Gram-positive anaerobic cocci

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Chapter 7

Antimicrobial susceptibility of clinically relevant gram-positive anaerobic cocci, collected in a 3-year period in the Netherlands

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Abstract

The susceptibility of 14 species of 115 gram-positive anaerobic cocci (GPAC) was determined for 14 antibiotics. To assure correct identification, strains were genotypically identified by fluorescent *in situ* hybridisation and sequencing. Susceptibility differences (MIC_{50} and MIC_{90}) for penicillin G, clindamycin, tigecycline, levofloxacin, amoxicillin-clavulanic acid, cefoxitin, ertapenem, meropenem, metronidazole, and doxycycline were found for the three clinically most relevant GPAC species; *Finegoldia magna*, *Parvimonas micra*, and *Peptoniphilus harei*.

Introduction

Gram-positive anaerobic cocci (GPAC) are part of the commensal microbiota and account for about one-third of the anaerobic isolates recovered from clinical materials [14]. It is a heterogeneous group, which in the last decade has undergone an extensive taxonomic change. The species *Peptostreptococcus micros* and *Peptostreptococcus magnus* were transferred to two new genera, *Micromonas* and *Finegoldia*, respectively, with each being the only species present in their respective genus [15]. The genus *Micromonas* has recently been replaced by *Parvimonas*, with *Parvimonas* (*Pa.*) *micra* the only species present [19]. Ezaki et al. [7] divided the remaining peptostreptococci in three phylogenetic groups, *Peptoniphilus* gen. nov., *Anaerococcus* gen. nov. and *Gallicola* gen. nov, with *Gallicola barnesae* being the only species present in the latter genus. The species left in the genus *Peptostreptococcus* is *P. anaerobius*, and a recently described new species *P. stomatis* [6]. Song et al. [18] described three new species, *Peptoniphilus* (*Pn.*) *gorbachii* sp. nov., *Pn. olsenii* sp. nov. and *Anaerococcus murdochii* sp. nov. The most commonly found GPAC in clinical material are *F. magna*, *Pa. micra*, *Pn. harei* [20] and *P. anaerobius* [21]. Data on antimicrobial susceptibility of the different species of GPAC is often based on GPAC in general, eventhough several authors describe a difference in antimicrobial susceptibility between species [3-5, 11, 12, 17]. In these studies, the strains were identified phenotypically. However, for some species it is difficult to obtain a reliable phenotypic identification e.g. in the past *Pn. harei* has been often misidentified as *Pn. asaccharolyticus* [20] probably caused by the fact that these two species share the same biochemical characteristics [10].

In this study, we have assessed the susceptibility of 115 isolates of GPAC, against 14 different antibiotics. Isolates were genotypically identified using fluorescent *in situ* hybridisation (FISH) [20] or sequencing, thus obtaining a more accurate insight in the distribution of susceptible and resistant strains within the different species.

Material and methods

Isolates

Strains were obtained from the diagnostic laboratory of the University Medical Center Groningen and collected in the years 2002-2004. All strains were isolated from human clinical samples from a variety of anatomical sites, e.g. from abdominal, head and neck and soft tissue infections. Strains were stored at -80 ºC and subcultured on Brucella Blood Agar (BBA) prior to susceptibility testing.

Identification

Strains were genotypically identified using 16S rRNA based probes [20] and sequencing. Shortly, bacterial cells were harvested from BBA using a sterile loop and fixed in 1:1 phosphate-buffered saline (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 per liter) and ethanol 96 % v/v. Fixed cells were spotted on slides, and if necessary permeabilized using proteinase-K. Strains were hybridized using probes directed against *F. magna*, *Pa. micra*, *Pn. harei*, *P. anaerobius, A. vaginalis*, *Pn. asaccharolyticus*, *A. lactolyticus* and *Pn. ivorii*. The addition of new species to the genera *Peptoniphilus* and *Anaerococcus* [18], showed that the probes directed against *A. lactolyticus* and *Pn. harei* were also positive with *A. murdochii* and *Pn. gorbachii*, respectively (data not shown). Strains which were negative with the probes or positive with the probes directed against *A. lactolyticus* and *Pn. harei* were sequenced. DNA was isolated as described previously [2] and the 16S genes were amplified and sequenced using universal 16S rRNA- specific primers [9]. Sequences were compared to those in the GenBank database by performing a BLAST-search from the National Center of Biotechnology Information [1].

