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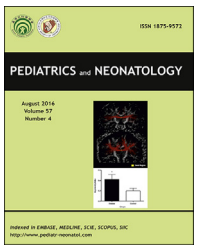

Original Article

Carbapenem-resistant *Enterobacteriaceae* infection in children less than one year old in an Asian medical center

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Key Words
carbapenem-resistant *Enterobacteriaceae*;
children;
colonization;
infection

Background: The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) is a threat to public health worldwide. This study aimed to determine the risk factors and outcomes for CRE colonization and infection in infants.
Methods: Children aged <1 year hospitalized with CRE pathogens isolated from January 2016 to June 2019 were retrospectively analyzed. Demographic and clinical data were examined.
Results: A total of 48 infections were identified in 70 infants aged <1 year, and 66.7% (32/48) of these infants were born preterm. The infection rate in infants aged <1 month was higher than that of others (P = 0.005). The most commonly isolated CRE was *Klebsiella pneumoniae* (60.4%, 29/48), followed by *Enterobacter cloacae* complex (18.8%, 9/48). Sputum (37.5%, 18/48), blood (27.1%, 13/48), and urine (25.0%, 12/48) were the most common clinical samples. Urinary tract infection was common in infants aged 6–12 months. CRE infection was associated with mechanical ventilation (P = 0.037), central venous catheter (CVC) insertion (P = 0.034), and congenital heart disease (P = 0.027). The hospital stay of patients with CRE infection was

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longer (median, 75 days; SD, 66.4 days), and their all-cause mortality (6.4%) was higher than those with colonization.
Conclusions: CRE infection was common in infants aged <1 month, and patients usually had longer hospitalization. Carbapenemase production was not common. Mechanical ventilation, CVC insertion, and congenital heart disease were associated with a higher risk of CRE acquisition in infants aged <1 year.

1. Introduction

Antimicrobial resistance likely poses a great risk to poor health outcomes.[1] Carbapenems were previously thought to be the preferred treatment for multidrug-resistant (MDR) Gram-negative bacterial infection. However, with the increasing prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) in both adults and children, few therapeutic options are available. CRE is linked to high morbidity and mortality rates,[1–3] thereby posing a serious threat to humans.[1–4]

CRE may cause an infection or colonization in a clinical setting. This organism can be present in or on the human body without causing any signs or diseases. However, colonizing CRE strains can cause infections or spread to other patients in healthcare facilities.[5]

Although CRE infection can affect individuals of different ages, its incidence in children has been rarely explored. In one investigation in Delhi, pathogens isolated from neonatal units reported a high level of antimicrobial resistance; among them, *Acinetobacter* species are the most widespread.[6] In another study conducted in Colorado, USA, the risk factors and epidemiologic predictors of CRE bloodstream infection (BSI), particularly in infants aged <12 months (17%), caused by the New Delhi metallo-β-lactamase (NDM-1) resistance mechanism have been explored. Central venous catheter (CVC) placement, previous carbapenem use, and intensive care unit (ICU) admission have been linked to BSI with NDM-1-producing organisms and other MDR strains.[7]

Few studies have focused on children, particularly those aged <1 year. As such, this study was conducted to characterize the risk factors of CRE infection among children aged <1 year and hospitalized in a medical center and to describe the most common infection sites and outcomes in this population.

2. Methods

2.1. Sample collections and pathogen identification

A retrospective analysis was conducted at Chang Gung Memorial Hospital (CGMH) in Linkou, which is a university-affiliated medical center that provides primary to tertiary care in northern Taiwan. Patients with a clinically culture-confirmed CRE from January 2016 to June 2019 were considered in the study. All the hospitalized children aged <1 year were eligible and thus enrolled in this study. The cases were identified through a review of microbiology laboratory records. A patient who had a record of several CRE isolates at the same time or during the same hospitalization was included as one episode only.

The patients were divided into infection and colonization groups in accordance with the American Thoracic Society, Infectious Diseases Society of America, and Centers for Disease Control and Prevention guidelines (Table 1).[5,8,9] An infection was defined as the presence of clinical signs or symptoms and laboratory findings compatible with a clinical infection based on the isolated CRE in a relevant clinical specimen, whereas colonization was defined as having positive culture but no clinical signs and did not match the aforementioned parameters.

