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Carbapenem-resistant Enterobacterales colonization and subsequent infection in a neonatal intensive care unit in Shanghai, China

L. Yin [a,†], L. He [b,†], J. Miao [a], W. Yang [a], X. Wang [a], J. Ma [a], N. Wu [a], Y. Cao [c,*], C. Wang [d]

[a] Department of Nosocomial Infection Control, Children's Hospital of Fudan University, Shanghai, China
[b] Clinical Microbiology Laboratory, Children's Hospital of Fudan University, Shanghai, China
[c] Neonatal Intensive Care Unit, Children's Hospital of Fudan University, Shanghai, China
[d] Department of Nosocomial Infection Control and the Clinical Microbiology Laboratory, Children's Hospital of Fudan University, Shanghai, China

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SUMMARY

Background: Colonization has been reported to play an important role in carbapenem-resistant Enterobacterales (CRE) infection; however, the extent to which carriers develop clinical CRE infection and related risk factors in neonatal intensive care unit (NICU) patients is unclear.

Aim: To investigate the frequency of CRE colonization and its contribution to infections in NICU patients.

Methods: CRE colonization screening and CRE infection surveillance were performed in the NICU in 2017 and 2018.

Findings: Among 1230 unique NICU patients who were screened for CRE colonization, 144 patients tested positive (11.7%, 144/1230), with 9.2% (110/1197) in the intestinal tract, which was higher than that in the upper respiratory tract (6.6%, 62/945) (P=0.026). Gestational age, low birth weight and prolonged hospitalization were risk factors for CRE colonization (all P<0.001). Diversilab homology monitoring found an overall 17.4% (25/144) risk of infection among patients colonized with CRE. For carbapenem-resistant Klebsiella pneumoniae (CR-KP) and carbapenem-resistant Escherichia coli (CR-ECO), the risks were 19.1% (21/110) and 13.8% (4/29), respectively. The independent risk factors for CR-KP clinical infection among CR-KP carriers were receiving mechanical ventilation (odds ratio (OR), 10.177; 95% confidence interval (CI), 2.667–38.830; P=0.013), a high level of neonatal nutritional risk assessment (OR, 0.251; 95% CI, 0.072–0.881; P=0.031) and a high neonatal acute physiology II (SNAP-II) score (OR, 0.256; 95% CI, 0.882–1.034; P=0.025).

Conclusions: The colonization of CRE may increase the incidence of corresponding CRE infection in NICU patients. Receiving mechanical ventilation, malnutrition and critical conditions with high SNAP-II scores were independent risk factors for subsequent CR-KP clinical infection.

* Corresponding author. Address: Department of Nosocomial Infection Control and the Clinical Microbiology Laboratory, Children's Hospital of Fudan University, Shanghai, China. Tel.: +86 13701699545.
E-mail address: chuanqing523@163.com (Y. Cao).
† These authors contributed equally to this work.

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Introduction

Infections by carbapenem-resistant Enterobacterales (CRE) are considered a healthcare challenge because CRE isolates are usually extensively drug resistant and associated with high morbidity and mortality [1,2]. Patients hospitalized in the intensive care unit (ICU) setting are at a particularly high risk of newly acquired CRE during their hospital stay due to multiple pre-existing medical conditions, compromised immune systems, lengthy unit stays, and significant rates of device and antibiotic utilization establish an ideal milieu for antibiotic resistance [3,4].

In a previous study we found that, among the children in our hospital, those in the neonatal intensive care unit (NICU) had the highest proportion of CRE nosocomial infections, and neonatal and non-neonatal patients showed different CRE molecular characteristics [5]. Therefore, identifying the risk for CRE infection in NICU patients and classifying genotypes are priorities in this vulnerable population. Although many experimental research reports have shown that colonization is a prerequisite for infection [6], the extent to which colonized patients develop infection with CRE is unclear in NICU patients, with most studies only evaluating adult patients and with rates ranging from 7.6% to 44.4% [7]. Meanwhile, most cases are symptomatic infections that lack verification of homology test results, which are important data to guide decision-making regarding infection-control interventions, such as screening and contact precautions for colonized patients.

Therefore, we undertook a retrospective study to understand the risk of infection following colonization with CRE in NICU patients. Furthermore, we characterized and compared the resistance genotypes as well as the sequence evolution of the CRE colonization and clinical infection isolates to evaluate whether the clinical infection carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) strains were clonally identical to CR-KP colonization isolates.

Methods

Study design and definitions

This was a single-center, cross-sectional, retrospective study performed at the NICU of the Children's Hospital of Fudan University, a tertiary-care teaching hospital of 800 beds. Over the years, the unit with the highest CRE nosocomial infection incidence was the NICU (1.3%) [5]. We evaluated all consecutive patients admitted to the NICU, and CRE intestinal and upper respiratory tract colonization screening and CRE infection surveillance were performed from January 2017 to December 2018, as previously described [5].

The positive rectal or pharyngeal culture isolates, identified as resistant to either of the carbapenems (etapenem, imipenem, and meropenem), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [8], were recognized as CRE strains. Patients were identified as having CRE colonization only if they had a positive rectal and/or pharyngeal culture but did not demonstrate signs and/or symptoms. Patients were identified as having CRE infection if they had at least one clinically positive culture and demonstrated signs and/or symptoms.

