Infection and Drug Resistance

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ORIGINAL RESEARCH

Fecal Carriage and Molecular Epidemiology of Carbapenem-Resistant *Enterobacteriaceae* from Inpatient Children in a Pediatric Hospital of Shanghai

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Purpose: To determine the epidemiology characteristics of intestinal colonization of carbapenem-resistant *Enterobacteriaceae* (CRE) among inpatients in a pediatric hospital in China.

Methods: A retrospective study was conducted from April to December 2019. Medical records were reviewed to extract the clinical information. Antimicrobial susceptibility was performed by broth microdilution method. Drug resistance determinants and plasmid types were analyzed using polymerase chain reaction (PCR) assays. Multilocus sequence typing (MLST) and Enterobacterial repetitive intergenic consensus sequences PCR (ERIC-PCR) were employed to determine the genetic relationships between strains.

Results: A total of 90 CRE strains were isolated, with a fecal carriage rate of 8.6% (90/1052), and mainly distributed in *E. aerogenes* (n=30), *K. pneumoniae* (n=25) and *E. coli* (n=23). More than 50% of CRE colonizers had a history of invasive procedures and antibiotic exposures. As high as 91.1% (82/90) of CRE isolates carried carbapenemase genes, with $bla_{\text{NDM-5}}$ (n=56) being the most common, and mainly found in *E. aerogenes* (51.8%, 29/56) and *E. coli* (32.1%, 18/56) isolates, which primarily belonged to ST4 (100%, 29/29) and ST692 (55.6%, 10/18), respectively. Followed by $bla_{\text{KPC-2}}$ (n=12), and all found in *K. pneumoniae* ST11 isolates. Other carbapenemase genes including $bla_{\text{NDM-1}}$, $bla_{\text{IMP-4}}$ and $bla_{\text{IMP-26}}$. Meanwhile, ESBL genes ($bla_{\text{CTX-M}}$, $bla_{\text{TEM-1}}$ and bla_{SHV}) and AmpC genes ($bla_{\text{DHA-1}}$ and bla_{EBC}) were also detected. All CRE isolates showed high resistance to cephalosporins and carbapenemases (97.8%-100.0%) but remained susceptible to tigecycline (98.9%). IncX3 was a major plasmid type in NDM-containing strains (91.3%), and 91.7% of KPC-2-producing *K. pneumoniae* harboring IncFII and IncFIB plasmids. The ERIC-PCR revealed that several strains with identical STs were genetically similar.

Conclusion: This study revealed a major intestinal colonization of ST4 NDM-5 *E. aerogenes*, ST11 KPC-2 *K. pneumoniae* and ST692 NDM-5 *E. coli* strains among inpatients in a pediatric hospital. Infection control measures should be implemented immediately to prevent the spread of these strains in clinical settings.

Keywords: intestinal colonization, hospitalized children, ST4, *E. aerogenes*, NICU, ERIC-PCR

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Introduction

Carbapenems used to be the most effective agents for the treatment of multi-drug resistant (MDR) bacterial infections, but the emergence and dissemination of carbapenem resistance have been increasingly reported across the world since

1990s.[1,2] The global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is one of the most severe threats to human health in clinical settings. According to the nationwide surveillance of bacterial resistance data in China, the resistance rate of *K. pneumoniae* to imipenem and meropenem increased to 25% and 26.3% in 2018, respectively, from 3.0% and 2.9% in 2005.[3] In Europe, the prevalence of carbapenem-resistant *K. pneumoniae* (CR-KPN) has risen to 60% in Greece and 40% in Italy already.[4,5] The production of carbapenemases (KPC, NDM, and OXA-48-like) is the major mechanism among CRE isolates,[6,7] and the rapid worldwide spread of CRE largely attributes to the dissemination of carbapenemase enzymes.[8]

In the last 10–15 years, CRE has become a major cause of healthcare-associated infections (HAI).[9] Intestinal colonization with CRE has been considered as a significant risk factor for subsequent infection, a previous cohort study demonstrated that nearly 50% CRE-colonized patients developed a CRE infection within 30 days compared to non-colonized patients, and the odds of infection increased by 10.8 times.[10] As we all know, patients with CRE colonization or infection have been associated with higher healthcare costs, prolonged hospital stays, treatment failures and mortality, as well as wide usage of broad-spectrum antimicrobial agents.[11] Several studies have been conducted to investigate the epidemiology characteristics of intestinal colonization of CRE strains in hospitalized adults.[12,13] However, the fecal carriage of CRE in hospitalized children has not been well studied, especially in China. Therefore, our study was conducted to determine the clinical characteristics, antimicrobial resistance profiles and molecular epidemiology of intestinal colonization of CRE among inpatients in a pediatric hospital in Shanghai, China.