The demographic and clinical data of the colonization and infection groups were examined. The following clinical information was obtained: age; sex; underlying conditions (such as premature birth); cardiac, lung, and renal diseases; identified organisms; isolate source; ICU admission; antimicrobial agents used; hospital admission date and cause; and patient outcomes (such as mortality and length of stay). Overall mortality was defined as death from any cause within 1 month of the commencement of CRE infection. The clinical characteristics were also analyzed by grouping children at 3-month intervals up to the age of 12 months.

This study was ethically approved by the Institutional Review Board of CGMH (202000754B0). Informed consent was waived because this is a retrospective study using anonymous clinical data.

2.2. Antimicrobial susceptibility testing and carbapenemase detection

The matrix-assisted laser desorption/ionization-time of flight mass-spectrometry analyzer (Bruker Daltonics, Bremen, Germany) with the Bruker BioTyper 3.0 System software (Bruker Daltonics) was used to identify all the isolates collected. The antimicrobial susceptibility of the isolates was tested by the disk diffusion method. If the CRE strains were isolated from sterile sites, the minimum inhibitory concentration (MIC) was determined in accordance with the MIC breakpoints of Clinical and Laboratory Standards Institutes (CLSI).[10,11] A modified Hodge test (MHT, before May 2019) and a modified carbapenem inactivation technique (mCIM, after May 2019) were conducted to screen carbapenemases in all the isolates of carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* from all sites.

Table 1 The definitions of colonization and infection in the ATS, IDSA, and CDC guidelines.

Guidelines	Groups		Remarks
	Colonization	Infection	
ATS	All of the other culture episodes than infection sources (as described in right-hand column) were designated colonizations. Detection of at least two isolates of an organism separated by at least 3 months in a year.	If the organism can be isolated from blood or any other sterile source. Evidence of systemic inflammation on the day the positive culture documented can be defined as an abnormal systemic white cell count (either $>10 \times 10^3$ or $>4 \times 10^3$ cells/μL) and/or an abnormal body temperature (either >99.5 °F or >96 °F). For nonsurgical wounds, the definition required evidence of systemic inflammation on the day of culture and documentation of infection.	Reference[8]
IDSA	Non-blood cultures can be classified colonization.	A positive blood culture can be classified as a true infection	Reference[9]
CDC	The organism can be found in or on the body without symptoms of disease. CRE colonization can be prolonged (>6 months)	The organism can cause symptoms of disease.	Reference[5]

Abbreviation: CRE, carbapenem-resistant Enterobacterales; ATS, American Thoracic Society; IDSA, Infectious Diseases Society of America; CDC, Centers for Disease Control and Prevention, the United States of America.

2.3. MHT

In accordance with the CLSI guidelines,[10,11] 0.5 McFarland turbidity standard suspension of bacterial organisms (carbapenem-susceptible *E. coli* ATCC 25922 as an indicator organism) was prepared to test the culture media performance. A carbapenem antibiotic susceptibility disk was placed in the center of the test area, and the test organism was streaked in a straight line from the edge of the disk to the edge of the plate. After 16—24 h of incubation at 35 °C, the results were examined. A positive result was indicated by a clover leaf-like indentation forming along the growth streak of the test organism within the disk diffusion zone. This finding indicated that the test microorganism generated carbapenemase, a carbapenem inactivator.

2.4. mCIM test

Through the direct colony suspension method in accordance with the CLSI guidelines,[10] the suspension with 1 μL of a loopful of a test organism that was emulsified in tryptic soy broth (TSB) and incubated for 4 h was immersed in a 10-μg carbapenem antimicrobial susceptibility disk. Thereafter, it was incubated at 35 °C for 18—24 h. The immersed disk was removed from the TSB and placed onto a Mueller Hinton agar plate coated with a 0.5-McFarland standard suspension of the indicator organism (*E. coli* ATCC 25922) via standard disk diffusion. A carbapenemase-positive result was defined as a diameter of the inhibition zone size of 6—15 mm or colonies inside the 16—18 mm zone region, whereas a negative one was described as a diameter of the inhibition zone of ≥19 mm.

