Çocuk Dergisi 2017; 17(4):169-173
doi:10.5222/j.child.2017.169

Araştırma

Screening of Rectal Swabs for Carbapenem Non-susceptible *Bacteroides fragilis* Group Bacteria in Hospitalized Children

Ayşe KARAASLAN*, Nurver Ulger TOPRAK, Gülşen AKKOÇ*, Öncü AKGÜL**, Eda KEPENEKLİ KADAYİFCİ*, Sevliya ÖCAL DEMİR*, Nurhayat YAKUT*, Serkan ATICI*, Ahmet SOYSAL*, Mustafa BAKIR*, Güner SÖYLETİR****

Screening of Rectal Swabs for Carbapenem Non-susceptible Bacteroides fragilis Group Bacteria in Hospitalized Children

Objective: *The aim of this study was to investigate the carbapenem resistance profiles and risk factors for colonization with carbapenem-resistant Bacteroides group (CR-B) in pediatric patients hospitalized in a tertiary university hospital in Turkey.*

Material and Methods: *A prospective case-control study was performed. Rectal swab specimens (n=1361) were collected from 680 hospitalized pediatric patients. All anaerobic colonies isolated from selective medium were identified using MALDI-TOF (Vitek MS, bioMérieux) automated system and antibiotic susceptibility testing for meropenem by e-test. The presence of the cfiA gene was investigated by PCR method.*

Results: *A total of 1361 rectal swab specimens were collected from all 680 hospitalized children. During the screening period 80 Bacteroides fragilis group (BFG) microorganisms with a MIC>0.125 mg/L (12%) were isolated from rectal swab specimens of 680 patients. Seven different BFG species were recovered, the main species were B.fragilis (n=29; 36%), B.ovatus (n=16; 20%), B.vulgatus (n=13; 16%), P.distasonis (n=12; 15%), B.theoiotomicron (n=5; 6%), B.uniformis (n=4; 5%) and B.caccae (n=1; 1%). The presence of cfiA gene was detected in 25 (31%) isolates, while 29 (36%) isolates were not susceptible to meropenem (MIC>2 mg/L).*

Conclusion: *The high carriage rate in our hospitalized children for carbapenem-non-susceptible Bacteroides fragilis group creates a great risk for serious infections and mortality and deserves significant attention.*

Keywords: *Bacteroides fragilis group, carbapenem non-susceptible, children*

J Child 2017; 17(4):169-173

Hastanede Yatan Çocukların Rektal Sürüntülerinde Karbapenem Duyarlı Olmayan Bacteroides fragilis Grup Bakteri Taraması

Amaç: *Bu çalışmanın amacı, Türkiye'de üçüncü düzey bir üniversite hastanesinde yatmakta olan çocuk hastalarda karbapenem dirençli Bacteroides grup (KD-B) bakteriler ile kolonizasyon için risk faktörlerini ve karbapenem direnç profilini araştırmaktı.*

Gereç ve Yöntem: *Prospektif vaka-kontrol çalışması uygulandı. Hastanede yatmakta olan 680 çocuk hastadan 1361 rektal swab toplandı. Selektif mediumdan izole edilen tüm anaerobik kolonilere identifikasyon için MALDI-TOF (Vitek MS, bioMérieux) otomatize sistem ve meropenem duyarlılığı için e-test uygulandı. cfiA geni PCR yöntemiyle araştırıldı.*

Bulgular: *Hastanede yatmakta olan 680 çocuk hastadan 1361 rektal swab toplandı. Tarama süresince MIC>0.125 mg/L olan 80 Bacteroides fragilis group (BFG) bakteri izole edildi. Yedi farklı tür BFG saptandı, sıklık sırasına göre şöyleydi: B.fragilis (n=29; %36), B.ovatus (n=16; %20), B.vulgatus (n=13; %16), P.distasonis (n=12; %15), B.theoiotomicron (n=5; %6), B.uniformis (n=4; %5) ve B.caccae (n=1; %1). 25 izolatda (%31) cfiA geni saptanırken, meropeneme hassas olmama (MIC>2 mg/L) 29 izolatda (%36) belirlendi.*

