Original Article

Molecular typing and drug resistance analysis of carbapenem-resistant *Klebsiella pneumoniae* from paediatric patients in China

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Abstract
Introduction: There are few studies on paediatric carbapenem-resistant *Klebsiella pneumoniae* (CRKP) in China. The present study investigated the molecular epidemiological and drug resistance characteristics of CRKP from paediatric patients in China to provide a reference for the prevention and control of CRKP infection.
Methodology: In total, 20 nonrepetitive clinical CRKP isolates were collected between February 2019 and February 2020 in a tertiary hospital in China. Strain identification and drug susceptibility testing were carried out using the VITEK® 2 Compact Bacterial Identification and Monitoring System. Sequence typing, phylogenetic relationships, and antibiotic resistance-associated genes were analysed by whole genome sequencing (WGS).
Results: sequence typing (MLST) and Core genome multilocus sequence typing (cgMLST) analysis revealed the most frequently represented were ST2407-CT3536 (30%), ST76-CT5893 (25%), and ST309-CT7864 (25%). All 20 CRKP isolates were divided into three clusters. All isolates were highly resistant to a variety of β-lactams and were highly susceptible to quinolones, aminoglycosides, and sulphonamides. All isolates mainly carried the carbapenem resistance genes bla_{NDM-1} and bla_{KPC-2}, among which 10 isolates carried both bla_{NDM-1} and bla_{KPC-2} simultaneously.
Conclusions: Sequence typing, phylogenetic relationships, and antibiotic resistance genes can be determined using WGS technology. This can guide CRKP infection control and clinical treatment for paediatric patients.

Key words: Whole genome sequencing; paediatrics; carbapenem-resistant *Klebsiella pneumoniae*; molecular typing; drug resistance.

Introduction

Since the emergence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates in 2001 from North Carolina, United States, the isolation rate of this strain has continued to rise worldwide [1]. CRKP is largely associated with hospital-acquired infections, especially in children. The China Antimicrobial Surveillance Network (CHINET) showed that CRKP in children increased from 2.2% in 2005 to 25.4% in 2017 and then showed a gradually decreasing trend year by year until 2020 [2, 3]. Due to its high pathogenicity and limited antibiotic treatment, CRKP infection has a significantly higher mortality rate than infection with carbapenem-susceptible *Klebsiella pneumoniae* (CSKP) [4]. In 2017, the World Health Organization recognized CRKP as a serious threat to human health

and listed it as one of the priority pathogens for which new antibiotics urgently need to be developed [5].

Although there are several mechanisms in CRKP, carbapenemase production is the most frequent. Many carbapenemase-encoding genes (bla_{KPC}, bla_{NDM}, bla_{OXA-48}, or bla_{VIM}) are plasmid-borne and can be horizontally transferred through plasmids, prompting the rapid development of CSKP into CRKP [6]. CRKP subsequently leads to a wider resistance spectrum and a higher resistance rate in the population, and the prevention and control of nosocomial infection becomes more difficult. According to the report, silent colonization of patients may result in nosocomial infection outbreaks in CRKP [7]. In view of the easy diffusion and strong concealment of CRKP, accurately identifying the source and route of CRKP transmission

in hospitals is an important tool for nosocomial infection prevention and control.

With the promotion, progress, and decreased costs of sequencing technology, whole genome sequencing (WGS) is increasingly used in nosocomial infection control. It can provide information on pathogen identification, epidemiological analysis, and drug resistance genes [8]. Due to the immaturity of paediatric patients, the disease progresses rapidly after CRKP infection. Therefore, a large amount of basic data analysis is required to prevent and control CRKP. At present, there are few studies on paediatric CRKP in China. Thus, this study collected CRKP isolates from a paediatric department of a tertiary hospital in Guizhou, China, and used WGS technology to deeply understand the molecular epidemiology and drug resistance mechanism of paediatric CRKP. Thus, this study provides data support for clinical treatment and infection control measures of paediatric CRKP.