Methods

Samples Collection and Strains Screening

The study was performed from April to December 2019 retrospectively in Shanghai Children's Hospital, which is a 700-bed specialized pediatric teaching hospital, serving a population of more than 2.4 million children annually, and nearly 50,000 are inpatients. We consulted electronic medical record of each inpatient for clinical information. This study was reviewed and approved by the Ethics Committee of Shanghai Children's Hospital in accordance with the Declaration of Helsinki and its amendments or comparable ethical standards. The patient informed consent was waived because our study only focused on the bacterial isolates and did not have an influence on the patients.

Fecal swabs were consecutively collected from hospitalized children who received the fecal culture testing, and stored in Cary-Blair transport medium (Hopebio, Qingdao, China) in 4°C condition up to 1 day prior to testing. For each patient, only the first fecal sample was considered. The CRE isolates were detected by inoculating in home-made MacConkey agar supplemented with meropenem at 1μg/mL and then incubated at 35±2°C in a 5% CO_2 incubator for 24–48 h, the growth of at least one colony per agar plate was considered as a positive result. All strains screened were stored at −80°C in 40% glycerol broth medium for further analysis.

CRE Identification and Antimicrobial Susceptibility Testing

All screened strains were identified by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry using MALDI Biotyper (Bruker Daltonik GmbH, Bremen, Germany). A panel of 17 antimicrobial agents were used to determine the antimicrobial susceptibility of all CRE isolates, including cefotaxime, ceftazidime, cefoperazone, cefepime, ertapenem, imipenem, meropenem, ciprofloxacin, cefoperazone-sulbactam, piperacillin–tazobactam, amikacin, gentamicin, trimethoprim-sulfamethoxazole, aztreonam, ceftazidime-avibactam, fosfomycin, colistin and tigecycline. The minimum inhibitory concentrations (MICs) was determined by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints, and *E. coli* strain ATCC 25922 was used for quality control. The interpretive criterion for colistin and tigecycline was based on the breakpoints of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Food and Drug Administration (FDA), respectively. MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories.[14]

Detection of Resistance Genes of CRE

Carbapenemase genes (bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{AIM}, bla_{GIM}, and bla_{SIM}), ESBL genes (bla_{CTX-M}, bla_{TEM} and bla_{SHV}), AmpC genes (bla_{MOX}, bla_{FOX}, bla_{DHA}, bla_{CIT}, bla_{AAC} and bla_{EBC}) and the colistin resistance genes (mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5) were identified by PCR assays. Primer design and

amplification conditions were based on the previous reports.[15–19] The amplified products were sequenced by Sangon Biotech (Shanghai) Co. Ltd., and then compared to the sequences available at the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Plasmid Replicon Typing

For all CRE isolates, the plasmids were typed based on their incompatibility group using PCR-based replicon typing (PBRT), and a total of 21 pairs of primers (HI1, HI2, I1, L/M, N, FIA, FIB, W, Y, P, FIC, A/C, T, FIIAs, F, K, B/O, X1, X2, X3 and X4) were amplified as previously described.[20,21] The amplified products were then electrophoresed in 1.5% agarose gels, stained with GeneGreen Nucleic Acid Gel Stain (TIANGEN, Beijing, China) and visualized under ultraviolet light using the Gel Doc 2000 system (Bio-Rad).

Multilocus Sequence Typing (MLST)

MLST was performed using the schemes hosted on the Pub MLST website (https://pubmlst.org/) and Institute Pasteur MLST website (https://bigsdb.pasteur.fr/). The housekeeping genes of *E. aerogenes* and *E. Cloacae* (*dnaA, fusA, gyrB, leuS, pyrG, rplB* and *rpoB), K. pneumoniae* (*ropB, gapA, mdh, pgi, phoE, infB* and *tonB*) and *E. coli* (*dinB, icdA, pabB, polB, putB, trpA, trpB* and *uidA*) were amplified and sequenced, then the STs were obtained by comparing the sequences in the MLST database.

Enterobacterial Repetitive Intergenic Consensus Sequences PCR (ERIC-PCR)

The ERIC-PCR was applied using the ERIC-F (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC-R (5′-AAGTAAGTGACTGGGGTGAGCG-3′) primers for *E. aerogenes, K. pneumoniae* and *E. coli* isolates, and the amplification conditions were adjusted according to the reports published previously.[22,23] The gel electrophoresis image was analyzed by BioNumerics7.6 version software (Applied Maths; NV Keistraat, Sint-Martens-Latem, Belgium). Dice coefficients were used to calculate the similarity of ERIC-PCR patterns, of which optimization set at 1% and tolerance at 1%. Dendrograms were constructed by the unweighted pair group method with arithmetic averages (UPGMA) and isolates were categorized into the same cluster with a cutoff value of 90% similarity. Identical strains were defined as isolates with 100% similarity.

Statistical Analysis

Data were described as median and interquartile ranges (25th and 75th percentile), the number of cases or percentages. The quantitative data were compared by *t*-test and variance analysis, categorical data were evaluated by Chi-square or Fisher's exact test. A P-value of <0.05 was considered statistically significant, and all statistical analysis was performed using SPSS version 26.0 software (IBM, Armonk, NY). The antibiotic resistance data were analyzed with WHONET 5.6.

