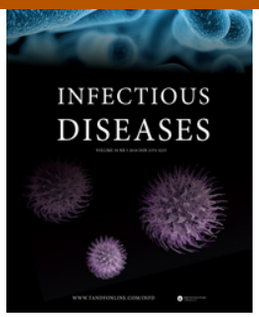

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ORIGINAL ARTICLE

A multicentre survey of the antibiotic susceptibility of clinical *Bacteroides* species from Hungary

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ABSTRACT

Background: The species of the *Bacteroides fragilis* group are important components of human microbiota, but as opportunistic pathogens they can be the causative agents of severe infections.

Methods: The major aims of our investigation were the evaluation of the susceptibility of 400 different Hungarian *B. fragilis* group isolates to 10 antibiotics by the agar dilution method, the comparison of our resistance data with previous national and international antibiotic resistance data and the comparison of present data in regional aspect. The MIC-values on 10 antibiotics of all the strains were determined with the agar dilution method by CLSI. The presence of the *cfiA* gene in Division II *B. fragilis* strains was confirmed by RT-PCR.

Results: We detected a relatively high resistance rate of ampicillin, moxifloxacin, clindamycin and tetracycline, but amoxicillin/clavulanic acid, metronidazole, tigecycline and chloramphenicol showed excellent activity. In this study, we found that 6.75% of the isolates were resistant to cefoxitin and 7% to meropenem, while 8.58% of our *B. fragilis* strains harboured the *cfiA* gene. Most of the meropenem resistant strains were isolated in one of the participating centres. In the case of meropenem, cefoxitin, clindamycin and high-level-ampicillin-resistant strains, we found significant regional differences.

Discussion: Most of the results of our study were concordant with previous national and international data, with the exception of amoxicillin/clavulanic acid, cefoxitin and meropenem.

Conclusions: Our study highlighted the importance of the periodic monitoring of the antimicrobial susceptibility of *Bacteroides* species providing important information for the appropriate therapy.

KEYWORDS
Bacteroides species
agar dilution
antibiotic susceptibility test

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Background

The *Bacteroides fragilis* group species are very important members of human microbiota; however, as opportunistic pathogens these clinical isolates can cause severe or sometimes fatal infections (e.g. skin and soft tissue, wound, intraabdominal infections, sepsis and abscesses) [1]. β-lactam/β-lactamase inhibitor combinations, cephamycins, carbapenems, clindamycin, fourth-generation fluoroquinolones and 5-nitroimidazoles are used for the therapy of *Bacteroides sp.* infections and antibiotic susceptibility tests are performed for the above-mentioned drugs in these types of infections [2]. In the last few decades, the increasing antimicrobial resistance among *Bacteroides* isolates is a growing problem worldwide [2,3]. The routine antimicrobial susceptibility testing of anaerobic isolates is only recommended by the Clinical and Laboratory Standards Institute (CLSI), in particular circumstances [4]: (i) relapse after initially successful therapy, (ii) failing to response to the empirical therapy, (iii) samples taken from physiologically sterile body sites, (iv) few available antibiotic susceptibility data, (v) often resistant isolate to antianaebic drugs and (vi) required prolonged therapy [4,5]. Susceptibility testing is recommended in case of epidemiological surveillance and highly virulence bacteria (e.g. *Bacteroides*, *Prevotella*, *Fusobacterium*, *Clostridium spp.*, *Bilophila wadsworthia* and *Sutterella wadsworthensis*) [4,5]. The antimicrobial susceptibility of *Bacteroides* isolates varies among different species and depend on geographical location [6,7]. For these reasons, the accurate identification of isolates, regular performance of antimicrobial susceptibility surveys and a knowledge of local antimicrobial resistance data are essential. A comprehensive antimicrobial survey among *B. fragilis* group isolates in Hungary had not been performed for over 20 years.

Aims and methods

Aims

The objectives of our investigation were as follows: (1) evaluation of the susceptibility of 400 different Hungarian *B. fragilis* group isolates to 10 antibiotics by the agar dilution method, (2) analysis of any certain differences in the resistance rate among the species, (3) comparison of regional resistance data, (4) comparison of present and former Hungarian data and (5) the data of this survey with that of international studies.