To determine risk factors for CR-KP infection among colonized patients, we conducted a retrospective case—control study. We compared the case group (patients infected by CR-KP of the same species as the colonizing strain among carriers) with patients in a control group who had CR-KP colonization but did not develop a subsequent clinical infection (1:2.3 case-to-control ratio) during the period of hospitalization. Forty-eight hours was defined as the 'subsequent' infection between the screening specimen and a clinical specimen. All clinical information for CR-KP patients was systematically reviewed from electronic medical records including sex, birth weight, gestational age, date of NICU admission, previous surgery, and invasive procedures, including mechanical ventilation, umbilical venous catheter (UV), umbilical arteriosus catheter (UA), peripherally inserted central catheter (PICC), nasogastric tube insertions, and antibiotic exposure. Neonatal nutritional risk assessment (improved evaluation scale based on STRONGkids [9] in our hospital; Table I) and the Score for Neonatal Acute Physiology II (SNAP-II) [10] were also completed on admission.

To determine hospital length of stay (LOS), all groups were followed up from admission until discharge from the hospital or death.

Patients excluded from the analysis included all subjects who failed to have at least one surveillance culture within 48 hours after patient admission or patients with evidence of colonization or clinical infection due to CRE prior to active surveillance culture after admission to our hospital.

CRE surveillance

Since 2017, as part of bundle interventions to reduce CRE infections, intestinal and upper respiratory tract CRE colonization screening was performed by pharyngeal swab and rectal swab CRE culture within 48 h of patient admission and was implemented weekly during the course of the admission in the NICU [5]. Routine CRE infection surveillance was analysed using culture and antimicrobial susceptibility tests. Strains were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) biotyper mass spectrometry (Bruker Company, Germany). Antimicrobial susceptibility tests (ASTs) were performed by automatic Vitek 2 compact machines. In addition, the AST breakpoint criteria of 2017 and 2018 CLSI M100-S27 were adopted [8]. The standard strains *Escherichia coli* ATCC25922 and *E. coli* ATCC35218 (enzyme-producing strains) were used as quality-control strains for antimicrobial susceptibility tests. All clinical information for CRE-positive patients was systematically reviewed from electronic medical records.

Table I
Neonatal nutritional risk assessment*

Low risk	1. Premature or full-term infants with body weight >1.8 kg who can start feeding within 3 days after birth 2. Premature infants whose growth rate reaches the expected value and their condition is relatively stable
Moderate risk	1. Body weight 1.0—1.5 kg within 1 month after birth 2. Body weight 1.5—1.8 kg within 2 weeks after birth 3. The body weight of 100 people ranked from small to large and P3 (3%) was the third person's data 4. The weight gain continued to be lower than expected for more than 2 weeks 5. Congenital abnormality of genetic metabolism and malformation of digestive tract 6. Newly diagnosed NEC, BPD metabolic osteopathy, cholestasis 7. Enteral nutrition cannot be established within 1 week after surgery
High risk	1. Body weight <1.0 kg and current weight <1.5 kg 2. Continuous total parenteral nutrition for more than 2 weeks 3. Months old: >2 months still need continuous parenteral nutrition (>2 weeks)

* Improved evaluation scale based on STRONGkids in our hospital.

Molecular detection of resistance genes and homology analysis of strains

Carbapenemase, extended spectrum β-lactamase and plasmid-mediated AmpC genes were investigated by polymerase chain reaction (PCR) with primers described in our previous study [5,11]. PCR amplicons were sequenced, and the resulting DNA sequences were compared with those available in the NCBI GenBank database using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multi-locus sequence typing (MLST) was carried out according to protocols available at the MLST Pasteur website (https://bigsdb.pasteur.fr/index.html). Diversilab typing was performed as previously described [12].

Statistical analysis

All data are expressed as the means ± standard deviation for continuous variables or percentages for categorical variables. Univariate analysis of risk factors was performed using χ^2/Fisher and unpaired Student's *t*-test to identify the risk factors for CR-KP infection. A *P*-value of <0.05 was regarded as indicative of statistical significance. Multivariable logistic regression analysis was performed on statistically significant risk factors for CR-KP infection to identify independent risk factors. All statistical analyses were performed with the statistical software SPSS 23.0.

Ethics statement

Only bacterial isolates recovered from routine screening and diagnostic laboratory tests were assessed in this study without direct use of clinical specimens. Patient consent was not required. The study was approved by the Ethics Committee of the Children's Hospital of Fudan University, Shanghai, China (approval number (2019)192).

Results

The clinical characteristics of CRE-colonized patients

During the study period, 3200 unique patients were admitted to the NICU, and 1230 unique patients were screened for CRE colonization. In total, 144 patients tested positive for CRE colonization, and the total CRE colonization incidence was 11.7% (144/1230), with 9.2% (110/1197) in the intestinal tract, higher than that in the upper respiratory tract (6.6%, 62/945) (*P*=0.026). Forty-four patients (30.6%, 44/144) were positive for CRE colonization on admission within 48 hours, and the colonization incidence was 3.6% (44/1230). In total, 548 unique patients were negative on their admission screens and remained in the NICU long enough to contribute additional swabs; 100 (18.2%) of them had a subsequent CRE-positive screening. The median time to CRE colonization was 13 days (interquartile range (IQR), five to 36 days).

The CRE colonization incidence increased with decreased gestational age and birth weight (Table II). Meanwhile, the CRE colonization incidence significantly increased with an increased hospital stay (Table II).

