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Analysis of Romanian *Bacteroides* isolates for antibiotic resistance levels and the corresponding antibiotic resistance genes

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ABSTRACT

As part of an ESCMID Study Group on Anaerobic Infections (ESGAI) project, a study was conducted to measure the antibiotic susceptibilities and corresponding gene contents of 53 *Bacteroides fragilis* group strains isolated in Romania. The antibiotic resistance data was comparable with the data found for other East-European countries. Here, no resistant isolate was found for imipenem, metronidazole and tigecycline. An increasing role of the *cepA*, *cfxA* and *cfiA* genes was observed in their corresponding antibiotic resistances. Moreover, no isolate was found that harbored the *cfiA* gene with a possible activating IS element. Clindamycin resistance was low, similarly to that the rate for the ermF gene. However, we did find some isolates with *nimB*, *ermB*, *msrSA*, *linA*, *satG*, *tetX*, *tetM* and *bexA* genes. This study was the first to provide antibiotic resistance data for clinical *Bacteroides* strains from Romania.

1. Introduction

Anaerobic pathogens may cause severe infections and the most frequently isolated strains belong to *Bacteroides fragilis* and to some related species. These important species, are normally intestinal commensals that exert many useful interactions in the gut. In addition, clinical *Bacteroides* isolates have the highest antibiotic resistance levels and the highest number of antibiotic resistance mechanisms among all human pathogenic anaerobic bacteria. However, their special culturing requirements make their diagnostic processes and antimicrobial testing tedious and time consuming. Therefore regular geographical and temporal records of antimicrobial susceptibilities are essential for their empirical therapies. Regular resistance surveys have been conducted over the years in the USA [1], in Europe [2] and also in some other developed countries [3,4]. Thus the general picture that has emerged is that *Bacteroides* are almost completely resistant to 'regular β-lactams' (penicillins, cephalosporins) and tetracyclines, moderate resistance can be found for cefoxitin, amoxicillin/clavulanic acid, clindamycin and moxifloxacin, and the resistance is very low for carbapenems, piperacillin/tazobactam, metronidazole and tigecycline. The latest susceptibility survey for Europe has been completed with the identification of corresponding antibiotic resistance genes [5] along with a detailed antibiotic resistance mechanism analysis for metronidazole-resistant, *nim*-positive strains and strains with elevated imipenem resistance levels [6]. The particular antibiotic resistance genes that are suspected or proved to be behind the main antibiotic resistance phenotypes are as follows: *cepA* for 'regular β-lactams' (penicillins, cephalosporins), *cfxA* for cephamycins, *cfiA* for carbapenems, *ermF* for the MLSB (macrolide, lincosamide, streptogramin) group of antibiotics, *nim* genes for 5-nitroimidazole resistances, and *tetQ* for tetracyclines. Besides these, other less frequent antibiotic resistance genes may also be found among clinically important *Bacteroides* strains like *tetX* for tetracyclines and glycylcyclines, *msrSA* for streptogramins or *bexA* for fluoroquinolones. An interesting aspect of the antibiotic resistance mechanisms of *Bacteroides* is that special insertion sequence (IS) elements may activate the resistance genes to induce high resistance levels.

Romania did not send *Bacteroides* strains to the latest European survey, partly because anaerobic diagnostics is only performed to a limited extent. However, since 2008 at Targu-Mures (a university town in Transylvania) a good anaerobic diagnostics facility has been operating using good connections with the ESGAI and the National

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Table 1
Comparison of the susceptibility values detected in this study with those for Europe.

Group	Descriptor	Ampicillin	Amoxicillin/clavulanic acid	Piperacillin/tazobactam	Cefoxitin	Clindamycin	Imipenem	Metronidazole	Moxifloxacin	Tetracycline	Tigecycline
European	Range	1–>256	0.016–>256	0.016–>256	1–>256	0.016–>256	0.002–>32	0.016–256	<0.125–32	–	0.016–32
Bacteroides	MIC_{50}	32	1	4	16	2	0.5	0.5	1	–	0.25
fragilis group	MIC_{90}	>256	16	32	128	>256	1	1	16	–	2
strains ($n = 824$)	Resistance (%)	98.2	10.4	10.3	17.2[a]	32.4	0.85[a]	0.5	13.6[a]	–	1.7[a]
Romanian	Range	1–>256	0.25–64	0.032–32	2–128	0.032–>256	0.032–2	0.125–1	0.125–>32	<0.5–256	0.032–8
Bacteroides	MIC_{50}	32	2	4	16	1	0.125	0.25	1	32	0.25
fragilis group	MIC_{90}	>256	16	16	64	4	1	0.5	>32	64	0.5
strains ($n = 53$)	Resistance (%)	96.3	13.0	5.6	14.8[a]	9.4	0	0	14.8[a]	75.9[a]	0[a]
European	Range	1–>256	0.016–>256	0.016–>256	1–>256	0.016–>256	0.002–>32	0.016–32	<0.125–64	–	0.016–32
Bacteroides	MIC_{50}	32	1	2	16	1	0.25	0.5	0.5	–	0.5
fragilis strains	MIC_{90}	>256	16	16	256	256	0.5	1	8	–	2
($n = 600$)	Resistance (%)	97.4	8.7	6.5	13.7[a]	28.5	1.2	0.5	14.0[a]	–	1.8[a]
Romanian	Range	2–>256	0.25–64	0.032–32	4–64	0.064–>256	0.032–2	0.125–0.5	0.125–>32	<0.5–256	0.125–8
Bacteroides	MIC_{50}	32	2	2	16	1	0.125	0.25	1	32	0.25
fragilis strains	MIC_{90}	>256	16	8	32	2	1	0.5	>32	64	0.5
($n = 36$)	Resistance (%)	97.3	13.5	2.7[b]	2.7[a,b]	2.8[b]	0	0	13.5[a]	73.0[a]	0[a]
Romanian	Range	1–>256	0.25–16	2–32	2–128	0.032–>256	0.064–1	0.25–1	0.5–>32	<0.5–64	0.032–2
non-fragilis	MIC_{50}	16	2	16	32	4	0.25	0.5	2	32	0.125
Bacteroides	MIC_{90}	>256	16	32	64	256	1	0.5	>32	64	1
strains ($n = 17$)	Resistance (%)	94.1	11.8	11.8[b]	41.2[a,b]	23.5[b]	0	0	17.6[a]	82.4[a]	0[a]

