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In vitro activity of cefiderocol, a siderophore cephalosporin, against carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in China

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ABSTRACT Cefiderocol is a siderophore cephalosporin that binds ferric iron and utilizes iron transporters to cross the cell membrane. Hypervirulent *Klebsiella pneumoniae* (hvKp) is known to produce more siderophores; in this case, the uptake of cefiderocol may be decreased. Therefore, the objective of this study was to evaluate the *in vitro* activity of cefiderocol against hvKp isolates. A total of 320 carbapenem-resistant *K. pneumoniae* (CRKp) isolates were collected in China between 2014 and 2022, including 171 carbapenem-resistant hvKp (CR-hvKp) and 149 carbapenem-resistant classical *K. pneumoniae* (CR-cKp). Quantitative detection of siderophores showed that the average siderophore production of CR-hvKp (234.6 mg/L) was significantly higher than that of CR-cKp (68.9 mg/L, $P < 0.001$). The overall cefiderocol resistance rate of CR-hvKp and CR-cKp was 5.8% (10/171) and 2.7% (4/149), respectively. The non-susceptible rates of both cefiderocol and siderophore production of CR-hvKp isolates were higher than those of CR-cKp in either NDM-1- or KPC-2-producing groups. The MIC90 and MIC50 for CR-hvKp and CR-cKp were 8 mg/L and 2 mg/L and 4 mg/L and 1 mg/L, respectively. The cumulative cefiderocol MIC distribution for CR-hvKp was significantly lower than that of CR-cKp isolates ($P = 0.003$). KL64 and KL47 consisted of 53.9% (83/154) and 75.7% (53/70) of the ST11 CR-hvKp and CR-cKp, respectively, and the former had significantly higher siderophore production. In summary, cefiderocol might be less effective against CR-hvKp compared with CR-cKp isolates, highlighting the need for caution regarding the prevalence of cefiderocol-resistant *K. pneumoniae* strains, particularly in CR-hvKp isolates.

KEYWORDS Carbapenem-resistant *Klebsiella pneumoniae*, cefiderocol, siderophore, hypervirulent *Klebsiella pneumoniae*, KPC-2, NDM-1

Carbapenem-resistant *Klebsiella pneumoniae* (CRKp) is one of the most important pathogens threatening human health (1, 2), being resistant to almost all β-lactam antibiotics with limited treatment options (3). Studies have shown that the failure rate of clinical treatment of patients with carbapenem-resistant *Enterobacterales* (CRE) infections is two to three times that of non-CRE infections (4), and the overall mortality rates of CRKp infections worldwide are 33–42% (5, 6). KPC and NDM are the most prevalent carbapenemases of CRKp in China, accounting for more than 96% of the total carbapenemases (7, 8).

Cefiderocol is a novel siderophore cephalosporin, recently approved in the United States and the European Union (9). The novel antibiotic agent has a chlorocatechol sidechain, which mimics catecholate-type siderophores (e.g., enterobactin) that chelate iron, allowing it to enter bacterial cells through iron transporter channels (9). To date, several international surveillance studies concerning the *in vitro* antimicrobial activity of cefiderocol demonstrated that it was a promising alternative against a wide range

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of carbapenem-resistant bacteria (10–12), although there are some limitations requiring further investigation, including user-unfriendly antimicrobial susceptibility testing (AST), different-to-interpret results and reduced activity against VIM-producing *Enterobacter* spp. (13–15).

Iron is an essential growth factor for most bacteria, including hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp). The primary mechanism of iron acquisition in *K. pneumoniae* is through the production of siderophores, which secrete, bind iron, and reenter in the bacterial cell through siderophore receptors (16). Daoud et al. found that the susceptibility of cefiderocol is positively correlated with enterobactin receptor expression; if the bacteria harbor multiple iron acquisition systems, the uptake of cefiderocol will be reduced (17). The virulence determinants of hvKp include four siderophore systems for iron acquisition, that is, enterobactin, salmochelin, yersiniabactin, and aerobactin. Among them, salmochelin and aerobactin are hvKp-specific virulence factors (18), and aerobactin accounts for up to 90% of total siderophore production (19). This study aimed at evaluating the *in vitro* activity of cefiderocol against CRKp showing, or not, a hypervirulent genotype.

MATERIALS AND METHODS

Source of CRKp isolates and AST

A total of 320 non-duplicate CRKp clinical isolates were collected from six hospitals in different cities in China between 2014 and 2022. The AST of piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, tobramycin, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole was performed using the Vitek-2 system. The minimum inhibitory concentration (MIC) of cefiderocol was determined with the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (20). All the susceptibility tests were performed in triplicate. The results were interpreted according to the CLSI 2022 guidelines (20). The breakpoints for cefiderocol tested against *Enterobacterales* are as follows: susceptible (S), ≤4 mg/L; intermediate (I), 8 mg/L; and resistant (R), ≥16 mg/L.