The probability from colonization to subsequent infection

Of the 144 patients who tested positive for CRE colonization (110 CR-KP, 29 carbapenem-resistant *Escherichia coli* (CR-ECO), and five carbapenem-resistant *Enterobacter cloacae* (CR-ECL)), 29 (23 CR-KP, six CR-ECO) patients had a subsequent symptomatic infection with positive clinically isolated strains at any time during their hospital stay.

To determine whether infections were caused by the patients' own colonizing bacteria, the 29 clonally related

Table II
Outcome of patients with carbapenem-resistant Enterobacterales (CRE) colonization

Variables	Incidences (CRE-positive number/ screening patient number)	*P*
Gender		
Male	12.2 (88/720)	0.505
Female	11.0 (56/510)	
Specimen type		
Rectal swab	9.2 (110/1197)	0.026
Pharyngeal swab	6.6 (62/945)	
Hospitalization time		
≤48 hours	3.6 (44/1230)	< 0.001
>48 hours	18.2 (100/548)	
Birth weight		
< 1000 g	24.0 (29/120)	< 0.001
1000–1499 g	19.4 (63/324)	
1500–2499 g	7.6 (31/411)	
2500–4000 g	5.7 (19/342)	
> 4000 g	6.7 (2/33)	
Gestational age		
< 28 weeks	25.5 (52/205)	< 0.001
≥28 weeks and < 32 weeks	19.2 (54/280)	
≥32 weeks and < 37weeks	5.6 (20/360)	
37–42 weeks	4.9 (18/365)	
> 42 weeks	0.0 (0/20)	
Intestinal tract colonization incidencein different length of hospital stay		
≤48 hours	3.3 (35/1033)	< 0.001
3–7 days	12.7 (8/63)	
8–14 days	20.4 (58/285)	
> 14	35.4 (62/175)	
Upper respiratory tract colonization incidence in different length of hospital stay		
≤48 hours	6.0 (45/743)	< 0.001
3–7 days	11.2 (6/50)	
8–14 days	12.0 (15/128)	
> 14	33.7 (36/108)	

The bold values are actually represent the p value < 0.05, which has statistical significance.

colonization and clinical infection counterparts were further analysed by MLST and Diversilab.

In total, four sequence types (STs) were identified in the CR-KP strains, with ST17, ST278, ST45 and ST846 in 30, 13, two and one strains, respectively (Figure 1). Six STs were identified in the CR-ECO strains, with ST692, ST833, ST58, ST73, ST33 and ST650 in five, two, two, one, one and one strains, respectively (Figure 2).

Diversilab homology monitoring found that 91.3% (21 of 23) of patients with CR-KP infection and 66.7% (four of six) of patients with CR-ECO infection were colonized by clonally related strains, showing an overall 17.4% (25/144) risk of infection with CRE among patients colonized with CRE. For CR-KP and CR-ECO, the risks were 19.1% (21/110) and 13.8% (4/29) among colonized patients, respectively. Furthermore, there were two types of CR-KP clonal dissemination; 28 ST17 and 12 ST278 CR-KP isolates, including both colonization and clinical infection isolates, from 14 and six patients, respectively, were identified in the same lineage with only small distances between them, all of which carried the bla_{NDM-1} gene encoding carbapenemase (Figure 1).

The total hospital stay for CRE-infected patients was 35 ± 8.8 days, and the average hospital stay from colonization to infection (day 1 was the date the initial rectal swab or pharyngeal swab was obtained) was 22.9 ± 5.8 days. The most common site of CR-KP infection was in the urinary tract, identified in 11 patients, followed by the lung (eight patients) and bloodstream (two patients). For CR-ECO, there were two cases in the urinary tract and lung. Four CR-KP-infected patients died, and the mortality rate was 19.0% (4/21).

Risk factors from colonization to subsequent infection of CR-KP

Of the 110 patients colonized with CR-KP, 21 had a subsequent symptomatic infection with clonally related colonization strains, 49 patients only had colonization during hospitalization, and the remaining 40 patients were associated with clinical infection at the time of colonization.

To determine risk factors for CR-KP infection among colonized patients, we compared the 21 CR-KP infection patients (case group) with the 49 patients who only had colonization during hospitalization (control group).

The average gestational age (30.3 vs 32.1 weeks) and birth weight (1708.8 vs 1840.8 grams) in the case group were lower than those in the control group, although there was no statistical significance ($P>0.05$). The total length of hospital stay (61.1 vs 42.0 days) and the length of stay after colonization (51.9 vs 32.2 days) in the case group were significantly longer than those in the control group ($P<0.05$). On univariate analysis, case group patients were more likely to have a history of hospitalization (61.9% vs 32.7%, $P=0.023$), being mechanically ventilated (81.0% vs 20.4%, $P<0.001$), having a nasogastric tube (66.7% vs 40.8%, $P=0.047$), having previous surgery (23.8% vs 4.1%, $P=0.037$), and having high neonatal nutritional risk assessment ($P=0.018$) and SNAP-II scores (17.2% vs 9.8%, $P=0.002$) (Table III). Multivariate analysis revealed that the variables that remained independent risk factors for CR-KP clinical infection among CR-KP carriers were having mechanical ventilation (odds ratio (OR), 10.177; 95% confidence interval (CI), 2.667–38.830; $P=0.013$) and high neonatal nutritional risk assessment (OR, 0.251; 95% CI, 0.072–0.881; $P=0.031$) and SNAP-II scores (OR, 0.256; 95% CI, 0.882–1.034; $P=0.025$) (Table IV).

