An analysis of the antibiotic resistance gene content of intestinal Bacteroides isolated using a novel chromogenic agar

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Background: Recently, we determined the antibiotic susceptibilities and antibiotic resistance gene content of clinical Bacteroides strains in an ESGAI study. Therefore, we aimed to investigate the antibiotic resistance gene content of intestinal Bacteroides isolates and compare it to those from clinical samples in order to follow changes that drive the occurrence of antibiotic-resistant Bacteroides in clinical samples. Comparison of the antibiotic resistance gene contents of Bacteroides isolates of antibiotic non-treated (healthy) and carbapenem treated (ICU) individuals was also investigated.

Material/methods: Faecal samples of healthy and ICU patients were collected in Hungary and Slovenia. They were suspended in BHI medium and serial dilutions were plated on a novel selective Bacteroides chromogenic medium (BCM) with or without meropenem (4 mg/L), and incubated anaerobically for 48 h. Isolates with different colony morphologies were subcultured and their species identities were determined by MALDI-TOF MS. The cepA, cfxA, cfiA and ermF gene contents were determined by RT-PCR.

Results: 108 B. fragilis group strains (25 B. ovatus, 20 B. vulgatus, 15 B. thetaiotaomicron, 17 B. fragilis, 8 Parabacteroides distasonis, 7 B. uniformis, 5 P. johnsoniae, 3 B. caccae, 3 B. intestinalis, 3 B. stercoris, 1 B. finegoldii and 1 B. nordii) were isolated from stools of 28 individuals (22 healthy and 6 ICU patients). On BCM only a few Enterococcus faecalis were isolated, showing that the new medium was highly selective for the Bacteroides fragilis group. The colony counts were ~100-fold less on meropenem-supplemented plates than on BCM plates without meropenem. All but one of the 17 B. fragilis were cepA-positive and cfiA-negative, and 1 B. fragilis isolate was cepA-negative and cfiA-positive, while all the non-fragilis Bacteroides (NFB) were uniformly cepA and cfiA-negative. The cfxA gene prevalence was higher in the total Bacteroides (58.6 vs. 18.0 %, p<0.001), the B. fragilis (33.3 vs. 14.8 %, p=0.003) and the NFB (62.3 vs. 30.3 %, p=0.006) strains, when comparing faecal isolates with previously analysed clinical isolates. The ermF gene prevalence was higher in the total Bacteroides (54.3 vs. 14.9 %, p<0.0001), the B. fragilis (33.3 vs. 22.6 %, p=0.011) and the NFB (69.3 vs. 30.3 %, p<0.001) strains, when comparing faecal isolates with previously analysed clinical isolates.

Conclusions: The utility of a novel Bacteroides-specific chromogenic medium was demonstrated. Meropenem treatment substantially reduced the normal Bacteroides flora in ICU patients. The cfxA and ermF genes were more prevalent in recent intestinal Bacteroides isolates than in previous clinical strains.