Enterobacteriaceae species are common cause of infections in both community and healthcare settings worldwide. Carbapenem are the mainstay of therapy for infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). The emergence and dissemination of carbapenem resistance among Enterobacteriaceae in all over the world represents a serious threat to public health and significantly limits the treatment options for life threatening infections.1 Although colistin is currently considered as a last-resort treatment for infections caused by multi-drug resistant (MDR) bacteria, colistin resistance in Enterobacteriaceae species has been reported in several countries around the world and its prevalence has continued to increase, thus becoming a great healthcare concern.2 5 It is important for healthcare facilities to understand how common CRE are in their institutions. Recognizing the risk factors of carbapenem and colistin-resistant Enterobacteriaceae infections is essential in order to conserve carbapenem and colistin, since there are no new antibiotics to treat multidrug-resistant Enterobacteriaceae infections.

Key words: carbapenem-resistance, colistin-resistance, Enterobacteriaceae, outcome, risk factors.
MDR Enterobacteriaceae infections. Interventions to control CRE are evolving as more data and experience become available. Although several factors that increase the risk of infection with CRE have been reported, there is limited data on the epidemiology, risk factors, treatment, and outcomes in pediatric populations with colistin-resistant Enterobacteriaceae infections. In this study it was aimed to characterize the clinical features of patients with carbapenem and colistin-resistant Enterobacteriaceae infections. The other goals were to identify the risk factors, investigate the outcomes and determine the frequency of colistin and carbapenem resistance in clinical isolates of Enterobacteriaceae species.

Material and Methods

The clinical and microbiological data of all patients with isolation of Enterobacteriaceae from different specimens between December 2013 and December 2017 were retrospectively evaluated in our university hospital. Only patients determined to have an active infection were included in the study. Patient demographics, comorbidities, dates of admission, outcomes, medications, history of surgery, use of mechanical ventilation, and procedures applied during the hospitalization were included in the study. To identify CRE definition CDC surveillance reports in 2015 were used. According to CDC reports CRE was identified in two situations; 1-Resistant to any carbapenem antimicrobial (i.e. minimum inhibitory concentrations of ≥4 mcg/ml for doripenem, meropenem, or imipenem or ≥2 mcg/ml for ertapenem) 2- For bacteria that have intrinsic imipenem non-susceptibility (i.e. Morganella morganii, Proteus spp., Providencia spp.), resistance to carbapenems other than imipenem. Identification and susceptibility testing of clinical isolates were performed using automated laboratory system; Vitek-2 (bioMérieux). Minimum inhibitory concentrations (MICs) were interpreted according to European Committee on Antimicrobial Susceptibility testing (EUCAST) breakpoints. Empiric antimicrobial therapy was identified as the antibiotics those used before the index blood culture results and those used at least 48 hours. Healthcare-associated infections were diagnosed according to Centers for Disease Control and Prevention definitions. This study was approved by the Institutional Ethics Committee (5th October 2018; report number 81/5). All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Statistical analysis

An \( \chi^2 \) test or Fisher exact test was used to compare the categorical variables, while Student t-test or Mann-Whitney U test was used to compare the continuous variables, as appropriate. Univariate and multivariate logistic regression analyses were used to assess factors associated with in-hospital mortality and CRE infections. In-hospital mortality was compared between the CRE group and CSE group by using Kaplan-Meier estimation and compared using the long-rank test. A p-value <0.05 was considered statistically significant for all tests. All analyses were performed with SPSS Statistics (version 21.0. IBM Corp, Armonk, New York).

Results

Demographics and clinical history of the patients with CRE

One-hundred-fifty patients with Enterobacteriaceae infections were included in the study; 76 were male (50.7%), 74 were female (49.3%). In total 62 patients (41.3%) were infected with CRE. Twenty-eight (45.2%) of the CRE isolates were in an ICU ward (neonatal and pediatric). The wards in which patients with CRE infections were isolated are shown in Table I.
Carbapenem resistance was observed in 47 of 98 (47.9%) Klebsiella species, in eight of 23 (34.7%) E. coli species, in three of ten (30%) Enterobacter species, in one of two (50%) Citrobacter species and in three of eight (37.5%) Stenotrophomonas species. CRE was isolated from blood in 51 (82.3%) patients, from respiratory tract in eight (12.9%), from urine in one patient (1.6%) and from wound specimens in two patients (3.2%). Ventilator-associated pneumonia (VAP) was observed in 22 patients (35.5%) and pneumonia other than VAP was observed in six patients (9.7%).