Antimicrobial resistance characteristics and resistance genes of CRE colonization and infection strains

The resistance characteristics of 46 CR-KP and 12 CR-ECO isolates isolated from 29 (23 CR-KP, six CR-ECO) patients who

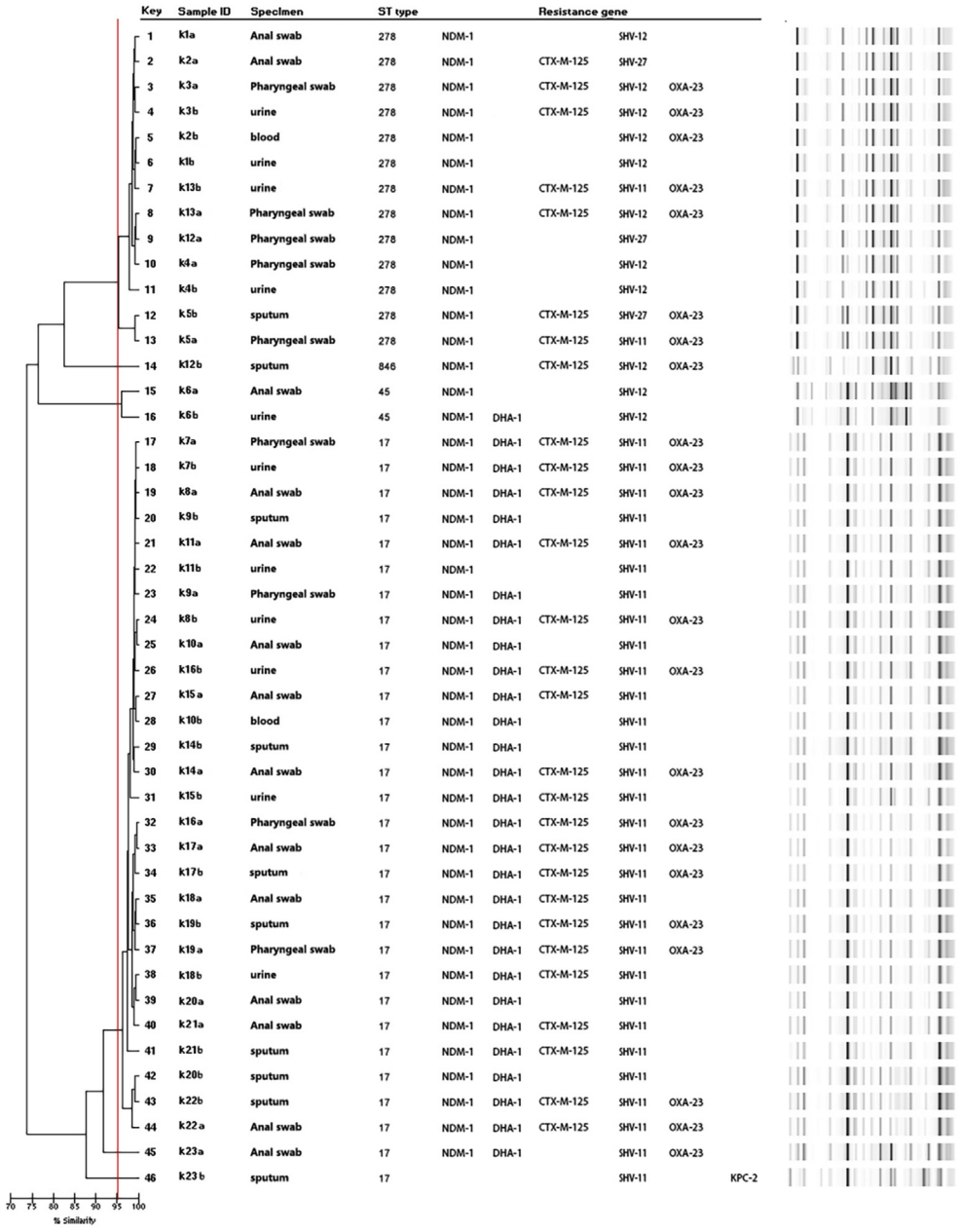

Figure 1. Diversilab patterns and gene distribution of 46 carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) colonization and infected isolates. The red line delineates the similarity score of 95% set by the manufacturer.

had a subsequent symptomatic infection with positive clinically isolated strains are shown in Table IV. The majority of CR-KP and CR-ECO strains were resistant to multiple antibiotics, with the exception of aminoglycosides and quinolones. The resistance rates to ertapenem, meropenem and imipenem were more than 70%, and the colonized and infected strains showed similar characteristics of drug resistance (Table V).

In total, eight types of resistance genes were detected in CR-KP strains, and 10 kinds of resistance genes were detected in CR-ECO strains (Table VI). The predominant carbapenemase genes were bla_{NDM-1} (97.8%, 45/46) in CR-KP strains and bla_{NDM-5} (69.2%, 9/12) in CR-ECO strains (Table VI). Among the 29 patients who developed subsequent infection from colonization, 16 CR-KP and three CR-ECO patients showed completely consistent resistance genes between colonized strains and clinically infected strains (Figures 1 and 2).

Discussion

In this study, we screened 1230 patients for CRE and found that 11.7% (144/1230) were colonized with CRE. In addition, 17.4% (25/144) of those carriers developed CRE clinical infection. A subsequent retrospective case—control study identified that receiving mechanical ventilation, malnutrition and critical conditions were independent risk factors for subsequent CR-KP infection.