Determination of resistant and virulent factors

Whole-genome sequencing (WGS) was carried out using Illumina HiSeq 2500 platform. Raw reads were filtered to remove low-quality sequences and adaptors using skewer (21). *De novo* assembly was conducted using SPAdes Genome Assembler v3.14.0 (22). Gene prediction was performed using Prokka 1.12 (23). Carbapenemase, sequence type, capsular serotype, and virulent factors were annotated with Kleborate v2.3.2 (24). The nucleotide sequences of *fepA*, *iroN*, *fiu*, and *cirA* receptor genes were retrieved from the database from Pasteur Institute (https://bigsdb.pasteur.fr/klebsiella/), translated into amino acid sequences and then made into a local database. Assembled genomes were annotated as the presence of the four genes using BLASTX at the cutoff value of 1e-7, a sequence identity of 90%, and a coverage of 99%. *K. pneumoniae* strains showing a hypervirulent genotype, with a virulence score ≥3 (harboring aerobactin and/or yersiniabactin and colibactin) according to Lam et al. (24) and carrying *rmpA/rmpA2* genes, were defined as hvKp.

Qualitative and quantitative siderophore detections

Qualitative detection of siderophore was performed according to a previous study with slight modification (18). In brief, an iron-free modified King B (MKB) plate was prepared with glycerol 15 mL, K_2HPO_4 2.5 g, hydrolyzed casein 5 g, $MgSO_4{\cdot}7H_2O$ 2.5 g, and agar 20 g in 1 L deionized water. Chrome azurol S (CAS) plate was prepared as follows: 18 g agar was dissolved in 1 L deionized water and steam-sterilized at 121°C. When the medium was cooled to about 60°C, 20% sucrose 10 mL, 1 mM $MgSO_4$ 20 mL, 10% hydrolyzed casein 30 mL, 0.1 M phosphate buffer 100 mL, and CAS solution (containing

1 mM CAS, 0.1 mM $FeCl_3$, and 2 mM hexadecyl trimethyl ammonium bromide) 100 mL were added. *K. pneumoniae* strains were sub-cultured on MKB plate for 18–24 h at 37°C and were adjusted to McFarland 4.0. Afterwards, 5 µL of bacterial suspensions was inoculated onto CAS plates and incubated at 37°C for 18–24 h. A color change of the colony from blue to orange indicates siderophore production. The width of the orange circle was then recorded.

The siderophore quantification assays were performed using a SideroTec Assay Kit (Accuplex Diagnostics Ltd., Co. Kildare, Ireland) in 11 sessions. Importantly, this kit is a universal assay that will react with all classes of siderophore regardless of chemistry or origin. *K. pneumoniae* strains NTUH-K2044 and ATCC 700603 were used as positive and negative controls for each session, respectively. Both qualitative and quantitative assays were performed in triplicate.

Arnow assay

The catechol production was quantified using Arnow assay as previously described (25). Briefly, the strains were cultured overnight in M9 medium, and the OD_{600} was measured. Bacterium-free supernatants were obtained by filtration using 0.2 µM filters. Catechol was quantified by combining the equal volume of the sample, 0.5 N HCl, nitrite-molybdate reagent (consisting of 10% [wt/vol] sodium nitrate and sodium molybdate), and 1 N NaOH. Then, the mixture was incubated for 5 min to complete the reaction. Absorbance was measured at 510 nm with uninoculated media used as a blank [a known 2,3-dihydroxybenzoic acid (2,3-DHBA) concentration was used as a standard], and the sample was adjusted for bacterial growth by normalizing to the OD_{600} value of each culture. The Arnow assay was conducted in eight sessions. *K. pneumoniae* strains NTUH-K2044 and ATCC 700603 were used as controls for each session.

Statistical analysis

Statistically significant differences in the cumulative percentage of MICs and siderophore concentrations were calculated using Student's *t*-test or Wilcoxon rank-sum test in GraphPad Prism 9.5.1. The qualitative data were compared using the χ test or Fisher test when the expected value of any cell in the contingency table was less than 5. $P < 0.05$ was considered statistically significant.

RESULTS

Overall genotypic characteristics

A total of 320 CRKp strains, isolated from different sources including sputum, bronchoalveolar lavage fluid, urine, blood, and biopsy tissue, were collected and sequenced. The sequence type and capsular serotype were analyzed using the assembled genomes. A total of 44 different sequence types were found, including ST11 (224, 70%), ST340 (16, 5%), ST35 (7, 2.2%), ST65 (7, 2.2%), ST37 (5, 5%), ST307 (5, 5%), ST8520 (5, 5%), and others (Fig. 1A; Table S1). A total of 37 different capsular serotypes were also identified, including KL47 (126, 39.4%) and KL64 (94, 29.4%), followed by KL55 (18, 5.6%), KL25 (9, 2.8%), KL2 (9, 2.8%), and others (Table S1).