Results

Samples Collection and CRE Distribution

A total of 1612 consecutive and non-duplicate fecal samples were collected from inpatients between April to December in 2019, getting rid of 560 samples from patients hospitalized for less than 48 h, there were totally 90 CRE strains isolated as a carriage rate of 8.6% (90/1052), including *E. aerogenes* (n=30, 33.3%), *K. pneumoniae* (n=25, 27.8%), *E. coli* (n=23, 25.6%), *E. cloacae* (n=9), *K. oxytoca* (n=2) and *C. freundii* (n=1). The CRE strains mainly distributed in neonatal intensive care unit (NICU) (45.6%, 41/90), pediatric intensive care unit (PICU) (22.2%, 20/90) and gastroenterology ward (20.0%,18/90) (Figure 1). It is worth mentioning that, compared to *E. aerogenes* isolates, more *K. pneumoniae* strains were isolated from PICU than NICU (P<0.05), and 43.5% (10/23) and 30.4% (8/23) of *E. coli* isolates originated from NICU and PICU, respectively. In addition, we also found CRE isolates in respiratory, hematology, special consultation, nephrology and general surgery wards.

Clinical Characteristics

As shown in Table 1, the median age of patients colonized with CRE isolates was 2 months (interquartile range, 0.7–12 months), male to female ratio was 1.8 to 1. Compared to patients carried *E. aerogenes* and *E. coli* isolates, children who colonized with *K. pneumoniae* had a longer hospitalization time (P<0.05). The median length from admission to CRE detected was 12 days. Many children were accompanied by severe underlying disease such as gastrointestinal diseases (22.2%), Immunocompromised (12.2%), neonatal respiratory distress syndrome (NRDS) (12.2%) and chronic heart disease (CHD) (4.4%). More than 50% CRE

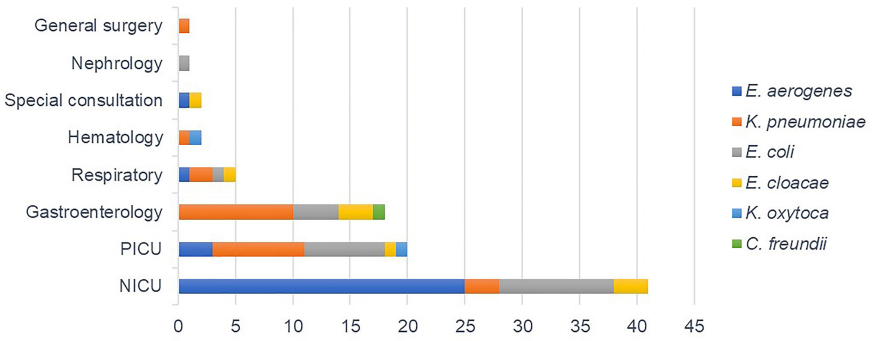

Figure 1 Distribution of CRE strains in different departments.
Abbreviations: PICU, pediatric intensive care unit; NICU, neonatal intensive care unit.

colonizers had a history of invasive procedures and antibiotic exposures. In addition, the total cure and death rate were 81.1% and 6.7%, respectively.

We also retrospectively reviewed the clinical information of patients with CRE colonized in NICU (Table 2) and observed an interesting phenomenon that the birth weight of *E. coli* colonizers (Median, 1360 g) were lighter than the *E. aerogenes* colonizers (Median, 1630g) (P<0.05), and the gestational age was also younger (P<0.05), the Apgar Score at 5 min was 8. Breastfeeding was the main feeding options in newborns (68.3%), and 5 neonates at birth were also found to be colonized with CRE. During pregnancy, the diseases of pre-eclampsia (24.4%) and gestational diabetes (22.0%) were more common, and dexamethasone was the most frequently used agent (19.5%), followed by antihypertensives (12.2%) and antibiotics (9.8%). A large proportion of neonates were born via cesarean delivery (61.0%).

Carbapenemase and Other Resistance Genes

As high as 91.1% (82/90) of CRE isolates carried carbapenemase genes, with $bla_{\text{NDM-5}}$ (n=56) being the predominant carbapenemase genotype. The $bla_{\text{NDM-5}}$ gene was most common in *E. aerogenes* (51.8%, 29/56) and *E. coli* isolates (32.1%, 18/56), which was also observed in *K. pneumoniae* (n=6) and *E. cloacae* isolates (n=3). The second most common gene was $bla_{\text{KPC-2}}$ (n=12), which was all found in *K. pneumoniae* isolates. In addition, 6 strains harbored $bla_{\text{NDM-1}}$, including 2 *E. coli*, 2 *E. cloacae*, 1 *K. oxytoca* and 1 *C. freundii* isolates. Moreover, 2 *K. pneumoniae* isolates carried $bla_{\text{IMP-4}}$, and 4 strains possessed both $bla_{\text{NDM-1}}$ and $bla_{\text{IMP-4}}$, containing *K. pneumoniae* (n=2), *E. cloacae* (n=2) and *K. oxytoca* (n=2) isolates, but the $bla_{\text{IMP-26}}$ was only identified in *E. cloacae* isolates.

