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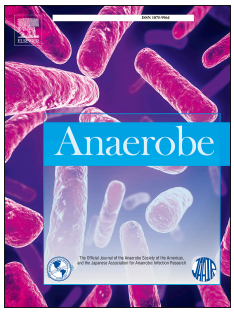

A comparison of the antimicrobial resistance of fecal *Bacteroides* isolates and assessment of the composition of the intestinal microbiotas of carbapenem-treated and non-treated persons from Belgium and Hungary

József Sóki, Ingrid Wybo, Roland Wirth, Edit Hajdú, Mária Matuz, Katalin Burián, on behalf of the ESCMID Study Group on Anaerobic Infections

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A comparison of the antimicrobial resistance of fecal *Bacteroides* isolates and assessment of the composition of the intestinal microbiotas of carbapenem-treated and non-treated persons from Belgium and Hungary

József Sóki[a]*, Ingrid Wybo[b], Roland Wirth[c], Edit Hajdú[d], Mária Matuz[e] and Katalin Burián[a,f] on behalf of the ESCMID Study Group on Anaerobic Infections

[a] Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

[b] Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel,Vrije Universiteit Brussel, Belgium

[c] Department of Biotechnology, Faculty of Sciences and Informatics, University of Szeged, Szeged Hungary

[d] Division of Infectious Diseases, First Department of Internal Medicine, Faculty of Medicine, University of Szeged, Szeged, Hungary

[e] Department of Clinical Pharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary

[f] Department of Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary

Running title: Antibiotic resistance of *Bacteroides* and microbiomes of carbapenem-treated persons

Keywords: antibiotic resistance, *Bacteroide*s, *B. fragilis*, carbapenems, microbiome

* Corresponding author:

Dr. József Sóki

Institute of Clinical Microbiology, Albert Szent-Györgyi Clinical Centre, Faculty of Medicine, University of Szeged

Semmelweis 6

H-6726 Szeged, Hungary

Tel.: 36 62 545399

Fax: 36 62 545712

e-mail: soki.jozsef@med.u-szeged.hu

Abstract

The antimicrobial susceptibilities of *Bacteroides* strains isolated from the feces of imipenem-treated patients from Belgium and Hungary were compared with those isolated from the normal microbiota from these two and five other European countries and assessed. Of the 10 antibiotics tested, highly significant differences were found with cefoxitin (decrease for Belgium and for this two and the five countries from the previous study), clindamycin (decrease for Belgium and for this two and the five countries from the previous study) and moxifloxacin (increase for Belgium and for this two and the five countries from the previous study) relative to normal microbiota strains reported earlier. Imipenem treatment brought about modest, but notable differences in the compositions of the microbiomes where there was less diversity in the treated group relative to the non-treated group.

Bacteroides species are important and, also common participants of the normal human gut microbiota. Together or separately their phylum (Bacteroidetes), order (Bacteroides), and genera (*Bacteroides* and the similar *Parabacteroides* species) usually comprise 15-30 %-s colonic microbiome suggested from the results of earlier studies [1, 2]. With these, they exert important interactions with the same (digestive) or other organ systems like immune, cardiovascular, and central nervous systems. In the gut, they digest carbohydrates or glycoproteins such as mucin, produce short-chain fatty acids, conjugate bile, and they can exert immune-modulatory effects through the capsular polysaccharides (CPS) of *Bacteroides fragilis* [1]. *B. fragilis* may produce an enterotoxin that mainly affects children, it can harm the mucosal epithelium, and hence it has a tumorigenic potential [3]. The immunomodulatory effects of the CPSs of *B. fragilis* outside the gut can induce abscesses and *B. fragilis* also has a high oxygen tolerance [4, 5]. With these, they can be opportunistic pathogens and may be the most prevalent taxon in infections caused by anaerobic bacteria. They are also the most antibiotic-resistant anaerobes that have many resistance mechanisms and they have the highest resistance rates compared with other known anaerobic pathogens. To facilitate their antibiotic treatment, resistance surveys are conducted to guide empiric therapies and direct antimicrobial susceptibility tests (most often Etest) can be used for targeted therapies [6]. Our knowledge of the antimicrobial susceptibilities of the clinical isolates is quite good, at least in the developed countries, but it is more limited when it comes to the normal microbiota isolates. Therefore, after the 2010 European *Bacteroides* antimicrobial susceptibility survey [7], a new study on fecal *Bacteroides* strains isolated between 2014-2016 was carried out [8]. This study included persons who were treated or not treated with carbapenems (in Belgium and Hungary to carry out antimicrobial susceptibility tests) and these results are now presented along with the normal microbiome compositions of patients who were treated or not treated with imipenem in Hungary.