Susceptibility testing

The antimicrobial susceptibility against penicillin G, amoxicilin-clavulanic acid, cefotetan, cefoxitin, ertapenem, meropenem, levofloxacin, moxifloxacin, clindamycin, metronidazole, linezolid, chloramphenicol, doxycycline and tigecycline was determined using E-test (AB Biodisk, Sweden). Suspensions of approximately 2 McFarland were made in pre-reduced Brucella broth and applied onto a pre-reduced BBA. All culture handlings were performed in an anaerobic chamber. Plates with E-test strips were incubated for 48 hrs at 37 ºC, in an anaerobic chamber before reading the minimal inhibitory concentration (MIC). In each batch a quality control strain *Bacteroides fragilis* ATCC 25285 was included.

A difference in susceptibility was defined as at least 2 dilution steps (with one dilution step being a difference of two-fold dilutions with a precision of a 0.5 dilution) difference between the MIC's of the different species.

Results

The quality control strain *B. fragilis* ATCC 25285 was tested 10 times with all 14 antibiotics. The obtained MIC's are summarized in Table 1.

Table 1. MIC-values of the quality control tests on *Bacteroides fragilis* ATCC 25285

	MIC-value (no. of tests)	Expected range[1]
Penicillin G	12 (2), 16 (7), 24 (1)	8-32
Amoxicillin/clavulanic acid	0.19 (2), 0.25 (5), 0.38 (3)	0.125-0.5[†]
Cefotetan	6 (7), 8 (3)	4-16
Cefoxitin	4 (1), 6 (7), 8 (2)	4-16
Ertapenem	0.125 (4), 0.19 (6)	0.064-0.25
Meropenem	0.094 (2), 0.125 (4), 0.19 (4)	0.064-0.25
Levofloxacin	1 (1), 1.5 (9)	1[*]
Moxifloxacin	0.19 (1), 0.25 (1), 0.38 (6), 0.5 (2)	0.125-0.5
Clindamycin	1.5 (2), 2 (4), 3 (4)	0.5-2
Metronidazole	0.25 (4), 0.38 (4), 0.5 (2)	0.25-1
Linezolid	4 (1), 6 (5), 8 (3), 12 (1)	2-8[*]
Chloramphenicol	6 (3), 8 (7)	2-8
Doxycycline	0.25 (3), 0.38 (5), 0.5 (2)	0.25-0.5[*]
Tigecycline	0.25 (2), 0.5 (2), 0.75 (6)	0.125-1[*]

[1] The expected range is derived from CLSI for *B. fragilis* for reference agar dilution testing, unless indicated with a * or †.

† Expected range derived from the manufacturer.

* Expected range derived from literature.

All results of the clinical isolates are summarized in Table 2 and Table 3. The MIC_{50} and MIC_{90} were only calculated for species of which more than 10 strains were present in the study, i.e. *F. magna*, *Pa. micra* and *Pn. harei*. Comparing the MIC_{50} and MIC_{90} of these 3 species with each other, *F. magna* shows the highest MIC_{50} and MIC_{90} values for penicillin G, amoxicillin-clavulanic acid, clindamycin,and tigecycline. It has the highest MIC_{50} values for cefotetan, cefoxitin, meropenem, linezolid and chloramphenicol and the highest MIC_{90} values for levofloxacin and moxifloxacin. *Pa. micra* has the lowest MIC_{50} and MIC_{90} for levofloxacin, metronidazole and doxycycline. It has the lowest MIC_{90} for amoxicillin-clavulanic acid. *Pn. harei* has the highest MIC_{50} for levofloxacin and doxycycline. It has the lowest MIC_{50} and MIC_{90} for cefoxitin, ertapenem and meropenem and the lowest MIC_{90} for chloramphenicol.

