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Original Article

Evaluation of Carbapenem-resistant *Enterobacteriaceae* Bloodstream Infections in the Children with Gastrointestinal Carbapenem-resistant *Enterobacteriaceae* Colonisation

E ***Kiymet***, E ***Böncüoğlu***, İ ***Çağlar***, R ***Çolak***, AA ***Kara***, N ***Demiray***, T ***Erdem***, TG ***Yildirim***, H ***Ağin***, N ***Bayram***, Ş ***Çalkavur***, İ ***Devrim***

Abstract Carbapenem-resistant *Enterobacteriaceae* (CRE) has emerged as a significant public health threat worldwide. Aim of the study was to evaluate the number of CRE-associated bloodstream infections (BSIs) episodes in patients with previously CRE colonisation at paediatric and neonatal intensive care units. For this purpose, a retrospective cohort of all children, with CRE colonisation detected during routine surveillance in the intensive care units between June 2018-January 2019 was evaluated. Among 496 patients who were screened for CRE colonisation, 79 (15.9%) CRE-colonised patients included in the study. Forty-five (56.9%) patients were detected CRE-colonised in the routine screening cultures prior to the hospitalisation. A total of 14 (n:14/79, 17.7%) CRE infections were observed. Carbapenem-resistant *Enterobacteriaceae*-colonised children had developed 3.7% (n:3/79) BSIs during follow-up. Although prevalence of CRE-related bacteraemia was relatively low in colonised children, it is necessary to screen of rectal CRE colonisation due to high mortality of infection.

Key words *Carbapenem-resistant Enterobacteriaceae; Colonisation; Children; Intensive care unit; Neonatal intensive care unit*

Department of Paediatric Infectious Diseases, University of Health Sciences Dr. Behçet Uz Children's Hospital, İzmir, Turkey
E **Kiymet** *MD*
E **Böncüoğlu** *MD*
İ **Çağlar** *MD*
AA **Kara** *MD*
N **Bayram** *MD*
İ **Devrim** *MD*

Department of Neonatology, University of Health Sciences Dr. Behçet Uz Children's Hospital, İzmir, Turkey
R **Çolak** *MD*
Ş **Çalkavur** *MD*
TG **Yildirim** *MD*

Department of Infection Control Committee, University of Health Sciences Dr. Behçet Uz Children's Hospital, İzmir, Turkey
N **Demiray** *BSN*

Department of Microbiology, University of Health Sciences Dr. Behçet Uz Children's Hospital, İzmir, Turkey
T **Erdem** *MD*

Department of Children Intensive Care Unit, University of Health Sciences Dr. Behçet Uz Children's Hospital, İzmir, Turkey
H **Ağin** *MD*

Correspondence to: Dr E **Kiymet**
Email: elifkiymet_1264@hotmail.com

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Introduction

Infections due to the antimicrobial-resistant microorganisms were estimated to cause the death of approximately 700,000 people each year worldwide and according to some experts' predictions, this number could rise to 10 million by 2050.[1] Carbapenem-resistant *Enterobacteriaceae* (CRE) as like Carbapenem-resistant Acinetobacter baumannii and Carbapenem-resistant Pseudomonas aeruginosa is among the top tier of the World Health Organization list of antibiotic-resistant "priority pathogens" that pose the greatest threat to human health.[1]

In the 1980s, carbapenem class antibiotics have introduced as the last line of defence against multidrug-resistant Gram-negative organisms. However, resistance due to mostly production of carbapenemase expanded in the *Enterobacteriaceae* by the early 2000s. Carbapenem-resistant *Enterobacteriaceae* have emerged as a significant public health threat.[2] The resistance of CRE to carbapenems could be generally based on two mechanisms: some CRE possess a carbapenemase and some CRE possess a β-lactamase which, when combined with porin mutations, can render an organism non-susceptible to carbapenems.[3]

The presence of CRE in the community poses an urgent public health threat. According to a meta-analysis, community-associated CRE prevalence is ranging from 0.04% to 29.5%, with United State (US) based studies alone ranging from 5.6-10.8%.[4] According to the National Healthcare Safety Network report, among the nosocomial pathogens reported during 2006-2007 from 463 hospitals throughout the US, 10-11% of all Klebsiella pneumoniae isolates causing central line-associated blood stream infection (BSIs) or urinary tract infections were resistant to carbapenems.[5]