Patients treated with meropenem (the standard regimen of 1g-2g *iv*/8 h) in Belgium (n=6) and imipenem (the standard regimen of 500 mg *iv*/6 h) in Hungary (n=7) were included in the study (ethical permissions: Regional Research Ethical Board of the University of Szeged, permission no.

70/2015-SZTE and Commissie Medische Ethiek O.G.016 Reflectiegroep Biomedische Ethiek in Hungary and Belgium, respectively).

Strain isolation, cultivation, storage, antibiotic susceptibility testing, and the statistical evaluation were performed as described previously and we compared our new data with those obtained in the previous one [8]. The number of subjects/patients and the isolated strains are listed in Table 1 for the earlier and the present study.

DNA samples from feces of the Hungarian patients, with or without imipenem treatment, for next generation sequencing were isolated using the Qiagen Stool DNA Mini Kit. 16S rDNA amplification and sequencing (V3-V4 region, 466 bp) were carried out by the Illumina 150 bp paired-end method (Novogene, Hong Kong), the datasets were uploaded to the EZ Bio Cloud (www.ezbiocloud.net) database and they were evaluated as described earlier using a local in-house application [9, 10]. Comparative datasets on the antibiotic resistance levels of *Bacteroides* strains isolated from normal microbiota and carbapenem-treated patients can be seen in Table 2. Statistically significant differences were obtained for cefoxitin (increased from about 16% to 51%), clindamycin (decreased from about 49% to 31%), and moxifloxacin (increased from about 10% to 41%) in cases where the merged data of Belgium and Hungary or the data for the five European countries of the previous study were analyzed [8]. The significance levels for these data values lay in the range of <0.001-0.006, which are beyond doubt. There were also some differences that were seen in the Belgian strains. For instance with tetracycline, an increase (from 59.5% to 89.5%) in the resistance rate was observed with a moderate significance ($p=0.044$), but for moxifloxacin it was more pronounced (an increase from 5.4% to 52.6%, $p<0.0001$) and for clindamycin a pronounced but adverse effect (from 75.7% to 31.6%, $p=0.004$) was noted (Table 2). Interestingly, no significant difference was found for imipenem, contrary that carbapenems were applied, but the actual values displayed a high variance (0 to 10.5% in Belgium and 2 to 10.3% for all countries, Table 2). Møller Hansen *et al.* studied the short-term effects of antibiotic treatments (piperacillin/tazobactam, meropenem, metronidazole, and clindamycin) on the enrichment of antibiotic-resistant *Bacteroides* in the gut in of patients from

Denmark. A significant increase (p=0.0001), contrary to our expectations, was found for the carbapenem meropenem (but here the number of the strains involved differed significantly i.e. 197 vs. 10 patients and 357 vs. 39 strains in Denmark and this study, respectively) [11].

We should mention that in our study clinical-to-normal flora differences were experienced for amoxicillin/clavulanic acid, cefoxitin, imipenem, clindamycin and moxifloxacin, where the resistance levels for cefoxitin, imipenem and clindamycin increased, and for moxifloxacin only species-specific differences were found[8]. This latter study offered a possible explanation for this, namely that the origin of the strains might differ – more precisely, the clinical strains originating from the mucosa and the fecal strains originating from the lumen where vertical and horizontal strain/gene spread may also differ[8]. At present it is thought that imipenem might act as an influencer of the proportion of antibiotic-resistant strains in the microbiota. An enhancement of the conjugation frequencies was described for the tetracycline resistance conjugative transposons and mobilizable elements of *Bacteroides* by tetracycline [12] and Tn*916* of Firmicutes by ribosome targeting antibiotics [13]. It is conceivable that imipenem has similar effects as well since it can induce resistance in some 'silently' resistant, *cfiA*-positive *B. fragilis* strains (according to our preliminary, unpublished observations). In our investigations, detecting the prevalence of corresponding resistance genes/genetic elements in our laboratory may take us closer to finding the reasons for the above-mentioned differences.

