Intestinal Carriage of Carbapenemase-Producing *Enterobacteriaceae* Members in Immunocompromised Children During COVID-19 Pandemic

Nasim Almasian Tehrani [1, 2], Masoud Alebouyeh [2], Shahnaz Armin [2], Neda Soleimani [1], Abdollah Karimi [2], Bibishahin Shamsian [3], Shiva Nazari [3] and Leila Azimi [2, *]

[1]Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
[2]Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: leilaazimi1982@gmail.com

Received 2022 May 11; **Revised** 2022 October 07; **Accepted** 2022 October 10.

Abstract

Background: Hospital-acquired infection with carbapenem-resistant *Enterobacteriaceae* (CRE) is a global concern. The administration of antibiotics among the infected and non-infected immunocompromised children with SARS-CoV-2 is associated with an increased risk of intestinal CRE colonization and bacteremia during hospitalization.

Objectives: The present study aimed to detect the correlation between the intestinal colonization of carbapenemase encoding Enterobacteriaceae with SARS-CoV-2 infection and antibiotic prescription among immunocompromised children admitted to the oncology and Bone Marrow Transplantation (BMT) wards.

Methods: Stool samples were collected from the immunocompromised children, and the members of *Enterobacteriaceae* were isolated using standard microbiological laboratory methods. Carbapenem resistance isolates were initially characterized by the disc diffusion method according to CLSI 2021 and further confirmed by the PCR assay. SARS-CoV-2 infection was also recorded according to documented real-time PCR results.

Results: In this study, 102 *Enterobacteriaceae* isolates were collected from the stool samples. The isolates were from *Escherichia* spp. (59/102, 57.8%), *Klebsiella* spp. (34/102, 33.3%), *Enterobacter* spp. (5/102, 4.9%), *Citrobacter* spp. (2/102, 1.9%), and *Serratia* spp. (2/102, 1.9%). The carbapenem resistance phenotype was detected among 42.37%, 73.52%, 40%, 50%, and 100% of *Escherichia* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp., respectively. Moreover, bla_{OXA-48} (49.1%) and bla_{NDM-1} (29.4%), as well as bla_{VIM} (19.6%) and bla_{KPC} (17.6%) were common in the CRE isolates. SARS-CoV-2 infection was detected in 50% of the participants; however, it was confirmed in 65.45% (36/55) of the intestinal CRE carriers. The administration of antibiotics, mainly broad-spectrum antibiotics, had a significant correlation with the CRE colonization in both the infected and non-infected children with SARS-CoV-2 infection.

Conclusions: Regardless of the COVID-19 status, prolonged hospitalization and antibiotic prescription are major risk factors associated with the CRE intestinal colonization in immunocompromised children.

Keywords: Children, Hospital-Acquired Infections, Carbapenem Resistant *Enterobacteriaceae*

1. Background

Immunocompromised children with underlying diseases are at higher risk of hospital-acquired infection (1). These patients experience multiple hospitalization courses and receive antibiotics with likely effects on their gut microbiota composition. The richness of resistant variants of these bacteria in the intestine during hospitalization is mainly associated with extraintestinal infections and increased mortality risk.

The increased use of antibiotics has been reported in clinical settings during the COVID-19 pandemic. Among the resistant bacteria, carbapenem-resistant *Enterobacteriaceae* (CRE) is of primary concern. In these bacteria, Carbapenemases can hydrolyze all beta-lactam antibiotics, including carbapenems, as the last line of medication in severe nosocomial infections (2). Carbapenemase genes are encoded on transferable genetic elements, and their horizontal transfer makes them potentially responsible for outbreaks. The intestinal colonization of CRE is a risk factor for severe hospital infections and could serve as a reservoir for the transmission of these pathogens in clinical set-

tings ([2], [3]). CRE is a major global concern leading to an increased mortality rate ([4], [5]). Carbapenemases are classified by their molecular structure (Ambler classification) in three groups ([4], [6]). *Escherichia coli* with NDM-1 is a human health-threatening pathogen, especially in vulnerable patients with underlying diseases, long-term hospitalization, and immune deficiencies ([7], [8]).