Gastrointestinal colonisation with CRE is an important step for the development of infection.[6,7] Most common risk factors for colonisation and are antipseudomonal antibiotic exposure within the previous three months, prior surgery, and mechanical ventilation.[7,8] The risk factors for CRE gut colonisation have been identified as the duration of hospitalisation, nasogastric tube, nasogastric feeding, lack of breastfeeding, top feeding, ventilation, and antibiotic administration.[9]

The aim of the current study was to evaluate the episodes of healthcare-associated infections by CRE that developed in CRE-colonised patients at paediatric and neonatal intensive care units (ICUs).

Methods

This retrospective study was conducted between June 2018 to January 2019 in the paediatric and neonatal ICUs at University of Health Sciences Dr. Behçet Uz Children's Hospital, a 350-bed referral centre for paediatric infectious diseases in the Aegean Region of Turkey. The current hospital has a 24-bed paediatric ICU and a 58-bed neonatal ICU. Patients with gastrointestinal CRE colonisation detected during routine surveillance in the intensive care units were included in this study.

Definitions: Carbapenem-resistant *Enterobacteriaceae* colonisation was defined as gastrointestinal tract carriage determined by the rectal swab in patients without any clinical sign of infection.[10] Carbapenem-resistant *Enterobacteriaceae* associated BSI was defined as isolation of CRE from the peripheral blood culture with fever or other signs of infection. Microorganism growth in sterile samples (cerebrospinal fluid, urine, deep tracheal aspiration) was used to identify infections other than BSI in patients who have clinical findings of infection. The other type of infections was classified as respiratory infections, central nervous system infections, urinary tract infections, abdominal, and surgical infections. Clinical cultures with CRE-positive were classified as hospital-onset if the culture was taken after the third day of hospital admission.[10]

At the study centre; rectal sample screening for CRE was performed at admission and weekly in all ICUs using conventional cultures. The "red flag" precautions were performed for patients CRE-colonised and strict infection control policies were applied as part of patient management.

All the children with gastrointestinal CRE colonisation were included in this study. Two trained reviewers (IC and EB) reviewed patients' data via using computerised medical files and laboratory data. The bloodstream infections and aetiologic agents were recorded to a prepared form. The proportion of patients who subsequently developed CRE bloodstream infections within the study cohort were evaluated.

The investigators called and questioned all patients' caregivers about the health status of individuals and hospitalisation instead of our hospital after discharge by the phone.

Microbiological Tests: Rectal swabs were directly inoculated onto a chromogenic agar plate (ChromID Carba agar bioMérieux, France). This agar is a selective

chromogenic medium containing three chromogenic substrates which enable the presumptive identification of the most frequently isolated Carbapenemase-producing *Enterobacteriaceae* from laboratory samples. Inoculated plates were incubated at 36°C aerobically for 72h.

Identification and antibiotic susceptibility tests for pathogens resulting bacteraemia were performed using the BD Phoenix™ automated identification and susceptibility testing system (BD Diagnostics, Sparks, MD, USA) via Gram-negative identification card, NMIC/ID-400. Antibiotic susceptibility testing was performed according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Genetic analysis for carbapenemase resistance is not available in our hospital.

Statistical Analysis: Statistical analysis was performed by using the Statistical Package for the Social Science (SPSS) software. The distribution of numeric variables was tested by both graphical methods and the Shapiro–Wilk test. P-value <0.05 was considered to be statistically significant.

Results

During the study period, a total of 496 neonates and children were screened for CRE colonisation and 79 (15.9%) of the patients were found to be colonised with CRE. The median age of the patients was 42.5 days ranging from 2 days of life to 6 years of age. Forty-two patients (53.2%) were male; 37 (46.8%) were female. Most of the patients (n=45, 56.9%) were referred to the study hospital from different hospitals and were CRE-positive prior to the hospitalisation in the screening cultures.