The type of carbapenemase was also determined in line with WGS data. The results showed that KPC-2 (230, 71.9%), KPC-3 (1, 0.3%), NDM-1 (57, 17.8%), NDM-5 (11, 3.4%), NDM-4 and OXA-181 (1, 0.3%), NDM-4 and OXA-48 (1, 0.3%), and IMP-4 (2, 0.6%) were found in 303 of the 320 CRKp isolates, and carbapenemase was not detected in the other 17 isolates. The NDM-1- and KPC-2-producing CRKp isolates accounted for 89.7% (287/320) of the total isolates and were further analyzed.

The virulent factors were analyzed. Summarily, 253 yersiniabactin (*ybt*), 9 colibactin (*clb*), 181 aerobactin (*iucA*), 27 salmochelin (*iroB*), and 176 hypermucoviscous regulators (*rmpA*/rmp*A2*) were annotated. In addition, the enterobactin (*fepA*) and salmochelin (*iroN*) receptors, as well as the other two known catecholate siderophore receptors (*fiu*

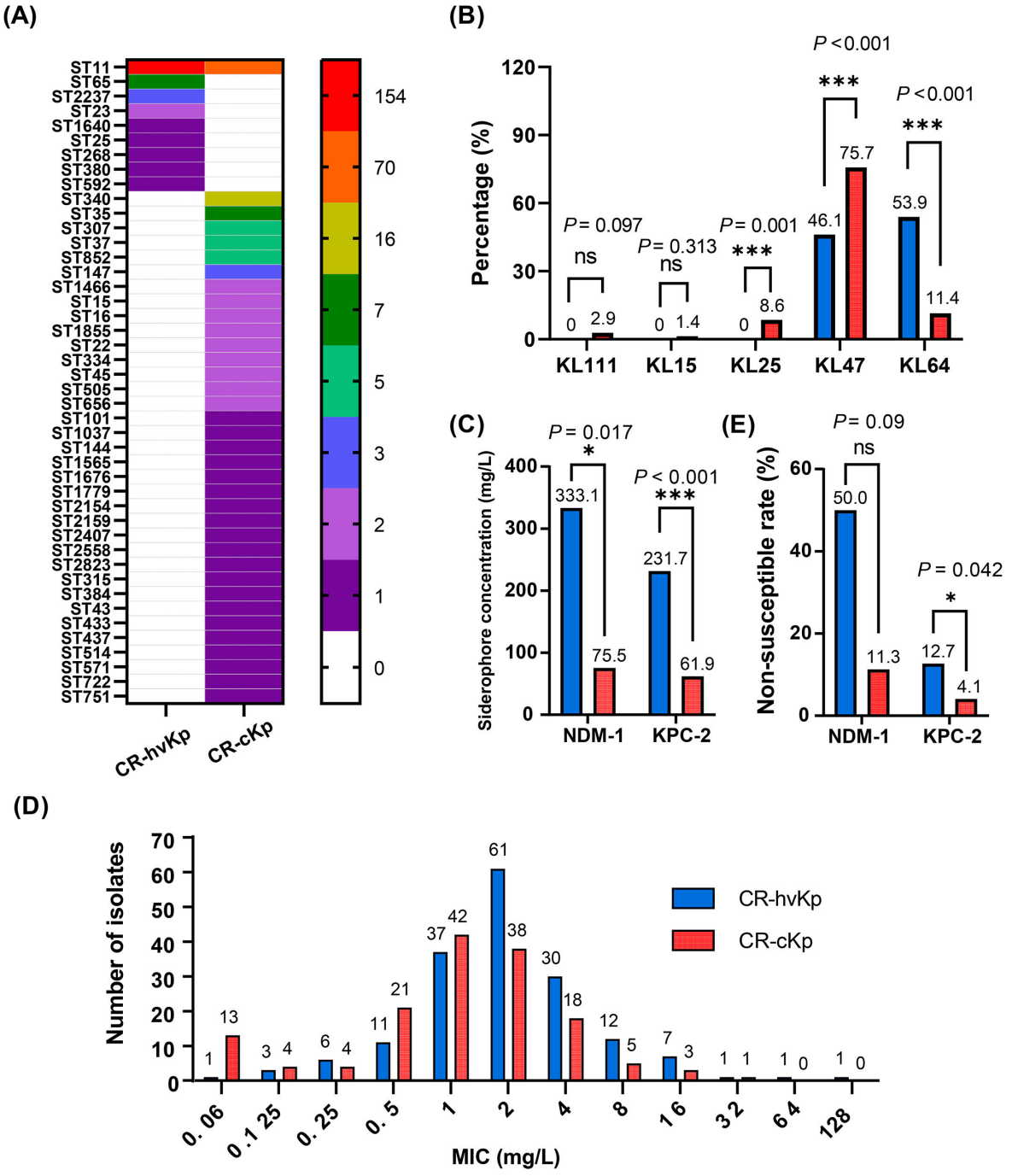

FIG 1 The phenotypic and genotypic comparation between CR-hvKp and CR-cKp isolates in specific genetic background. (A) Distribution of sequence types among CR-hvKp and CR-cKp isolates. (B) Capsular serotypes in ST11 CR-hvKp and CR-cKp isolates. (C) Siderophore concentration of isolates in NDM-1- or KPC-2-producing groups. (D) MIC distribution of CR-hvKp and CR-cKp isolates. (E) Non-susceptible rate of cefiderocol in NDM-1- or KPC-2-producing groups. The significance level was as follows: $^{*}P < 0.05$, $^{***}P \leq 0.001$.

and *cirA*) were also annotated. The results showed that *fepA*, *iroN*, *fiu*, and *cirA* were found in 318, 25, 317, and 317 of the 320 collected isolates, respectively.