2. Objectives

The rates of fecal carriage of CRE vary across the world and patients with different health conditions. As a risk factor in HAIs, the fecal carriage of CRE is integral in vulnerable pediatric patients and accounts for comorbidity with SARS-CoV-2 and therapeutic regimens ([9], [10]). To find such a correlation in clinical settings, this study aimed to investigate the correlation between the intestinal carriages of CRE with the diversity of carbapenemase genes, SARS-CoV-infection status, and the types of antibiotics prescribed in immunocompromised children during hospitalization at oncology and hematology wards.

3. Methods

3.1. Patients and Samples

This study was conducted from August 21 to January 20 in Tehran, Iran. To this end, 102 stool samples were collected from immunocompromised pediatric patients admitted to the oncology and BMT wards of the hospital. The patients' information was obtained from questionnaires and hospital documents. All the stool samples were brought to the laboratory at least 2 hours after sampling. Selective cultures such as MacConkey agar (Pronadisa, CONDA, Spain) and biochemical tests such as TSI, IMViC, ureases, and lysine decarboxylase were used for *Enterobacteriaceae* characterization. Moreover, the COVID-19-related data of the patients were collected from the hospital documents.

3.2. Antibiotic Susceptibility Testing

The standard disk diffusion method was used to determine the sensitivity/resistance of *Enterobacteriaceae* isolates against imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), gentamycin (10 μg), ceftazidime (30 μg), levofloxacin (5 μg), cefepime (30 μg), cefotaxime (30 μg), aztreonam (30 μg), and tigecycline (15 μg). Moreover, Muller Hinton agar (BD, USA), culturing 24 hours of isolates, diluting in 3 milliliters of physiological serum, and making standard McFarland were also used. The antimicrobial susceptibility was measured using the inhibition zone in the CLSI chart ([11]).

3.3. Detection of Carbapenemase Genes

Carbapenemase genes (bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, and bla_{VIM}) were assayed by PCR. Specific primers (SIGMA, Germany, and UK) were also used, and their sequences are presented in Table 1.

4. Results

4.1. Patients

Among the participants, there were 54% (55/102) males and 46% (47/102) females, most of whom (43.13%, 44/102) were 1 - 5 years old. Moreover, 88% (90/102) of the patients had undergone treatment with broad-spectrum antibiotics such as carbapenems, cephalosporins, aminoglycosides, ciprofloxacin, and azithromycin. Table 2 presents the patients' demographic information.

4.2. CRE Frequency in Intestinal Samples Isolated from Immunocompromised Children

During the study period, 102 clinical *Enterobacteriaceae* isolates were collected, the frequencies of which was as follows: *Escherichia* spp. (59/102, 57.8%), *Klebsiella* spp. (34/102, 33.3%), *Enterobacter* spp. (5/102, 4.9%), *Citrobacter* spp. (2/102, 1.9%), and *Serratia* spp. (2/102, 1.9%). According to the antimicrobial susceptibility tests, the strains showed the highest and the lowest resistance rates against ceftazidime (74.5%, 76/102) and tigecycline (13.72%, 14/102), respectively. Table 3 shows antimicrobial-resistant patterns.

The highest rate resistance to antibiotics was recorded in *Escherichia* spp. to ceftazidime and cefotaxime (76.27%), *Klebsiella* spp. to ceftazidime and cefotaxime (73.5%), *Enterobacter* spp. to ceftazidime and aztreonam (60%), *Citrobacter* spp. to all antibiotics except for meropenem and amikacin (50%), and *Serratia* spp. to all antibiotics except for gentamycin and tigecycline (100%).

4.3. Frequency of Carbapenemase Genes in Intestinal CRE Isolated from Immunocompromised Children

Carbapenem resistance was observed in 54% (55/102) of the strains, the frequencies of which was as follows: *Escherichia* spp. (42.37%, 25/59); *Klebsiella* spp. (73.52%, 25/34); *Enterobacter* spp. (40%, 2/5); *Citrobacter* spp. (50%, 1/2), and *Serratia* spp. (100%, 2/2). Out of the 55 CRE strains, the frequencies of carbapenemase were as follows bla_{OXA-48} (90.9%, 50/55), bla_{NDM-1} (54.54%, 30/55), bla_{VIM} (36.36%, 20/55), and bla_{KPC} (32.72%, 18/55). Figure 1 illustrates the frequency of the resistance genes among the CRE strains.