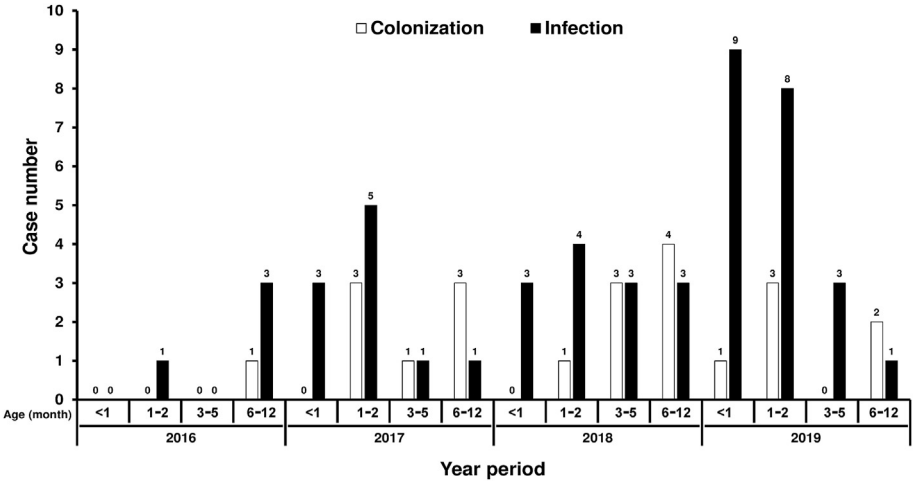

Figure 1 Patients in the infection and colonization groups over the years and stratified by age. Isolation of carbapenem-resistant *Enterobacteriaceae* gradually increased yearly.

2.5. Statistical analysis

The collected data were analyzed in Excel (Microsoft Office 365). Pearson's chi-square test was used to determine the significance of the compared correlation coefficients. A P value < 0.05 was statistically considered to be significant.

3. Results

3.1. Demographics of the patients

In the 3.5-year study period, 70 patients with a clinical culture confirmation for carbapenem-resistant gram-negative pathogens were identified. The majority of the patients were preterm neonates (66.7%). Of these patients, 48 (68.6%) satisfied the criteria for definite infection, and the remaining patients were considered to have colonization. Furthermore, 35 (72.9%) and 10 (45.5%) boys were in the infection and colonization groups in the age range of 0–1 year, and their mean age was 2.9 (0–5.8) months compared with 5.4 (2.1–8.5) months, respectively. Infections caused by CRE appeared to increase from 2016 to 2019 (Fig. 1). Among the 48 patients in the infection group, 4 (8.3%) in 2016, 10 (20.8%) in 2017, 13 (27.10%) in 2018, and 21 (43.8%) in 2019 were identified. Table 2 provides the demographic and clinical characteristics of the patients. As regards the proportion of CRE infections, the number of patients aged <1 month in the infection group was significantly higher than those in the colonization group ($P = 0.005$).

3.2. Bacterial species and carbapenemase production

In Table 2 and Fig. 2, the most frequently isolated CRE in the infection group were *K. pneumoniae* (60.4%) and *Enterobacter cloacae* complex (18.8%). *Serratia liquefaciens* (6.3%), *E. coli* (4.2%), and *Morganella morganii* (2.1%) were also detected in this study. The anatomical sources of CRE infections were the sputum (37.5%, 18/48), blood (27.1%, 13/48), urine specimens (25.0%, 12/48), pus (4.2%, 2/48), bronchoalveolar lavage sample (2.1%), cerebrospinal fluid (2.1%), and tissue sample (2.1%) from a patient who had osteomyelitis and underwent debridement. After stratification by age group, the number of CRE isolates in the sputum or blood culture was higher than that in other infection sites in patients aged <3 months and children aged 6–12 months. Urinary tract infection (UTI) is the most common infection.