Bacterial strains and cultivation

In our study, 400 *B. fragilis* group isolates, collected between 2014 and 2016 by four Hungarian clinical microbiological centres (Centre 1: Semmelweis University, Budapest, Hungary; Centre 2: SYNLAB Ltd., Budapest, Hungary; Centre 3: University of Debrecen, Debrecen, Hungary; Centre 4: University of Szeged, Szeged, Hungary) were investigated by our team. The strains ($n = 10$) obtained from the University of Pécs were investigated with the isolates from the Centre 1. The collection criteria were the isolation of 100 clinically relevant, non-repeating samples by each centres. The strains were stored in brain heart infusion (BHI) broth supplemented with 20% glycerol at −80 °C. All of the isolates were cultured on Schaedler agar (bioMérieux, Marcy l'Etoile, France) for 48 hours, at 37 °C in an anaerobic chamber (Perkin Elmer, Beaconsfield, UK) under anaerobic conditions (85% N_2, 10% CO_2, 5% H_2). The identification at the species level was performed in Centre 4 with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonik, Bremen, Germany) using Biotyper Version 3.0 software. The *B. fragilis* strains were categorized as Division I (*cfiA* gene negative strains) and Division II (positive for the *cfiA* carbapenemase gene) by MALDI-TOF MS, as described by Fenyvesi et al. earlier [8].

Agar dilution method

The minimal inhibitory concentration (MIC) values for 10 antibiotics were determined with the agar dilution method according to the CLSI recommendations [4]. The tested antibiotics were ampicillin, cefoxitin, tetracycline, tigecycline, chloramphenicol (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), amoxicillin/clavulanic acid (GlaxoSmithKline, Brentford, UK), meropenem, moxifloxacin (Fresenius Kabi, Mihla, Germany), clindamycin (Pfizer, New York, NY) and metronidazole (TEVA, Petach Tikva, Israel). The tested ranges of the antibiotics were the following: ampicillin (2–256 mg/l), amoxicillin/calvulanic acid (0.064/0.032–16/8 mg/l), cefoxitin (0.5–256 mg/l), meropenem (0.064–16 mg/l), clindamycin (0.064–256 mg/l), metronidazole (0.064–8 mg/l), moxifloxacin (0.064–32 mg/l), tetracycline (0.125–256 mg/l), tigecycline (0.064–32 mg/l) and chloramphenicol (0.125–32 mg/l). We used fixed concentration of amoxicillin/clavulanic acid for stock solution (10/2.5 mg/ml). For the interpretation of the MIC-value, either European Committee on Antimicrobial Susceptibility Testing (EUCAST) (ampicillin, amoxicillin/clavulanic acid, meropenem, clindamycin and metronidazole) or CLSI guidelines (cefoxitin, moxifloxacin, tetracycline and chloramphenicol) were used [4,9]. As the tigecycline breakpoints among *Bacteroides* species

Table 1. Antimicrobial activities of 10 antibiotics applied on *Bacteroides fragilis* group isolates.

