RESEARCH ARTICLE

Ozlem Aydemir [1]
Mehmet Koroglu [1]
Gokcen Ormanoglu [1]
Tugba Ayhanci [1]
Yusuf Aydemir [2]
Ertugrul Guclu [3]

[1] Department of Microbiology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[2] Department of Pulmonology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[3] Department of Infectıon Disease and Microbiology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye

Corresponding Author:
Ozlem Aydemir
mail: akkozlem@hotmail.com

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konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Carbapenem-Resistant *Enterobacterales* Infections and Their Association with Rectal Colonization

ABSTRACT

Objective: Carbapenem-resistant *Enterobacterales* (CRE) infections have limited treatment options, and these infections are associated with high mortality rates. Asymptomatic carriers colonized with CRE contribute to the spread of CRE in hospitals. It was aimed to determine the frequency of CRE isolates detected in our center, carbapenemase ratios in these strains, carbapenemase genes, antibiotic resistance profiles, rectal CRE colonization rates and to evaluate various clinical features of CRE infections.

Methods: *Enterobacterales* species isolated from various specimens and *Enterobacterales* species isolated from rectal swab specimens sent for colonization screening were examined. Patients with CRE colonization in rectal swab samples were examined for the development of CRE infection at a later time. CRE isolates were examined for carbapenemase production and the presence of carbapenemase gene.

Results: 14521 *Enterobacterales* (10161 *E. coli* and 4195 *K. pneumoniae*, 165 *Citrobacter*) isolates were examined. Carbapenem resistance was detected in 8.9% of these strains. CRE was detected in 4.7% of 15695 rectal swab samples evaluated for colonization. In 23.4% of the patients with CRE colonization, CRE growth was detected in other samples besides the rectal swab in the later period. It was observed that CRE infections developed on average 21 days after colonization.

Conclusions: CRE infections have started to emerge as a factor not only in hospitalized patients but also in community-acquired infections. Our study also showed that CRE colonization could be a significant risk factor for the development of infection. Therefore, early screening detection to detect colonization can help prevent or limit CRE infections with appropriate isolation methods.

Keywords: Carbapenem-Resistant *Enterobacterales*, Colonizations, Carbapenemase.

Karbapenem Dirençli *Enterobacterales* Enfeksiyonları ve Rektal Kolonizasyon ile İlişkisi

ÖZET

Amaç: Karbapenem dirençli *Enterobacterales* (CRE) enfeksiyonlarının tedavi seçenekleri sınırlıdır ve bu enfeksiyonlar yüksek mortalite oranları ile ilişkilidir. CRE ile kolonize olan asemptomatik taşıyıcılar, CRE'nin hastanelerde yayılmasına katkıda bulunur. Bu çalışmada: merkezimizde saptanan CRE izolatlarının sıklığının, bu suşlardaki karbapenemaz oranlarının, karbapenemaz genlerinin, antibiyotik direnç profillerinin, rektal CRE kolonizasyon oranlarının belirlenmesi ve CRE enfeksiyonlarının çeşitli klinik özelliklerinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çeşitli örneklerden izole edilen *Enterobacterales* türleri ve kolonizasyon taraması için gönderilen rektal sürüntü örneklerinden izole edilen *Enterobacterales* türleri incelenmiştir. Rektal sürüntü örneklerinde CRE kolonizasyonu olan hastalar daha sonra CRE enfeksiyonu gelişimi açısından incelendi. CRE izolatları karbapenemaz üretimi ve karbapenemaz geni varlığı açısından incelenmiştir.

Bulgular: 14521 *Enterobacterales* (10.161 *E. coli* ve 4195 *K. pneumoniae*, 165 *Citrobacter*) izolatı incelendi. Bu suşların %8.9'unda karbapenem direnci saptanmıştır. Kolonizasyon için değerlendirilen 15695 rektal sürüntü örneğinin %4,7'sinde CRE saptanmıştır. CRE kolonizasyonu olan hastaların %23,4'ünde ilerleyen dönemde rektal sürüntü dışında diğer örneklerde de CRE üremesi saptandı. CRE enfeksiyonlarının kolonizasyondan ortalama 21 gün sonra geliştiği gözlendi.