Meanwhile, ESBL genes such as $bla_{\text{CTX-M-14}}$ (n=62), $bla_{\text{TEM-1}}$ (n=61) and $bla_{\text{CTX-M-15}}$ (n=51) were also detected in these CRE isolates, and $bla_{\text{SHV-11}}$ and $bla_{\text{SHV-1}}$ were mainly found in *K. pneumoniae* isolates (21/30), besides, $bla_{\text{SHV-12}}$ (n=4) and $bla_{\text{SHV-5}}$ (n=1) were only observed in *E. cloacae* isolates. What's more, 39 CRE strains carried AmpC genes, including $bla_{\text{DHA-1}}$ found in *K. pneumoniae* (n=16) and *E. cloacae* (n=5) isolates, and *blaEBC* found in *E. aerogenes* (n=17) and *E. coli* (n=1) isolates, respectively. Eight out of 90 CRE isolates including 3 *K. pneumoniae*, 3 *E. coli*, 1 *E. aerogenes* and 1 *E. cloacae* without carbapenemase genes, but we detected ESBL and (or) AmpC genes in these strains, such as $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-11}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{DHA-1}}$. As high as 41.1% (n=37) of the CRE isolates harbored carbapenemase, ESBL and together with AmpC genes, consisting of 17 *E. aerogenes* (56.7%), 14 *K. pneumoniae* (56.0%), 5 *E. cloacae* and 1 *E. coli*. However, no mcr gene was identified in this study.

In vitro Antimicrobial Susceptibility

All CRE isolates showed high resistance to the third or fourth-generation cephalosporins and carbapenemases (97.8%-100.0%) (Table 3), as well as cefoperazone-sulbactam (98.9%) and piperacillin–tazobactam (98.9%). The resistance rate of ciprofloxacin and trimethoprim-sulfamethoxazole was 93.3% and 53.3%, respectively. A small portion of Metallo-β-lactamases (MBL)-producing CRE isolates showed resistance to amikacin (15.7%) and gentamicin (31.4%), but the result was quite opposite among KPC-2 containing strains (83.3% and 91.7%) (p<0.05). In addition, 12.9% (9/70) of MBL-containing strains were found to be sensitive to aztreonam. Ceftazidime-avibactam showed better activity against the KPC-2-producing CRE

Table 1 The Clinical Characteristics of CRE Carriers

Characteristics	Total (n=90)	*E. aerogenes* Carriers (n=30)	*K. pneumoniae* carriers (n=25)	*E. coli* Carriers (n=23)	P-value*
Age(m)[a]	2(0.7–12)	0.9(0.4–1)	16(5–60)	3(0.7–12.5)	0.000
Male gender	58(64.4%)	16(53.3%)	17(68.0%)	15(65.2%)	0.490
Length of stay (d)[a]	31(16–74)	30(15–74)	35(25–77)	25(9–77)	0.012
Length from admission to CRE detected(d)[a]	12(3–34)	15(3–32)	8(3–48)	9(3–26)	0.075
Underlying Condition					
Gastrointestinal Diseases	20(22.2%)	5(16.7%)	6(24.0%)	5(21.7%)	0.787
Immunocompromised	11(12.2%)	3(10.0%)	4(16.0%)	2(8.7%)	0.691
NRDS	7(12.2%)	1(3.3%)	2(8.0%)	3(13.0%)	0.420
CHD	4(4.4%)	1(3.3%)	0(0.0%)	1(4.3%)	0.600
Invasive Procedures b					
Intubation/Mechanical ventilate	52(57.8%)	21(70.0%)	11(44.0%)	16(69.6%)	0.112
Lumbar puncture	34(37.8%)	14(46.7%)	9(36.0%)	8(34.8%)	0.612
Central venous catheter	27(30.0%)	11(36.7%)	5(20.0%)	9(39.1%)	0.363
Surgery	6(6.7%)	3(10.0%)	1(4.0%)	1(4.3%)	0.592
Urinary catheterization	5(5.4%)	2(6.7%)	2(8.0%)	1(4.3%)	0.873
Abdominal drainage	2(2.2%)	0(0.0%)	1(4.0%)	0(0.0%)	0.342
Antibiotic Exposures c					
β-lactam/β-lactamase inhibitor	47(52.2%)	13(43.3%)	10(40.0%)	8(34.8%)	0.809
Cephalosporins	32(35.6%)	11(36.7%)	9(36.0%)	9(39.1%)	0.973
Carbapenems	15(16.7%)	5(16.7%)	6(24.0%)	2(8.7%)	0.364
Fluoroquinolones	6(6.7%)	4(13.3%)	2(8.0%)	0(0.0%)	0.195
Vancomycin	6(6.7%)	0(0.0%)	4(16.0%)	1(4.3%)	0.049
Fosfomycin	5(5.6%)	2(6.7%)	3(12.0%)	0(0.0%)	0.237
Outcome					
Cure	73(81.1%)	25(83.3%)	18(72.0%)	20(87.0%)	0.381
Death	6(6.7%)	3(10.0%)	3(12.0%)	0(0.0%)	0.247