Methodology

Bacterial isolates

All 20 CRKP isolates from paediatric patients were obtained from a tertiary-care university hospital in Guizhou, China, from February 2019 to February 2020. Duplicate strains in the same part of the same patient were excluded.

Antimicrobial susceptibility testing

The identification and drug sensitivity testing of the 20 CRKP isolates were carried out by the VITEK® 2 Compact Bacterial Identification and Monitoring System. The judgement standard was in accordance with the 2022 standard of the American Clinical and Laboratory Standards Institute (CLSI) [9]. *K. pneumoniae* resistant to any carbapenems was identified as CRKP. The control strain was *Escherichia coli* ATCC 25922.

Extraction of bacterial genomic DNA

A single colony of CRKP was picked and added to Luria-Bertani medium for shaking overnight at 37 °C and 220 r/min. Then, 4 mL of bacterial solution was collected, and genomic DNA was extracted according to the manufacturer's instructions of the TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China). The recovered product was verified by agarose gel electrophoresis, and its concentration was determined.

Whole genome sequencing

Second-generation DNA library construction and sequencing were completed by Shanghai Sangon Biological Company. WGS of 20 CRKP isolates was performed on the Illumina HiSeq 2500 sequencing platform.

Sequence typing

Core genome multilocus sequence typing (cgMLST) is a high-resolution typing method constructed with thousands of core genes as sequence typing markers based on a large number of strains. The multilocus sequence typing (MLST) was identified using the PubMLST database. To further distinguish the isolates, cgMLST was performed with the SeqSphere+ software to assign complex types (CTs).

Phylogenetic tree construction

Taking *K. pneumoniae* MGH78578 as the reference strain, the genomes of 20 CRKP isolates were compared with the reference strain to determine the site differences of core genome single nucleotide polymorphisms (cgSNP). The phylogenetic tree was constructed by IQtree software based on the cgSNP differences between strains [10].

Drug resistance gene analysis

The genome sequences of 20 CRKP isolates were compared with the Comprehensive Antibiotic Resistance Database (CARD) database using BLAST. The annotation results were obtained by combining genes and their corresponding drug resistance function annotation information.

Results

Patients and Isolates

Among the 20 CRKP isolates, 13 strains were from neonates (13/20, 65%), and the rest were from 2- to 5-month-old infants. Among the specimens, 13 strains were isolated from sputum (13/20, 65%); 2 strains were isolated from throat swabs, 2 from blood, and 2 from urine (2/20, 10% each); and 1 strain was isolated from oral secretions (1/20, 5%).

CRKP susceptibility

For β-lactam antibiotics, the resistance rate to ampicillin-sulbactam, cefazolin, ceftazidime, ceftriaxone, imipenem, meropenem and ertapenem was 100%; to aztreonam and cefepime was 90% (18/20); to piperacillin-tazobactam was 80% (16/20); and to cefotetan was 55% (11/20). For aminoglycoside antibiotics, the resistance rate to gentamicin was only 10% (2/20), and was completely sensitive to tobramycin and amikacin. About quinolone antibiotics, the strains were completely sensitive to levofloxacin

and intermediate to ciprofloxacin. The resistance rate to trimethoprim-sulfamethoxazole was 25% (5/20).

Whole genome sequencing

The amount of data obtained from each sample was not less than 1 G, the data quality was Q30 > 90%, and the average sequencing depth was > 150x.

CRKP sequence typing

The 20 CRKP isolates were divided into 5 distinct sequence types (STs), including 6 strains belonging to ST2407 (6/20, 30%), 6 strains to ST76 (6/20, 30%), 5 strains to ST309 (5/20, 25%), 2 strains to ST414 (2/20, 10%), and 1 strain to ST534 (1/20, 5%). Typing by cgMLST revealed 6 distinct CTs, including four new CTs (CT7864, CT7865, CT7866, and CT7868). The 6 distinct CTs, including 6 strains belonging to CT3536 (6/20, 30%), 5 strains to CT7864 (5/20, 25%), 5 strains to CT5893 (5/20, 25%), 2 strains to CT7866 (2/20, 10%), 1 strain to CT7865 and 1 strain to CT7868 (1/20, 5% each), were shown in Figure 1.