	MIC (mg/ml)			% of isolates		
Antimicrobial agents	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Bacteroides fragilis group ($n = 400$)						
Ampicillin	2 to >256	64	>256	0.75	1.25	98.00
Amoxicillin/clavulanic acid	0.064–32	0.5	8	87.00	8.50	4.50
Cefoxitin	0.5–256	8	32	77.00	16.25	6.75
Meropenem	0.064–32	0.5	4	88.75	4.25	7.00
Clindamycin	0.064 to >256	2	>256	63.25	0.00	36.75
Metronidazole	0.064–16	0.5	1	99.75	0.00	0.25
Moxifloxacin	0.064–64	1	8	75.00	6.50	18.50
Tetracycline	0.125–256	16	64	28.75	6.00	65.25
Tigecycline	0.064–64	0.5	4	94.75	3.75	1.50
Chloramphenicol	0.125–16	4	8	99.50	0.50	0.00
B. fragilis ($n = 233$)						
Ampicillin	2–512	64	>256	0.00	0.00	100.00
Amoxicillin/clavulanic acid	0.064–32	0.5	4	91.87	6.43	1.70
Cefoxitin	1–256	8	16	90.56	6.00	3.44
Meropenem	0.064–32	0.5	8	84.98	5.15	9.87
Clindamycin	0.064 to >256	1	>256	74.25	0.00	25.75
Metronidazole	0.125–4	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.125–64	0.5	8	76.40	8.15	15.45
Tetracycline	0.125–256	32	64	25.75	3.00	71.25
Tigecycline	0.064–32	0.5	4	94.85	4.30	0.85
Chloramphenicol	0.25–8	4	8	100.00	0.00	0.00
B. thetaiotaomicron ($n = 79$)						
Ampicillin	2–512	128	>256	1.27	0.00	98.73
Amoxicillin/clavulanic acid	0.064–32	0.25	8	83.54	13.92	2.53
Cefoxitin	0.5–256	16	32	53.16	37.97	8.87
Meropenem	0.125–32	1	2	92.41	6.33	1.26
Clindamycin	0.064 to >256	8	>256	44.30	0.00	55.70
Metronidazole	0.064–4	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.064–32	1	16	75.96	5.06	18.98
Tetracycline	0.125–256	16	64	40.51	5.06	54.43
Tigecycline	0.064–64	0.5	4	94.94	3.80	1.26
Chloramphenicol	0.125–16	4	8	98.74	1.26	0.00
B. vulgatus ($n = 26$)						
Ampicillin	4–512	128	>256	0.00	0.00	100.00
Amoxicillin/clavulanic acid	0.125–16	1	16	69.23	23.08	7.70
Cefoxitin	0.5–128	4	64	80.76	7.69	11.55
Meropenem	0.125–32	1	4	84.62	11.54	3.84
Clindamycin	0.064 to >256	4	>256	50.00	0.00	50.00
Metronidazole	0.125–2	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.25–64	2	32	50.00	0.00	50.00
Tetracycline	0.125–64	16	64	19.24	7.69	73.07
Tigecycline	0.064–8	0.25	2	96.16	3.84	0.00
Chloramphenicol	0.5–8	4	8	100.00	0.00	0.00
B. ovatus ($n = 24$)						
Ampicillin	2–512	256	>256	8.33	0.00	91.67
Amoxicillin/clavulanic acid	0.064–32	2	16	79.16	12.50	8.33
Cefoxitin	2–128	32	64	41.67	41.67	16.66
Meropenem	0.125–32	1	16	75.00	8.34	16.66
Clindamycin	0.064 to >256	8	>256	45.83	0.00	54.17
Metronidazole	0.125–8	0.5	2	95.83	0.00	4.17
Moxifloxacin	0.25–32	1	32	79.16	0.00	20.84
Tetracycline	0.125–32	8	32	29.16	20.84	50.00
Tigecycline	0.064–8	0.25	4	95.83	4.16	0.00
Chloramphenicol	2–8	8	8	100.00	0.00	0.00
P. distasonis($n = 15$)						
Ampicillin	8 to >256	>256	>256	0.00	0.00	100.00
Amoxicillin/clavulanic acid	0.125–32	4	16	53.33	20.00	26.67
Cefoxitin	2–128	16	128	60.00	20.00	20.00
Meropenem	0.25–4	0.5	4	86.67	13.33	0.00
Clindamycin	0.5 to >256	4	>256	66.67	0.00	33.33
Metronidazole	0.25–2	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.25–2	0.5	1	100.00	0.00	0.00
Tetracycline	0.25–32	16	32	20.00	20.00	60.00
Tigecycline	0.125–4	0.5	2	100.00	0.00	0.00
Chloramphenicol	4–8	8	8	100.00	0.00	0.00
B. uniformis($n = 11$)						
Ampicillin	32 to >256	128	>256	0.00	0.00	100.00
Amoxicillin/clavulanic acid	0.125–2	0.25	2	100.00	0.00	0.00
Cefoxitin	1–64	8	32	81.80	9.10	9.10
Meropenem	0.25–4	0.5	1	90.90	9.10	0.00
Clindamycin	0.064 to >256	>256<	>256	45.45	0.00	54.55