Table 1. This Study Used Nucleic Acid Primer Sequencing of CRE Genes to Diagnose CRE Strains

Carbapenemase Genes	Base Pairs	Sequence (5´-3')	TM°C	PCR Conditions	Reference
OXA-48	392	F CCAAGCATTTTTACCCGCATCK-ACC	65.5	One cycle of initial denaturation at 95°C for 1 min, 30 cycles of denaturation at 95°C for 30 sec, Annealing at 55°C for 30 sec, Extension at 75°C for 1 min; and 1 cycle of final extension at 72 for 7 min.	(12)
		R GYTTGACCATACGCTGRCTGCG	61		
NDM-1	129	F CCCCGCCACACCAGTGA-CANCTC	75.6	One cycle of initial denaturation at 95°C for 1 min, 32 cycles of denaturation at 95°C for 30 sec, Annealing at 61°C for 30 sec, Extension at 7°C for 1 min; and 1 cycle of final extension at 72°C for 5 min.	(13)
		RGTAGTGCTCAGTGTGGGCAT	63		
VIM	390	F GATGGTGTTTGGTCGCATA	55.5	One cycle of initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 30 sec, Annealing at 61°C for 40 sec, Extension at 72°C for 1 min; and 1 cycle of final extension at 72°C for 7 min.	(14)
		R CGAATGCGCAGCACCAG	57.2		
KPC	636	F CTGTCTTGTCTCTCATGGCC	60.5	One cycle of initial denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 35 sec, Annealing at 62°C for 35 sec, Extension at 72°C for 32 sec; and 1 cycle of final extension at 72°C for 5 min.	(15)
		R CCTCGCTGTGCTTGTCATCC	62.5		
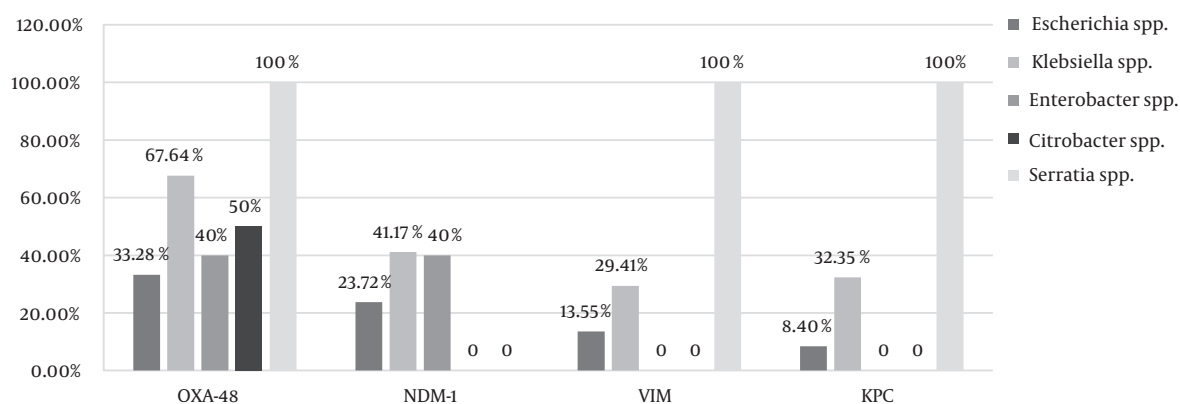
Figure 1. Frequency of carbapenemase-producing genes in CRE isolated from stool samples of immunocompromised children.

4.4. Intestinal CRE Carriage in Children with COVID-19 and Broad-Spectrum Antibiotic Usage

In this study, 50% (51/102) of the immunocompromised patients had laboratory-confirmed COVID-19. Of patients with intestinal CRE colonization 54% (55/102), 65.45% (36/55) were COVID-19 positive after admission in the oncology and BMT wards. Out of patients with COVID-19 (50%, 51/102), 94.11% (48/51) underwent broad-spectrum antibiotics treatment. Table 4 presents the status of CRE carriers, COVID-19, and broad-spectrum antibiotic usage.

5. Discussion

The rate of hospital admissions in both infectious disease and pediatric intensive care unit (PICU) wards has been increasing because of the COVID-19 pandemic. This had enhanced the likelihood of COVID-19 diffusion among other hospital wards (16). The description of broad-spectrum antibiotics such as carbapenems in these patients is associated with some side-effects, including the multiplication of carbapenemase genes, and the admission in different wards can also lead to the CRE outbreaks (17). The fecal carriage and intestinal colonization of CRE can raise the risks of secondary infections and increase mortality rates (18). CRE has revealed different drug resistance mechanisms, making the early detection and control of the CRE infections challenging (19).