Table III
Univariate analysis of risk factors associated with subsequential carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) clinical infection among CR-KP carriers

Variables	Case group [*] ($N = 21$)	Control group [†] ($N = 49$)	*P*
Gestational age, weeks	30.3 ± 3.9	32.1 ± 4.3	0.077
Birth weight, g	1708.8 ± 442.2	1840.8 ± 421.3	0.540
Low birth weight, *N* (%)	17 (81.0)	37 (75.5)	0.852
Premature, *N* (%)	19 (90.5)	40 (81.6)	0.566
Natural labor, *N* (%)	10 (47.6)	20 (40.8)	0.598
Receipt of glucocorticoid before birth, *N* (%)	10 (47.6)	21 (42.9)	0.713
Male sex, *N* (%)	13 (61.9)	26 (53.1)	0.495
Improvement prognosis, *N* (%)	19 (90.5)	47 (95.9)	0.736
Antimicrobial Exposure, *N* (%)	17 (81.0)	33 (67.3)	0.248
Prior exposure to carbapenems, *N* (%)	7 (33.3)	10 (20.4)	0.248
Maternal diseases during pregnancy[‡], *N* (%)	14 (66.7)	31 (63.3)	0.785
Previous hospitalization, *N* (%)	13 (61.9)	16 (32.7)	**0.023**
Receive intrusive operations, *N* (%)			
Mechanical ventilation	17 (81.0)	10 (20.4)	**<0.001**
Nasogastric tube	14 (66.7)	20 (40.8)	**0.047**
Umbilical venous catheter	2 (9.5)	3 (6.1)	1.000
Umbilical arteriosus catheter	2 (9.5)	0 (0.0)	0.159
Peripherally inserted central catheter	6 (28.6)	9 (18.4)	0.340
Previous surgery, *N* (%)	5 (23.8)	2 (4.1)	**0.037**
SNAP-II	17.2 ± 11.26	9.8 ± 7.32	**0.002**
Neonatal nutritional risk assessment, *N* (%)			
Low risk	1 (4.7)	18 (36.7)	**0.018**
Moderate risk	14 (66.7)	19 (38.8)	
High risk	6 (28.6)	12 (24.5)	
Total length of hospital stay, days	61.1 ± 15.6	42.0 ± 16.9	**0.015**
Hospital stay after colonization, days	51.9 ± 19.3	32.2 ± 14.3	**0.003**

The bold values are actually represent the p value < 0.05, which has statistical significance.
SNAP-II, Score for Neonatal Acute Physiology II.
[*] Patients who had subsequent infection of CR-KP from colonization during hospitalization.
[†] Patients who only screened positive but not had subsequent infection of CR-KP during hospitalization.
[‡] Including infection, diabetes, hypertension, hypothyroidism and hyperthyroidism.

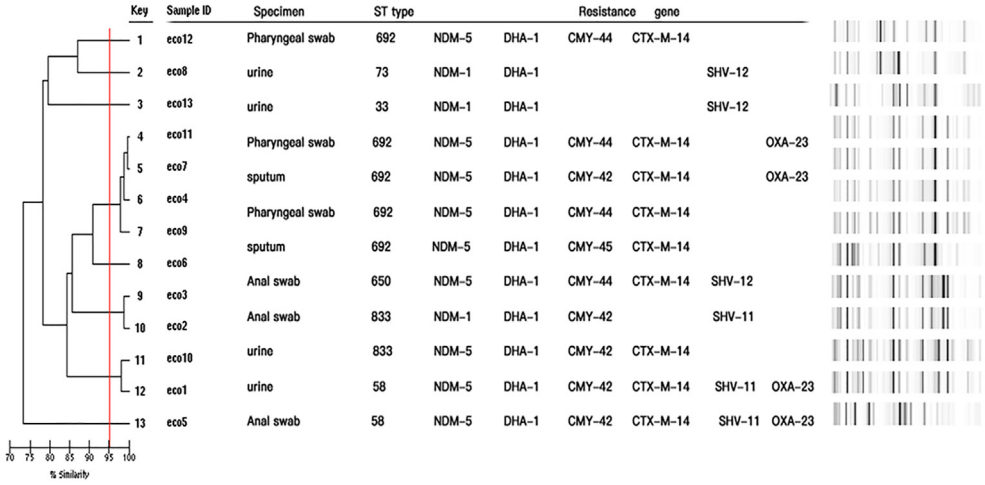

Figure 2. DiversiLab patterns and Gene distribution of 24 carbapenem-resistant *Escherichia coli* (CR-ECO) colonization and infected isolates. The red line delineates the similarity score of 95% set by the manufacturer.

Table IV
Multivariate analysis of risk factors associated with subsequential carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) clinical infection among CR-KP carriers

Covariate	OR (95% CI)	*P*
Previous surgery	1.857 (0.269—11.637)	0.509
Neonatal nutritional risk assessment	0.251 (0.072—0.881)	**0.031**
SNAP-II	0.256 (0.882—1.034)	**0.025**
Nasogastric tube	0.288 (0.073—1.127)	0.723
Mechanical ventilation	10.177 (2.667—38.830)	**0.013**
Previous hospitalization	0.826 (0.275—2.480)	0.733

CI, confidence interval; OR, odds ratio; SNAP-II,Score for Neonatal Acute Physiology II.