Sixty (75.9%) of the patients were hospitalised at neonatal ICU while 19 (24.1%) were hospitalised at paediatric ICU. The median time of CRE colonisation was 1 month changing from 1 day to 7 months.

During the study period, a total of 14 healthcare-associated CRE infections (17.7%) were observed. Among 14 infections, 8 (57.1%) were urinary tract infections which followed by 3 BSIs (21.4%), 1 (7.1%) meningitis, 1 (7.1%) ventilator-associated pneumonia, and 1 (7.1%) surgical site infections. The descriptive features of the patients were shown in Table 1. Although most of the patients who had been diagnosed as nosocomial urinary tract infections were at the neonatal ICU, all of the BSIs due to CRE were at paediatric ICU. Among 79 patients, 15 (18.9%) patients had a total of 22 BSI attacks. Among 22 attacks of BSI, 12 (54.5%) of the causative agents were belonging to the *Enterobacteriaceae* family included 10 Klebsiella species and two Serratia species. The list of aetiologic agents was reviewed in Table 2. Overall, among CRE-colonised 79 patients, a total of 3 (3.7%) patients were had CRE-associated BSIs.

Table 1 General characteristics of patients with Carbapenem-resistant *Enterobacteriaceae* associated infection

Case	Age	Sex	Unit	Infection	Bacteria	Comorbidity	Prognosis
1	4 months	M	NICU	Surgical site infection	*Klebsiella pneumoniae*	CHD	Survived
2	6 days	F	NICU	VAP	*Klebsiella pneumoniae*	Prematurity	Exitus
3	7 days	F	NICU	Meningitis	*Klebsiella pneumoniae*	Prematurity	Survived
4	6 years	M	PICU	BSI	*Klebsiella pneumoniae*	CP	Survived
5	3 years	M	PICU	BSI	*Klebsiella pneumoniae*	CP, Meningomyelocele	Exitus
6	10 months	M	PICU	BSI	*Serratia marcescens*	CP	Survived
7	20 days	M	NICU	UTI	*Klebsiella pneumoniae*	Asphyxia	Survived
8	2 months	M	NICU	UTI	*Klebsiella pneumoniae*	Prematurity	Survived
9	6 months	M	NICU	UTI	*Klebsiella pneumoniae*	Prematurity	Survived
10	42 days	F	NICU	UTI	*Klebsiella oxytoca*	Prematurity	Survived
11	2.5 months	M	PICU	UTI	*Klebsiella pneumoniae*	CHD	Survived
12	5 months	F	NICU	UTI	*Klebsiella pneumoniae*	CHD	Survived
13	8 months	M	NICU	UTI	*Klebsiella pneumoniae*	Prematurity	Survived
14	6 months	F	PICU	UTI	*Klebsiella pneumoniae*	ID	Survived

M: male, F: female, NICU: neonatal intensive care unit, PICU: paediatric intensive care unit, VAP: ventilator associated pneumonia, BSI: blood stream infection, UTI: urinary tract infection, CHD: congenital heart disease, CP: Cerebral palsy, ID: Immunodeficiency

The clinical features of our patients who had CRE-associated BSI following CRE colonisation were different from each other. The first patient was a 10-month-old boy with a diagnosis of cerebral palsy due to premature birth. The second patient was a 6-year-old boy with cerebral palsy and hypogonadotropic hypogonadism. The last patient was a 3-year-old boy with meningomyelocele and cerebral palsy. All these patients were hospitalised at paediatric ICU and the last one did not survive from the CRE-associated BSI.

Discussion

In recent years, healthcare-related infections caused by CRE have been emerging and more and more reports of CRE infections have been reported worldwide.[11] The data about CRE colonisation and infection, associated risk factors generally are limited to adult studies,[12-14] and limited number of studies focusing on children are present.[15] Carbapenem-resistant *Enterobacteriaceae* has been identified as the cause of healthcare-associated infections in adult patients. Infections due to CRE in adult populations have been associated with poor clinical outcomes, including mortality rates as high as 40-65%.[10,12-14] Paediatric data about CRE colonisation and especially about consecutive or associated BSI following colonisation is limited.[15,16] There are a few studies about CRE colonisation in neonates. Karaaslan et al demonstrated that 9% of patients were colonised with CRE at neonatal ICU in Turkey.[17] During our study period, a total of 496 neonates and children were screened for CRE colonisation and 79 (15.9 %) of the patients were found to be colonised with CRE. Among CRE-colonised 79 patients, a total of 3 (3.7%) patients were had CRE-associated BSIs.