Accordingly, we focused on the correlation between the intestinal carriage of CRE and the possible effects of carbapenemase genes on immunocompromised children

Table 2. Demographic Information of Immunocompromised Children

Variables	No. (%) (N = 102)
Gender	
Male	55 (54)
Female	47 (46)
Age (y)	
≤ 5	44 (43.13)
6 - 10	41 (40.19)
≥ 11	17 (16.66)
Ward	
BMT	23 (22.5)
Oncology	79 (77.5)
Underlying disease	
AML	31 (30.3)
ALL	13 (12.7)
Neuroblastoma	10 (9.8)
Others	48 (47.05)
Antimicrobial prophylaxis	
Broad spectrum antibiotics	90 (88)
Non-broad spectrum antibiotics	12 (12)
COVID-19 status	
Positive	51 (50)
Negative	51 (50)
Death	
Yes	24 (23.5)
No	78 (76.5)

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia.

during the COVID-19 pandemic.

The findings revealed that 65.45% of the immunocompromised pediatrics with the intestinal carriage of CRE were COVID-19 positive after hospitalization. Most of the patients were males (54%), and AML was the most frequent underlying disease among the patients. COVID-19 played an integral role in hospital prolongation and increasing death rates in intestinal CRE carriers.

Another study was conducted in the UK in 2022 to examine COVID-19 in immunocompromised children, and the findings indicated no mortality and the low risk of severe infections [1]. The differences between this study and the present study might have underestimated the pivotal role of antibiotics administration and its association with CRE carriage and COVID-19.

Escherichia spp. was the most frequent strains isolated from immunocompromised children (57.8%) and also the

most frequent carbapenem-resistant strain isolated from the intestinal CRE carriage of COVID-19 positive patients (68%). The second most frequent strain was *Klebsiella* spp. (33.3%) in 60% of COVID-19 positive CRE carriages. In a study carried out in 2021 in the USA, the frequency of CRE strains isolated from confirmed COVID-19 patients was 41.9%, and the most frequent species was *Klebsiella pneumonia* (90.3%) [17]. The differences between the percentages of the CRE isolates could be attributed to differences in the samples as the target samples in the former study were feces; however, the latter one worked on blood and respiratory samples. Moreover, the frequency of *Escherichia* spp. was higher than the other kinds of samples.

Among intestinal CRE isolated from the intestinal track of the immunocompromised children, OXA-48 had the highest prevalence in this study. The first case of bla_{OXA-48} detection in Iran was reported by Azimi et al., who reported bla_{OXA-48} in 96% of imipenem-resistant *K. pneumonia* isolated from the immunodeficient patients [20, 21]. According to a systematic review by Nasiri et al., bla_{OXA48} gene was the most common cause of carbapenem resistance in *K. pneumoniae* and *E. coli* in Iran [22]. Pérez-Blanco et al. founded the higher frequency of *OXA-48* in *K. pneumoniae* compared to other species, including *K. pneumonia* (93.6%), *Escherichia coli* (2.3%), *Enterobacter* spp. (1.7%), both *K. pneumoniae* and *E. coli* (0.5%), and *Raoultella* spp. or *Citrobacter* spp. (1.8%) [23]. The high prevalence of OXA-48 among CRE in many studies can prove that carbapenem strains harboring OXA-48 has been boosting in Iran and becoming the endemic carbapenemase, thereby highlighting the significance of the strength detection and prevention control of CRE [22].

The detection of *NDM-1* in *K. pneumonia* was first reported in 2013 in Iran [24]. According to a study in 2019, *NDM-1* was detected among 100% of the CRE isolates in Brazil, suggesting the high prevalence of *NDM-1* in this country [25]. In our study, *NDM-1* was detected in 54.54% of the CRE strains. According to the data about harboring NDM-1 plasmid, a carrier of several other resistant genes, we can conclude that the spread of this challenging carbapenemase is in a medium range in Iran. On the other hand, the findings of this study suggest that NDM-1 has been increasing across the country in recent years. The high prevalence of *NDM-1* in bacteria in hospitals can be a major challenge in treating and controlling infectious diseases [24].

The emergence of *VIM*-producing *K. pneumoniae* in Iran is also a concern. VIM-producing bacteria constitute the prevalent multidrug-resistant population of *K. pneumoniae* in Iran [26]. In a study in 2019, 73% of *Enterobacteriaceae* isolates, especially *Enterobacter* spp., collected from clinical and environmental samples, were *VIM* carriers [27].