(*continued*)

Table 1. Continued

Antimicrobial agents	MIC (mg/ml)			% of isolates		
	Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant
Metronidazole	0.125–0.5	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.5–32	0.25	1	72.72	9.10	18.18
Tetracycline	0.125–32	8	32	54.55	18.18	27.27
Tigecycline	0.064–2	0.5	2	100.00	0.00	0.00
Chloramphenicol	4–8	8	8	100.00	0.00	0.00
Other *Bacteroides* species[a] ($n = 12$)						
Ampicillin	2 to >256	64	64	0.00	0.00	100.00
Amoxicillin/clavulanic acid	0.125–4	0.5	4	100.00	0.00	0.00
Cefoxitin	1–64	16	32	41.67	41.67	16.67
Meropenem	0.125–2	0.5	1	100.00	0.00	0.00
Clindamycin	0.125 to >256	4	>256	41.67	0.00	58.33
Metronidazole	0.125–2	0.5	1	100.00	0.00	0.00
Moxifloxacin	0.064–64	2	16	66.67	8.33	25.00
Tetracycline	0.25–128	16	128	16.67	8.33	75.00
Tigecycline	0.125–32	0.25	16	75.00	0.00	25.00
Chloramphenicol	0.25–16	4	8	91.67	8.33	0.00

[a]*B. stercoris* (1), *B. cellulosilyticus* (1), *B. caccae* (4), *B. intestinalis* (1), *B. salyersiae* (2), *B. nordii* (2), *P. goldsteinii* (1).

have not yet been established either by EUCAST or CLSI, the breakpoints published by Nagy et al. were applied for the interpretation [3]. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were used as control strains.

RT-PCR

The presence of the *cfiA* gene in Division II *B. fragilis* strains was confirmed by RT-PCR, as described by Eitel et al. [10].

Statistical evaluation

The data were analysed by using Fischer's exact and Spearman's correlation tests in the SigmaPlot 12 program and the significance level was set to 0.05 (i.e. $p < .05$). The antibiotic resistance data values were analysed via the chi-square test (χ^2-test) in SigmaPlot 12.

Results

Isolates

In our study, 400 clinically relevant *Bacteroides* strains were investigated. A sum of 397 samples were taken after 48 hours of admission to hospital; and three samples by a General Practitioner (GP). Here, 13% of the isolates were isolated from a pure culture and 87% from a mixed culture. A total of 43.5% of the patients were female, 56.5% were male and they had an average age of 59.3 years (4–101 years). The majority of the isolates was *B. fragilis* (58.3%), followed by *B. thetaiotaomicron* (19.8%), *B. vulgatus* (6.5%), *B. ovatus* (6.0%), *P. distasonis* (3.8%), *B. uniformis* (2.8%); and other *B. fragilis* group species (*B. caccae, B. nordii, B. salyersiae, B. stercoris, B. cellulosilyticus, B. intestinalis, P. goldsteinii*) were also identified in low rates (0.3–1%). The most common sample types were wound (44.8%) and intraabdominal samples (42.7%); while extraabdominal abscess (3.8%), blood culture (4.2%) and other types (gynaecological samples, middle ear, cerebrospinal fluid, pericardial fluid) (4.5%) were less frequent. Half of the strains were collected in Surgery, 12.7% in an Intensive Care Unit (ICU), 12.5% in Internal Medicine, 5.8% in Pediatrics, 5.0% in Obstetrics and Gynaecology, and the remaining samples were collected in other departments (1.0–4.5%).