Sonuç: CRE enfeksiyonları sadece hastanede yatan hastalarda değil toplum kökenli enfeksiyonlarda da bir etken olarak karşımıza çıkmaya başlamıştır. Çalışmamız ayrıca CRE kolonizasyonunun enfeksiyon gelişimi için önemli bir risk faktörü olabileceğini göstermiştir. Bu nedenle, kolonizasyonu saptamak için erken tarama tespiti, uygun izolasyon yöntemleriyle CRE enfeksiyonlarını önlemeye veya sınırlamaya yardımcı olabilir.

Anahtar Kelimeler: Karbapenem Dirençli *Enterobacterales*, Kolonizasyon, Karbapenemaz

INTRODUCTION

Carbapenem-resistant *Enterobacterales* (CRE) first appeared in the 1980s and has spread worldwide (1). CRE infections have limited treatment options and these infections are associated with high mortality rates (2). Asymptomatic carriers colonized with CRE contribute to the spread of CRE in hospitals. The gastrointestinal tract is the most critical reservoir for hospitalized patients, resulting in cross-contamination and infections. Therefore, CRE colonization has been recognized as an important risk factor for subsequent infection (3). It has been reported that approximately 50% more CRE infections develop in patients colonized with CRE than in non-colonized patients.

In patients colonized or infected with CRE; it has been reported that health care costs increased, length of hospital stay increased, mortality rates increased, and treatment failures were more common (4). Therefore, identifying colonized patients/carriers of CRE and taking the necessary infection control measures are crucial to halt the spread of CRE (1).

Carbapenem resistance in *Enterobacterales* generally results from the production of β-lactamases such as KPC and New Delhi metallo β-lactamase (NDM), extended-spectrum β-lactamase (ESBL), AmpC lactamase (AmpC) and/or outer membrane protein loss. The genes that cause carbapenem resistance are usually found on plasmids. This resistance can be spread by clonal expansion or by horizontal transfer of genes to naive bacteria (2). Carbapenemase-producing strains spread more quickly than non-carbapenemase-producing strains. More intensive infection control measures are required against these strains. Therefore, it is appropriate to examine carbapenem-resistant strains for carbapenemases production (5). The Centers for Disease Control and Prevention (CDC) recommends that clinical laboratories actively screen isolates for carbapenemase production in carbapenem-resistant *Enterobacterales* strains (6).

However, tests to determine the underlying mechanism of carbapenem resistance are not routinely performed by most clinical microbiology laboratories today. It is known that the efficacy of new antibiotics developed and put into use against resistant strains varies according to carbapenemase production and carbapenemase species. This highlights the importance of detecting carbapenemase production and carbapenemase species in CRE (7).

In this study, it was aimed to determine the frequency of CRE isolates detected in our center in the last five years, carbapenemase ratios in these strains, carbapenemase genes, antibiotic resistance profiles, rectal CRE colonization rates in intensive care units, and to evaluate various clinical features of CRE infections.

MATERIAL AND METHODS

Carbapenem-resistant *Enterobacterales* species isolated from blood, tracheal aspirate, bronchoalveolar lavage (BAL), wound, urine, and sterile body fluid samples sent from intensive care and clinics between January 2017 and December 2021 were included in the study. CRE strains detected in the repetitive sample of the same patient were excluded from the study. However, strains that grew in different samples were included in the study.

In our hospital, patients hospitalized in intensive care units are routinely screened for CRE colonization from rectal swab samples on the first day of the week. In case of recurrent CRE growth in the rectal swab sample of the same patient, only the first growths were included in the study. The first CRE growth detected in the rectal swab sample of the patient was evaluated as colonization. It was examined whether there was growth in different samples of the patients who had growth in the rectal swab sample at a later time.

*Enterobacterale*s species were screened for carbapenem resistance. Eosin metilen blue (EMB) agar medium containing 2 mg/L ertapenem, which we prepared ourselves, was used for CRE screening. Antimicrobial susceptibility tests were performed with the VITEK 2® [Biomerieux, Marcy l'Etoile, France] automated system. Carbapenem resistance was confirmed by using the ertapenem gradient strip test [BioMérieux, Marcy l'Etoile, France] in strains found to have carbapenem resistance with VITEK 2®. To determine carbapenemase production, the Carbapenemaz inactivation test (CIM) was performed in accordance with the recommendations of Clinical & Laboratory Standards Institute (CLSI). Among the carbapenemase genes, blaIMP-1, blaKPC, blaNDM-1, blaOXA-48 and blaVIM genes were determined using the Gene-Xpert® System Carba R® kit [Cepheid, Sunnyvale, USA]. Ethics committee approval was not required because our study was conducted as a retrospective file review.