Sonuç: *Çalışmamızda, hastanede yatan çocuklarda karbapenem hassas olmayan Bacteroides fragilis grup bakteriler ile kolonizasyon oranları yüksek bulunmuştur ki, bu durum ciddi enfeksiyonların oluşması, artmış mortalite açısından önemlidir ve dikkate alınmalıdır.*

Anahtar kelimeler: *Bacteroides fragilis grup, karbapenem hassas olmama, çocuklar*

Çocuk Dergisi 2017; 17(4):169-173

INTRODUCTION

Bacteroides species are anaerobic, non-spore-forming, gram-negative rods which are the dominating constitutive bacteria of the human intestinal flora and also the major anaerobic pathogen of the suppu-

Alındığı tarih: 23.11.2017
Kabul tarihi: 26.01.2018
*Marmara Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Çocuk Enfeksiyon Hastalıkları Bilim Dalı
**Marmara Üniversitesi Tıp Fakültesi, Mikrobiyoloji Bölümü
Yazışma adresi: Prof. Dr. Ahmet Soysal, Küçükbakkalköy Mah. Vedat Günyol Cad. 28-30, Ataşehir 34865 İstanbul
e-posta: drahmetsoysal20@gmail.com

rative infections in intraabdominal cavity and pelvis. Also, bacteremia and bone and joint infections have rarely been reported with *Bacteroides* spp. in children [1]. *Bacteroides* spp. are potentially resistant to a wide diversity of antimicrobial agents. The most effective antimicrobials against *Bacteroides* spp. are in limited number such as, beta-lactam/beta-lactamase inhibitor combinations, metronidazole and carbapenems [2]. Clinically, *B.fragilis* is the most important species, because it is still the mostly isolated virulent bacteria from clinical isolates [1]. However, the carbapenem-non-susceptible *Bacteroides fragilis* bacteria have been reported in several countries recently with a consequence of treatment failure and mortality [3-5]. The mortality rates increase to 60%, if a verified *B.fragilis* infection is not treated [6]. It has been put forward that the generation of the imipenem-hydrolyzing metallo-beta-lactamase enzyme which is encoded by the *cfiA* gene is responsible for carbapenem resistance among *B.fragilis* strains [7].

In the present study, we investigated the risk factors and carbapenem resistance profiles of *Bacteroides* spp. with rectal colonization in hospitalized pediatric patients in a tertiary university hospital. As far as we know, this is the first study that investigated risk factors of colonization with *Bacteroides* spp. in children.

MATERIALS and METHODS

The study was performed in a tertiary university hospital in Istanbul, Turkey. The hospital has a 649-bed capacity, including all major pediatric departments and services, neonatal, and pediatric intensive care units. In the study period, the hospital infection control committee had started to implement rectal culture surveillance for carbapenem- resistant gram-negative bacilli (CR-GNB) colonization in all pediatric units because of CR-GNB infections were documented in intensive care units. Rectal swabs were obtained weekly from all hospitalized patients admitted to the hospital. Carbapenem- resistant *Bacteroides* spp. were investigated in specimens. Rectal swab specimens (n=1361) were collected from 680 hospitalized pediatric patients, between 2/3/2014, and 5/24/2014 of February, and also between 5/24/2014,and 8/28/2015.

Demographic characteristics of the patients, possible risk factors, such as length of antibiotic usage, intensive care unit (ICU) stay, use of nasogastric catheters, ventilators, use and duration of intravenous (IV) treatments or urinary catheters, therapeutic interventions and underlying chronic disease(s) were recorded retrospectively. A case-control study was conducted, comparing colonized patients of *Bacteroides* spp. with non-colonized patients at a ratio of 1:1. The control patients were randomly chosen among patients who stayed in the same ward during the study period. We accepted colonization with *Bacteroides* group in patients whom the bacteria could be yielded because of high MIC value (MIC >0.125 mg/L) obtained with meropenem. Also the patients colonized with Bacteroides group with meropenem MIC >2 mg/L were accepted as meropenem non-susceptibility group. Colonized patients showed no any symptom of infection due to *Bacteroides* species.

The study protocol was approved by the Ethics Committee of Marmara University Medical School.

