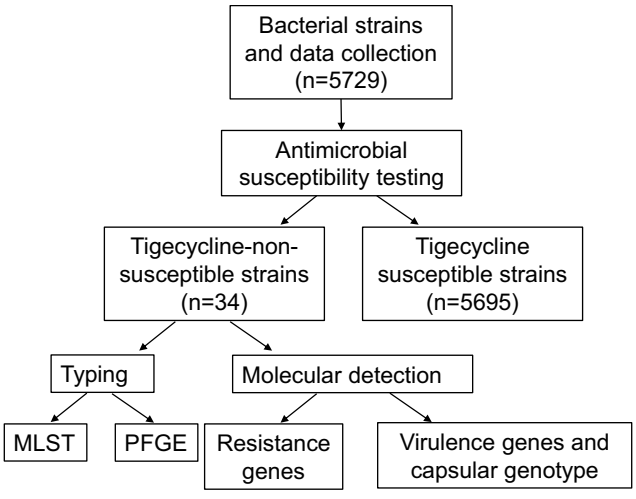

Figure 1 A concise flow chart about this study.

Antibiograms

The antibiotic resistance results of TNSKP strains to 10 antibiotics are shown in Table 1. We can see that 34 strains showed low susceptibility to all of the 10 antibiotics, including piperacillin/tazobactam (11.8%), ceftazidime (2.9%), ceftriaxone (2.9%), ertapenem (11.8%), imipenem (14.7%), meropenem (14.7%), amikacin (17.6%), and ciprofloxacin (5.9%); acid susceptibility rates to amoxicillin/clavulanic were zero. Among the 34 tested TNSKP strains, 30 (30/34, 88.2%) were also CRKP strains. According to the interpretive criteria for tigecycline (≤2 μg/mL, susceptible; 4 μg/mL, intermediate; ≥8 μg/mL, resistant), there were three (3/34, 8.8%) intermediate strains; the others were resistant.

Analysis of Resistance Determinants in TNSKP Strains

To investigate the resistance mechanism of TNSKP, potential tigecycline resistance determinants were identified in 34 strains by PCR and sequencing. The genes *tet*(*A*) and *rpsJ* were detected in most of the TNSKP strains except for strain No. 44; *tet*(*X*) was found in six strains (17.6%), whereas *tet*(*L*) and *tet*(*M*) were not detected in all of the TNSKP strains. Three strains (8.8%) were detected with *ramR* expression; similarly, *oqxR* was found in three (8.8%) isolates. One strain with S29L substitutions in *ramR* and another two strains with a termination codon appeared in advance. Meanwhile, mutations in *rpsJ* and *oqxR* were not identified in this study (Table 2).

A total of five carbapenemases were detected in this study; 96.7% (29/30) of Tigecycline non-susceptible carbapenem-resistant *K. pneumoniae* (TNSCRKP) were bla_{kpc} positive (Table 2); and bla_{VIM}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48} were not detected in any of the strains. Among the 34 strains, it was detected that 29 strains carried bla_{SHV-12}, 2 strains carried both $bla_{SHV-158}$ and $bla_{SHV-187}$, 30 strains carried bla_{TEM-1}, and 1 strain carried $bla_{TEM-235}$. A total of 31 strains carried $bla_{CTX-M-65}$, 1 strain carried

both $bla_{CTX\text{-}M\text{-}3}$ and $bla_{CTX\text{-}M\text{-}14}$, and 31 strains carried $bla_{LAP\text{-}2}$. For strain No. 11 only, no carbapenemases were detected while in CRKP.

Virulence Analysis

All strains in the study carried the *mrkD* gene, followed by *kpn* (97.1%, 33/34) and *uge* (91.2%, 31/34). Only one strain carried the *rmpA* gene, while *aero* and *iroN* were not detected among any of the strains. Two or more genes coexisted in these isolates; the combination of *uge, mrkD*, and *kpn* was the most common prototype observed, and there were 29 isolates of this type. Three isolates were a combination of *mrkD* and *kpn*, while one combination of *mrkD* and *uge* was found. There was only one strain, No. 25, that was a combination of *rmpA, uge, mrkD*, and *kpn* (Table 2).

Isolates Typing and PFGE

In total, five different multilocus sequence types were detected; ST11 was the most prevalent type (85.3%, 29/34), followed by ST37 (5.9%, 2/34), and only one strain each of ST3627, ST2593, and ST2601 (Figure 2).