Antibiotic susceptibility

The data of antimicrobial susceptibility, the MIC ranges, the MIC_{50} and MIC_{90} values are summarized in Table 1. A total of 98% of the strains were resistant to ampicillin and the overall resistance rate to moxifloxacin was 18.5%. A sum of 36.75% of the isolates displayed resistance to clindamycin. CLSI breakpoints indicated a high resistance of 65.25% to tetracycline. On the other hand, only 4.5% displayed resistance to amoxicillin/clavulanic acid. Metronidazole remained very active against *Bacteroides* species, with only one strain demonstrating resistance (0.25%). Most of the isolates (94.75%) were susceptible to tigecycline, only four strains being resistant to this antibiotic agent. Chloramphenicol also remained very active against *Bacteroides* species; with 99.5% of the strains were fully susceptible, and no resistant isolates was found. The rate of cefoxitin resistant

Table 2. Antibiotic susceptibility of *B. fragilis* group isolates obtained from the different Hungarian centres.

		% of isolates		
Antimicrobial agents	MIC (mg/l) range	Susceptible	Intermediate	Resistant
Centre 1 ($n = 100$)				
Ampicillin	4–512	0.00	5.00	95.00
≥64 mg/l				62.00
Amoxicillin/clavulanic acid	0.064–16	92.00	5.00	3.00
Cefoxitin	1–128	77.00	16.00	7.00
Meropenem	0.125–32	95.00	1.00	4.00
Clindamycin	0.125 to >256	52.00	0.00	48.00
Metronidazole	0.125–4	100.00	0.00	0.00
Moxifloxacin	0.25–32	71.00	7.00	22.00
Tetracycline	0.125–128	31.00	6.00	63.00
Tigecycline	0.125–32	89.00	7.00	4.00
Chloramphenicol	0.25–8	100.00	0.00	0.00
Centre 2 ($n = 100$)				
Ampicillin	2–512	1.00	0.00	99.00
≥64 mg/l				57.00
Amoxicillin/clavulanic acid	0.064–32	89.00	9.00	2.00
Cefoxitin	1–128	77.00	19.00	4.00
Meropenem	0.25–32	92.00	3.00	5.00
Clindamycin	0.064 to >256	63.00	0.00	37.00
Metronidazole	0.064–4	100.00	0.00	0.00
Moxifloxacin	0.125–32	72.00	9.00	19.00
Tetracycline	0.125–256	27.00	10.00	63.00
Tigecycline	0.064–16	97.00	1.00	2.00
Chloramphenicol	2–16	98.00	2.00	0.00
Centre 3 ($n = 100$)				
Ampicillin	2 to >256	2.00	0.00	98.00
≥64 mg/l				73.00
Amoxicillin/clavulanic acid	0.125–32	89.00	8.00	3.00
Cefoxitin	0.5–128	80.00	17.00	3.00
Meropenem	0.125–32	87.00	6.00	7.00
Clindamycin	0.064 to >256	73.00	0.00	27.00
Metronidazole	0.064–16	99.00	0.00	1.00
Moxifloxacin	0.125–64	78.00	5.00	17.00
Tetracycline	0.25–128	35.00	4.00	61.00
Tigecycline	0.064–8	99.00	1.00	0.00
Chloramphenicol	0.125–8	100.00	0.00	0.00
Centre 4 ($n = 100$)				
Ampicillin	16 to >256	0.00	0.00	100.00
≥64 mg/l				91.00
Amoxicillin/clavulanic acid	0.5–16	77.00	14.00	6.00
Cefoxitin	1–256	74.00	13.00	13.00
Meropenem	0.064–32	81.00	7.00	12.00
Clindamycin	0.064 to >256	65.00	0.00	35.00
Metronidazole	0.125–4	100.00	0.00	0.00
Moxifloxacin	0.25–64	79.00	5.00	16.00
Tetracycline	0.125–128	22.00	4.00	74.00
Tigecycline	0.064–8	95.00	5.00	0.00
Chloramphenicol	4–8	100.00	0.00	0.00

Centre 1: Semmelweis University, Budapest; Centre 2: SYNLAB Ltd., Budapest; Centre 3: University of Debrecen; Centre 4: University of Szeged.

strains was 6.75% and we found the meropenem resistance rate of 7% (Table 1).