According to the definition described above, 171 isolates were CR-hvKp and the other 149 were carbapenem-resistant classical *K. pneumoniae* (CR-cKp). In the CR-hvKp isolates, the dominant sequence type was ST11 (154, 90.1%), followed by ST65 (7, 4.1%), ST2237 (3, 1.8%), ST23 (2, 1.2%), ST1640 (1, 0.6%), ST268 (1, 0.6%), ST25 (1, 0.6%), ST380 (1, 0.6%),

and ST592 (1, 0.6%). The CR-cKp isolates had more diverse sequence types, and the dominant sequence type was also ST11 (70, 47%), followed by ST340 (16, 10.7%), ST35 (7, 4.7%), ST307 (5, 3.4%), ST37 (5, 3.4%), ST852 (5, 3.4%), ST147 (3, 2%), and others (Table S1). The 224 ST11 CRKp isolates were further analyzed for the capsular serotype. Notably, KL15, KL25, and KL111 were only found in CR-cKp isolates; KL47 was mainly found in CR-cKp isolates (75.7% vs 46.1%, $P < 0.001$); and KL64 was mainly found in CR-hvKp isolates (53.9% vs 11.4%, $P < 0.001$), as shown in Fig. 1B.

In addition, the KL64 isolates had significantly higher average siderophore concentration (226.5 mg/L vs 157 mg/L, $P = 0.001$) and non-susceptible rate against cefiderocol (14.3% vs 7.3%, $P = 0.093$).

Siderophore production

Siderophore qualitative detection was conducted to characterize these isolates. The average width of the orange circle representing siderophore production for CR-hvKp was 2.6 cm, which was significantly larger than that of CR-cKp (1.3 cm, $P < 0.001$), as shown in Fig. 2A. We then performed the quantitative detection of siderophore using SideroTec Assay. The average concentration of siderophore produced by CR-hvKp was 234.6 mg/L, while that by CR-cKp was 68.9 mg/L (Fig. 2B). A significant difference was observed between them ($P < 0.001$). In addition, the siderophore concentrations of 320 CRKp were divided by those of reference cKp strain ATCC 700603 in each of the 11 sessions, and the result showed that 93.6% (160/171) and 65.8% (98/149) of the CR-hvKp and CR-cKp strains, respectively, showed higher siderophore production than that of strain ATCC 700603.

In both NDM-1- and KPC-2-producing CRKp groups, CR-hvKp isolates had significantly higher siderophore concentrations than CR-cKp isolates, with 333.1 mg/L vs 75.5 mg/L ($P = 0.017$) and with 231.7 mg/L vs 61.9 mg/L ($P < 0.001$), as shown in Fig. 1C. However, in CR-hvKp and CR-cKp groups, the siderophore concentrations of NDM-1-producing isolates were numerically higher than those of KPC-2-producing isolates, though it did not reach a significant level ($P = 0.144$ and $P = 0.183$). In addition, siderophore receptors *fepA*, *iroN*, *fiu*, and *cirA* were detected in all the four NDM-1-producing CR-hvKp isolates, while in the 157 KPC-2-producing CR-hvKp isolates, the positive rates for the four receptors were 100% (157/157), 5.7% (9/157), 99.4% (156/157), and 99.4% (156/157), respectively. A further subgroup analysis of the yield of siderophore was carried out in CR-hvKp isolates. The results showed that the average siderophore production for strains with or without *iroN* was 212.2 mg/L and 237.8 mg/L ($P = 0.43$), respectively. In 157 KPC-2-producing CR-hvKp isolates, the average siderophore production for strains with or without *iroN* was 184.8 mg/L and 234.5 mg/L ($P = 0.495$), respectively.

Arnow assay

Arnow assay showed that *K. pneumoniae* strain NTUH-K2044 had lower catechol production (an average of 44.6 mg/L) than *K. pneumoniae* strain ATCC 700603 (an average of 66.3 mg/L), though not to a significant level. However, the CR-hvKp isolates secrete more catechol than CR-cKp isolates (64.2 mg/L vs 55.8 mg/L, $P = 0.004$), as seen in Fig. S1. According to the genetic characteristics described above, the total CRKp isolates were composed of 224 ST11 and 96 non-ST11 isolates. The catechol production among the two subgroups was further analyzed. Specifically, of the 224 ST11 CRKp isolates, CR-hvKp had significantly lower catechol production than CR-cKp (65.9 mg/L vs 73.4 mg/L, $P = 0.029$). On the contrary, in the non-ST11 groups, the CR-cKp had significantly lower catechol production than CR-hvKp (39.8 mg/L vs 49.5 mg/L, $P = 0.019$). The catechol production in non-ST11 CR-cKp isolates was too low, which greatly reduced the overall catechol yield of the total CR-cKp. Besides, the catechol production of the four NDM-1-producing CR-hvKp was 51.2 mg/L, which was lower than that of the 157 KPC-2-producing CR-hvKp (65.3 mg/L, $P = 0.23$), though not to a significant level.