In this current study, the rate of the CRE-colonised patients was 56.9% at admission and all of them were referred to the study hospital from other healthcare centres. There is no paediatric study evaluating the prevalence of CRE colonisation at admission. However, a previous study including 1007 adult patients reported that 36 (3.6%) of the patients had *Enterobacteriaceae* colonisation at admission.[18] The high rate in our study may be due to higher risk of colonisation of the new-born patients.

The studies about the relationship between CRE colonisation and CRE infection, were limited. In our study, most of the CRE-associated infections were urinary tract infections observed after the CRE colonisation. In a large multicentre study in children reported 34 CRE related infections, including mostly sepsis (41.2%), followed by respiratory (26.5%) and urinary tract infections (20.6%).[15] In that recent study, in addition to patients at paediatric ICU and neonatal ICU, the haematology-oncology patients also were included in the study group and nearly half of the infected patients had underlying conditions such as haematologic/oncologic disease, transplantation and immunosuppression conditions. The difference in rates of infection types could be due to each hospitals' distinctive characteristics such as bed capacity, the patients' comorbidities. Also, as in patients with haematological-

Table 2 Distribution of causative microorganisms for bloodstream infections in children with gastrointestinal Carbapenem-resistant *Enterobacteriaceae* colonisation

Causative agents for bloodstream infections	Number (%)
Klebsiella pneumonia	9 (40.9)
Klebsiella oxytoca	1 (4.5)
Staphylococcus haemolyticus	1 (4.5)
Pseudomonas aeruginosa	5 (23)
Enterococcus faecalis	1 (4.5)
Serratia marcescens	2 (9.1)
Staphylococcus epidermidis	1 (4.5)
Stenotrophomonas maltophilia	2 (9.1)
Total	22 (100)

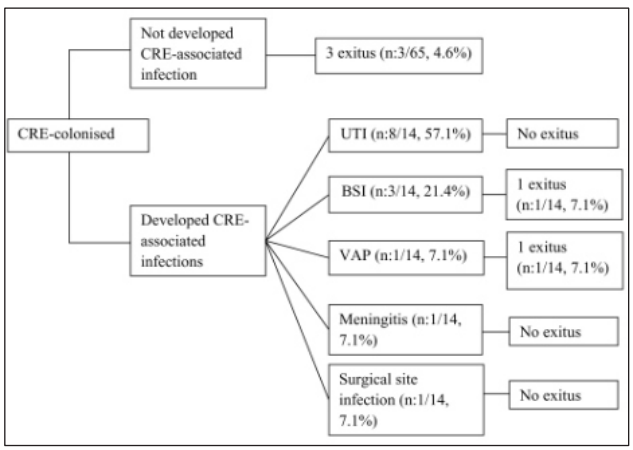

Figure 1 Mortality in patients with Carbapenem-resistant *Enterobacteriaceae* colonisation.

CRE: Carbapenem-resistant *Enterobacteriaceae*, UTI: urinary tract infection, BSI: blood stream infection, VAP: ventilator associated pneumonia.

oncological diseases and immunosuppression conditions, bacteraemia may be more common.

Li et al reported that the most common organism was isolated *K. pneumoniae* (78 cases, 79.6%) in adults with CRE associated BSIs.[19] In our study; the most frequently isolated were also *K. pneumonia* as consistent with other previous paediatric report.[20]

In one study from Israel focusing on adult ICU patients, *Enterobacteriaceae* infections were reported to occur in 28.8% of CRE carriers[21] which was relatively high compared to our study. During our study period, a total of 14 healthcare-associated CRE infections (17.7%) were observed. It is possible to find variations between the infection rates because of the patient groups are at different ages.