Comparing the resistance rates of the different species, 91.67–100% of the species were resistant to ampicillin. *P. distasonis* strains had the highest resistance rates to amoxicillin/clavulanic acid (26.67%) and cefoxitin (20%). A total of 16.66% of the *B. ovatus* strains were resistant to meropenem; whilst all of the *P. distasonis*, *B. uniformis* and other *Bacteroides* species were susceptible to this drug. The resistance rate of 55.7% for clindamycin was found among *B. thetaiotaomicron*, whilst 25.75% of the *B. fragilis* isolates were resistant to this drug.

Antibiotic susceptibility data values of the centres are summarized in Table 2. A significant difference among highly ampicillin resistant (≥64 mg/l) strains was observed between centre pairs: Centres 1 and 4 ($p < .001$); Centres 2 and 4 ($p < .001$); Centres 2 and 4 ($p = .002$). Comparing the cefoxitin resistant rates of the centres, the difference was significant between Centre 3 (3.00%) and Centre 4 (13.00%) ($p < .001$). We detected a relatively high difference in meropenem susceptibility data. A total of 28 meropenem resistant strains were found, 25 of them were *B. fragilis* (89.28%, 25/28). Interestingly, in Centre 4, we identified 11 *B. fragilis* (39.28%, 11/28) and one *B. ovatus* (3.57%, 1/28) meropenem resistant strains. All of these *B. fragilis* strains were identified as a member of the Division II by MALDI-TOF MS and harboured the *cfiA* gene proved by RT-PCR. In

Centre 4, we found 11 high-level-meropenem-resistant strains (MIC $\geq$ 16 mg/l) and two *cfiA*-positive strains with MIC-values of 4 mg/l and 8 mg/l. In other centres, the rate of meropenem resistant strains was lower (4.0–7.0%) (Table 1) and all of them were high-level-meropenem-resistant (MIC $\geq$ 16 mg/l). A significant difference in meropenem resistance was observed between Centres 1 and 4 ($p < .001$) (Table 3). Clindamycin resistance displayed a relatively strong geographical difference, which was significant between Centre 1 (48.0%) and Centre 3 (27.00%) ($p = .003$). The highest resistance rate to tetracycline was found among the strains isolated in Centre 1 (63.0%); while in Centre 4 this rate was 74.0%; but the difference was not significant ($p = .121$) (Table 3). With a correlation analysis, a strong correlation ($p < .05$) was observed with the following antimicrobial drug pairs on the rate of resistant strains: ampicillin and amoxicillin/clavulanic acid; cefoxitin and amoxicillin/clavulanic acid; tetracycline and tigecycline. We analysed the antibiotic susceptibility data based on the clinical source, but we did not find any significant correlation (i.e. $p < .05$).

Table 3. Meropenem MIC values of *cfiA*-positive and -negative *B. fragilis* and *Bacteroides* non-fragilis isolates.

Meropenem MIC (mg/l)	Centre 1	Centre 2	Centre 3	Centre 4
cfiA-positive *B. fragilis*				
≥16	1	2	3	11
8				1
4				1
<4	1			
cfiA-negative *B. fragilis*				
≥16	2	3	2	
8				
4				
<4				
Non-fragilis *Bacteroides*				
≥16			2[a]	1[b]
8				
4				
<4				

[a] *B. ovatus*, *B. thetaiotaomicron*.
[b] *B. ovatus*.