The microbiome examinations by 16S rDNA amplicon sequencing and principal component analysis of the fecal samples taken from Hungarian patients revealed no marked differences between the normal and imipenem-treated individuals (Fig S1). As expected, the usual Firmicutes and Bacteroidetes species were found in the core microbiomes (Fig. S2) and the ten top species displayed a near-even distribution between the two groups (Fig. S3). However, out of the simpler α-diversity measures, (using the Shannon and Chao formulas which use different calculations to be able to compare different species compositions), with the Chao formula there was a difference with a low significance value (p=0.02, Fig. S4). This may be because in the imipenem-treated group there was a tendency for one taxon to dominate (e.g. *B. fragilis*, *Clostridioides difficile*, enterococci), as was

noticed in an inspection of the species composition bars (not shown). Imipenem attains a good concentration (0-8 µg/ml) [14, 15] in the intestine during use in *iv* treatments but still, from the low disturbance of the intestinal microbiome with imipenem observed in this study, a resilience to this drug due to the production of carbapenemase can be expected. As regards the latter, we note that there is a significant proportion of carbapenem resistant strains in the gut (about 1% of the *Bacteroides* which are not exclusively *B. fragilis*) that can mediate this resilience [8]. Although antibiotic treatment may have short and long-term effects on the composition of the intestinal microbiota especially for cultivable bacteria [16], now it seems from several studies that it has some resilience to such disturbance indeed. This latter phenomenon can vary between microbiotas of different individuals, it may depend on the diet, the content of the main species, and different environmental factors [17-19]. It was also expected that the differences in antibiotic resistance values might be due more to imipenem than the composition of the microbiotas.

In summary, it can be stated that carbapenem treatment induced antibiotic resistance level changes of *Bacteroides* isolates obtained from the intestinal microbiotas of the treated individuals. In spite of this, the whole microbiotas of the imipenem-treated patients displayed no marked adverse effects, which can be attributed to the resilience of the intestinal microbiota. However, in view of the low number of individuals involved in this present study, we think that larger sample sizes sould provide more reliable results.

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Transparency declarations

The authors hereby declare that they have no conflict of interest.

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Table 1. Patients and *Bacteroides* strains

	Belgium		Hungary		Both[a]		All[b]
	NM[c]	Treated[c]	NM	Treated	NM	Treated	
Number of patients	5	6	12(+1)[d]	4(+3)[d]	17	10	42
Species distribution of Isolates							
All	40	19	62	20	102	39	241
B. fragilis	2	1	14	-	16	1	24
B. thetaiotaomicron	1	4	9	4	10	8	19
B. ovatus/xylanisolvens	8	4	14	6	22	10	48
B. vulgatus/dorei	6	4	9	5	15	9	36
B. uniformis	1	5	5	1	6	6	14
P. distasonis	3	-	5	2	8	2	12
Other[d]	19	1	20	2	39	3	49

[a] Merged values of Belgium and Hungary. [b] All the strains from the five European countries including the isolates from the imipenem-treated patients. [c] NM – Normal microbiota, Treated – imipenem treated patients. [d] The number of patients are shown in parentheses where *Bacteroides* isolation has not been carried out, and their fecal samples were only used for DNA extraction to perform microbiome composition sequencing. [d] We placed the following species *B. cacae*, *B. celulosilyticus*, *B. clarus*, *B. coprocola*, *B. eggerthii*, *B. faecis, B. finegoldii*, *B. nordi*, *B.stercoris*, *Parabacteroides johnsonii* and *P. merdae* in the 'other' category.