Jaiswal et al[22] reported that CRE bacteraemia is occurred exclusively in colonised with CRE and CRE colonisation was found the most significant risk factor for CRE bacteraemia.[22] The incidence of CRE bacteraemia in CRE-colonised patients was reported to be 18% in this study.[22] A recent study including paediatric haematology-oncology patients reported that a total of 5 (26%) patients had bacteraemia among 19 CRE-colonised patients.[16] On the contrary, in our study, 3.7% (n:3/79) of CRE-colonised patients had bacteraemia due to CRE during follow-up. The high rate of bacteraemia associated with CRE in those two studies may be due to the immunosuppression since these patients had leukaemia or transplantation, while our patients had different risk factors such as prematurity, cerebral palsy or congenital heart disease, instead of immunosuppression.

Carbapenem-resistant *Enterobacteriaceae* is a crucial problem for clinicians because of difficulties in management and treatment. Bloodstream infections with CRE were found associated with significantly higher mortality rates than observed with Carbapenem-susceptible *Enterobacteriaceae* (38% vs 12%; $p<0.001$).[23] It was shown that CRE infection was associated with an increased risk of mortality. The previous meta-analysis found that a significant association between CRE infection and mortality, odds ratio ranged from 2.85 to 3.73.[24] In our study, two of 14 patients with CRE-associated infection have died. Both had a comorbid disease such as prematurity and cerebral palsy. On this basis, having a comorbid disease can be a key factor for poor prognosis and CRE-infection. However, when evaluating this result, it should be taken into consideration that our study design is retrospective and the study group is small.

This study has limitations due to its retrospective design. For this reason, it could not be shown a clonal relationship with colonisation and bacteraemia with pulsed-field gel electrophoresis. Thus we could not directly link the CRE infections with colonisation but our study gives important data about a topic in which limited studies were present in the children. Secondly, we only registered the hospitalisations in our hospital, however, we did have a phone call to the family for further hospitalisations to different centres, to increase the coverage of the patients. Thirdly we had only focused on CRE related bacteraemia and did not evaluate the other health-care related infection types related to CRE. Also we must emphasise that due to the retrospective study design, risk factors for colonisation and infection could not be evaluated effectively.

In conclusion, among CRE-colonised 79 patients, a total of 3 (3.7%) patients had developed CRE-associated BSIs during follow-up. Although we had a relatively low prevalence of CRE bacteraemia in children and infants, the high mortality expectation especially with a comorbid disease, supported the screening of rectal CRE colonisation. The road to CRE bacteraemia from CRE colonisation can change from hospital to hospital, due to the individual differences of the hospitals including healthcare workers' compliance to contact precautions and the awareness of the health-care centres.

Ethics Approval and Consent to Participate

This study was approved by the Ethical Committee of University of Health Science İzmir, Dr. Behçet Uz Paediatrics and Surgery Training and Research Hospital, (approval number: 13399118-799, approval date: 19.06.2019). The patients or legal guardians of the participants provided signed informed consent forms for inclusion in the study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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References