All the isolates of *K. pneumoniae* and *E. coli* were tested for carbapenemase production either via an MHT test before May 2019 or an mCIM test after May 2019. Carbapenemase production was not detected in any of the isolates in the infection group. In the mCIM test, only two isolates tested positive in the colonization group. No antimicrobial agents were used during the hospitalizations in the two cases because of the lack of clinical symptoms.

Table 2 Demographic data of the age, gender, underlying condition, hospitalization status, outcome, and species of carbapenem-resistant organism isolates.

	Colonization N = 22	Infection N = 48	*P* value
Gender			
Male	10 (45.5)	35 (72.9)	0.008
Age (month)			0.016
<1	1 (4.5)	15 (31.3)	0.005
1–3	7 (31.8)	18 (37.5)	
3–5	4 (18.2)	7 (14.6)	
6–12	10 (45.5)	8 (16.7)	
Pre-hospitalization			
Previous admission within 30 days	1 (4.6%)	6 (12.5%)	0.420
Previous admission within 3 months	3 (13.6)	9 (18.8%)	0.741
Underlying disease			
Preterm	9 (40.9)	32 (66.7)	0.007
Congenital heart disease/PDA	2 (9.1)	13 (27.1)	0.027
PDA	2 (9.1)	11	0.09
PDA w/or w/o other CHD	2	12	0.049
Other CHD, w/o PDA	0	1	1
Hematology problems	0	1 (2.1)	1
Gastrointestinal disease	0	5 (10.4)	0.313
Renal disease	0	8 (16.7)	0.419
Hydrocephalus s/p OP w/shunt	1 (4.5)	4 (8.3)	1
Hospitalization			
Ward			0.002
PICU	1 (4.5)	2 (4.2)	
NICU	6 (27.3)	33 (68.8)	
General ward	15 (68.2)	13 (27.0)	
Initial diagnosis on admission			0.042
Comorbidity with infection disease	13 (59.1)	14 (29.2)	
Non-infection disease	9 (40.9)	34 (70.8)	
Isolated pathogens			
CR *C. freundii*	1 (4.5)	0	
CR *E. coli*	4 (18.2)	2 (4.2)	
CR *K. aerogenes* (previously *Enter. aerogenes*)	2 (9.0)	4 (8.4)	
CR *K. pneumoniae*	7 (31.8)	29 (60.4)	
[Including CR *K. pneumoniae*, mCIM (+)]	[2 (9.0)]	0	
CR *Enter. cloacae* complex	8 (36.4)	9 (18.8)	
CR *M. morganii*	0	1 (2.1)	
CR *S. liquefaciens*	0	3 (6.3)	
Time of positive culture after admission			
≤72hrs	14 (63.6)	11 (23.4)	
3–5 days	1 (4.5)	0	

Table 2 (*continued*)

	Colonization N = 22	Infection N = 48	*P* value
>5 days	7 (31.8)	37 (77.1)	
Duration of antibiotics treatment			
Days, median ± SD	0	9.5	
SD		8.1	
Factors associated with infection			
Ventilator	5 (22.7)	25 (52.1)	0.037
ETT	5 (22.7)	24 (50.0)	0.063
Tracheostomy tube	0	1 (2.1)	1
V–P shunt	0	1 (2.1)	1
Port-A	0	1 (2.1)	1
A-line	0	1 (2.1)	1
CVC	5 (22.7)	26 (54.2)	0.034
Gastrostomy, ileostomy, or colonostomy	0	3 (6.3)	0.548
Indwelling urinary catheter/cycstostomy or vesicostomy	1 (4.5)	4 (8.3)	1
PD tube	0	2 (4.2)	0.519
Length of hospital stay: days, median ± SD	5	75	
SD	57.6	65.8	
Before positive culture: days, median	0	23.5	
SD	34.6	39.5	
After positive culture: days, median	6	40.5	
SD	28.0	47.6	
Status discharge			
Alive	21 (95.5)	45 (91.7)	1
Mortality (≤1 month)	0	0	1
Re-admission ≤90 days	2 (9.1)	10 (20.8)	0.381
Dead	1 (4.5)	3 (6.3)	