CRKP phylogenetic tree

As shown in Figure 1, the 20 CRKP isolates were divided into three clusters. Among them, cluster 1 contained 7 strains, KP-zy10, KP-zy4, KP-zy6, KP-zy7, KP-zy5, KP-zy12 and KP-zy11; cluster 2 contained 7 strains, KP-zy17, KP-zy14, KP-zy1, KP-zy2, KP-zy20, KP-zy16 and KP-zy18; and cluster 3 contained 6 strains, KP-zy19, KP-zy15, KP-zy13, KP-zy8, KP-zy3 and KP-zy9.

Distribution and characteristics of drug resistance genes

By comparing the genome sequences of 20 CRKP isolates with the CARD database, the drug resistance genes carried by all strains were identified. As shown in Figure 1, the CRKP strains mainly carried the carbapenem resistance genes bla_{NDM-1} (15/20, 75%) and bla_{KPC-2} (14/20, 70%); the aminoglycoside resistance genes *aad*A (5/20, 25%), *amr*A (20/20, 100%), *aac(6')-IId-cr* (4/20, 20%), *aac(3')-IIb* (3/20, 15%) and *aac(3')-IIa* (3/20, 15%); the β-lactam resistance genes bla_{TEM-1} (19/20, 95%), $bla_{CTX-M-15}$ (16/20, 80%), $bla_{CTX-M-65}$ (19/20, 95%), bla_{SHV-1} (20/20, 100%), bla_{SHV-4} (6/20, 30%), bla_{DHA-1} (8/20, 40%) and bla_{LEN-12} (20/20, 100%); the fluoroquinolone resistance genes *qnr*B4 (8/20, 40%), *qnr*S8 (8/20, 40%), *gyr*B (19/20, 95%), *par*C (20/20, 100%), and *par*E (20/20, 100%); the sulfonamide resistance genes *sul*1 (8/20, 40%) and *sul*2 (8/20, 40%); the fosfomycin resistance gene *fos*A (20/20, 100%); the trimethoprim resistance genes *dfr*A3 (20/20, 100%) and *dfr*A22 (20/20, 100%); the tetracycline resistance gene *tet* (D) (20/20, 100%); and the macrolide resistance genes *mph* (A) (3/20, 15%), *mac* (B) (20/20, 100%) and *msr* (E) (3/20, 15%).

Discussion

CRKP is one of the most harmful nosocomial pathogens, accounting for more than 70% of carbapenem-resistant *Enterobacteriaceae* (CRE) in Chinese hospitals [11, 12]. The burden of CRKP increased 6.16 times during 2007–2015 in terms of the number of infections and deaths in a European study of

