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

Thomas Vognbjerg Sydenham[a], Betina Hebbelstrup Jensen[b], Andreas Munk Petersen[c,d], Karen Angeliki Krogfelt[b], Ulrik Stenz Justesen[a]

[a]Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark, [b]Department of Microbiology & Infection Control, Statens Serum Institut, Copenhagen, Denmark, [c]Department of Clinical Microbiology, Hvidovre University Hospital, Copenhagen, Denmark, [d]Department of Gastroenterology, Hvidovre University Hospital, Copenhagen, Denmark

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 *B. fragilis* group isolated from 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 μg/ml) and kanamycin (100 μg/ml), with colistin 150 μg 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 ≥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 μg disk (Oxoid)), piperacillin tazobactam (TZP) (30/6 μg disk), metronidazole (MTZ) (5 μg disk) and clindamycin (CLI) (2 μg 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 anaerobe 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.

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

***B. fragilis* group spp.**	**n**	**MEM**	**TZP**	**MTZ[a]**	**CLI[a]**
B. ovatus	101	2.0% (0/2)	1.0% (1/0)	0.0%	29.7% (-/30)
B. fragilis	96	4.2% (1/3)	0.0%	0.0%	9.4% (-/9)
B. vulgatus	66	0.0%	1.5% (0/1)	0.0%	21.2% (-/14)
B. thetaiotaomicron	54	0.0%	24.1% (6/7)	0.0%	31.5% (-/17)
Other[b]	29	0.0%	3.45% (1/0)	0.0%	20.7% (-/6)
Total	346	1.7% (1/5)	4.6% (8/8)	0.0%	22.0% (-/76)

I, intermediate-susceptible; R, resistant; MEM, meropenem; TZP, piperacillin/tazobactam; MTZ, metronidazole; CLI, clindamycin. Numbers of I and R are given in parenthesis
EUCAST breakpoints: MEM (S ≤ 2 mg/L; R > 8 mg/L), TZP (S ≤ 8 mg/L; R > 16 mg/L), MTZ and CLI: (S ≤ 4 mg/L < R)
a) There is no intermediate category for metronidazole and clindamycin
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 goldsteinii (n=1), B. cellulosilyticus (n=1)*.

Addition since abstract submission

After abstract submission, bioMérieux has issued a field safefty 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 charactaristics leeding to false susceptible results /very major errors.

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 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%).

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.

References

1. Justesen *et al* Int J Antimicrob Agents 2013
2. Hebbelstrup *et al* Acta Paediatr 2016
3- Luu *et al* ECCMID 2013

*Corresponding and presenting author:
Thomas Vognbjerg Sydenham.
e-mail: thomas.sydenham@dadlnet.dk

Poster P0681
ECCMID 2016