All patients were hospitalized for >48 hours before their CRE infection. Underlying medical conditions were reported in 54 (87.1%) of 62 patients with CRE infection. The most common condition was cardiac disease (17 patients, 27.4%), 14 (22.5%) had oncologic processes, nine (14.5%) had pulmonary disease, nine (14.5%) had a history of prematurity, four (6.4%) patients had necrotizing enterocolitis and/or short-bowel syndrome. A history of surgery was reported in 31 (50%) of 62 patients; 15 (48.3%) of the procedures were gastrointestinal, and 10 (32.2%) were cardiac procedures. An indwelling device was reported in 60 (96.8%) of 62 children.

Of 62 patients with CRE infections, bacteremia was observed in 51 patients (82.2%). There was no statistical difference in terms of gender between CRE and carbapenem-susceptible Enterobacteriaceae (CSE) groups (p = 0.25). The median age of patients in CRE group was 29 days (range: 4 days – 2.71 years) and in the CSE group was 1.03 year (range: 5 days - 22.7 years). Patients in the CRE group were younger than the patients in the CSE group (p = 0.004). Demographics and clinical findings of patients with CRE are shown in Table II.

### Risk factors of CRE infections

Although patients with CRE infections were younger and had higher rates of central catheter compared to the CSE group, in regression analysis neither age nor central line was associated with the risk of CRE infections. Risk factors of CRE infections are demonstrated in Table III. Prior to the index culture, empiric polyantibiotic therapy for Gram negative bacteria was detected in 31 (50%) patients with CRE vs. 16 (18.2%) patients with CSE. Prior exposure to polyantibiotic therapy for Gram negative bacteria was found as a risk factor of CRE (p = 0.001). The median length of hospital stay prior to positive cultures with CRE infections was longer than the length of hospital stay in patients with CSE infections (p = 0.003).

Logistic regression analysis revealed that three variables were independently associated with the isolation of CRE strains: length of hospitalization prior to index culture (OR, 1.02; 95% CI, 1.01 -1.04, p=0.003), previous polyantibiotic therapy (OR, 6.4; 95% CI, 3.07-13.6, p=0.001) and history of surgical intervention during admission (OR, 2.46; 95% CI, 1.2-5.1, p=0.005).

| Department                        | CRE (n= 62) | CSE (n= 88) | Total (n= 150) |
|-----------------------------------|-------------|-------------|---------------|
| Pediatric intensive care unit, n (%)| 14 (22.6)   | 17 (19.3)   | 31 (20.7)     |
| Neonatal intensive care unit, n (%)| 14 (22.6)   | 48 (54.5)   | 62 (41.3)     |
| Pediatric hematology and oncology, n (%)| 15 (24.2)   | 17 (19.3)   | 32 (21.3)     |
| Pediatric cardiovascular surgery, n (%)| 7 (11.3)    | 2 (2.3)     | 9 (6)         |
| Burn unit, n (%)                  | 2 (3.2)     | 0 (0)       | 2 (1.3)       |
| Pediatric surgery, n (%)          | 1 (1.6)     | 1 (1.1)     | 2 (1.3)       |
| Other pediatric wards, n (%)      | 9 (14.5)    | 3 (3.4)     | 12 (8)        |

CRE: carbamapen-resistant Enterobacteriaceae, CSE: carbapenem-sensitive Enterobacteriaceae
Demographics and clinical history of the patients with colistin-resistant Enterobacteriaceae infections

Twenty-three patients had colistin-resistant Enterobacteriaceae infections. The median age of the children with colistin-resistant Enterobacteriaceae infections was 150 days (range, 5 days -11.8 years). Nine (39%) patients were male and 14 (61%) patients were female. The median length of hospital stay prior to positive culture results of colistin-resistant species was 26 days (range, 5-150 days; interquartile range, 11-46 days). The rate of colistin resistance in Enterobacteriaceae species increased markedly from 4.9% in 2014 to 25% in 2017. In 2014 two out of 41 Enterobacteriaceae infections were colistin-resistant (4.9%). In 2015 eight out of 37 infections (17.8%), in 2016 six (18.2%) were colistin-resistant and in 2017 seven (25%) were colistin-resistant. Nineteen (82%) patients were in an ICU ward (neonatal and pediatric). Underlying medical conditions were
reported in all patients with colistin-resistant Enterobacteriaceae infections. The most common condition was cardiac disease (nine patients, 39%), six (26%) had pulmonary disease, five (21.7%) had a history of prematurity, three patients (13%) had necrotizing enterocolitis and/or short-bowel syndrome. A history of surgery was reported in 11 (47.8%) out of 23 patients. Central line was reported in 22 (95.7%) patients. Mechanical ventilation prior to index culture was reported in 21 (91.3%) patients. Urinary catheter was reported in 15 (65.2%) patients. Source of isolates were blood in 20 (87%) patients. Sixteen (69.6%) colistin-resistant strains were Klebsiella species, six (26.1%) were Serratia species, one (4.3%) was E.coli species. Demographics and clinical findings of patients with CRE are shown in Table IV.