Figure 1. The phylogenetic tree and the distribution of antibiotic resistance genes of the 20 CRKP strains.
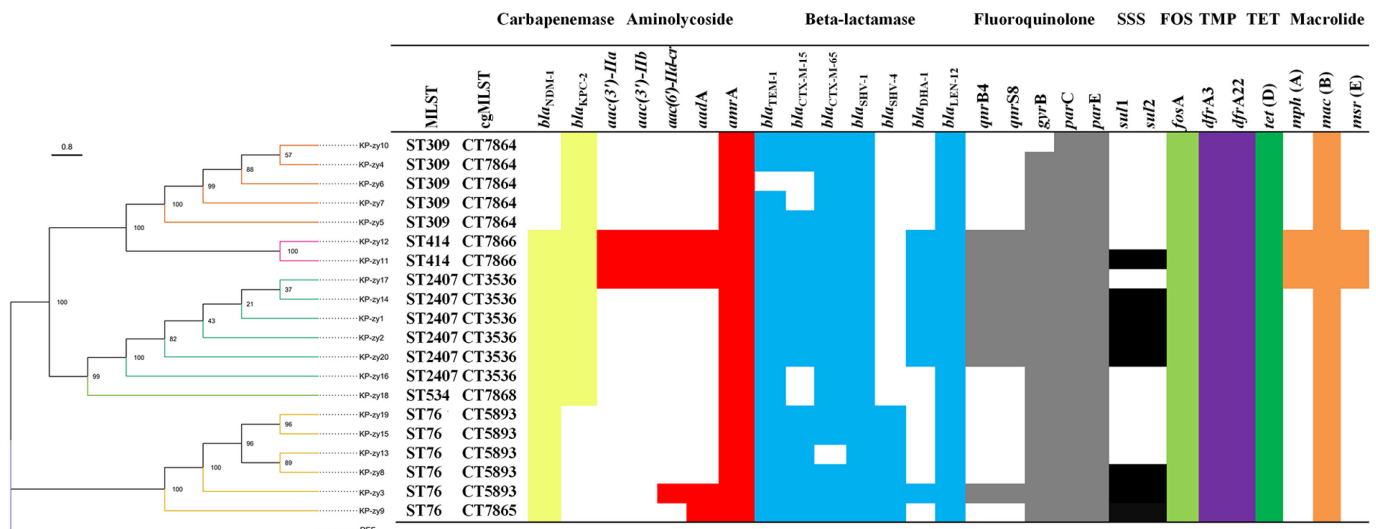
SSS: sulfonamide; FOS: fosfomycin; TMP: trimethoprim; TET: tetracycline.

attributable deaths caused by infections with antibiotic-resistant bacteria [13]. CRKP spreads rapidly around the world, and its resistance rate to carbapenems continues to increase. According to CHINET surveillance from 2005 to 2017, the resistance rate of *K. pneumoniae* to carbapenems continued to rise, and its resistance rates to imipenem and meropenem increased rapidly from 3.0% and 2.9% in 2005 to 25.3% and 26.8% in 2019, respectively [12]. The increasing drug resistance rate has led to limited drugs for the treatment of CRKP infection in the clinic, and fewer types of drugs can be used for children [14]. Therefore, the prevention and control of CRKP infection in children deserves more attention. At present, nosocomial infection managers mainly track and trace the outbreaks and transmission routes of nosocomial pathogens by homology analysis through pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) [15]. These traditional methods are time-consuming, complicated, and low-resolution. When highly similar clones appear, it is often impossible to distinguish the small differences between them. However, the emergence of WGS technology has solved this problem. Using this technology, the difference between nearly a thousand SNP sites can be found between the genomes of strains with very similar homology, and the possibility and route of transmission among these strains can be accurately determined [16].

In this study, the main STs of 20 CRKP isolates were ST2407, ST76, and ST309, which was consistent with the reports from other children's hospitals in China [17, 18]. By combining clinical epidemiological data and a phylogenetic tree, it was found that the 20 CRKP isolates belonged to three clusters. All seven isolates in cluster 1 were from neonatology, and the separation time of KP-zy4, KP-zy5, and KP-zy6 samples differed by only two days at most. cluster 2 contained seven isolates, of which five were from the general paediatric ward, and the other two were from the paediatric intensive care unit. All six isolates in cluster 3 were from the paediatric intensive care unit. There is a possibility of spatiotemporal cross-infection within the cluster. Studies have shown that the most common sources of nosocomial infections are patients, medical staff and the environment, among which CRKP-contaminated environments and the hands of medical staff can lead to infection epidemics [19, 20]. Regardless of the cluster, most patients were in the same department, and there was overlap between cleaning staff and medical staff, thus increasing the risk of CRKP transmission.