A-line, arterial line; CHD, coronary heart disease; *C. freundii*, *Citrobacter freundii*; CR, carbapenem resistance-isolate; CVC, central venous catheter; Enter. aerogenes, Enterbacter aerogenes; *E. coli*, *Escherichia coli*; ETT, endotracheal tube; K. aerogenes, Klebsiella aerogenes; *K. pneumoniae*, *Klebsiella pneumoniae*; *M. morganii*, *Morganella morganii*; NICU, neurologic intensive care unit; Port-A, port-a-catheter; PD, peritoneal dialysis; PDA, patent ductus arteriosus; PICU, pediatric intensive care unit; SD, standard deviation; *S. liquefaciens*, *Serratia liquefaciens*; V–P shunt, ventriculo-peritoneal shunt; w/, with; w/o, without.

3.3. Characteristics associated with CRE infection

Table 2 summarizes the epidemiological, microbiological, and clinical characteristics of the patients. Notable differences were noted in the baseline characteristics between the two groups. CRE infections were significantly more common in patients born preterm ($P = 0.007$), male patients ($P = 0.008$), patients with previous exposure to invasive procedures (including mechanical ventilation [$P = 0.037$] and CVC use [$P = 0.034$]), and patients with a congenital heart disease ($P = 0.027$).

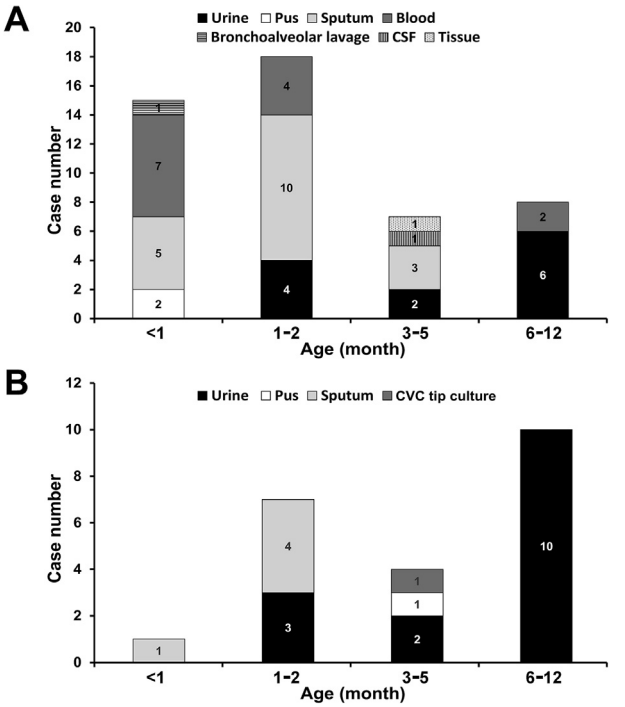

Figure 2 Incidence number of carbapenem-resistant organisms in colonization (A) and infection (B) groups classified by age and isolation sites.

Table 2 summarizes the admission diagnosis of the patients Some CRE colonization cases (13/22, 59.1%) were reported as infectious diseases due to viral infections (in most cases, bronchiolitis or roseola infantum) and bacterial infections (including pertussis), and the CRE strains were only isolated from urine cultures. As a result, most of the children were only managed without antimicrobial agents for viral infections. However, the medical records on previous antimicrobial uses were only available from family descriptions on hospital admissions in the previous 30 days or 3 months. Records revealed that only 1 (4.6%) patient and 6 (12.5%) patients in the colonization and infection groups, respectively, had been hospitalized in the previous 30 days and 3 (13.6%) patients and 9 (18.8%) patients in the previous 3 months (Table 2).

Table 2 also summarizes the initial diagnosis on admission. Some CRE colonization cases were reported to occur as infectious diseases, resulting in comorbidity with viral infection (in most cases, such as bronchiolitis or roseola infantum) or other bacterial infections, such as pertussis, which is incompatible with CRE growth culture findings (because all of their cultures were urine cultures). The time of positive culture after admission, as presented in Table 2, did not apply equally to hospital-acquired infections. CRE infection and colonization cases included patients who had suspicious (but not certainly infectious) symptoms at the time of administration. Some patients admitted for another reason may develop fever or suspicious symptoms after few days after admission. Therefore, culture may be scheduled within a few days of admission. Those with less evidence of infection (such as having incompatible clinical findings or favoring viral disease) will be classified as colonization, mostly identified within 72 h (63.6%),

Table 3 Risk factors and outcomes of carbapenem-resistant Gram-negative organisms in pediatric populations in the selected publications.