RESULTS

A total of 10,161 *E. coli* and 4195 *K. pneumoniae*, 165 *Citrobacter* growths were detected during the study period. It was observed that 1305 (8.9%) of these strains were carbapenem-resistant. When the distribution of CRE strains at the species level is examined, 250 (19.2%) were *E. coli*, 1046 (80.1%) were *K. pneumoniae*,11 (0.8%) were *Citrobacter spp.* was detected. When the distribution of CRE rates by years is evaluated; between 2017 and 2021, CRE rates were respectively; It was determined as 6.4%, 8.1%, 7.9%, 7.8%, and 14.1%. The distribution of CRE strains at the species level by years is shown in Table 1.

When the samples with CRE growth were examined; It was determined that 344 (26.3%) were

Table 1. Distribution of CRE isolates at the species level by years.

Year	*E. coli*	CR *E. coli*		*K.pneumoniae*	CR *K. pneumoniae*		*Citrobacter spp*	*CR Citrobacter spp*		Total CR	
	n	n	%	n	n	%	n	n	%	n	%
2017	2039	48	2.3	777	133	17.1	41	3	7.3	184	6.4
2018	1600	32	2	530	142	26.7	40	3	7.5	177	8.1
2019	2440	66	2.7	1020	208	20.3	31	2	6.4	276	7.9
2020	2012	61	3.0	837	164	19.5	25	2	8	227	7.8
2021	2070	43	2.0	1031	399	38.5	28	1	3.5	443	14.1
toplam	10161	250	2.4	4195	1046	24.9	165	11	6.6	1305	8.9

CR: carbapenem resistance

blood, 560 (42.9%) urine, 176 (13.4%) tracheal aspirate, and BAL, 225 (17.3%) other samples [wound, peritoneum, sterile, catheter].

A total of 1305 CRE strains were detected from 1225 patients. In 80 (6.5%) of the patients, CRE growth was observed in more than one sample.

Of the patients with CRE, 629 (51.4%) were treated in intensive care units, 461 (37.6%) were treated in wards, and 135 (11%) were admitted to polyclinics. Of the patients, 572 (46.7%) were male and 653 (53.3%) were female. The mean age of the patients was 65.7.

During the study period, 15695 rectal swab samples taken from hospitalized patients and sent to the laboratory for screening for CRE colonization were examined. CRE growth was detected in 745 (4.7%) of these rectal swab samples. All of the patients with CRE growth were hospitalized in the intensive care unit. Of the patients, 348 (46.8%) were female, and 397 (53.2%) were male. The mean age of the patients was 68.5. It was determined that these patients had CRE colonization. While 175 (23.4%) of these 745 patients who were evaluated as CRE colonization were hospitalized, CRE growth was detected in other samples other than rectal swab in the later period (in one or more samples).

In our study, 175 (13.4%) of 1305 CRE strains that we identified and evaluated as causative agents of CRE infection were infections that developed after rectal colonization. In patients with rectal colonization, CRE infections developing after colonization were observed to develop on average 21 days after colonization. Of the patients with colonization, 14 (8%) blood, 67 (38.2%) urine, 13 (7.4%) tracheal aspirate, 12 (6.8%) wound, 68 (39%) more than one CRE growth was detected in the sample.

Carbapenemase production was detected in 78% of the strains. The carbapenemase gene was investigated with Gene-Xpert® System Carba R® kit in 126 (91 positive, 35 negative) strains whose carbapenemase production was investigated by CIM. Carbapenemase gene was detected by Gene-Xpert® System Carba R® in 90 of the strains with carbapenemase production. When the distribution of carbapenemase genes detected from CRE isolates is examined; blaKPC was detected in 10 strains (11.1%), blaNDM-1 in 31 strains (34.4%), blaNDM+OXA-48 in 14 strains (15.5%), and blaOXA-48 in 35 strains (38.8%).