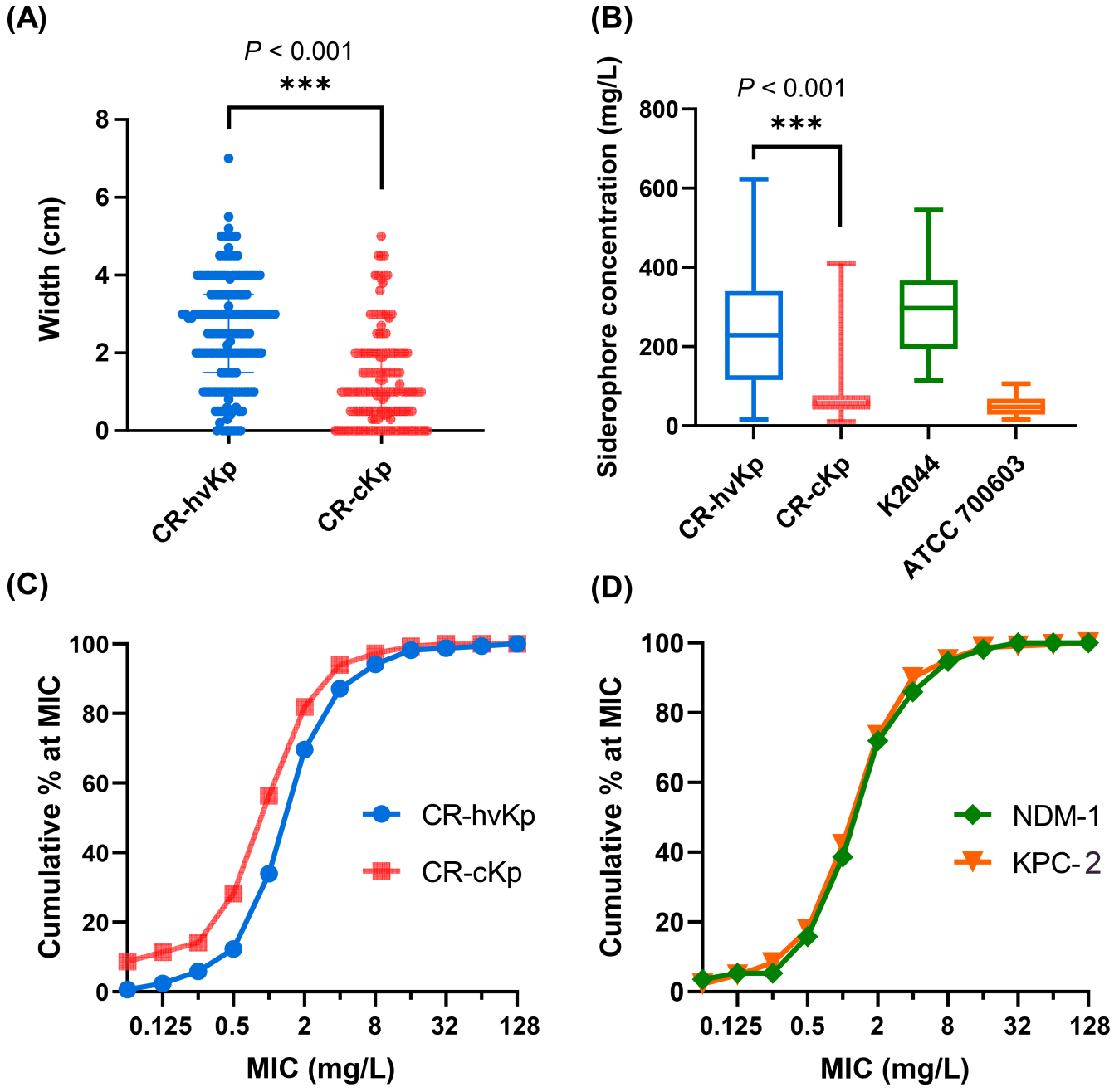

FIG 2 Siderophore detection and antimicrobial susceptibility results of cefiderocol between CR-hvKp and CR-cKp isolates. (A) Qualitative and (B) quantitative detection of siderophores in 320 *K. pneumoniae* isolates. (C) Cumulative percentage MIC distribution of cefiderocol between CR-hvKp and CR-cKp isolates. (D) Cumulative percentage MIC distribution of cefiderocol between NDM-1- and KPC-2-producing *K. pneumoniae* isolates. The significance level was as follows: ***$P < 0.001$.