Table 2. MIC's and range for GPAC against 14 antibiotics.

Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}	Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}
F. magna (31)	Penicillin G	0.023 - 0.38	0.125	0.25	*Pn. harei* (16)	Penicillin G	0.016 – 0.19	0.023	0.032
	Amoxicillin-clavulanic acid	0.094 – 2	0.25	0.5		Amoxicillin/clavulanic acid	0.016 – 0.38	0.023	0.25
	Cefotetan	0.25 – 4	2	2		Cefotetan	0.38 - 8	0.5	1
	Cefoxitin	0.38 – 3	1	1.5		Cefoxitin	0.023 – 1.5	0.094	0.5
	Ertapenem	0.016 – 0.19	0.064	0.125		Ertapenem	0.006 – 0.023	0.012	0.016
	Meropenem	0.064 – 0.25	0.125	0.19		Meropenem	0.004 – 0.032	0.008	0.032
	Levofloxacin	0.094 – 64	0.75	64		Levofloxacin	2 – 64	4	6
	Moxifloxacin	0.047 – 64	0.19	6		Moxifloxacin	0.125 – 1.5	0.19	0.38
	Clindamycin	0.125 - >256	1	3		Clindamycin	0.094 – 1.5	0.25	1.5
	Metronidazole	0.094 – 1.5	0.38	1		Metronidazole	0.032 – 2	0.38	1.5
	Linezolid	2 – 6	3	3		Linezolid	0.5 – 2	0.75	1.5
	Chloramphenicol	4 – 16	6	8		Chloramphenicol	1.5 – 4	3	3
	Doxycycline	0.75 – 24	2	24		Doxycycline	0.064 – 24	8	16
	Tigecycline	0.064 – 1	0.25	0.75		Tigecycline	0.023 – 0.25	0.094	0.25
Pa. micra (27)	Penicillin G	0.016 – 0.125	0.016	0.047	*A. vaginalis* (8)	Penicillin G	0.016 – 0.094		
	Amoxicillin/clavulanic acid	0.016 – 0.75	0.032	0.094		Amoxicillin/clavulanic acid	0.016 – 0.125		
	Cefotetan	0.125 - 2	0.38	1.5		Cefotetan	0.094 – 0.5		
	Cefoxitin	0.125 - 3	0.5	2		Cefoxitin	0.032 – 0.125		
	Ertapenem	0.008 – 0.19	0.047	0.125		Ertapenem	0.023 – 0.19		
	Meropenem	0.008 – 0.38	0.047	0.19		Meropenem	0.006 – 0.125		
	Levofloxacin	0.125 – 3	0.25	0.5		Levofloxacin	24 – 64		
	Moxifloxacin	0.094 – 1.5	0.19	0.38		Moxifloxacin	0.5 – 2		
	Clindamycin	0.047 – 2	0.38	1.5		Clindamycin	0.023 - >256		
	Metronidazole	0.032 - >256	0.094	0.25		Metronidazole	0.047 – 0.5		
	Linezolid	0.125 – 3	1	3		Linezolid	0.38 – 1.5		
	Chloramphenicol	0.75 – 6	3	6		Chloramphenicol	1.5 – 3		
	Doxycycline	0.047 – 4	0.125	1		Doxycycline	0.125 – 16		
	Tigecycline	0.016 – 0.38	0.064	0.125		Tigecycline	0.047 – 1.5		

Table 2. Continued.

Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}	Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}
Pn. ivorii (5)	Penicillin G	0.016 – 0.047			*Pn. lacrimalis* (4)	Penicillin G	0.016 – 0.125		
	Amoxicillin/clavulanic acid	0.016 – 0.032				Amoxicillin/clavulanic acid	0.016 – 0.25		
	Cefotetan	0.125 – 1				Cefotetan	0.016 – 0.38		
	Cefoxitin	0.125 – 0.75				Cefoxitin	0.016 – 0.25		
	Ertapenem	0.004 – 0.032				Ertapenem	0.002 – 0.012		
	Meropenem	0.002 – 0.016				Meropenem	0.002 – 0.016		
	Levofloxacin	0.38 – 64				Levofloxacin	3 – 8		
	Moxifloxacin	0.094 – 64				Moxifloxacin	0.002 – 0.38		
	Clindamycin	0.094 – 2				Clindamycin	0.016 – 0.38		
	Metronidazole	0.094 – 0.25				Metronidazole	0.023 – 0.38		
	Linezolid	0.19 – 2				Linezolid	0.19 – 2		
	Chloramphenicol	1 – 3				Chloramphenicol	0.75 – 3		
	Doxycycline	0.064 – 16				Doxycycline	0.125 – 4		
	Tigecycline	0.032 – 0.25				Tigecycline	0.023 – 0.25		
P. anaerobius (4)	Penicillin G	0.064 – 2			*Pn. gorbachii* (4)	Penicillin G	0.016 – 0.19		
	Amoxicillin/clavulanic acid	0.125 – 4				Amoxicillin/clavulanic acid	0.016 – 0.064		
	Cefotetan	0.5 – 24				Cefotetan	0.5 – 1.5		
	Cefoxitin	0.19 – 3				Cefoxitin	0.064 – 0.5		
	Ertapenem	0.032 – 0.75				Ertapenem	0.012 – 0.023		
	Meropenem	0.023 – 1				Meropenem	0.004 – 0.064		
	Levofloxacin	0.38 – 1.5				Levofloxacin	3 – 64		
	Moxifloxacin	0.19 – 0.25				Moxifloxacin	0.19 – 0.5		
	Clindamycin	0.032 – 1				Clindamycin	0.125 – 0.75		
	Metronidazole	0.032 – 0.25				Metronidazole	0.023 – 0.5		
	Linezolid	0.38 – 1.5				Linezolid	0.75 – 1.5		
	Chloramphenicol	1 – 3				Chloramphenicol	2 – 3		
	Doxycycline	0.5 – 4				Doxycycline	0.064 – 0.38		
	Tigecycline	0.064 – 0.125				Tigecycline	0.016 – 0.094		

Table 2. Continued.

Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}	Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}
A. murdochii (3)	Penicillin G	0.016 – 0.75			*A. tetradius* (2)	Penicillin G	0.023 – 0.032		
	Amoxicillin/clavulanic acid	0.032 – 0.25				Amoxicillin/clavulanic acid	0.032 – 0.064		
	Cefotetan	0.75 – 8				Cefotetan	0.25 – 0.5		
	Cefoxitin	0.125 – 1				Cefoxitin	0.19 – 0.38		
	Ertapenem	0.19 – 2				Ertapenem	0.094 – 0.125		
	Meropenem	0.125 – 0.75				Meropenem	0.094 – 0.125		
	Levofloxacin	1.5 – 4				Levofloxacin	2 – 3		
	Moxifloxacin	0.25				Moxifloxacin	0.19 – 0.38		
	Clindamycin	0.016 – 0.5				Clindamycin	1 – 4		
	Metronidazole	0.19 – 0.5				Metronidazole	0.25 – 0.75		
	Linezolid	0.38 – 0.75				Linezolid	1 – 1.5		
	Chloramphenicol	1 – 3				Chloramphenicol	3 – 3		
	Doxycycline	0.25 – 16				Doxycycline	2 – 8		
	Tigecycline	0.047				Tigecycline	0.125 – 0.19		
At. parvulum (4)	Penicillin G	0.094 – 0.25			*Pn. octavius* (1)	Penicillin G	0.125		
	Amoxicillin/clavulanic acid	0.064 – 0.25				Amoxicillin/clavulanic acid	0.064		
	Cefotetan	2 – 8				Cefotetan	0.5		
	Cefoxitin	1.5 – 3				Cefoxitin	0.25		
	Ertapenem	0.032 – 0.19				Ertapenem	0.094		
	Meropenem	0.125 – 0.25				Meropenem	0.094		
	Levofloxacin	0.38 – 0.5				Levofloxacin	4		
	Moxifloxacin	0.19 – 0.38				Moxifloxacin	0.5		
	Clindamycin	1.5 – 6				Clindamycin	0.047		
	Metronidazole	0.19 – 0.5				Metronidazole	0.38		
	Linezolid	0.75 – 2				Linezolid	0.75		
	Chloramphenicol	4 – 16				Chloramphenicol	2		
	Doxycycline	1 – 2				Doxycycline	0.19		
	Tigecycline	0.064 – 0.5				Tigecycline	0.064		

Table 2. Continued.

Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}	Organism (n)[a]	Antibiotic	MIC range	MIC_{50}	MIC_{90}
R. gnavus (1)	Penicillin G	1			GPAC (4)	Penicillin G	0.023 – 0.125		
	Amoxicillin/clavulanic acid	0.19				Amoxicillin/clavulanic acid	0.016 – 0.094		
	Cefotetan	32				Cefotetan	1 – 4		
	Cefoxitin	4				Cefoxitin	0.125 – 1		
	Ertapenem	0.38				Ertapenem	0.006 – 2		
	Meropenem	0.125				Meropenem	0.008 – 0.75		
	Levofloxacin	64				Levofloxacin	0.5 – 2		
	Moxifloxacin	6				Moxifloxacin	0.064 – 0.38		
	Clindamycin	0.38				Clindamycin	0.094 – 0.125		
	Metronidazole	0.094				Metronidazole	0.064 – 0.38		
	Linezolid	2				Linezolid	0.5 – 1		
	Chloramphenicol	3				Chloramphenicol	1.5 – 3		
	Doxycycline	0.25				Doxycycline	0.094 – 1		
	Tigecycline	0.094				Tigecycline	0.023 – 0.19		
A. lactolyticus (1)	Penicillin G	0.125							
	Amoxicillin/clavulanic acid	0.125							
	Cefotetan	2							
	Cefoxitin	0.5							
	Ertapenem	1							
	Meropenem	0.38							
	Levofloxacin	6							
	Moxifloxacin	0.19							
	Clindamycin	0.047							
	Metronidazole	0.25							
	Linezolid	0.38							
	Chloramphenicol	1							
	Doxycycline	0.38							
	Tigecycline	0.094							

[a]Genus abbrevations: *P., Peptostreptococcus*; *A., Anaerococcus*; *Pa., Parvimonas*; *Pn., Peptoniphilus*; *At., Atopobium*; *R., Ruminococcus*.

Table 3. The overall resistance of GPAC against 14 antibiotics[a].

Antibiotic	Range	MIC_{50}	MIC_{90}
Penicillin G	0.016 – 2	0.047	0.19
Amoxicillin/clavulanic acid	0.016 – 4	0.094	0.38
Cefotetan	0.016 – 32	0.75	3
Cefoxitin	0.016 – 4	0.5	2
Ertapenem	0.002 – 2	0.064	0.19
Meropenem	0.002 – 1	0.064	0.25
Levofloxacin	0.094 – 64	0.75	64
Moxifloxacin	0.002 – 64	0.25	1.5
Clindamycin	0.016 - >256	0.38	2
Metronidazole	0.023 - >256	0.19	0.75
Linezolid	0.125 – 6	1.5	3
Chloramphenicol	0.75 – 16	3	8
Doxycycline	0.047 – 24	1	16
Tigecycline	0.016 – 1.5	0.094	0.38

[a] The overall resistance of GPAC (n=115) against various antiobiotics is indicated.

Discussion

Since GPAC can show poor growth we used a McFarland 2 inoculum. The MIC-values obtained with the quality control strain *B. fragilis* ATCC 25285 show that most of these values are within the expected range. Comparison between a McFarland 1 and 2 inoculum using the quality control strain gave the same MIC-value (data not shown). However, 4 of the 10 MIC values obtained for clindamycin were just above the expected range using McFarland 2. Since GPAC show poor growth when compared to *B. fragilis*, this is not expected to affect our set of data. A practical approach is to use a higher McFarland turbidity as recommended by the manufacturer of E-test.