Notes: [a] Median and interquartile ranges (25th and 75th percentile); [b] Invasive testing or treatment before CRE detected; [c] At least 1 antibiotic dose used before CRE detected. * A comparison between three groups. A P-value of <0.05 was considered statistically significant. The P-value (sig) of age between *E. aerogenes* and *K. pneumoniae* carriers, *E. aerogenes* and *E. coli* carriers, *K. pneumoniae and E. coli* carriers were 0.000, 0.006, and 0.018, respectively. The P-value (sig) of Length of stay between *E. aerogenes* and *K. pneumoniae* carriers, *E. aerogenes* and *E. coli* carriers, *K. pneumoniae and E. coli* carriers were 0.019, 0.800, and 0.028, respectively.

Abbreviations: m, month; d, day; PICU, pediatric intensive care unit; NICU, neonatal intensive care unit; NRDS, neonatal respiratory distress syndrome; CHD, Chronic Heart Disease.

isolates than the MBL-producing strains (p<0.05). The in vitro activity of fosfomycin and colistin and tigecycline against CRE isolates were 66.7%, 73.3% and 98.9%, respectively. The results are shown in Table 3. All CRE isolates were categorized into MDR in this study.

Plasmid Replicon Types

For all CRE isolates, the IncX3 was the most common plasmid replicon type (76.7%, 69/90), and mainly found in NDM-containing strains (91.3%, 63/69) (Figure 2), followed by IncFII plasmid (37.8%, 34/90), and others including IncN (n=29), IncFIB (n=15), IncHI2 (n=2) and IncA/C (n=2). IncX3, IncN and IncFII were found together in 19 out of 56 NDM-5-producing isolates. As high as 91.7% (11/12) of KPC-2-producing strains simultaneously harbored the plasmids of IncFIB and IncFII, IncHI2 (n=2) was also detected. IncN was the unique plasmid found in the IMP-producing strains; however, IncX3, IncA/C and IncFIB plasmids were both detected in NDM-1 carrying strains. In addition, there were five strains without plasmid in this study.

STs and ERIC Patterns

As depicted in Figure 2, all NDM-5 producing *E. aerogenes* isolates belonged to ST4 and were classified into 12 different clusters (A1-12). Several strains among cluster A1, A4, A6, A9 and A11 had identical ERIC patterns and mainly distributed in NICU. Another *E. aerogenes* without carbapenemase gene were belonged to ST37. For *K. pneumoniae* isolates,

Table 2 The Clinical Characteristics of CRE Carriers in NICU

Characteristics	Total (n=41)	*E. aerogenes* Carriers (n=25)	*E. coli* Carriers (n=10)	P-value
Birth weight (g)[d]	1590(1260–1822)	1630(1172–1985)	1360(1260–1590)	0.002
Gestational age (w)	31(30–33)	31(30–34)	31(29–32)	0.003
Apgar score at 5 minutes[d]	8(5–9)	8(5–9)	8(6.5–9)	0.624
Feeding options				
Breast feeding	28(68.3%)	12(48.0%)	5(50.0%)	1.000
Mixed feeding	8(19.5%)	5(20.0%)	2(20.0%)	1.000
Not started to feed	5(12.2%)	2(8.0%)	2(20.0%)	0.674
Disease during pregnancy				
Pre-eclampsia	10(24.4%)	6(24.0%)	3(30.0%)	1.000
Gestational diabetes	9(22.0%)	7(28.0%)	2(20.0%)	0.951
PROM	4(9.8%)	1(4.0%)	2(20.0%)	0.390
Hypothyroidism	2(4.9%)	1(4.0%)	1(10.0%)	1.000
Drug use during pregnancy				
Dexamethasone	8(19.5%)	4(16.0%)	2(20.0%)	1.000
Antihypertensives	5(12.2%)	3(12.0%)	2(20.0%)	0.939
Antibiotics	4(9.8%)	3(12.0%)	0(0.0%)	0.633
Insulin	3(7.3%)	2(8.0%)	1(10.0%)	1.000
Euthyrox	2(4.9%)	1(4.0%)	1(10.0%)	1.000
Others	7(17.1%)	5(20.0%)	2(20.0%)	1.000
Delivery method				
Cesarean delivery	25(61.0%)	15(60.0%)	6(60.0%)	1.000
Natural vaginal delivery	15(36.6%)	9(36.0%)	4(40.0%)	1.000

Notes: [d]Median and interquartile ranges (25th and 75th percentile); A P-value of <0.05 was considered statistically significant.
Abbreviations: w, week; PROM: premature rupture of membranes.