Discussion

In our study, we found a very high ampicillin resistance rate (98.0%) due to the widely distributed β-lactamase producing genes among *Bacteroides* isolates, which is concordant with the results published by Nagy et al. (2008–2009) (97.4%) [3]. Only 4.5% of our isolates were resistant to amoxicillin/clavulanic acid, while Nagy et al. (2008–2009) reported a rate of 8.7% [3] and Wybo et al. (2011–2012) a rate of 14% [11]. Some 6.75% of the strains exhibit a resistance to cefoxitin, which is much lower than reported in previous surveys (15.2–17.2%) [3,6]. The background of decreased amoxicillin/clavulanic acid and cefoxitin resistance rate may be the different collection sites, change of the antibiotic usage, the different number of the isolates. The background of the reduction of cefoxitin resistance rate can be its very low usage. Interestingly, the consumption of amoxicillin/clavulanic acid is high and this was the first choice in *Bacteroides* infections, the resistant rate remained quite low. However, the comparison of data of present and previous Hungarian studies is quite difficult because of the different methods (microbroth dilution [12] vs. agar dilution [3]), different breakpoints and number of isolates. The rate of cefoxitin resistance depends on the different species: for instance, 3.44% of the *B. fragilis* and 20% of the *P. distasonis* strains were resistant. This finding is in agreement with Snydman et al. (2010–2012), who found that 3.7% of the *B. fragilis* and 14.7% of the *P. distasonis* were resistant to cefoxitin [13]. In general, carbapenems show high activity against anaerobic bacteria, but there is a threat of increasing carbapenem resistance rate [14,15]. A meropenem resistance rate of 0.5% for the *B. fragilis* group isolates was reported in an American study [16], and in Europe it was 1.3% [17]; however, Liu et al. (2000–2007) found a resistance rate of 12% of *B. fragilis* strains in Taiwan [15]. In our study, we found an overall species resistance level of 7% to meropenem, and 9.87% of *B. fragilis* strains resistant to meropenem, which rates were relatively higher in comparison to that of reported by American and European studies [16,17]. Studies have reported a prevalence of *cfiA*-positivity of between 2.4 and 5.7% [18–20]; and 8.58% of 233 *B. fragilis* strains harboured the *cfiA* gene (Table 3). The difference of the meropenem resistance rates among the centres can be the different prevalence of the *cfiA* gene and the local antibiotic administration. According to the literature, the meropenem resistance mechanism of *cfiA*-negative *B. fragilis* strains can be the alteration of the penicillin binding proteins (PBPs) and/or decreased permeability [1]. We noticed an elevated overall resistance level of 36.75% to clindamycin, which varied among the different species. This rate was lowest among *B. fragilis* isolates (25.75%) and highest among *B. thetaiotaomicron* (55.7%) strains. Others have reported a clindamycin resistance rate of between 27% and 37.6% [3,21,22]. We found that clindamycin resistance displayed a relatively strong geographical difference, which is concordant with the results published by Nagy et al.: in the Southern European countries, the mean rate of clindamycin resistant strains was 37.6%; however, in Northern Europe, it was found to be 81.4% [3]. Despite the

frequent usage of metronidazole, this drug still showed excellent activity against *Bacteroides* isolates, only one strain was resistant to metronidazole (0.25%). The overall resistance rate to metronidazole among *Bacteroides* isolates remained low (<1%) [3,5,6]. Among the different *Bacteroides* species, the moxifloxacin resistance rate varies considerably; all of the *P. distasonis* ($n = 15$) isolates were susceptible, but 15.45% of the *B. fragilis* ($n = 233$) and 50% of the *B. vulgatus* ($n = 26$) strains were resistant to moxifloxacin. Considerable differences in moxifloxacin susceptibility between species were observed by Snydman et al. (2010–2012): the resistance rate varied from 38.9% in *P. distasonis* to over 70% of *B. ovatus*, *B. vulgatus* and other *Bacteroides spp.* (*B. caccae*, *B. eggerthii*, etc.) [12]. Nagy et al. reported significant regional differences of the rates of moxifloxacin resistance strains from Southern (92.45%) and Northern European (70.1%) countries [3] and we found particular geographical differences (Centre 3: 3% vs. Centre 4: 13%) in Hungary. We detected an overall resistance rate to tetracycline of 65.25%, but there was also a great variation; with 27.27% of the *B. uniformis* ($n = 11$) isolates, and 75% of the other *Bacteroides* isolates ($n = 12$) were resistant to this drug, and an overall rate of 65.25%. Tigecycline was very active, only three resistant strains were isolated (1.5%), which result was consistent with the results published by Nagy et al. (2008–2009) (1.7%) [3]. The effectiveness of chloramphenicol remained excellent, and with the exception of one intermediate susceptible strain, all were susceptible to chloramphenicol. Our result was concordant with other studies: Wybo et al. reported a susceptibility rate of 99% of 2004 [11], and Nitzan et al. found that 98.5% (2012) of anaerobic isolates were susceptible to chloramphenicol [23].