1. Willyard C. The drug-resistant bacteria that pose the greatest health threats. Nature 2017;543:15.
2. Temkin E, Adler A, Lerner A, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: biology, epidemiology, and management. Ann N Y Acad Sci 2014;1323:22-42.
3. Goodman KE, Simner PJ, Tamma PD, Milstone AM. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant *Enterobacteriaceae* (CRE). Expert Rev Anti Infect Ther 2016;14:95-108.
4. Kelly AM, Mathema B, Larson EL. Carbapenem-resistant *Enterobacteriaceae* in the community: a scoping review. Int J Antimicrob Agents 2017;50:127-34.
5. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 2008;29:996-1011.
6. Bonten MJ, Weinstein RA. The role of colonization in the pathogenesis of nosocomial infections. Infect Control Hosp Epidemiol 1996;17:193-200.
7. Chiotos K, Tamma PD, Flett KB, et al. Multicenter Study of the Risk Factors for Colonization or Infection with Carbapenem-resistant *Enterobacteriaceae* in Children. Antimicrob Agents Chemother 2017;61:e01440-17.
8. Díaz A, Ortiz DC, Trujillo M, et al. Clinical Characteristics of Carbapenem-resistant Klebsiella pneumoniae Infections in Ill and Colonized Children in Colombia. Pediatr Infect Dis J 2016;35:237-41.
9. Singh NP, Choudhury DD, Gupta K, et al. Predictors for gut colonization of carbapenem-resistant *Enterobacteriaceae* in neonates in a neonatal intensive care unit. Am J Infect Control 2018;46:e31-5.
10. Centers for Disease Control and Prevention. Vital Signs: Carbapenem-Resistant *Enterobacteriaceae*. MMWR Morb Mortal Wkly Rep 2013;62:165-70. (https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6209a3.htm, last access date:04/02/2021)
11. Centers for Disease Control and Prevention. Facility Guidance for Control of Carbapenem-resistant *Enterobacteriaceae* (CRE). November 2015 Update - CRE Toolkit. 2015. (https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf. last access date:04/02/2021)
12. Satlin MJ, Jenkins SG, Walsh TJ. The global challenge of carbapenem-resistant *Enterobacteriaceae* in transplant recipients and patients with hematologic malignancies. Clin Infect Dis 2014;58:1274-83.
13. Borer A, Saidel-Odes L, Riesenberg K, et al. Attributable Mortality Rate for Carbapenem-Resistant Klebsiella pneumoniae Bacteremia. Infect Control Hosp Epidemiol 2014;30:972-6.
14. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-Resistant *Enterobacteriaceae*: Epidemiology and Prevention. Clin Infect Dis 2011;53:60-7.
15. Montagnani C, Prato M, Scolfaro C, et al. Italian Society of Pediatric Infectious Diseases. Carbapenem-resistant *Enterobacteriaceae* Infections in Children: An Italian Retrospective Multicenter Study. Pediatr Infect Dis J 2016;35: 862-8.
16. Giannini MA, Gilliam C, Owings A, Glover B, Gipson M, Hakim H. Does Colonization with Carbapenem-Resistant *Enterobacteriaceae* Correlate to Infection? Am J Infect Control 2017;45:S37.
17. Karaaslan A, Soysal A, Gelmez GA, Kadayifci EK, Söyletir G, Bakir M. Molecular characterization and risk factors for carbapenem-resistant Gram-negative bacilli colonization in children: emergence of NDM-producing Acinetobacter baumannii in a newborn intensive care unit in Turkey. J Hosp Infect 2016;92:67-72.
18. Dautzenberg MJD, Wekesa AN, Gniadkowski M, et al. Mastering Hospital Antimicrobial Resistance in Europe Work Package 3 Study Team. The association between colonization with carbapenemase-producing *enterobacteriaceae* and overall ICU mortality: an observational cohort study. Crit Care Med 2015;43:1170-7.
19. Li C, Li Y, Zhao Z, Liu Q, Li B. Treatment options and clinical outcomes for carbapenem-resistant *Enterobacteriaceae* bloodstream infection in a Chinese university hospital. J Infect Public Health 2019;12:26-31.
20. Folgori L, Livadiotti S, Carletti M, Bielicki J. Epidemiology and Clinical Outcomes of Multidrug-resistant, Gram-negative Bloodstream Infections in a European Tertiary Pediatric Hospital During a 12-month Period. Pediatr Infect Dis J 2014;33:929-32.
21. Chiotos K, Han JH, Tamma PD. Carbapenem-Resistant *Enterobacteriaceae* Infections in Children. Curr Infect Dis Rep 2016;18:2.
22. Jaiswal SR, Gupta S, Kumar RS, et al. Gut Colonization with Carbapenem-resistant *Enterobacteriaceae* Adversely Impacts the Outcome in Patients with Hematological Malignancies: Results of a Prospective Study. Mediterr J Hematol Infect Dis 2018;10:1-8.
23. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 2008;29:1099-106.
24. Soontaros S, Leelakanok N. Association between carbapenem-resistant *Enterobacteriaceae* and death: A systematic review and meta-analysis. Am J Infect Control 2019;47:1200-12.