The *wzi* capsular gene was sequenced to assign a capsular type (K-type) to each isolate. In this study, two K-types were identified. A total of 88.2% (30/34) of the isolates were typed as K14.K64, while 8.8% (3/34) were typed as K15.K17.K50.K51.K52. One strain, No. 63, was not linked to any K-type (Figure 2).

According to the results of PFGE, the similarity of the strains ranged between 60% and 100%. Among them, the 34 strains were divided into six different PFGE clusters (clusters A to F) at an 82% similarity cut-off (Figure 2). Obviously, the dominant strain species was cluster A, which contained a total of 28 strains (82.4%, 28/34), followed by cluster D (5.9%, 2/34); each of the remaining four cluster (B/C/E/F) contained only one strain; 29 ST11 isolates mainly belonged to cluster A.

Discussion

The prevalence of carbapenem-resistant *K. pneumoniae* has been increasing globally, making antimicrobial treatment difficult and leading to higher disease-related mortality.[16,17] In this study, we collected 5729 *K. pneumoniae* strains from our hospital over four years; carbapenem resistance rates in *K. pneumoniae* reached 32.3%, which is higher than the average level in Jiangxi province according to the China Antibiotic Resistance Surveillance System in 2019 (http://www.carss.cn/). This may be related to the large number of severe patients in our hospital, and because of long hospital stays, most of the TNSKP strains were from the intensive care unit. With the increasing administration of tigecycline in clinical settings, tigecycline-non-susceptible strains will become increasingly prominent. The TNSKP rates were below 8.5% at present in our hospital, but we should pay more attention to this issue, in light of the rising trend detected over our four-year study period. Meanwhile, we found that

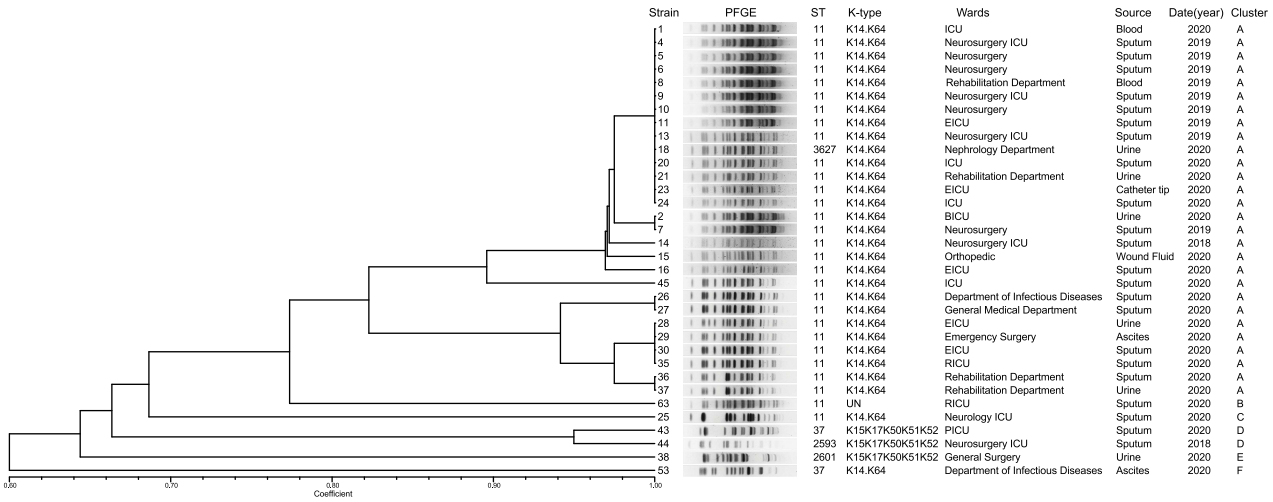

Figure 2 PFGE results for 34 TNSKP strains. The adjacent information shown on the right represents ST, K-type of the sequenced isolates, wards, source, and cluster in turn.

Abbreviations: PFGE, pulsed-field gel electrophoresis; UN, unknown; ICU, intensive care unit; EICU, emergency intensive care unit; BICU, burn intensive care unit; RICU, respiratory intensive care unit; PICU, pediatric intensive care unit.