Microbiological methods

Few studies have investigated carbapenemase-producing *Bacteroides fragilis* group bacteria in stool samples or rectal swabs. Although a range of different culture media has been proposed to determine carbapenemase activity of aerobic or facultative anaerobic bacteria, there is no specific media for those of BFG bacteria. In this present study, we modified kanamycin vancomycin- laked blood agar supplemented with 0.125 mg/L meropenem (the screening cut-off MIC values).

Each rectal swab was transferred into 1 ml Mueller Hinton broth. An inoculum volume of 100 μL was immediately transferred onto the first kanamycin-vancomycin-leaked blood agar plus 0.125 mg/L meropenem (KVLBA-CARBA) directly and 100 μL onto second KVLBA-CARBA plate after overnight anaerobic incubation in 5 ml Brucella Broth (suplemented with vitamin K and hemin) with a 10 μg meropenem disk as a selective medium (BB+CARBA). After 48- hour incubation period in anaerobic atmosphere, different colonies were subcultured for aerotolerance testing. All anaerobic colonies were identi-

fied using MALDI-TOF (Vitek MS, bioMérieux) automated system and antibiotic susceptibility testing for meropenem by E-test method. MIC:>2 mg/L of meropenem was accepted as meropenem non-susceptibility according to EUCAST guidelines [8]. The presence of the *cfiA* gene was investigated by PCR using GBI-1 F 5'- CCCAACTCTCGGACAAAGTG-3 and GBI-2 R 5'-AGTGAATCGGTGAATCCATG-3 primers [9].

RESULTS

A total of 1361 rectal swab specimens were collected from all 680 hospitalized children between 2/3/2014, and 5/24/2014, and also between 8/28/2014, and 3/9/2015 -24th of May 2014 and 28th August 2014-9th March 2015. During the screening period 80 BFG organisms (12%) were isolated from rectal swab specimens of 680 patients. Seven different BFG species were recovered, the main species were *B.fragilis* (n=29; 36%), *B.ovatus* (n=16; 20%), *B.vulgatus* (n=13; 16%), *P.distasonis* (n=12; 16%), *B.theoiotomicron* (n=5; 6%), *B.uniformis* (n=4; 5%) and *B.caccae* (n=1; 1%). *CfiA* gene was detected in 25 (31%), and the meropenem non-susceptibility (MIC>2 mg/L) in 29 (36%) isolates. All of the *cfiA* gene positive strains (n=25) were *B.fragilis* and 18 (72%) of these strains were meropenem-non-susceptible. Eleven *Bacteroides fragilis* group strains [*B.fragilis* (n=2), *P.distasonis* (n=3), *B.vulgatus* (n=3), *B.ovatus* (n=1), *B.uniformis* (n=1), *B.caccae* (n=1)] without *cfiA* were meropenem non-susceptible. All the organisms detected and the MIC values of meropenem are shown in Table 1.

Forty-two (53%) males and thirty-eight (47%) females with a mean (± SD) age of 76.5±66.6 months (range 0-254, median 59 months) were colonized with *Bacteroides fragilis* group. Among 80 patients, 58 (72.5%) received antibiotic therapy. We investigated risk factors such as carbapenem and other anti-anaerobic use, length of antibiotic use, intensive care unit (ICU) stay, use of nasogastric catheters, ventilators, use and duration of intravenous (IV) treatment or urinary catheters and underlying chronic disease(s) for colonization with *Bacteroides* group. We did not identify any risk factors between *Bacteroides* group with MIC>2 vs *Bacteroides* group with MIC≤2, between *cfiA* (+) *B.fragilis* vs *cfiA* (-) *B.fragilis*, between *B.fragilis* with MIC>2 vs *B.fragilis* MIC≤2 and between non-*B.fragilis* spp. with MIC>2 vs non-*B.fragilis* spp.with MIC≤2.

DISCUSSION

Bacteroides fragilis is the most common anaerobe of the human colon and it is responsible for different type of infections such as intra-abdominal infections, bacteremia and bone/joint infections in children [1]. Accurate and prompt treatment is very important for outcome of these diseases and delayed treatment regimen is associated with mortality [10]. In the past two decades, resistance to antimicrobial agents, such as carbapenems, metronidazole and beta-lactam/beta-lactamase inhibitor combinations has been notified [11]. The carbapenemase metallo-beta-lactamase gene (*cfiA*) is produced by *B.fragilis* and contributes to carbapenem resistance [7]. In our study, *cfiA* was detected in 31% (n=25) of bacteria, and all of them

Table 1. All the organisms detected and the MIC values of Meropenem.