ST11 (40%,10/25) was the dominant type and all found in KPC-2-producing strains, which was categorized into 4 clusters (B1-B4), with cluster B1 and B3 belonged to PICU (n=3) and gastroenterology ward (n=4), respectively, followed by ST48 which was detected in two NDM-5 producing *K. pneumoniae* isolates in gastroenterology ward and divided into cluster B10. Almost all strains in cluster B1, B3 and B10 were defined as the identical strains. Others including ST681, ST692, ST215, ST441, ST40, ST1428, ST690, ST17, ST1308, ST419, ST595 and ST2735. A total of seven STs were found among *E. coli* isolates, with ST692 (55.6%,10/18) being the prime type in NDM-5 containing strains and grouped into 3 different clusters (C10-C12), which were mainly prevalent in NICU (n=6), PICU (n=2) and gastroenterology ward (n=2), with many identical strains found as well. In addition, ST662 (n=3), ST2 (n=3), ST39 (n=2), ST35 (n=2), ST58 (n=2) and ST3 (n=1) were also detected. Meanwhile, diverse STs were identified between 9 *E. Cloacae* strains, including ST419, ST524, ST1318, ST920, ST391, ST45, ST39 and ST45.

Discussion

The retrospective epidemiological study was conducted for determining the prevalence and molecular epidemiology of inpatients with CRE intestinal colonization in a pediatric hospital of Shanghai, which helps us to figure out the current situation of fecal carriage of CRE among hospitalized children in china and take more scientific infection prevention and control measures. The fecal carriage rate of inpatients was 8.6% in our study, which was higher than two hospitals in Hunan (8.5%) and Fujian (6.6%), China.[24,25] Worldwide, the colonization rate ranged from 18.9% to 69.5% in hospitalized patients.[26–28] However, in outpatients, the rate of colonization with CRE in fecal sample collected from children was only 3.6% in our hospital in 2016.[29] Consistent with the previous reports, CRE colonization in patients from community settings was infrequent. Healthcare facilities are usually considered as reservoirs of transmission[30] of CRE, and the healthcare providers are partly to blame in CRE acquisition among hospitalized patients.[31] Moreover, antibiotic exposures and

Table 3 Antimicrobial Susceptibility and MIC Distributions of CRE Isolates

Antibiotics	Total(n=90)			MBL-producers(n=70)			KPC-2 -producers(n=12)		
	MIC50 (μg/mL)	MIC90 (μg/mL)	R (%)	MIC50 (μg/mL)	MIC90 (μg/mL)	R (%)	MIC50 (μg/mL)	MIC90 (μg/mL)	R (%)
CAZ	>256	>256	100.0	>256	>256	100.0	>256	>256	100.0
CTX	>128	>128	100.0	>128	>128	100.0	>128	>128	100.0
FEP	>256	>256	98.9	>256	>256	100.0	128	>256	100.0
ETP	32	>128	100.0	16	128	100.0	64	>128	100.0
IPM	32	128	97.8	32	128	97.1	32	128	100.0
MEM	32	128	98.9	32	128	100.0	64	256	100.0
CSL	>256	>256	98.9	>256	>256	100.0	>256	>256	100.0
TZP	>256/4	>256/4	98.9	>256/4	>256/4	100.0	>256/4	>256/4	100.0
CIP	8	64	93.3	4	64	94.3	64	128	100.0
SXT	16/304	>32/608	53.3	2/38	16/304	50.0	16/304	>32/608	66.7
AMK	<2	>256	25.6	<2	>256	15.7	>256	>256	83.3
GEN	<1	>128	40.0	<1	>128	31.4	>128	>128	91.7
ATM	128	>256	88.9	128	>256	85.7	128	>256	100.0
CZA	64/4	>128/4	82.2	64/4	>128/4	92.9	2/4	>128/4	33.3
FOS	32	>512	22.2	32	>512	18.6	64	>512	33.3
COL	1	>4	26.7	1	>4	25.7	2	>4	33.3
TGC	1	1	1.1	1	1	1.4	1	1	0.0

Abbreviations: CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CSL, cefoperazone-Sulbactam; TZP, piperacillin–tazobactam; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; AMK, amikacin; GEN, gentamicin; ATM, aztreonam; CZA, ceftazidime-avibactam; FOS, fosfomycin; COL, colistin; TGC, tigecycline; R, resistant.

invasive practices such as mechanical ventilation, percutaneous intervention and surgery increase the probability of CRE colonization and infection,[32,33] and more than half CRE colonizers had such experiences before CRE being detected in this study. The CRE strains mainly distributed in NICU and PICU in our hospital, which might be related to critical underlying diseases, prolonged hospitalization, long-term application of carbapenems and other antimicrobial agents in these departments.[34] In addition, five neonates were identified to carry CRE at birth, which may be attributed to the mother-to-infant transmission,[35] and this remained to be further explored.