Table 1 Antibiotic Susceptibilities of TNSKP Strains (n=34)

Strain No	MIC (μg/mL)[a]									
	Amoxicillin/Clavulanic Acid	Piperacillin/ Tazobactam	Ceftazidime	Ceftriaxone	Ertapenem	Imipenem	Meropenem	Amikacin	Ciprofloxacin	Tigecycline
1	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
2	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
4	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
5	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
6	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
7	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
8	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
9	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
10	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
11	≥32/R	16/S	≥16/R	≥32/R	>4/R	≤0.5/S	≤0.5/S	≤8/S	≥4/R	≥8/R
13	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
14	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
15	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
16	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
18	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
20	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
21	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
23	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
24	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
25	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
26	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
27	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
28	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
29	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
30	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
35	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	16/S	≥4/R	≥8/R
36	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
37	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
38	≥32/R	8/S	8/I	≥32/R	≤0.25/S	≤0.5/S	≤0.5/S	≤8/S	≥4/R	4/I
43	≥32/R	≥128/R	≥16/R	≥32/R	≤0.25/S	≤0.5/S	≤0.5/S	≤8/S	≤0.25/S	≥8/R
44	≥32/R	8/S	≤2/S	≤1/S	≤0.25/S	≤0.5/S	≤0.5/S	≤8/S	≤0.25/S	4/I
45	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R
53	≥32/R	8/S	≥16/R	≥32/R	≤0.25/S	≤0.5/S	≤0.5/S	≤8/S	≥4/R	4/I
63	≥32/R	≥128/R	≥16/R	≥32/R	>4/R	>8/R	>8/R	≥64/R	≥4/R	≥8/R

Note: [a]MICs of amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, ceftriaxone, ertapenem, imipenem, meropenem, amikacin, ciprofloxacin, and tigecycline were determined using the broth microdilution methodology.
Abbreviations: S, susceptible; I, intermediate; R, resistant.

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Table 2 Resistance Determinants and Virulence Genotype Were Examined in the Present Study

Strain No.	Resistance Determinants (Mutations Occurring in the Protein Sequence)												Virulence Genotype					
	tet(A)	tet(X)	tet(L)	tet(M)	rpsJ	oqxR	ramR	Carbapenem	β-Lactamase Resistance				rmpA	aero	uge	iroN	mrkD	kpn
1	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
2	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
4	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
5	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
6	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
7	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
8	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
9	+	–	–	–	+	–	–	bla_{KPC-2}	$bla_{SHV-158}$	–	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
10	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
11	+	–	–	–	+	–	–	–	$bla_{SHV-158}$	–	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
13	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
14	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
15	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
16	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
18	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
20	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
21	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
23	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
24	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
25	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	+	–	+	–	+	+
26	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
27	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
28	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
29	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
30	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
35	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
36	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
37	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
38	+	–	–	–	+	+	–	–	$bla_{SHV-187}$	bla_{TEM-1}	$bla_{CTX-M-3}$	–	–	–	–	–	+	+
43	+	–	–	–	+	+	+(Q160stop)	–	$bla_{SHV-187}$	$bla_{TEM-235}$	$bla_{CTX-M-14}$	–	–	–	–	–	+	+
44	–	–	–	–	–	+	+(Q6stop)	–	–	–	–	–	–	–	+	–	+	–
45	+	+	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+
53	+	–	–	–	+	–	+(S29L)	–	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	–	–	+	+
63	+	–	–	–	+	–	–	bla_{KPC-2}	bla_{SHV-12}	bla_{TEM-1}	$bla_{CTX-M-65}$	bla_{LAP-2}	–	–	+	–	+	+

Notes: +, presence of PCR product and no change in the nucleotide/amino acid sequences; –, absence of PCR product.

most of the TNSKP strains were also resistant to carbapenems, which is consistent with other studies;[18] hence, the antimicrobial susceptibility testing confirmed low susceptibility to all of the 10 antibiotics. According to the antibiotic resistance results of tigecycline, there were three intermediate strains (strains No. 38, 44, and 53), and among them two strains with mutations in *ramR* (strains No. 44 and 53), and two strains were detected with *oqxR* expression (strains No. 38 and 44). To our knowledge, the AcrAB-TolC efflux pump associated with the inactivation of the repressor *ramR* caused by mutations is a major cause of tigecycline resistance.[19] In addition to this, the OqxAB efflux pump is downregulated by the repressor *oqxR*, which was also associated with tigecycline resistance.[20] Thus, our results are in accordance with the above. Except for efflux-mediated resistance mechanisms, there were other resistance mechanisms such as Tet proteins, including mutated *tet*(*A*), *tet*(*L*), *tet*(*X*), and t*et*(*M*), and the *rpsJ* gene, which was associated with an alteration in the tigecycline target site of the ribosomal protein S10.[12] In this study, 31/34 (91.2%) strains were tigecycline-resistant, and all of them were detected as *tet*(*A*) and *rpsJ* gene-positive, although mutations were not identified. We suspect that they may be the main cause of the resistance mechanisms among these strains, but further studies are needed.