The antimicrobial susceptibility profiles

The antimicrobial activity of cefiderocol and comparator agents is shown in Table 1. Of these 320 CRKp isolates, 14 (4.4%) were resistant to cefiderocol, and the MIC90 and MIC50 were 4 mg/L and 2 mg/L, respectively. Besides, the non-susceptible rates of cefiderocol for CR-hvKp and CR-cKp were 12.9% (22/171) and 6% (9/149), respectively, with a *P*-value of 0.039. The MIC ranges, MIC90 and MIC50, for CR-hvKp and CR-cKp were summarized in Table 2 and Fig. 1D. Meanwhile, the cumulative percentage of CR-hvKp and CR-cKp isolates inhibited at various MICs of cefiderocol was shown in Fig. 2C, in

TABLE 1 Antimicrobial activity of cefiderocol and comparator agents against 320 CRKp isolates

Antimicrobial agent	MIC range (mg/L)	Resistance (%)
Cefiderocol	0.06 to ≥128	4.4
Piperacillin/tazobactam	32 to ≥128	100
Ceftazidime	16 to ≥64	100
Cefepime	2 to ≥64	97.2
Aztreonam	≤1 to ≥64	94.7
Imipenem	2 to ≥16	98.8
Meropenem	2 to ≥16	99.7
Amikacin	≤2 to ≥64	55.6
Tobramycin	≤1 to ≥16	63.4
Ciprofloxacin	≤0.25 to ≥4	93.8
Levofloxacin	≤0.25 to ≥8	90.9
Trimethoprim/sulfamethoxazole	≤20 to ≥320	46.3

TABLE 2 MIC50, MIC90, MIC ranges, and resistance percentages of cefiderocol against 320 CRKp isolates

Isolates (*n*)	MIC (mg/L)			CLSI (%)		
	MIC50	MIC90	MIC range	S	I	R
CR-hvKp (171)	2	8	0.06 to 128	149 (87.1)	12 (7)	10 (5.8)
CR-cKP (149)	1	4	0.06 to 32	140 (94)	5 (3.4)	4 (2.7)
NDM-1 (57)	2	8	0.06 to 32	49 (86)	5 (8.8)	3 (5.3)
CR-hvKp (4)	2	16	0.5 to 16	2 (50)	1 (25)	1 (25)
CR-cKp (53)	2	8	≤ 0.06 to 32	47 (88.7)	4 (7.5)	2 (3.8)
KPC-2 (230)	2	8	0.06 to 128	207 (90)	12 (5.2)	11 (4.8)
CR-hvKp (157)	2	8	0.125 to 128	137 (87.3)	11 (7)	9 (5.7)
CR-cKp (73)	1	4	≤ 0.06 to 16	70 (95.9)	1 (1.4)	2 (2.7)

which the cumulative curve of CR-hvKp was significantly lower than that of CR-cKp isolates ($P = 0.003$).

The non-susceptible rates of NDM-1- and KPC-2-producing isolates in CR-hvKp and CR-cKp groups were 50% (2/4) vs 12.7% (20/157) and 11.3% (6/53) vs 4.1% (3/73), respectively, and no significant differences were found. The cumulative percentage of NDM-1- and KPC-2-producing *K. pneumoniae* inhibited at various MICs as shown in Fig. 2D indicated also no significant differences. Of the 57 NDM-1-producing CRKp isolates, 4 (7%) and 53 (93%) were CR-hvKp and CR-cKp, respectively. Notably, the MIC50 and MIC90 for CR-hvKp were 2 mg/L and 16 mg/L and those for CR-cKp were 2 mg/L and 8 mg/L (Table 2). A similar result was found in KPC-2-producing isolates. Of them, 68.3% (157/230) and 31.7% (73/230) belonged to CR-hvKp and CR-cKp, respectively. The MIC50 and MIC90 for KPC-2-producing CR-hvKp and CR-cKp were 2 mg/L and 8 mg/L and 1 mg/L and 4 mg/L, respectively. The non-susceptible rates of CR-hvKp and CR-cKp isolates in both NDM-1- and KPC-2-producing isolates were calculated, and CR-hvKp isolates showed higher non-susceptible rates than CR-cKp in both groups, as shown in Table 2 and Fig. 1E.

Among the 224 ST11 CRKp isolates, the MIC50 and MIC90 for the 154 CR-hvKp were 2 mg/L and 8 mg/L, and those for the 70 CR-cKp were 1 mg/L and 4 mg/L, respectively. In addition, the non-susceptible rates for CR-hvKp and CR-cKp were 13% and 2.9%, respectively.