The results of the phenotypic drug resistance in this study showed that, although CRKP was highly resistant to β-lactam antibiotics, it was most sensitive to aminoglycosides, quinolones, and sulfonamides. This was consistent with previous literature reports; thus, the above drugs can be selected for paediatric CRKP infection in hospitals [21, 22]. The results of drug resistance genes showed that all 20 CRKP isolates carried carbapenem resistance genes, of which five isolates only carried bla_{KPC-2}, six isolates only carried bla_{NDM-1}, and the remaining nine isolates carried both carbapenem resistance genes. This indicated that carbapenemase production is the main drug resistance mechanism of CRKP in the hospital, consistent with reports in China [23, 24]. The combined analysis of phenotypic drug resistance and the presence of drug resistance genes showed that the presence of aminoglycoside, quinolone, and sulfonamide drug resistance genes was not completely consistent with the results of phenotypic drug resistance analysis, and similar results were obtained in other studies [8, 25]. In vitro drug-susceptibility test results can guide which antibiotic to be used clinically, but the presence or absence of resistance genes confirmed by WGS did not exactly match the resistance phenotype results. The EUCAST committee also mentioned that, for most bacterial species, there is insufficient evidence to support the use of drug susceptibility phenotypes to guide clinical anti-infection decisions [26]. Therefore, the relationship between drug resistance gene information and clinical medication guidance still needs to be verified with more samples to obtain reliable data support.

As a special group, the high morbidity and mortality caused by CRKP infection in children have been a concern around the world. Most of the CRKP isolates that cause nosocomial infections in China carry bla_{KPC-2} or bla_{NDM-1}. They can lead to outbreaks of CRKP through plasmid-mediated horizontal transfer between different strains. To strengthen the control and spread of paediatric CRKP infection, we should accurately and timely grasp the aetiological information of patients, shorten the application time of antibiotics, reduce the types of antibiotics, and carry out the precise targeted treatment. Numerous studies have shown that carbapenem use is an independent risk factor for CRKP infection [27, 28]. Therefore, medical institutions should strictly control the use of carbapenem antibiotics. WGS can comprehensively grasp the drug resistance and drug resistance mechanism of bacteria. It can be used to track nosocomial infections and determine transmission routes, providing an important

method for preventing the spread of bacteria and reducing drug resistance in the clinic, which should be applied in the daily work of hospital infection management.

Conclusions

In this study, all 20 CRKP isolates carried carbapenem-resistance genes. They were divided into three clusters, implying three different common ancestors in the hospital. Sequence typing, phylogenetic relationships, and antibiotic resistance genes could be obtained using WGS technology. This can guide CRKP infection control and clinical treatment for paediatric patients.