At present, common carbapenemase genes, including bla_{VIM}, bla_{KPC}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48}, as well as *KPC*-type enzymes, and in particular *KPC-2*, are the most common carbapenemases in China.[21,22] As our data show, 96.7% (29/30) of Tigecycline non-susceptible carbapenem-resistant *K. pneumoniae* were detected with bla_{KPC-2}, which is consistent with previous reports. Only for strain No. 11 was no carbapenemase detected in this study. Strain No. 11 was ertapenem-resistant but imipenem- and meropenem-susceptible, and showed $bla_{SHV-158}$, $bla_{CTX-M-65}$, and bla_{LAP-2} beta-lactamases resistance; previous studies showed that the mechanisms of resistance in this form would be predominantly mediated by non-carbapenemase mechanisms, including deficiencies in the expression of porins Omp35 and Omp36[23] or the production of particular beta-lactamases.[24]

Among the TNSKP isolates, we detected the virulence-related genes *mrkD, kpn*, and *uge* with a positive rate more than 90%; these genes are associated with adhesins and lipopolysaccharides of *K. pneumoniae*.[25] Only one strain was positive for the *rmpA* gene, which is related to the high viscosity of *K. pneumoniae*;[26] *aero* and *iroN*, which are associated with iron carriers, were not detected in this study. In addition, in this experiment we determined capsular types by sequencing the *wzi* gene, and they were mainly the K14.K64 type, whereas previous studies reported the K47 type as dominant.[27] In this study, we did not find virulent capsular types, such as K1, K2, K5, K16, K20, K54, and K57, and thus these TNSKP isolates carried high resistance but low virulence.

Molecular typing of our strains showed the dominant sequence type of the TNSKP isolates to be ST11, which is in accordance with reports that ST11 is the most common type of CRKP associated with the spread of $bla_{\text{KPC-2}}$ in China.[28,29] Other STs, such as ST37, ST3627, ST2593, and ST2601, were also detected in 34 TNSKP strains, which was different from previous reports in Henan.[30] Our study is the first to report of ST3627, ST2593, and ST2601 *K. pneumoniae* with tigecycline-non-susceptible caused by clinical infection. PFGE results showed that the most prevalent clone type ST11-K14K64 (28/34, 82.4%) belongs to cluster A. In cluster A, the ST11-K14K64 isolates were mainly found in the ICU, neurosurgery ICU, and EICU, which indicated that there may have been a prevalence of ST11-K14K64 TNSKP in the ICU of our hospital from 2018 to 2020.

Conclusion

The rate of carbapenem-resistant *K. pneumoniae* in our hospital was higher than the average level in Jiangxi province, the tigecycline-non-susceptible *K. pneumoniae* was often found in carbapenem-resistant strains, and they were usually isolated from the intensive care unit. The ST11-K14K64 clone type carrying the bla_{KPC-2} was primarily detected in this study, and the *tet*(*A*) and *rpsJ* genes may contribute to the tigecycline-non-susceptible phenotype in the 34 TNSKP strains, which is worthy of further research. In all, we should take effective measures, such as active surveillance and effective segregation to prevent CRKP, thus reducing the emergence of TNSKP.

Abbreviations

CRKP, carbapenem-resistant *K. pneumoniae*; TNSKP, tigecycline-non-susceptible *K. pneumoniae*; TNSCRKP, tigecycline non-susceptible carbapenem-resistant *K. pneumoniae*; PFGE, pulsed-field gel electrophoresis; CLSI, the Clinical and Laboratory Standards Institute; FDA, Food and Drug Administration; MLST, multilocus

sequence typing; ST, sequence type; ICU, intensive care unit.

Data Sharing Statement

The data used and/or analyzed in this study are available from the Lingbing Zeng (E-mail: lingbing_zeng@163.com) and Junming Li (E-mail: lisir361@163.com) on reasonable request.

Ethics Approval

This study was approved by the Ethics Committee of the first affiliated hospital of Nanchang University (Code: 2014056). All participants in this study provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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