DISCUSSION

Cefiderocol is a siderophore cephalosporin with antibacterial activity against a broad range of gram-negative pathogens, including carbapenem-resistant isolates (11). Siderophore antibiotics bind ferric iron and utilize iron transporters to cross the cell membrane. HvKp is known to produce more siderophore, facilitating its survival in iron-deficient environments (16). However, a recent study found that when bacteria

harbor multiple iron acquisition systems, the uptake of cefiderocol would be reduced (17). Therefore, we hypothesized that less cefiderocol will be transported into hvKp than cKp isolates, causing less effectiveness of cefiderocol against the hvKp isolates (Fig. 3). Herein, we evaluated the *in vitro* activity of cefiderocol for hvKp and cKp.

Several phenotypic and genotypic markers have been used to identify the hypervirulent strains. Generally, the total siderophore production, or *iucA* and/or either *rmpA* or *rmpA2*, would be the most accurate and durable markers for hvKp (16, 26, 27). In this study, strains showing hypervirulent genotype, virulence score ≥3 according to reference (24), and the presence of *rmpA/rmpA2* genes were defined as hvKp. Nevertheless, the genetic biomarker is not a perfect surrogate for the hypervirulent phenotype.

We hypothesized that cefiderocol was less effective against CR-hvKp. In this study, CR-hvKp had a larger MIC range, MIC50, and MIC90 than CR-cKp. The overall resistance rate of cefiderocol of CR-hvKp (5.8%, 10/171) was higher than that of CR-cKp (2.7%, 4/149). In addition, in both NDM-1- and KPC-2-producing CRKp groups, the non-susceptible rates of cefiderocol and siderophore concentrations of CR-hvKp isolates were both higher than those of CR-cKp isolates. In ST11 CRKp group, the catechol production of CR-hvKp was significantly lower than that of CR-cKp. The findings above proved our hypothesis.

The *in vitro* activity of cefiderocol to CRKp isolates has been reported in several studies (28). Generally, cefiderocol has a high activity against *K. pneumoniae* strains, with most of the MIC90 values are ≤8 mg/L (29–31). Two studies from North America have reported resistance rates of 3% and 4.3% for 166 and 23 CRKp isolates, respectively (32, 33). A recent study from China found a resistance rate of 4.7% for 86 CRKp isolates (34).