Table 3. Antimicrobial-resistant Patterns of *Enterobacteriaceae* Members Isolated from Immunocompromised Children [a]

Antibiotics	*Escherichia* spp. N = 59	*Klebsiella* spp. N = 34	*Enterobacter* spp. N = 5	*Citrobacter* spp. N = 2	*Serratia* spp. N = 2
IMP (10 μg)	16 (27.11)	20 (58.82)	2 (40)	1 (50)	2 (100)
MEM (10 μg)	14 (23.72)	20 (58.82)	2 (40)	-	2 (100)
AN (30 μg)	11 (18.64)	13 (38.23)	2 (40)	-	2 (100)
GM (10 μg)	29 (49.15)	19 (55.88)	2 (40)	1 (50)	1 (50)
CAZ (30 μg)	45 (76.27)	25 (73.52)	3 (60)	1 (50)	2 (100)
LVX (5 μg)	25 (42.37)	15 (44.11)	-	1 (50)	2 (100)
CTX (30 μg)	45 (76.27)	25 (73.52)	2 (40)	1 (50)	2 (100)
CFM (30 μg)	31 (52.54)	19 (55.88)	2 (40)	1 (50)	2 (100)
AZT (30 μg)	41 (69.49)	23 (67.64)	3 (60)	1 (50)	2 (100)
TGC (15 μg)	3 (5.08)	8 (23.52)	2 (40)	1 (50)	-

Abbreviations: IMP, imipenem; MEM, meropenem; AN, amikacin; GM, gentamycin; CAZ, ceftazidime; LVX, levofloxacin; CFM, cefepime; CTX, cefotaxime; AZT, aztreonam; TGC, tigecycline.
[a] Values are expressed as No. (%).

Table 4. Frequency of Intestinal CRE Carriers with COVID-19 and Broad-Spectrum Antibiotic Usage [a]

CRE and Antibiotic Status	COVID-19 Positive; N = 51	COVID-19 Negative; N = 51	P-Value
CRE carriers and consumed broad-spectrum antibiotics	29 (56.86)	15 (29.41)	0.027
CRE carriers and not-consumed broad-spectrum antibiotics	2 (3.9)	4 (7.84)	
Not CRE carriers and consumed broad-spectrum antibiotics	19 (37.25)	27 (52.94)	
Not CRE carriers and not-consumed broad-spectrum antibiotics	1 (1.9)	5 (9.8)	

[a] Values are expressed as No. (%).

In the present study, we reported 36.36% of VIM carriage among CRE.

The correlation between CRE intestinal carriage with COVID-19 and administrational antibiotics was significant in this study. To the best of our knowledge, no similar study has addressed this issue.

Note that most of the patients who were CRE colonized had previous hospital admission and had consumed broad-spectrum antibiotics, especially ciprofloxacin and carbapenems. In the present study, the most frequently used prophylaxis antibiotics in these two wards were meropenem, vancomycin, and amikacin. It can be assumed that immune deficiency in patients admitted to the BMT and oncology wards is a remarkable risk factor for upcoming infections such as COVID-19 and that the circle of these infections finally would end in prolonged hospitalization period, which itself is one of the most dangerous hospital-acquired infection-inducing risk factors. Moreover, this process could lead to the spread of the CRE strains by the carriers during admission in different hospital wards. In this study, there was no control group of inpatient children; hence, further studies are required to detect the relationship between CRE colonization with previ-

ous hospital admissions, prophylaxis antibiotics diet, and COVID-19.

5.1. Conclusion

Regardless of the COVID-19 pandemic, prolonged hospitalization and antibiotic prescription are main risk factors associated with the CRE intestinal colonization in immunocompromised children. The high prevalence of bla_{OXA-48} in these isolates, especially in *Escherichia* spp., can raise therapeutic challenges and concerns about systemic infections in these patients. The intestinal carriage of KPC could increase the risk of secondary infection treatment failure in immunocompromised and COVID-19 positive patients. The co-occurrence of *NDM-1*, *OXA-48*, and *VIM*-producing *Enterobacteriaceae* is a concern and need further consideration to control their spread rate and highlight the significance of maximizing the infection control measures.

Acknowledgments

Footnotes

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