The most common site of screening for carriage was the gastrointestinal tract, specifically rectal swabs [13,14]. The initial carriage rate of CR-KP on admission to ICUs varied from less than 1% in South Korea [15] to more than 30% in Iran [16]. Furthermore, two Chinese-based studies reported, via random screening processes throughout the duration of ICU stays, frequencies of 6.5% [17] and 20.8% [18] in CR-KP carriage. In this study, we conducted active screening in both the upper respiratory tract and intestine and found that the total CRE colonization incidence was 11.7%, higher than that reported in neonates in an NICU in India (8.7, 26/300) [19] but lower than that in adults from Shanghai, in which intestinal or nasopharyngeal screening was carried out at the same time (15%, 37/243) [14]. Furthermore, we found that the CRE colonization incidence significantly increased with an increased hospital stay. Ruiz *et al.* also reported that the prevalence of colonization with multi-drug resistant *Klebsiella pneumonia* (MRKP) was more than 50% in patients who remained in the ICU for longer than three weeks [20], which suggested that the hospitalization environment increases the risk of colonization. Meanwhile, the CRE

Table VI
The resistant genes distribution of carbapenem-resistant Enterobacterales (CRE) colonization and subsequent infection isolates

Gene	CR-KP (%)		CR-ECO (%)	
	Infection	Colonization	Infection	Colonization
	(*N* = 23)	(*N* = 23)	(*N* = 6)	(*N* = 6)
Carbapenemase gene				
NDM-1	22 (95.7)	23 (100)	2 (33.3)	1 (16.7)
NDM-5	—	—	4 (66.7)	5 (83.3)
OXA-23	11 (47.8)	12 (52.2)	2 (33.3)	2 (33.3)
KPC-2	1 (4.3)	—	—	—
Extended spectrum β-lactamase gene				
SHV-11	16 (69.6)	16 (69.6)	1 (16.7)	2 (33.3)
SHV-12	6 (26.1)	5 (21.7)	2 (33.3)	1 (16.7)
SHV-27	1 (4.3)	2 (8.7)	—	—
CTX-M-125	13 (56.5)	15 (65.2)	—	—
CTX-M-14	—	—	4 (66.7)	5 (83.3)
Plasmid-mediated AmpC gene				
DHA-1	14 (60.9)	15 (65.2)	6 (100.0)	6 (100.0)
CMY-42	—	—	3 (50.0)	2 (33.3)
CMY-44	—	—	—	4 (66.7)
CMY-45	—	—	1 (16.7)	—

CR-ECO, carbapenem-resistant *Escherichia coli*; CR-KP, carbapenem-resistant *Klebsiella pneumoniae*.

Table V
Antibiotic resistance rates of carbapenem-resistant Enterobacterales (CRE) colonization and subsequent infection isolates (μg/mL)

Antibiotics	CR-KP (%)		CR-ECO (%)	
	Colonization (*N* = 23)	Infection (*N* = 23)	Colonization (*N* = 6)	Infection(*N* = 6)
Ampicillin	—*	—*	100	100
Ampicillin—sulbactam	100	100	83.3	83.3
Piperacillin—Tazobactam	91.3	95.7	66.7	66.7
Cefazolin	100	100	66.7	83.3
Cefuroxime	100	100	83.3	83.3
Ceftazidime	100	100	83.3	83.3
Ceftriaxone	100	100	100	100
Cefepime	60.9	65.2	83.3	83.3
Ertapenem	87	91.3	100	100
Imipenem	73.9	82.6	83.3	83.3
Meropenem	73.9	78.3	83.3	83.3
Amikacin	0	0	16.7	16.7
Gentamicin	0	0	50	66.7
Ciprofloxacin	0	0	66.7	66.7
Levofloxacin	0	0	66.7	83.3
Trimethoprim/sulfamethoxazole	4.3	8.7	66.7	66.7

CR-ECO, carbapenem-resistant *Escherichia coli*; CR-KP, carbapenem-resistant *Klebsiella pneumoniae*.
* Intrinsic resistance.

colonization incidence increased with decreased gestational age and birth weight, which suggested that premature delivery and low birth weight are risk factors for CRE colonization.

As CRE prevalence is increasing in paediatric populations, especially in NICU patients who had the highest proportion of CRE nosocomial infections and showed different CRE molecular characteristics from non-neonatal patients [5], identifying risk factors for CRE infection in those patients and classifying genotypes are priorities in this vulnerable population. Although there have been reports of CRE colonization in neonatal patients [21,22], the relevant reports specifically associated with progression from colonization to infection in NICU patients have not been elucidated. Compared with limited studies seeking to identify risk factors for progressing to clinical infection among adult CR-KP carriers, which are limited to faecal carriage [23,24] and mainly focused on certain types of infection [19,25,26], this study included patients originating from the upper respiratory tract and intestinal tract as well as various clinical infections. The results showed that colonization with CRE posed an overall 17.4% risk of subsequent infection. For most other studies, the rates ranged from 7.6% to 44.4% [7]. In this study, we found that the CRE colonization incidence increased with decreased gestational age and birth weight. However, when analysing the risk factors for the development of colonization into infection, we found that birth weight and gestational age were not statistically significant. For the first time, we found that nutritional status and critical status were independent risk factors for subsequent CRE infection in neonatal patients. Similar to adult studies [24], we also found that mechanical ventilation was an independent risk factor for subsequent CRE infection. This shows that, to reduce the incidence of nosocomial infections, stricter nursing operations and nosocomial infection control measures will be needed in those patients.