Enterobacteriaceae such as *E. coli* and *K. pneumoniae* are the part of the normal human intestinal flora but often responsible for HAI,[36] and had become the predominant CRE isolates according to the Nationwide Surveillance data of Clinical CRE Strains in China,[37,38] and were also detected in fecal samples in our study, mostly from patients in PICU and NICU. We should attach great importance to this, as ICU patients with these isolates were more likely to develop subsequent infection compared to patients without these isolates according to previous reports.[39,40] It is noteworthy that *E. aerogenes* were the most dominant colonized CRE strains in children in this research. According to a recent study reported by Fupin Hu, etc., in 2020,[41] the most prevalent CRE strains isolated from adult patients was *K. pneumoniae* (64.6%) in China from 2016 to 2018, and was consistent with the epidemic situation in our hospital before 2018 in clinic. While all 90 CRE strains were isolated from fecal samples of hospitalized children in 2019, and there was an outbreak of *E. aerogenes* between inpatient children (unpublished data). However, it still needs a further study on whether there is a causal relationship between clinical and

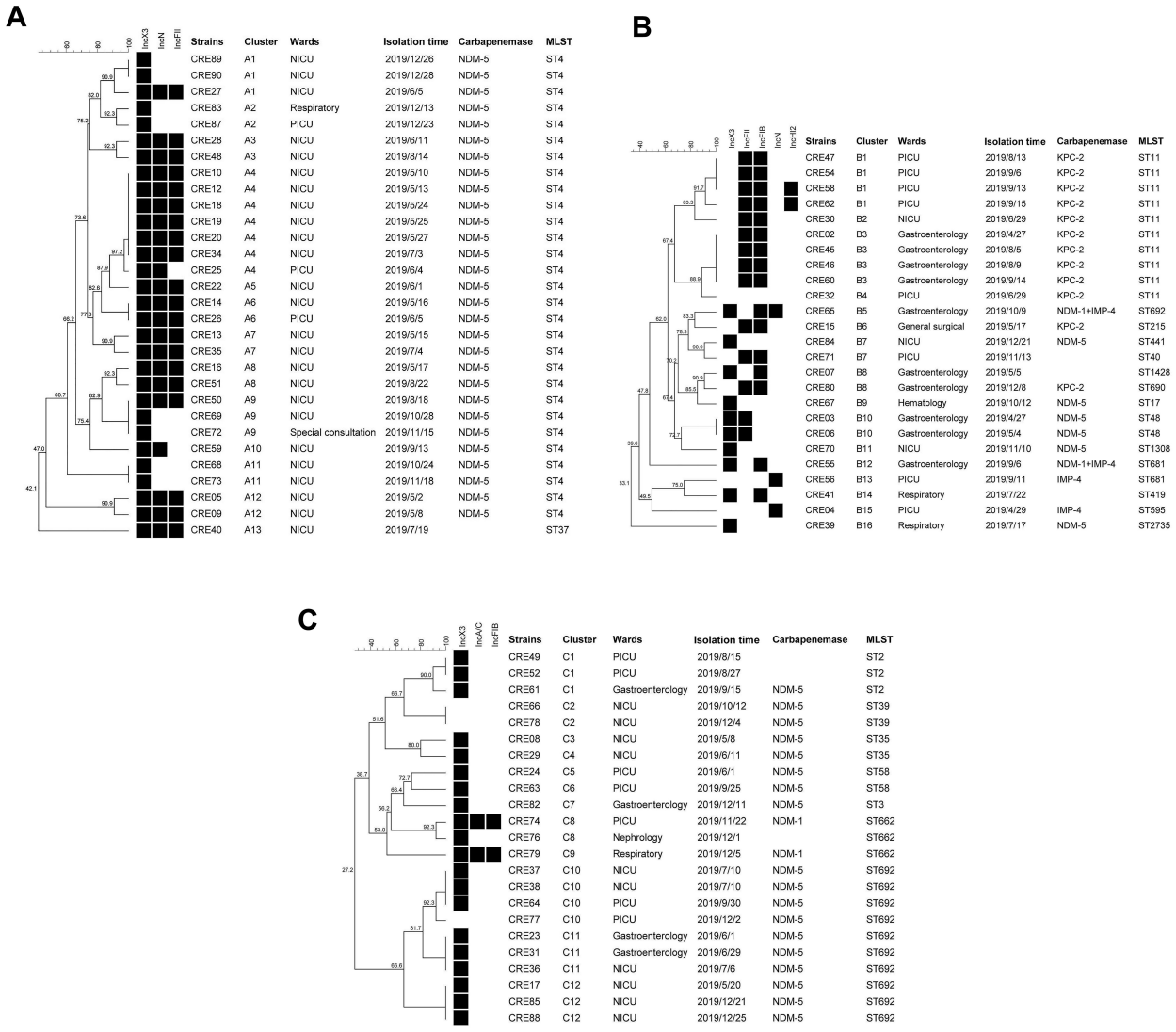

Figure 2 Dendrogram obtained from ERIC-PCR fingerprinting and Plasmid Replicon Profile of *E. aerogenes* (**A**), *K. pneumoniae* (**B**) and *E. coli* (**C**) isolates.

colonized CRE strains, which would be explored in our following research.