Risk factors of colistin-resistant Enterobacteriaceae infections

Mechanical ventilation prior to index culture were more common in patients with colistin-resistant group (21/23) than colistin-susceptible group (68/127) which was 91.3% vs. 53.5% respectively. Risk factors of colistin resistance are shown in Table III. In the regression analysis mechanical ventilation prior to index culture was determined as a risk factor of colistin resistance (OR, 9.4; 95% CI, 2-40.4; p = 0.004).

Urinary catheter was reported higher in the colistin-resistant group (15/23) than the colistin sensitive group (46/127) which was 65.2% vs. 36.2%, respectively. In logistic regression analysis urinary catheter was the risk factor of colistin resistance (OR, 0.32; 95% CI, 0.18-0.38; p = 0.012). The other independent risk factor of colistin resistance was underlying necrotizing enterocolitis and/or short-bowel syndrome (OR, 6.38; 95% CI, 1.16-35; p = 0.033).

Outcome in CRE and colistin-resistant Enterobacteriaceae infections

The in-hospital mortality of the CRE group was (28/62) 45.2%. There were no statistically significant difference in mortality rates between CRE and CSE groups which was 45.2% vs. 36.7% (p = 0.071). The median length of hospital stay after the index culture in CRE group was 21 days (range, 0-96 days) vs. 19 days (range, 0-160 days) in CSE group (p = 0.12) (Fig. 1). The in-hospital mortality rates of patients infected by colistin-susceptible Enterobacteriaceae was 36.2% (46/127) and colistin-resistant Enterobacteriaceae was 39.1% (9/23). In Kaplan-Meier analysis there were no statistically significant difference

| Table IV. Demographics and clinical findings of patients with and without colistin resistance Enterobacteriaceae. |
| Demographics and clinical findings | Colistin resistant (n = 23) | Colistin sensitive (n = 127) | P |
| Age, median (range) | 150 days (5 days – 1.8 years) | 165 days (4 days - 22.7 years) | 0.56 |
| Male, n (%) | 9 (39.1) | 67 (52.8) | 0.22 |
| Urinary catheter, n (%) | 15 (65.2) | 46 (36.2) | 0.009* |
| Underlying necrotizing enterocolitis, n (%) | 3 (13.1) | 4 (3.1) | 0.038* |
| Mechanical ventilation prior to index culture, n (%) | 21 (91.3) | 68 (53.5) | 0.001* |
| Piperacilin/tazobactam, n (%) | 1 (4.3) | 7 (5.5) | 1.0 |
| Carbapenem, n (%) | 5 (21.7) | 26 (20.5) | 1.0 |
| 3rd or 4th generation cephalosporin, n (%) | 2 (8.7) | 15 (11.8) | 0.66 |
| Aminoglycoside, n (%) | 3 (13) | 31 (24.4) | 0.23 |
| Carbapenem + aminoglycoside, n (%) | 3 (13) | 13 (10.2) | 0.36 |
| Carbapenem + except aminoglycoside (%) | 4 (17.4) | 11 (8.7) | 0.74 |
| Aminoglycoside + except carbapenem, n (%) | 5 (21.7) | 11 (8.7) | 0.035* |

*: p <0.05
in in-hospital mortality rates between the colistin-susceptible and colistin-resistant Enterobacteriaceae infections (p = 0.9). The median length of hospital stay after the index culture in colistin-resistant group was 20 days (range, 0 - 76 days) vs. 19 days (range, 0 - 160 days) in colistin-susceptible group (p = 0.80).

Discussion

Our 4-year surveillance clearly confirmed that infections caused by Enterobacteriaceae isolates are an important clinical problem and high rates of carbapenem and/or colistin resistance can be encountered with these infections. We also found a high rate of colistin resistance that emerged over a period of four years.