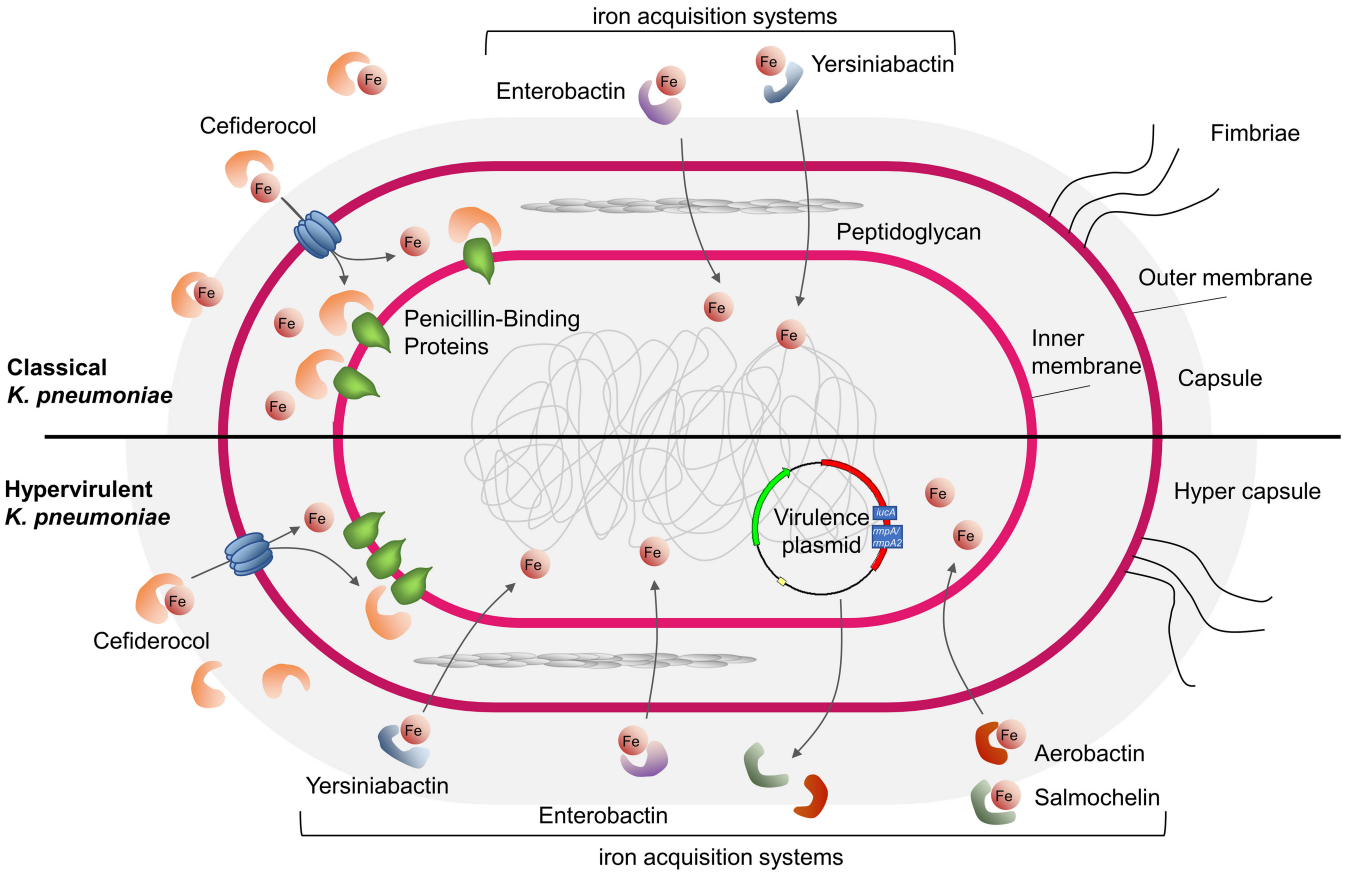

FIG 3 The probable transplantation mechanism of cefiderocol in *K. pneumoniae* strains. HvKp could produce four different siderophores, including enterobactin, salmochelin, yersiniabactin, and aerobactin. Whereas cKp produces only enterobactin and yersiniabactin. When bacteria harbor multiple iron acquisition systems, the uptake of cefiderocol was decreased, causing less effectiveness of cefiderocol against the hvKp isolates.

In our study, the overall resistance rate for 320 CRKp isolates was 4.4%, which was similar to the abovementioned reports. However, obvious differences in cefiderocol resistance rate were observed among isolates producing different phenotypes of β-lactamases. A study from the United Kingdom in 2020 reported that the resistance rates of cefiderocol for NDM- and KPC-producing isolates are 59% and 8.9%, respectively (the EUCAST breakpoints) (35). Another study from China showed that 569 KPC-producing isolates had a resistance rate of 0.4%, while 351 NDM-producing isolates had a resistance rate of 8% (the CLSI breakpoints) (36). In our study, the non-susceptible rates for NDM-1-producing isolates were also higher than those of KPC-2-producing isolates in both CR-hvKp and CR-cKp groups, which is consistent with the abovementioned reports. The numerical difference may be explained by the differences in siderophore production, geographical locations, and sample sizes (31).

In our study, the sequence types of the 320 CRKp isolates were diverse. Whereas the non-ST11 existed in either CR-hvKp or CR-cKp groups, ST11 could be found in both groups, accounting for the greatest number of isolates. In China, ST11 is the dominant sequence type of CRKp isolates (37), which is a single locus variant of ST258 (38). A variety of capsular serotypes have been identified in ST11 *K. pneumoniae* strains, with KL47 and KL64 being the dominant types in China (39–41). According to previous studies, KL64 is replacing KL47 as the most prevalent *K. pneumoniae* isolate in infected patients (42, 43). Notably, KL64 isolates contained more resistant and virulent factors (43). In this study, similar results were found that KL64 isolates were predominantly distributed in CR-hvKp isolates and had significantly higher siderophore yields than KL47 isolates.

The current study is limited by several factors. First, the CRKp isolates were collected from six centers in China, and more extensive multicenter studies are needed to get the representative conclusions. Second, ST11 consisted of the majority of CRKp isolates, which are mainly found in Asian countries, especially in China; therefore, the interpretation and extension of these results require great caution. Third, the definition of hvKp used in this study only takes into account the genotype, while the virulence profile of the phenotype is not included. As a result, there is a possibility of bias in the conclusions drawn from the definition. Fourth, the reason for the lower catechol production in these non-ST11 CR-cKp isolates needs further studied.

In summary, this study evaluated the *in vitro* activity of cefiderocol, a siderophore cephalosporin, against CRKp isolates. The results showed that cefiderocol is less effective against CR-hvKp than CR-cKp isolates and provided a reference for its clinical application in China.

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DATA AVAILABILITY

The sequenced data of the 320 collected isolates were deposited in the GenBank SRA database (bioproject: PRJNA996149; accession numbers of isolates: SAMN36519331-SAMN36519354, SAMN36519356-SAMN36519397, SAMN36663098-SAMN36663148, SAMN36687919-SAMN36688012, SAMN36585648-SAMN36585687, SAMN36664871-SAMN36664893, SAMN36607469-SAMN36607491, SAMN36679596-SAMN36679604, SAMN36679606-SAMN36679608, SAMN36770181-SAMN36770190).

ETHICAL APPROVAL

Permission for using the information in the medical records of the patient and the *K. pneumoniae* isolates for research purposes was approved by the Ethics Committee of the China-Japan Friendship Hospital (2022-KY-054).

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Figure S1 (AAC00735-23-s0001.docx). The catechol concentrations of these 320 CRKp isolates
Table S1 (AAC00735-23-s0002.xlsx). Siderophore concentration and genetic characteristics of 320 CRKp isolates

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