Author (Reference)	Geographic area[a]	Study period	Population	CRO/Total No	Risk for pathogens acquisition	Outcomes
This study	Northern Taiwan	2016/8/1—2019/6/30	Children aged 0～1 y	48/70	Mechanical ventilation, CVC insertion, and congenital heart disease	Longer hospital stay and increased all-cause mortality rate during the hospitalization.
Ballot et al. BMC Pediatrics. 2019 19:320	South Africa[15]	2013/1/1—2015/12/31	Neonate	291/2437	Prematurity, lower birth weight, maternal HIV infection, and oxygen on Day 28	Increased all-cause mortality
Kathleen Chiotos et al. Open Forum Infect Dis. 2018 Sep 10; 5 (10)	Boston, US[16]	2011/1/1—2016/7/1	Children <21 y	31/72	Hospitalized in the ICU and more often had health care-associated infections	Higher in 30-day of all-cause mortality
Kayoko Hayakawa et al. J Antimicrob Chemother 2020; 75: 697—708	Japan[17]	2016/10/1—2018/3/31	All age	88/7640	Nursing home or long-term care facility, longer prior length of hospital stay (LOS), and indwelling Foley or NG tube	Longer hospital stay
Kumar A et al. J Infect Dev Ctries. 2014 Aug 13; 8 (8):1049—54.	Delhi, India[18]	2010/1/1—2012/12/31	Neonate	33/65	Duration of ventilation, day of isolation post admission, prior antimicrobial use, and feeding with expressed breast milk	Increased all-cause mortality rate
Lili Fang et al. Pathog Dis. 2019 Jun 1; 77 (4)	Xiamen, China[19]	2015/1/1—2017/1/31	Age 0—91 y	47/141	Underlying pulmonary diseases and antibiotics used prior to culture within 30 days	Longer hospital stay and less in improvement
Montagnani C et al. Pediatr Infect Dis J. 2016; 35:862—868.	Italy[20]	2011/1/1—2014/3/1	Children aged 0—18 y	34/69 <24mo: 15/28	Longer prior length of hospital stays, duration of disease, and length of antibiotics treatment	Increased all-cause mortality and more often in presenting sequelae
Zhe Li et al. Chinese Medical Journal	Wenzhou, China[21]	2009/1/1—2018/12/31	Children aged 0—16 y	51/153 0—28 d: 19/57 29 d～1 y: 17.51	Previous exposure to third-generation cephalosporins, β-lactam/β-lactamase inhibitor, previous mechanical ventilation, and indwelling urethral catheter	Longer length of hospital stay, and increased in all-cause mortality

[a] Reference number.

whereas most of the bacteria were isolated from the CRE infection cases of >5 days after admission (77.1%).

3.4. Outcomes

The all-cause mortality in infants with CRE isolation was low. Among our patients, only three patients in the infection group and one patient in the colonization group died. Among patients with CRE infection, two showed *K. pneumoniae* BSI and pneumonia, and the other one was a case of *S. liquefaciens* BSI.

4. Discussion

CRE has emerged in many countries worldwide; consequently, it has significant morbidity and mortality.[12–14] In one study in Johannesburg, South Africa, which enrolled 291 neonates with multidrug-resistant *Enterobacteriaceae* (MDRE) infections and 2146 cases in the control group between 2013 and 2015, revealed a significant increase in MDRE, including CRE and a high all-cause mortality rate.[15] In another study in the USA, CRE infection seriously affected the mortality outcomes of children, including neonates and adolescents.[16] To enhance our understanding of the effect of CRE on young children, we conducted this study to assess the demographics and distribution of CRE among hospitalized infants aged <12 months. We discovered that the most common group was the one comprising patients aged <3 months (34 patients, 70.9%).