The inability to recognize CRE infections when they first occur in a health care facility leads to the loss of the chance of early treatment choices before these infections are transmitted more widely. For this reason it is important to be aware of the risk factors for CRE infections.6 In our study empiric polyantibiotic therapy for Gram negative bacteria, long hospital stay before index culture and history of surgical intervention were found as risk factors of CRE infections. Mechanical ventilation prior to index culture, urinary catheter exposure, underlying necrotizing enterocolitis and/or short-bowel syndrome was found as risk factors of colistin resistance.

One of the most concerning risk factors of CRE infections found in our study was the empiric polyantibiotic therapy for Gram negative bacteria. Use of antimicrobials including carbapenems, cephalosporins, and fluoroquinolones associated with CRE infections or carriage of CRE has been reported in the literature.12-14

In a 4-year case-control study involving 102 patients, the only common variable associated with CRE infections was the cumulative number of antibiotic exposures prior to CRE infections.8 In another case-control study from Greece both prior exposure to antibiotics and duration of the prior antibiotic treatment were identified as the risk factors of CRE.15

Injudicious use of broad-spectrum agents may lead to the development of clinical resistance during therapy.16 Broad spectrum antibiotics can destroy the sensitive flora, and lead to the colonization and proliferation of the resistant mutant strains.16

There is an evolving body of medical literature suggesting an important relationship between prior antimicrobial therapy and the subsequent identification of carbapenemase-producing bacteria. Our data was consistent with previous reports that empiric polyantibiotherapy which was mentioned in our study carbapenem plus aminoglycoside and aminoglycoside combination therapy other than carbapenem were consistent with higher rates of CRE infections. In order to get rid of the “broad-spectrum is best” approaches, we have to find novel ways to detect pathogens early.

Regarding the other risk factors other than antibiotic exposure, Patel et al.8 found that invasive infections with carbapenem-resistant K. pneumoniae were independently associated with longer length of stay when compared with patients with carbapenem-susceptible K.
pneumoniae. Also in our study long hospital stay before index culture was also found as a risk factor of CRE. During long hospital stay patients can be exposed to multiple invasive procedures.

Surgical intervention particularly gastrointestinal procedures were found as high-risk procedures for CRE infections. Since most, ESBL-producing Enterobacteriaceae reside in the gastrointestinal tract it was not surprising to find this relationship. Although this relationship raises questions such as whether patients should be screened for ESBL-producing Enterobacteriaceae prior to surgery or whether modified surgical antibiotic prophylaxis in areas with high ESBL-producing Enterobacteriaceae prevalence is needed. However this topic was beyond the scope of our study. Also there is no known answer for these situations. Invasive CRE infections are associated with worse outcomes as compared to CSE infections. CRE isolation has been associated with all-cause hospital mortality ranging from 29% to 52%.

In our study the rate was 45.2%. Although CRE infections are known to be associated with high mortality, we did not find any difference between the mortality rates of CRE and CSE groups. Also in the study by Bhargava et al. and Candevir Ulu et al. mortality was not statistically different between carbapenem-resistant and susceptible strains, which was similar to our study.

In the present study, the ratio of colistin-resistant infections in Enterobacteriaceae infections (32.3%) was similar to what was previous reported by Zarkotou et al. and Capone et al. which demonstrated 25% to 37% of resistance to colistin.

Although, colistin resistance has been associated with high mortality, there is controversy about the impact of resistance to colistin on prognosis. In our study mortality rates with colistin-resistant Enterobacteriaceae infections was not different from colistin-susceptible Enterobacteriaceae infections which was 45.2% and 45% respectively.

Our study has several limitations. Firstly, clinical data in this study were the single center experiences obtained from medical records of the patients retrospectively. Secondly, we used Vitex 2 for colistin MIC determination. Although, recently, a joint recommendation by CLSI and EUCAST released in 2016 recommended broth microdilution as a standard method for MIC testing of colistin, the use of broth microdilution methods for susceptibility may not be practical in laboratories depending on individual workloads and is rarely used in routine microbiology laboratories. Also Vitex 2 was previously reported as a good testing method for colistin MIC determination. 

In the literature there are evolving studies evaluating the commercial testing methods comparing to broth microdilution for colistin MIC determination.

Further investigations will increase our understanding of these serious infections and give us an opportunity to find practices for reducing the frequency and the mortality of these infections.

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