In this study, the predominant carbapenemase genes were bla_{NDM-1} in CR-KP strains and bla_{NDM-5} in CR-ECO strains. In addition, the majority of CR-KP and CR-ECO strains were resistant to multiple antibiotics, and the colonized and infected strains showed similar characteristics of drug resistance. Bla_{NDM-1} is the main reported CR-KP gene in neonatal patients [27], while bla_{KPC-2} has been reported in adults and older children [5,28]. bla_{NDM-1} was also the dominant carbapenemase gene in CR-KP isolates in our study, which is similar to our previous study [5,11] and other reports from China [25]. In addition, one CR-KP strain expressing the bla_{KPC-2} gene was found in our study. Compared with other carbapenemase genes, bla_{KPC} shows stronger virulence and transmission performance, with several hospital outbreaks (most often due to KPN with bla_{KPC-2}) reported in adult hospitals [28,29].

In this study, NDM-producing ST17 CR-KP was the most common MLST type (71.0%) in CR-KP isolates, followed by ST278, while ST278 (53.7%) was the predominant genotype from November 2015 to October 2016 [11]. This shows that the molecular type of the strains is also evolving, and active screening and dynamic monitoring of molecular typing are necessary. ST17 belongs to the well-known hypervirulent CC17 lineage. Due to its epidemiological relevance, type III/ST-17 has been defined as hypervirulent, and high invasiveness is presumably associated with additional virulence factors in addition to CPS [30].

In contrast to other previous studies, we also investigated the clonal relatedness between the colonization isolates and subsequent infection isolates using Diversilab, and 91.3% (21 of 23) of patients with CR-KP infection were colonized by clonally related strains, which further suggests that the colonization of CRE may serve as a reservoir for infection. In addition, horizontal transmission between patients was also detected, which generates an alert that strict infection control measures should be implemented.

The limitation of this study is that it was a single-center study, and the limitation of patient populations may have resulted in the conclusion lacking generalization. In addition, due to the limitation of retrospective studies, there was a lack of sampling and analysis of the surrounding environment/hand hygiene of the corresponding clone strains at the corresponding time; thus, it was impossible to determine the transmission route between strains.

In this study, we evaluated the prevalence associated with CRE carriage on admission to the NICU as well as that of CRE carriage weekly throughout the course of the NICU stay to assess the CRE colonization rate. Furthermore, we characterized and compared the resistance genotypes as well as the homology of the CRE colonization and clinical infection isolates to evaluate the risk from colonization to infection. In addition, we summarized the related risk factors for subsequent CR-KP infection, and for the first time, we found that nutritional status and critical status were independent risk factors for subsequent CR-KP infection in NICU patients.

In conclusion, the colonization of CRE can increase the incidence of corresponding CRE infection in NICU patients. Unlike in adult patients, preterm delivery and low birth weight are risk factors for colonization, while basic disease status and nutritional status play a more important role in the process of colonization to the subsequent infection. Apart from colonized strains, horizontal transmission was also detected, which generates an alert that strict infection control measures should be implemented.

Author's statement

Lijun Yin: Conceptualization, Methodology, Validation, Formal analysis, Writing - Original Draft
Leiyan He: Investigation, Resources
Jin Miao: Project administration
Weiqing Yang: Project administration
Xiaohua Wang: Project administration
Jian Ma: Project administration
Nana Wu: Formal analysis
Yun Cao: Conceptualization, Supervision
Chuanqing Wang: Conceptualization, Supervision, Project administration

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Conflict of interest statement

All authors declare that they have no conflicts of interest.

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References

[1] Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 2008;52(3):1028—33.

[2] Borer A, Saidel-Odes L, Riesenberg K, Eskira S, Peled N, Nativ R, et al. Attributable mortality rate for carbapenem-resistant Klebsiella pneumoniae bacteremia. Infect Control Hosp Epidemiol 2009;30(10):972—6.

[3] Martín-Loeches I, Diaz E, Vallés J. Risks for multidrug-resistant pathogens in the ICU. Curr Opin Crit Care 2014;20(5):516—24.

[4] McCann E, Srinivasan A, DeRyke CA, Ye G, DePestel DD, Murray J, et al. Carbapenem-Nonsusceptible Gram-Negative Pathogens in ICU and Non-ICU Settings in US Hospitals in 2017: A Multicenter Study. Open Forum Infect Dis 2018;5(10):ofy241.

[5] Yin L, He L, Miao J, Yang W, Wang X, Ma J, et al. Actively surveillance and appropriate patients placements' contact isolation dramatically decreased Carbapenem-Resistant Enterobacteriaceae infection and colonization in pediatric patients in China. J Hosp Infect 2020;105:P486—94.

[6] Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Front Cell Infect Microbiol 2018;8:4.

[7] Tischendorf J, de Avila RA, Safdar N. Risk of infection following colonization with carbapenem-resistant Enterobactericeae: a systematic review. Am J Infect Control 2016;44(5):539—43.

[8] Clinical and Laboratory Standards Institute. M100-S11, Performance standards for antimicrobial susceptibility testing. Clin Microbiol Newsl 2011;23(6):49.

[9] Lara-Pompa NE, Hill S, Williams J, Macdonald S, Fawbert K, Valente J, et al. Use of standardized body composition measurements and malnutrition screening tools to detect malnutrition risk and predict clinical outcomes in children with chronic conditions. Am J Clin Nutr 2020;112:1456—67.

[10] Richardson DK, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: simplified newborn illness severity and mortality risk scores. J Pediatr 2001;138(1):92—100.

