Noticeable levels of reduced antimicrobial susceptibility in *Bacteroides fragilis* group bacteria isolated from faecal samples from healthy children in Denmark

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**Introduction and purpose**

Reduced antimicrobial susceptibility is prevalent in bacteria of the *Bacteroides fragilis* group. Approximately 4-10% of clinical *B. fragilis* isolates show reduced susceptibility towards meropenem \(^1\). We ascertained the prevalence of antimicrobial resistance in meropenem \(^1\). We ascertained the prevalence of antimicrobial resistance in healthy children in Denmark, representing a relatively antibiotic ‘naïve’ population.

**Methods**

Faecal samples collected between years 2009 - 2012 from 174 children 0-6 years old \(^2\) were plated on Brucella blood agar supplemented with vancomycin \((7.5 \, \mu g/mL)\) and kanamycin \((100 \, \mu g/mL)\), with colistin \(150 \, \mu g\) tablets placed on the plates that were incubated for three days in an anaerobic atmosphere. Up to three morphologically distinct colonies were identified by MALDI-TOF MS (Biotyper). If a score \(\geq 2\) could not be achieved, 16S rRNA gene sequencing was performed. Disk diffusion was performed on Brucella blood agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany) using meropenem (MEM) \((10 \, \mu g \text{ disk (Oxoid)}\), piperacillin/tazobactam (TZP) \((30/6 \, \mu g \text{ disk})\), metronidazole (MTZ) \((5 \, \mu g \text{ disk})\) and clindamycin (CLI) \((2 \, \mu g \text{ disk})\) \(^3\). A 1 McFarland suspension was prepared and plates were incubated at 37°C (complying with the EUCAST 15-15-15 rule) in an anaerobic environment for 24 hours. MIC determination was performed using Etest (bioMérieux) or M.I.C.E (Oxoid) gradient strips and EUCAST breakpoints if disk diffusion zones were below 29, 23, 25 and 10 mm for MEM, TZP, MTZ and CLI respectively.

**Results**

346 non-duplicate isolates of *B. fragilis* group were isolated from 170 of 174 faecal samples. After screening for reduced susceptibility by disk diffusion MIC was determined for MEM, TZP, MTZ and CLI \(^4\) for 26, 144, 96 and 123 isolates using gradient strips (table 1). Reduced susceptibility towards MEM was 1.7% overall with the highest prevalence of 4.2% for *B. fragilis*. High levels of reduced susceptibility towards TZP was observed for *B. thetaiotaomicron*. No isolates with reduced susceptibility towards MTZ were found. Clindamycin resistance was lowest for *B. fragilis* (9.4%) and highest for *B. ovatus* (29.7%).

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**Table 1. Percentage of *B. fragilis* group isolates with reduced susceptibility (I/R)**

<table>
<thead>
<tr>
<th><em>B. fragilis</em> group spp.</th>
<th>n</th>
<th>MEM</th>
<th>TZP</th>
<th>MTZ(^a)</th>
<th>CLI(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ovatus</em></td>
<td>101</td>
<td>2.0%</td>
<td>1.0%</td>
<td>0.0%</td>
<td>29.7%</td>
</tr>
<tr>
<td><em>B. fragilis</em></td>
<td>96</td>
<td>4.2%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>9.4%</td>
</tr>
<tr>
<td><em>B. vulgaris</em></td>
<td>66</td>
<td>0.0%</td>
<td>1.5%</td>
<td>0.0%</td>
<td>21.2%</td>
</tr>
<tr>
<td><em>B. thetaiotaomicron</em></td>
<td>54</td>
<td>0.0%</td>
<td>24.1%</td>
<td>(6/7)</td>
<td>31.5%</td>
</tr>
<tr>
<td>Other(^b)</td>
<td>29</td>
<td>0.0%</td>
<td>3.45%</td>
<td>(1/0)</td>
<td>20.7%</td>
</tr>
<tr>
<td>Total</td>
<td>346</td>
<td>1.7%</td>
<td>4.6%</td>
<td>0.0%</td>
<td>22.0%</td>
</tr>
</tbody>
</table>

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\(^a\) Intermediate-susceptible; \(R\), resistant; MEM, meropenem; TZP, piperacillin/tazobactam; MTZ, metronidazole; CLI, clindamycin. Numbers of I and R are given in parenthesis

\(^b\) *B. uniformis* \((n=9)\), *Parabacteroides distasonis* \((n=7)\), *B. caccae* \((n=5)\), *B. salyersiae* \((n=3)\), *B. fluxus* \((n=1)\), *B. finegoldii* \((n=2)\), *Parabacteroides goldsteini* \((n=1)\), *B. cellulosityclicus* \((n=1)\).

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**Conclusions**

In this cohort of healthy children in day-care, the prevalence of reduced susceptibility towards meropenem was close to what is observed for clinical isolates, with rates for TZP and CLI also in range of this \(^3\). The lack of metronidazole resistant isolates was expected as this is still rare in the *B. fragilis* group. Children inherit their microbiome from their parents, and as such might be used as a proxy for screening the general population. This study suggest that strains with reduced susceptibility to important antibiotics are prevalent in the general population. Follow up studies in similar populations could be used to monitor antimicrobial resistance in the *B. fragilis* group in the general population microbiota.

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**References**

3- Luu et al ECCMID 2013

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**Addition since abstract submission**

After abstract submission, bioMérieux has issued a field safety notice regarding Etest PTZ for lot numbers used in this study. It is possible that performance of these lots have shifted compared to published performance characteristics leading to false susceptible results /very major errors.

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