References

1. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC (2001) Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 45: 1151-1161.
2. Guo Y, Hu FP, Zhu DM, Wang CQ, Wang AM, Zhang H, Wang C, Dong F, Zhen JH, Zhou SP, Zhou Y, Zhu L, Meng JH (2018) Antimicrobial resistance changes of carbapenem-resistant *Enterobacteriaceae* strains isolated from children. Chin. J. Pediatr 56: 907-914. [Article in Chinese]
3. Fu P, Xu H, Jing C, Deng J, Wang H, Hua C, Chen Y, Chen X, Zhang T, Zhang H, Chen Y, Yang J, Lin A, Wang S, Cao Q, Wang X, Deng H, Cao S, Hao J, Gao W, Huang Y, Yu H, Wang C (2021) Bacterial epidemiology and antimicrobial resistance profiles in children reported by the ISPED program in China, 2016 to 2020. Microbiol Spectr 9: 00283–21.
4. Xu L, Sun X, Ma X (2017) Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*. Ann Clin Microbiol Antimicrob 16: 1-12.
5. Tacconelli E, Sifakis F, Harbarth S, Schrijver R, van Mourik M, Voss A, Sharland M, Rajendran NB, Rodríguez-Baño J, Bielicki J, de Kraker M, Gandra S, Gastmeier P, Gilchrist K, Gikas A, Gladstone BP, Goossens H, Jafri H, Kahlmeter G, Leus F, Luxemburger C, Malhotra-Kumar S, Marasca G, McCarthy M, Navarro MD, Nuñez-Nuñez M, Oualim A, Price J, Robert J, Sommer H, von Cube M, Vuong C, Wiegand I, Witschi AT, Wolkewitz M (2018) Surveillance for control of antimicrobial resistance. Lancet Infect Dis 18: e99-e106.
6. Potter RF, D'Souza AW, Dantas G (2016) The rapid spread of carbapenem-resistant *Enterobacteriaceae*. Drug Resist Updat 29: 30-46.
7. Snitkin ES, Zelazny AM, Thomas PJ, Stock F, Nisc Comparative Sequencing Program Group, Henderson DK, Palmore TN, Segre JA (2012) Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. Sci Transl Med 4: 148ra116.
8. Chen C, Zhang Y, Yu SL, Zhou Y, Yang SY, Jin JL, Chen S, Cui P, Wu J, Jiang N, Zhang WH (2019) Tracking carbapenem-producing *Klebsiella pneumoniae* outbreak in an intensive care unit by whole genome sequencing. Front Cell Infect Microbiol 9 :281.
9. CLSI. Performance standards for antimicrobial susceptibility testing. 32nd ed.
10. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol 32: 268-274.
11. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, Xie L, Yang C, Ma X, Li H, Li W, Zhang X, Liao K, Man S, Wang S, Wen H, Li B, Guo Z, Tian J, Pei F, Liu L, Zhang L, Zou C, Hu T, Cai J, Yang H, Huang J, Jia X, Huang W, Cao B, Wang H (2018) Epidemiology of carbapenem-resistant *Enterobacteriaceae* infections: report from the China CRE network. Antimicrob Agents Chemother 62: e01882-17.
12. Hu FP, Guo Y, Zhu DM, Wang F, Jiang XF, Xu YC, Zhang XJ, Zhang ZX, Ji P, Xie Y, Kang M, Wang CQ, Wang AM, Xu Y, Huang Y, Sun Z, Chen Z, Ni Y, Sun J, Chu Y, Tian S, Hu Z, Li J, Yu Y, Lin J, Shan B, Du Y, Guo S, Wei L, Zou F, Zhang H, Wang C, Hu Y, Ai X, Zhuo C, Su D, Guo D, Zhao J, Yu H, Huang X, Liu W, Li Y, Jin Y, Shao C, Xu X, Yan C, Wang S, Chu Y, Zhang L, Ma J, Zhou S, Zhou Y, Zhu L, Meng

J, Dong F, Zheng H, Hu F, Shen H, Zhou W, Jia W, Li G, Wu J, Lu Y, Li J, Duan J, Kang J, Ma X, Zheng Y, Guo R, Zhu Y, Chen Y, Meng Q (2020) CHINET surveillance of bacterial resistance across tertiary hospitals in 2019. Chin. J. Infect. Chemother 20: 233-243. [Article in Chinese]

13. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-Cotinat M, Kretzschmar ME, Devleesschauwer B, Cecchini M, Ouakrim DA, Oliveira TC, Struelens MJ, Suetens C, Monnet DL (2019) Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis. Lancet Infect Dis 19: 56-66.

14. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH (2016) Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: Epidemiology, genetic context, treatment options, and detection methods. Front Microbiol 7: 895.

15. Zhan L, Wang S, Guo Y, Jin Y, Duan J, Hao Z, Lv J, Qi X, Hu L, Chen L, Kreiswirth BN, Zhang R, Pan J, Wang L, Yu F (2017) Outbreak by hypermucoviscous *Klebsiella pneumoniae* ST11 isolates with carbapenem resistance in a tertiary hospital in China. Front Cell Infect Microbiol 7: 182.

16. Jiang Y, Wei Z, Wang Y, Hua X, Feng Y, Yu Y (2015) Tracking a hospital outbreak of KPC-producing ST11 *Klebsiella pneumoniae* with whole genome sequencing. Clin Microbiol Infect 21: 1001-1007.