Only a limited comparison could be made among present and previous Hungarian *Bacteroides spp.* resistance data. However, according to the data reported by Nagy et al., the level of clindamycin resistance increased from 23% (1992) to 36.75% and moxifloxacin from 13.6% (2008–2009) to 18.50%, but the level of resistance to amoxicillin/clavulanic acid decreased from 15% (2008–2009) to 4.5% and cefoxitin from 24% (2008–2009) to 6.75% (Table 4) [3,13]. In general, we observed a strong correlation ($p < .05$) among the following three pairs: ampicillin and amoxicillin/clavulanic acid; cefoxitin and amoxicillin/clavulanic acid; and tetracycline and tigecycline. In the background, there could be common antibiotic resistance mechanisms: the β-lactamase production is the most common resistance mechanism for β-lactam antibiotics among *B. fragilis*

Table 4. Comparison of previous Hungarian resistance data and data taken from the present study of *B. fragilis* group isolates.

Percentage (%) of resistance strains at different timepoints			
Antimicrobial agents	1992 ($n = 200$) [25]	2010 ($n = 100$) [4]	Present study ($n = 400$)
Ampicillin	97	100	98.00
Amoxicillin/clavulanic acid	ND	15	4.50
Cefoxitin	11	24	6.75
Meropenem	ND	ND	7.00
Clindamycin	23	27	36.75
Metronidazole	0	1	0.25
Moxifloxacin	ND	13.6	18.50
Tetracycline	65	ND	65.25
Tigecycline	ND	1.7	1.50
Chloramphenicol	0	ND	0

ND: no data.

isolates; more than 90% of the isolates express at least one β-lactamase gene [1]. Among the important β-lactam resistance genes, Rogers et al. described the *cepA* cephalosporinase gene, the encoded enzyme hydrolyses penicillins and most of the cephalosporins (except for cefoxitin) [24]. Another resistance gene, the *cfxA* is responsible for the cefoxitin resistance and usually positioned on mobilizable transposons, e.g. Tn4555 [10]. The carbapenem resistance is associated with the chromosomal *cfiA* gene, which encodes Zn^{2+}-dependent metallo-β-lactamase. For the expression of *cfiA* gene, the presence of an IS element required in the upstream region (e.g. IS*613*, IS*1169*, IS*614B*, IS*4351*, IS*1186* or IS*1187*) [17]. The other resistance mechanisms are decreased permeability or the alteration of PBPs [1]. Against tetracycline, *Bacteroides* isolates can express active efflux, encoded by the *tetA, tetB, tetC, tetD, tetE*, etc. genes. Many *Bacteroides* strains express ribosomal protection proteins, encoded by *tetQ, tet32, tet36, tetM, tetO*, etc. genes. Bartha et al. reported elevated tigecycline MIC-value (≥8 mg/l) in *tetQ*-harbouring *B. fragilis* group isolates. The enzymatic modification is not important among anaerobic bacteria, because enzymes encoded by *tetX* and *tetX1* require both oxygen and NADPH for its activity; however, 75% of the *B. fragilis* group isolates with tigecycline MIC =4 mg/l harboured *tetX1* gene [25].

Conclusions

Currently, no valid, exact data about antibiotic prescribing practices are available in Hungary. The rational restriction of antibiotics can help the control of other diseases, e.g. *C. difficile* infection. In the past decade, the number of reports of β-lactam/β-lactamase inhibitor combinations, cefoxitin, moxifloxacin, tetracycline and

clindamycin resistant *B. fragilis* group isolates has increased worldwide [25,26]. The reasons for different resistance patterns maybe due to local antimicrobial chemotherapy administration, the distribution of antibiotic resistance genes, the variation between susceptibility testing methods, the differences in the interpretative breakpoints or the complete lack of them. The main conclusion of our survey and our results proved that the periodic monitoring of the antimicrobial susceptibility of *Bacteroides* species is essential to obtain accurate information on local and national rates of antimicrobial resistance, and that this is critical to guide appropriate therapy for patients.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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