		MEROPENEM MIC VALUE (mg/L)										
	Organisms	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**2**	**3**	**4**	**8**	**12**	**32**
cfiA (-)	*B.fragilis*	0	0	0	1	1	0	0	0	0	0	2
	B. ovatus	0	8	0	2	3	2	0	0	0	0	1
	B. vulgatus	1	4	2	2	0	1	0	0	0	0	3
	P. distasonis	1	4	0	3	1	0	0	0	1	1	1
	B.theaiotomicron	0	0	0	4	0	1	0	0	0	0	0
	B. uniformis	0	0	0	1	1	1	0	0	0	0	1
	B.caccae	0	0	0	0	0	0	0	0	0	0	1
	Total	2	16	2	13	6	5	0	0	1	1	9
cfiA (+)	B. fragilis	0	2	0	2	0	3	1	1	1	0	15
	Total	0	2	0	2	0	3	1	1	1	0	15

MIC: Minimum inhibitory concentration, B: Bacteroides

belonged to the *B.fragilis* spp. In our study, *cfiA* positive isolates were detected more frequently (7%) among clinical isolates relative to those previous reported in France (2%), Hungary (6%) and United Kingdom (7%) [12-14]. However our *cfiA* detection rate was similar to that mentioned (35%) in the report of Fernandez-Canigia et al [11] for Argentina. In our study, differently from other studies we analyzed samples from patients with rectal colonization. There are not many studies on this issue, so we could't compare our study results. In a previous study from our country, *cfiA* gene was detected in 27% of 66 *Bacteroides* isolates, similar to our study [15].

cfiaA and *ccrA* genes encode class B metallo-beta-lactamase enzyme which is responsible for resistance to carbapenem [16]. Some *Bacteroides fragilis* strains contain silent resistance genes and insertion sequences (IS) which are suffixed immediately upstream of the *cfiA*/*ccrA* gene, and can upregulate the expression of these carbapenemase genes. Moreover, increased efflux pump activity and changes in porins are possible reasons of resistance to carbapenems [1,17]. In this study we determined the carbapenem resistance profiles of *Bacteroides* species and the presence of the *cfiA* gene. We found that all of the *cfiA* gene positive strains (n=25) were *B.fragilis* and 18 (72%) of these strains were meropenem-non-susceptible. In other seven strains, IS can provide a promoter to express *cfiA* gene and these strains can become carbapenem resistant. Also in our study, 11 *Bacteroides* group strains (*B.fragilis* (n=2), *P.distasonis* (n=3), *B.vulgatus* (n=3), *B.ovatus* (n=1), *B.uniformis* (n=1), *B.caccae* (n=1)) without *cfiA* were meropenem non-susceptible. We think that changes in porins and increased efflux pump activity may be the possible reasons of carbapenem non-susceptibility.

As far as we know, there is no study investigating the risk factors for colonization with meropenem non-susceptible *Bacteroides* spp. in children. In this study, we investigated risk factors however we did not identify any risk factors between *Bacteroides* group with MIC>2 vs *Bacteroides* group with £2, *cfiA* (+) *B.fragilis* vs *cfiA* (-) *B.fragilis*, *B.fragilis* with MIC>2 vs *B.fragilis* MIC>2, non-*B.fragilis* species with MIC£2 vs non-*B.fragilis* species with MIC£2. Because of lack of relevant data, we could not compare our results with any study, for this reason further studies are needed.

In conclusion, high rate of colonization with meropenem non-susceptible *Bacteroides* spp.is an important and increasing health problem because they are probable agents of serious life-threatining infections. Further studies should be implemented to asses risk factors.

Conflicts of Interest

The authors declare that they have no conflicts of interests.