DISCUSSION

Despite all infection control measures, antibiotic resistance rates and infections caused by resistant bacteria are increasing all over the world (1,8,9). Resistance rates to meropenem and imipenem in *K. pneumoniae* strains in China; while it was 2.9% and 3.0% in 2005, in 2018; It has been reported to be 26.3% and 25% (10) It is seen that the rates of CRE vary between 1.1% and 60% worldwide (2,10-13). In our center, the rate of CRE was found to be 8.9% among all *Enterobacterales* species. While CRE infections were only encountered in hospitalized patients when they were first detected, community-acquired CRE infections have started to appear in recent years. Studies have reported that the prevalence of community-acquired CRE varies between 0% and 29.5% (14). 11.1% of CRE strains isolated in our study were isolated from the samples of patients admitted to outpatient clinics. The results of our study showed that CRE infections could be a risk factor not only for hospitalized patients but also for community-acquired infections.

The most common type of CRE isolated *K. pneumoniae*, followed by *E. coli* (2,15). It was determined that 250 (19.2%) of CRE strains detected in our center were *E. coli*, 1046 (80.1%) *K. pneumoniae*, 9 (0.7%) *Citrobacter spp.*

Carbapenem resistance seen in CRE strains can develop by different mechanisms. Among *Enterobacterales* species, the main mechanisms of carbapenem resistance are the production of carbapenemases such as KPC and NDM, ESBL, or AmpC beta-lactamase enzymes (16,17). Carbapenemase production in CRE strains ranges from 5% to 80% (16). This rate was found to be 78% in our center.

Carbapenemase resistance genes may vary depending on geographical differences (16-18). The most important mechanism of carbapenem resistance in *Enterobacterales* species is KPC-type

enzyme production. The KPC gene is usually transferred via plasmids. Therefore, there is a problem in controlling infections caused by KPC producing strains (16). In this study, blaKPC was found positive in 10 (11.1%) of the carbapenemase-secreting 91 CRE isolates in which carbapenemase gene regions were investigated by molecular method. NDM-1 poses a global health threat because it has high resistance rates and spreads rapidly and causes hospital epidemics (19). NDM rates have been reported to be between 3.3-54.5% in our country (15,20). NDM-1 was detected in 34.4% of CRE detected in our center, while NDM and OXA-48 were found to be associated in 15.5% of the strains. OXA-48-producing *Enterobacterales* species are spreading rapidly worldwide (21-23). Among the strains we examined in this study, the blaOXA-48 gene was found to have the highest rate (38.8%).

It is known that CRE infections usually develop before colonization (24). 1121 (86.4%) of 1305 CRE strains detected in our center were isolated as infectious agents. However, in 175 (23.4%) of the strains, rectal CRE colonization was detected first, and these strains were found to be infectious agents later on. Our study showed that rectal colonization with CRE is a significant risk factor for subsequent CRE infection.

Tischendorf et al. reported that infection developed in 16.5% of patients with colonization (24). McConvill et al. found that approximately 50% of patients colonized with CRE developed a CRE infection within 30 days and reported a 10.8-fold increase in infection rates compared to non-colonized patients. 9% of patients who developed rectal colonization developed CRE infection (4). After the worldwide spread of CRE, many centers have initiated infection control programs to limit the spread of CRE, the rate of infection, morbidity and mortality. Timely detection of carriers, separation of carriers from non-carriers, and activation of contact measures are important. In line with this information, our weekly routine screenings had continued since 2015, when the first CRE case was detected in our center, and rectal swab samples are taken from the patients hospitalized in intensive care units on the first day of the week and evaluated in the laboratories. We detected VRE colonization in our hospital at 4.7%. In 23.4% of the patients with CRE colonization, we encountered CRE strains as a factor in the later period.

The most important limitation of our study was that we could not search for carbapenemase detection methods and carbapenemase genes in all CRE strains.

In conclusion, CRE infections have started to emerge as a factor not only in hospitalized patients but also in community-acquired infections. Necessary measures should be taken in this regard as soon as possible. Our study also showed that CRE colonization could be a major risk factor for the development of infection. Therefore, early screening detection to detect colonization can help prevent or limit CRE infections with appropriate isolation methods. Detection of resistance mechanisms of CRE isolates will ensure that treatment measures are taken more accurately, and necessary measures will be taken to prevent the spread of pathogens.

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