Since the discovery of an ST14 *K. pneumoniae* with NDM-1 from a Swedish patient traveled to New Delhi in 2008,[42] NDM-type MBLs have been sporadic occurrences in many regions all over the world (except the Middle East and Balkan countries).[43] However, in china, bla_{NDM} was the dominant carbapenemase gene found in children group clinically, and most prevalent in *E. coli* isolates.[41] In our study, $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-1}}$ genes were both identified, with $bla_{\text{NDM-5}}$ being the main carbapenemase genotype, but detected more in *E. aerogenes* than in *E. coli* isolates. The $bla_{\text{KPC-2}}$ was found the second most frequently and all found in *K. pneumoniae* isolates, which had spread across China and many other countries, causing both outbreaks and endemicity in certain regions.[44] It is noteworthy that $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-5}}$ had been the predominant carbapenemase genes detected in CR-KPN isolates from clinical specimens in our hospital in 2018.[45] Besides, $bla_{\text{IMP-4}}$ and $bla_{\text{IMP-26}}$ were also observed, and 4 strains were identified to co-harboring $bla_{\text{NDM-1}}$ and $bla_{\text{IMP-4}}$, illustrating these genes spread in readily accessed between Enterobacteriaceae bacteria.

The mechanisms of carbapenem resistance in enterobacteriaceae include production of carbapenemases, β-lactamase (ESBLs and AmpC) activity combined with the mutation of porins, drug efflux pumps and alterations in penicillin-binding proteins. The first two are main

mechanisms. In 8 CRE isolates without carbapenemase gene, we detected ESBLs and (or) AmpC genes, which might be one of the major causes of carbapenem resistance, others need to be further explored. All these resistance mechanisms of CRE strains are posing great challenges to clinical treatment.

All CRE isolates showed high resistance to cephalosporins and carbapenemases, as well as cefoperazone-sulbactam and piperacillin–tazobactam, which might be due to the widespread use of these antibacterial agents in children. As far as we all know, ceftazidime-avibactam has activity against serine β-lactamases but does not possess activity against MBL-producing organisms,[46] therefore showing better activity against the KPC-2 producing CRE isolates than the MBL-producers ($p<0.05$). Fosfomycin, colistin and tigecycline, as the last-resort antibiotics in all to treat MDR infections,[47] wherein tigecycline shows the highest (98.9%) activity in vitro. The increasing prevalence of CRE strains are attributed to the dissemination of conservative mobile elements carrying bla_{NDM} or bla_{KPC-2} on plasmids in our country,[37] consistent with the uptrend worldwide among NDM-containing isolates from clinical samples recently,[45,48,49] IncX3 plasmid was the most common type in those strains. And our previous study revealed that the IncX3-type plasmid was responsible for the horizontal gene transfer of the bla_{NDM-5} gene among different Enterobacteriaceae isolates.[50] IncFIB and IncFII were the predominant plasmids found in KPC-2-producing *K. pneumoniae* isolates and had reported to mediate clinical transmission in different districts of China.[45,51] In our study, 100% of NDM-5 producing *E. aerogenes* and 55.6% of NDM-5 producing *E. coli* strains belonged to ST4 and ST692, respectively, and ST11 was the predominant clone-type in KPC-2 producing *K. pneumoniae* isolates. Several isolates of each species grouped into the same cluster were defined as the identical strains, indicating an ongoing nosocomial clonal transmission in our hospital in the same period, which may be acquired via physical contact with patients colonized or infected with CRE. Besides, two NDM-5 producing *K. pneumoniae* isolates belonged to ST48 also had the same ERIC pattern, and were reported to cause nosocomial outbreak in our hospital in 2017.[52]

CRE colonization is a prerequisite for development of CRE infection. According to a recent study conducted in Children's Hospital of Fudan University,[53] active CRE colonization surveillance and CRE positive patient propriety placement may decrease the CRE infection risk. Although the medical resources in many developing countries are limited, it is very important to take infection prevention and control measures to reduce the occurrence of nosocomial outbreak of CRE.

Conclusion

In conclusion, the study demonstrated a major intestinal colonization of ST4 NDM-5 *E. aerogenes*, ST11 KPC-2 *K. pneumoniae* and ST692 NDM-5 *E. coli* strains among hospitalized children in Shanghai, China. To reduce the nosocomial CRE infection and transmission, prevention and control measures should be strongly advocated and strictly implemented in clinical settings as soon as possible, especially in ICU.

Data Sharing Statement

All data generated and/or analyzed during the study are available from the corresponding author upon reasonable request.

Consent for Publication

All authors have consent for the manuscript publication.

Acknowledgments

We thank all members of the Clinical Laboratory of Shanghai Children's Hospital for their cooperation and technical help.

Funding

This work was supported by the Shanghai Municipal Key Clinical Specialty (shslczdzk06902) and Shanghai Municipal Health Commission (201940253). The sponsors had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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