17. Zhu J, Sun L, Ding B, Yang Y, Xu X, Liu W, Zhu D, Yang F, Zhang H, Hu F (2016) Outbreak of NDM-1-producing *Klebsiella pneumoniae* ST76 and ST37 isolates in neonates. Eur J Clin Microbiol Infect Dis 35: 611-618.

18. Fu Bo, Yin Dandan, Sun Chengtao, Shen Yingbo, Liu Dejun, Bai Rina, Zhang Rong, Shen Jianzhong, Hu Fupin, Wang Yang (2022) Clonal and horizontal transmission of bla_{NDM} among *Klebsiella pneumoniae* in children's intensive care units. Microbiol Spectr 10: e01574-21.

19. Magiorakos AP, Burns K, Rodriguez Bano J, Borg M, Daikos G, Dumpis U, Lucet JC, Moro ML, Tacconelli E, Simonsen GS, Szilagyi E, Voss A, Weber JT (2017) Infection prevention and control measures and tools for the prevention of entry of carbapenem-resistant Enterobacteriaceae into healthcare settings: guidance from the European centre for disease prevention and control. Antimicrob Resist Infect Control 6: 1-17.

20. Wei L, Wu L, Wen H, Feng Y, Zhu S, Liu Y, Tang L, Doughty E, van Schaik W, McNally A, Zong Z (2021) Spread of carbapenem-resistant *Klebsiella pneumoniae* in an intensive care unit: A whole-genome sequence-based prospective observational study. Microbiol Spectr 9: e00058-21.

21. Dong F, Zhang Y, Yao K, Lu J, Guo L, Lyu S, Yang Y, Wang Y, Zheng H, Song W, Liu G (2018) Epidemiology of carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections in a Chinese children's hospital: predominance of

New Delhi metallo-beta-lactamase-1. Microb Drug Resist 24: 154-160.

22. Zhou Y, Zhao Z, Zeng L, Peng J, Zhou S, Min L, Ke J, Liu J (2022) Surveillance of carbapenem-resistant *Klebsiella pneumoniae* in a paediatric hospital in China revealed the dynamics of carbapenemase and the prevalence of ST2735 *K. pneumoniae*. J Med Microbiol 71: 001482.

23. Sui W, Zhou H, Du P, Wang L, Qin T, Wang M, Ren H, Huang Y, Hou J, Chen C, Lu X (2018) Whole genome sequence revealed the fine transmission map of carbapenem-resistant *Klebsiella pneumonia* isolates within a nosocomial outbreak. Antimicrob Resist Infect Control 7: 1-11.

24. Yang Y, Liu J, Muhammad M, Liu H, Min Z, Lu J, Zhang L, Chai Z (2021) Factors behind the prevalence of carbapenem-resistant *Klebsiella pneumoniae* in pediatric wards. Medicine (Baltimore) 100: e27186.

25. Tamma PD, Fan Y, Bergman Y, Pertea G, Kazmi AQ, Lewis S, Carroll KC, Schatz MC, Timp W, Simner PJ (2019) Applying rapid whole-genome sequencing to predict phenotypic antimicrobial susceptibility testing results among carbapenem-resistant *Klebsiella pneumoniae* clinical isolates. Antimicrob Agents Chemother 63: e01923-18.

26. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Koser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen O, Woodford N (2017) The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST subcommittee. Clin Microbiol Infect 23: 2-22.

27. Lou T, Du X, Zhang P, Shi Q, Han X, Lan P, Yan R, Hu H, Wang Y, Wu X, Jiang Y, Yu Y (2022) Risk factors for infection and mortality caused by carbapenem-resistant *Klebsiella pneumoniae*: a large multicentre case-control and cohort study. J Infect 84: 637-647.

28. Li J, Li Y, Song N, Chen Y (2020) Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection: a meta-analysis. J Glob Antimicrob Resist 21: 306-313.

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