We conducted a literature review on the demographics and CRE infection-associated risk factors[15–20] and found that young children are a vulnerable group at risk of CRE infection. We could infer from those reports that BSI, pneumonia, and UTI were the three most common infections.[18,20,21] In our study, the common CRE infection sites are comparable with those described in previous studies (Table 3).[15–21] Infants aged <3 months are more likely to develop a respiratory tract infection or bacteremia, whereas infants aged 6—12 months are more likely to have a UTI because of CRE.

K. pneumoniae and *E. coli* are the most common reported CRE.[22] Another study involving 47 patients has revealed that *K. pneumoniae* is the most common cause of CRE infection (74.5%, 35 patients), followed by *E. cloacae* (8.5%), *Citrobacter freundii* (6.4%), and *E. coli* (6.4%).[18] Ballot et al.[15] conducted a pediatric study and revealed that *K. pneumoniae* appears to be the most prevalent isolated pathogen (66.2%, 308/465), followed by *E. cloacae* (10.5%, 49/465). In our study, the number of *K. pneumoniae* isolates detected in CRE remarkably increased (60.4%). *E. cloacae* complex is the second-most prevalent (18.8%). Although the ranking of *K. pneumoniae* and *E. cloacae* appeared different, *K. pneumoniae* and *E. cloacae* as well as *E. coli* were within the top 5 bacteria in those studies.[15–20,22] The reasons for the slight difference could be the specificity of horizontal carbapenemase gene transmission by conjugative plasmid or transposon among various bacteria.[23]

Carbapenemase-producing *K. pneumoniae* accounts for more than half of children with CRE infections[16,19]; as such, all isolates of *K. pneumoniae* and *E. coli* were tested for carbapenemase activity in our clinical microbiology laboratory by either the MHT or mCIM test. As a result, all pediatric CRE appeared to be non-carbapenemase producers. The reasons of the difference in the detection of carbapenemase-producing CRE between previous reports and our study could be (i) the different population studied and (ii) the enzymatic method (MHT or mCIM test) used in this study, which is different from the more sensitive genetic method, such as polymerase chain reaction. Other non-enzymatic drug resistance mechanisms, such as efflux pump system and impaired drug permeability, could be involved in CRE. However, further studies should determine whether CRE from children tend to be non-carbapenemase producers.

CRE infections significantly affect vulnerable children with underlying illnesses.[17,21,24–26] In the present study, we investigated the factors and procedures predisposing to CRE infection and found that mechanical ventilation, CVC insertion, and underlying diseases, including congenital heart disease, were the main factors associated with CRE infection.

In previous studies, the outcomes of patients with CRE infections varied. Mortality information, including in-hospital and crude mortality rates, disease duration, length of hospitalization stay, and hospitalization expenditures, has been used to assess outcomes. Previous studies have revealed a significantly longer of hospital stay and an increase in the mortality of adults and children.[4,16,17,19–21] Our findings were consistent with these studies, indicating that patients required longer hospital stay before and after positive culture growth, and most of the infections likely belonged to hospital-acquired infections because most of the bacteria were isolated days after admission. Additionally, our study found an increase in all-cause mortality.

As limitations, the study was conducted in a single center, so avoiding bias was difficult because of the retrospective study design. Determining whether CRE infection remarkably influenced the all-cause mortality in young children was also difficult because of the limited number of patients enrolled. Moreover, experiments were not conducted to detect carbapenemase genes in CRE isolates as a phenotypic test might give a false-negative result.

5. Conclusions

In this study, young infants were identified as a population at risk of CRE infections. To our knowledge, this study was the first to examine the epidemiology and clinical characteristics, risk factors, and outcomes of pediatric CRE infections in Taiwanese children aged <1 year. Children with CRE infection had a longer hospital stay because of a higher likelihood of mechanical ventilation, CVC insertion, and congenital heart disease. Furthermore, carbapenemase production was not common in isolated CRE cases. Given the increasing prevalence of resistant gram-negative bacterial infections, these organisms should be continuously monitored in young children.

Declaration of competing interest

None declared.

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