226 **Table 2.** A comparison of antibiotic susceptibilities of *Bacteroides* strains isolated from normal and
227 carbapenem-treated microbiotas

Antibiotic		Control				Treated				
	Countries	Range	MIC_{50}	MIC_{90}	R (%)	Range	MIC_{50}	MIC_{90}	R (%)	p[a]
Ampicillin	Belgium[b]	8->256	256	>256	100	16->256	>256	>256	100	-
	Hungary	2->256	128	>256	100	4->256	>256	>256	100	-
	Both[c]	2->256	128	>256	100	4->256[d]	>256	>256	100	-
	All	1->256	128	>256	96.6					n.s
Amoxicillin/clavulanate	Belgium	0.125->256	1	8	2.7	0.25-4	1	4	0	n.s.
	Hungary	0.125-32	1	8	7.9	0.064-2	0.5	0.5	0	n.s.
	Both	0.064-32	1	8	6.2	0.064-4	0.5	4	0	n.s.
	All	0.064-32	0.5	4	4.5					n.s.
Cefoxitin	Belgium	0.5-128	4	16	2.7	2-256	128	128	52.6	**<0.001**
	Hungary	0.5-128	32	64	25.0	1-128	32	128	50.0	n.s.
	Both	1-128	8	64	17.7	1-256	32	128	51.3	**0.003**
	All	0.5-256	16	64	14.9					**<0.001**
Imipenem	Belgium	0.125-2	0.5	1	0	0.5->32	1	32	10.5	n.s.
	Hungary	0.125-16	0.5	4	3.9	0.064-4	2	4	0	n.s.
	Both	0.064-16	0.5	2	2.7	0.064->32	1	16	10.3	n.s.
	All	0.032-32	0.5	2	2.0					n.s.
Clindamycin	Belgium	0.125->256	16	>256	75.7	0.25-32	16	32	31.6	**0.004**
	Hungary	0.064->256	2	>256	36.8	0.064->256	1	>256	30.0	n.s.
	Both	0.064->256	8	>256	50.4	0.064->256	1	>256	30.8	**0.004**
	All	0.064->256	4	>256	47.3					**0.006**
Metronidazole	Belgium	0.064-1	0.5	0.5	0	0.5-1	0.5	0.5	0	-
	Hungary	0.032-4	0.5	1	0	0.064-1	0.5	1	0	-
	Both	0.032-4	0.5	0	0	0.064-1	0.5	0	0	-
	All	0.032-4	0.5	1	0					-
Moxifloxacin	Belgium	0.125-32	1	2	5.4	0.5->32	16	32	52.6	**<0.001**
	Hungary	0.064-32	1	4	9.2	0.25->32	1	>32	15.0	n.s.
	Both	0.064-32	1	4	8.0	0.25->32	4	>32	41.0	**<0.001**
	All	0.064-64	1	8	11.4					**<0.001**
Tetracycline	Belgium	1->256	16	32	59.5	0.5-64	32	64	89.5	**0.014**
	Hungary	0.125-128	16	32	63.2	0.5-64	32	32	70.0	n.s.
	Both	0.125->256	16	32	62.8	0.5-64	32	64	82.1	**0.044**
	All	0.064->256	32	128	66.2					n.s.
Tigecycline	Belgium	0.064-32	0.5	8	2.7	0.032-16	1	8	5.0	n.s.
	Hungary	0.032-4	0.25	2	0	0.125-8	0.25	2	0	n.a.
	Both	0.032-32	0.5	2	0.9	0.032-16	0.25	8	2.6	n.s.
	All	0.032-32	0.5	4	1.5					n.s.
Chloramphenicol	Belgium	0.125-8	8	8	0	2-8	8	8	0	-
	Hungary	0.25-8	4	8	0	2-8	4	8	0	-
	Both	0.125-8	4	8	0	2-8	8	8	0	-
	All	0.125-8	4	8	0					-

228

229 [a] Significance of the differences. [b] These numerical replace those presented in Sóki *et al.* [8], which
230 otherwise does not affect the evaluations and conclusions given in that study. [c] Normal microbiota
231 values from the two (Belgium and Hungary) and all five European countries investigated earlier were
232 compared with the values of two countries in this study. [d] Merged values for Belgium and Hungary.

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Highlights

Bacteroides strains from the feces of imipenem-treated and non-treated patients were isolated.

Out of 10 antibiotics, cefoxitin, clindamycin, moxifloxacin, and tetracycline gave different resistance values between the above two groups of strains.

Imipenem-treatment caused a decrease in the diversity of the patients' microbiomes.

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József Sóki, Ingrid Wybo, Roland Wirth, Edit Hajdú, Mária Matuz, Katalin Burián

We declare no conflict of interests.

József Sóki
Corresponding author