[a] Here, CLSI breakpoints could be applied.
[b] The significance values for differences between *B. fragilis* and non-fragilis *Bacteroides* strains were 0.238 for piperacillin/tazobactam, <0.001 for cefoxitin and 0.032 for clindamycin.

Reference Laboratory for Anaerobic Pathogens at Szeged. The *Bacteroides* strains collected between 2010 and 2013 were subjected to the same analyses as those done for other European countries in the latest susceptibility and antibiotic resistance mechanism study [2]. The main goal was to provide antibiotic resistance data for Romania, as this has not yet been reported for this European country.

2. Material and methods

2.1. Bacterial strains and cultivation

53 *B. fragilis* group strains (36 *B. fragilis*, 7 *Bacteroides thetaiotaomicron*, 7 *Bacteroides ovatus* and 3 *Bacteroides vulgatus*) were collected in the period 2010 and 2013 at the Diagnostic Laboratory of the Emergency County Clinical Hospital at Targu-Mures in Romania, taken a wide variety of clinical samples, which were mainly abdominal, wound and blood culture cases. The routine isolations and identifications were performed according to methods described in the literature [7]. The species identifications were positively confirmed using MALDI-TOF MS at the Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary. For long-term storage, 20% glycerol stocks were used at −70 °C. In further analyses the strains were cultivated at 37 °C anaerobically on Columbia agar supplemented with 5% (v/v) sheep blood, 5 g/L hemin and 1 g/L vitamin K_1, or in BHIS broth (brain-heart infusion broth supplemented with 0.5% (w/v) yeast extract, 5 g/L hemin and 1 g/L vitamin K_1), in anaerobic environment (85% N_2, 10% H_2, 5% CO_2) for 48 h.

2.2. Antibiotic susceptibility and genetic testing

Antibiotic susceptibility tests were carried out by agar dilution as described earlier for ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, imipenem, clindamycin, moxifloxacin, metronidazole, tetracycline and tigecycline [2]. The antibiotic resistance genes (*cepA, cfxA, cfiA, ermB, ermF, ermG, linA, mefA, msrSA, bexA,nim, tetM, tetQ, tetX, tetX1* and *tet36*) and one IS element (IS*4351*) were detected by RealTime PCR [5]. Chi-squared analyses were carried out using Sigmaplot 12.0 (Systat Software, Inc.) after setting the significance threshold level to 0.05.

3. Results and discussion

3.1. Antibiotic resistance levels

Of the 53 strains examined, MALDI-TOF MS was able to confirm or update ($n = 5$) the original identifications. Data for the antibiotic resistance levels obtained are displayed in Table 1, it is grouped into (i) all strains, (ii) *B. fragilis* and (iii) NFB (non-fragilis *Bacteroides*), and where available are compared with the data from the last European *Bacteroides* antibiotic susceptibility study [2]. In general, the resistance data for Romania followed the European trends with high ampicillin resistance, moderate or low levels of resistance for cefoxitin, clindamycin, moxifloxacin and amoxicillin/clavulanate and very good data, i.e. very low levels of resistance (<6%) for imipenem, piperacillin/tazobactam, metronidazole and tigecycline. Tetracycline susceptibilities were also measured, and according to world-wide data the resistance was high and has reached 76%. Differencies among the southern and northern European countries were found earlier and also in the latest European *Bacteroides* antibiotic susceptibility study for cefoxitin, moxifloxacin and clindamycin, where cefoxitin and moxifloxacin resistances were higher in northern countries, but in these countries lower clindamycin levels were observed [2,8]. The resistance values obtained in this study for cefoxitin, moxifloxacin and clindamycin lay between the corresponding data values for Eastern European countries. A recent study from Bulgaria reported similarly low resistance values for cefoxitin (1%), clindamycin (3%) and metronidazole (0%) for *Bacteroides* fragilis group isolates [9]. No significant trends regarding the changes in the antimicrobial resistance levels over time were found. The Romanian *B. fragilis* strains exhibited lower resistance