[11] Yin D, Zhang L, Wang A, He L, Cao Y, Hu F, et al. Clinical and molecular epidemiologic characteristics of carbapenem-resistant Klebsiella pneumoniae infection/colonization among neonates in China. J Hosp Infect 2018;100(1):21—8.

[12] Werner G, Fleige C, Neumann B, Bender JK, Layer F, Klare I. Evaluation of DiversiLab®, MLST and PFGE typing for discriminating clinical Enterococcus faecium isolates. J Microbiol Meth 2015;118:81—4.

[13] Wiener-Well Y, Rudensky B, Yinnon AM, Kopuit P, Schlesinger Y, Broide E, et al. Carriage rate of carbapenem-resistant Klebsiella pneumoniae in hospitalised patients during a national outbreak. J Hosp Infect 2010;74(4):344—9.

[14] Qin X, Wu S, Hao M, Zhu J, Ding B, Yang Y, et al. The colonization of carbapenem-resistant Klebsiella pneumoniae: epidemiology, resistance mechanisms, and risk factors in patients admitted to intensive care units in China. J Infect Dis 2020;221(Supplement_2):S206—14.

[15] Kim J, Lee JY, Kim SI, Song W, Kim JS, Jung S, et al. Rates of fecal transmission of extended-spectrum β-lactamase-producing and carbapenem-resistant Enterobacteriaceae among patients in intensive care units in Korea. Ann Lab Med 2014;34(1):20—5.

[16] Solgi H, Badmasti F, Aminzadeh Z, Giske CG, Pourahmad M, Vaziri F, et al. Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: first report of co-production of bla NDM-7 and bla OXA-48. Eur J Clin Microbiol Infect Dis 2017;36(11):2127—35.

[17] Zhao ZC, Xu XH, Liu MB, Wu J, Lin J, Li B. Fecal carriage of carbapenem-resistant Enterobacteriaceae in a Chinese university hospital. Am J Infect Control 2014;42(5):e61—4.

[18] Shu LB, Lu Q, Sun RH, Lin LQ, Sun QL, Hu J, et al. Prevalence and phenotypic characterization of carbapenem-resistant Klebsiella pneumoniae strains recovered from sputum and fecal samples of ICU patients in Zhejiang Province, China. Infect Drug Resist 2018;12:11—8.

[19] Singh NP, Choudhury DD, Gupta K, Rai S, Batra P, Manchanda V, et al. Predictors for gut colonization of carbapenem-resistant Enterobacteriaceae in neonates in a neonatal intensive care unit. Am J Infect Control 2018;46(6):e31—5.

[20] Ruiz J, Gordon M, Villarreal E, Frasquet J, Sánchez MÁ, Martín M, et al. Influence of antibiotic pressure on multi-drug resistant Klebsiella pneumoniae colonisation in critically ill patients. Antimicrob Resist Infect Control 2019;8:38.

[21] Zhou J, Li G, Ma X, Yang Q, Yi J. Outbreak of colonization by carbapenemase-producing Klebsiella pneumoniae in a neonatal intensive care unit: Investigation, control measures and assessment. Am J Infect Control 2015;43(10):1122—4.

[22] Arhoune B, Oumokhtar B, Hmami F, Barguigua A, Timinouni M, El Fakir S, et al. Rectal carriage of extended-spectrum β-lactamase- and carbapenemase-producing Enterobacteriaceae among hospitalised neonates in a neonatal intensive care unit in Fez, Morocco. J Glob Antimicrob Resist 2017;8:90—6.

[23] Liu Q, Liu L, Li Y, Chen X, Yan Q, Liu WE. Fecal carriage and epidemiology of carbapenem-resistant Enterobacteriaceae among hospitalized patients in a university hospital. Infect Drug Resist 2019;12:3935—42.

[24] Chen X, Liu Q, Liu WE, Yan Q. Risk factors for subsequential carbapenem-resistant Klebsiella pneumoniae clinical infection among rectal carriers with carbapenem-resistant Klebsiella pneumoniae. Infect Drug Resist 2020;13:1299—305.

[25] Giannella M, Trecarichi EM, De Rosa FG, Del Bono V, Bassetti M, Lewis RE, et al. Risk factors for carbapenem-resistant Klebsiella pneumoniae bloodstream infection among rectal carriers: a prospective observational multicentre study. Clin Microbiol Infect 2014;20(12):1357—62.

[26] Dong F, Zhang Y, Yao K, Lu J, Guo L, Lyu S, et al. Epidemiology of carbapenem-resistant Klebsiella pneumoniae bloodstream infections in a Chinese children's hospital: predominance of New Delhi metallo-β-lactamase-1. Microb Drug Resist 2018;24(2):154—60.

[27] Qin S, Fu Y, Zhang Q, Qi H, Wen JG, Xu H, et al. High incidence and endemic spread of NDM-1-positive Enterobacteriaceae in Henan Province, China. Antimicrob Agents Chemother 2014;58(8):4275—82.

[28] Sun K, Chen X, Li C, Yu Z, Zhou Q, Yan Y. Clonal dissemination of multilocus sequence type 11 Klebsiella pneumoniae carbapenemase-producing K. pneumoniae in a Chinese teaching hospital. APMIS 2015;123(2):123—7.

[29] Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis 2009;9(4):228—36.

[30] Jones N, Oliver KA, Barry J, Harding RM, Bisharat N, Spratt BG, et al. Enhanced invasiveness of bovine-derived neonatal sequence type 17 group B streptococcus is independent of capsular serotype. Clin Infect Dis 2006;42(7):915—24.