Acknowledgements

All authors have participated in drafting of the manuscript and/or critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

REFERENCES

1. **Wexler HM.** Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 2007;20:593-621. https://doi.org/10.1128/CMR.00008-07
2. **Snydman DR, Jacobus NV, McDermott LA, et al**. Lessons learned from the anaerobe survey: historical perspective and review of the most recent data (2005-2007). *Clin Infect Dis* 2010;50:26-33. https://doi.org/10.1086/647940
3. **Goldstein EJ, Finegold SM, Harrell LJ, et al.** National survey on the susceptibility of Bacteroides fragilis group: report and analysis of trends in the United States from 1997 to 2004. *Antimicrob Agents Chemother* 2007;51:1649-55. https://doi.org/10.1128/AAC.01435-06
4. **Nagy E, Urbán E, Nord CE, et al.** Antimicrobial susceptibility of Bacteroides fragilis group isolates in Europe: 20 years of experience. *Clin Microbiol Infect* 2011;17:371-9. https://doi.org/10.1111/j.1469-0691.2010.03256.x
5. **Liu CY, Huang YT, Liao CH, Yen LC, Lin HY, Hsueh PR.** Increasing trends in antimicrobial resistance among clinically important anaerobes and Bacteroides fragilis isolates causing nosocomial infections: emerging resistance to carbapenems. *Antimicrob Agents Chemother* 2008;52:3161-8. https://doi.org/10.1128/AAC.00355-08
6. **Goldstein EJ.** Anaerobic bacteremia. *Clin Infect Dis* 1996; 23:97-101. https://doi.org/10.1093/clinids/23.Supplement_1.S97
7. **Khushi Payne TDJ, Fosberry A, Reading C.** Production of metal dependent b-lactamases by clinical strains of Bacteroides fragilis isolated before 1987. *J Antimicrob Chemother* 1996;37:345-50. https://doi.org/10.1093/jac/37.2.345

8. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). http://www.eucast.org/fileadmin/src/media/EUCASTfiles/Resistance_mechanisms/EUCASTdetection_of_resistance_mechanisms_v1.0_20131211.pdf
9. **Kato N, Yamazoe K, Han CG, Ohtsubo E.** New insertion sequence elements in the upstream region of cfiA in imipenem-resistant Bacteroides fragilis strains. *Antimicrob Agents Chemother* 2003;47:979-85. https://doi.org/10.1128/AAC.47.3.979-985.2003
10. **Nguyen MH, Yu VL, Morris AJ, et al.** Antimicrobial resistance and clinical outcome of Bacteroides bacteremia: findings of a multicenter prospective observational trial. *Clin Infect Dis* 2000;30:870-6. https://doi.org/10.1086/313805
11. **Fernández-Canigia L, Litterio M, Legaria MC, et al.** Anaerobe Surveillance Team. First national survey of antibiotic susceptibility of the Bacteroides fragilis group: emerging resistance to carbapenems in Argentina. *Antimicrob Agents Chemother* 2012;56:1309-14. https://doi.org/10.1128/AAC.05622-11
12. **Podglajen I, Breuil J, Casin I, Collatz E.** Genotypic identification of two groups within the species Bacteroides fragilis by ribotyping and by analysis of PCR-generated fragment patterns and insertion sequence content. *J Bacteriol* 1995;177:5270-5. https://doi.org/10.1128/jb.177.18.5270-5275.1995
13. **Soki J, Urban E, Szoke I, Fodor E, Nagy E.** Prevalence of the carbapenemase gene (cfiA) among clinical and normal flora isolates of Bacteroides species in Hungary. *J Med Microbiol* 2000;49:427-30. https://doi.org/10.1099/0022-1317-49-5-427
14. **Edwards R, Hawkyard CV, Garvey MT, Greenwood D.** Prevalence and degree of expression of the carbapenemase gene (cfiA) among clinical isolates of Bacteroides fragilis in Nottingham, UK. *J Antimicrob Chemother* 1999;43:273-6. https://doi.org/10.1093/jac/43.2.273
15. **Toprak NU, Uzunkaya OD, Sóki J, Soyletir G.** Susceptibility profiles and resistance genes for carbapenems (cfiA) and metronidazole (nim) among Bacteroides species in a Turkish University Hospital. *Anaerobe* 2012;18:169-71. https://doi.org/10.1016/j.anaerobe.2011.10.004
16. **Livermore DM, Woodford N.** Carbapenemases: a problem in waiting? *Curr Opin Microbiol* 2000;3:489-95. https://doi.org/10.1016/S1369-5274(00)00128-4