In this study strains were identified genotypically, since phenotypic identification is not always reliable for all species [20]. It is difficult to compare our results with other published resistance data, since authors may use different breakpoints. For example, some did use breakpoints advised by the Clinical and Laboratory Standards Institute (CLSI), while others used those advised by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Therefore, we have chosen to base a difference in susceptibility on the MIC_{50} and MIC_{90} values, instead of the percentage resistant strains. However, the interpretation of our results using CSLI and EUCAST breakpoints are shown in Table 4.

Table 4. . Percentages resistance of GPAC for 14 antibiotics using CLSI and EUCAST breakpoints.

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Penicillin G						
(interpretive criteria)	**0.5**[b]		**2**	**0.25**[c]		**0.5**
F. magna (31)	31			30	1 (3.2 %)	
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	3		1	3		1
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	2	1		2		1
At. parvulum (4)	4			4		
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)		1				1
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		
Amoxicillin-clavulanic acid						
(interpretive criteria)	**4**[b]		**16**	**4**[c]		**8**
F. magna (31)	31			31		
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			3		
At. parvulum (4)	4			4		
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		

Table 4. Continued.

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Cefotetan						
(interpretive criteria)	**16**[b]		**64**	**n.a.**[d]		**n.a.**
F. magna (31)	31					
Pa. micra (27)	27					
Pn. harei (16)	16					
A. vaginalis (8)	8					
Pn. ivorii (5)	5					
P. anaerobius (4)	3		1			
Pn. lacrimalis (4)	4					
Pn. gorbachii (4)	4					
A. murdochii (3)	3					
At. parvulum (4)	4					
A. tetradius (2)	2					
Pn. octavius (1)	1					
R. gnavus (1)			1			
A. lactolyticus (1)	1					
GPAC (4)	4					
Cefoxitin						
(interpretive criteria)	**16**[b]		**64**	**n.a.**		**n.a.**
F. magna (31)	31					
Pa. micra (27)	27					
Pn. harei (16)	16					
A. vaginalis (8)	8					
Pn. ivorii (5)	5					
P. anaerobius (4)	4					
Pn. lacrimalis (4)	4					
Pn. gorbachii (4)	4					
A. murdochii (3)	3					
At. parvulum (4)	4					
A. tetradius (2)	2					
Pn. octavius (1)	1					
R. gnavus (1)	1					
A. lactolyticus (1)	1					
GPAC (4)	4					

Table 4. Continued.

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Ertapenem						
(interpretive criteria)	**4**[b]		**16**	**1**[c]		**1**
F. magna (31)	31			31		
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			2		1
At. parvulum (4)	4			4		
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			3		1
Meropenem						
(interpretive criteria)	**4**[b]		**16**	**2**[c]		**8**
F. magna (31)	31			31		
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			3		
At. parvulum (4)	4			4		
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		

Table 4. Continued

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Moxifloxacin						
(interpretive criteria)	**2**[b]		**8**	**n.a.**		**n.a.**
F. magna (31)	27	1 (3.2 %)	3 (9.7 %)			
Pa. micra (27)	27					
Pn. harei (16)	16					
A. vaginalis (8)	8					
Pn. ivorii (5)	4		1			
P. anaerobius (4)	4					
Pn. lacrimalis (4)	4					
Pn. gorbachii (4)	4					
A. murdochii (3)	3					
At. parvulum (4)	4					
A. tetradius (2)	2					
Pn. octavius (1)	1					
R. gnavus (1)		1				
A. lactolyticus (1)	1					
GPAC (4)	4					
Clindamycin						
(interpretive criteria)	**2**[b]		**8**	**4**[c]		**4**
F. magna (31)	26	3 (9.7 %)	2 (6.5 %)	29		2 (6.5 %)
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	7		1	7		1
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			3		
At. parvulum (4)	2	2		3		1
A. tetradius (2)	1	1		2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		

Table 4. Continued

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Metronidazole						
(interpretive criteria)	**8**[b]		**32**	**4**[c]		**4**
F. magna (31)	31			31		
Pa. micra (27)	26		1 (3.7 %)	26		1 (3.7 %)
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			3		
At. parvulum (4)	4			4		
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		
Chloramphenicol						
(interpretive criteria)	**8**[b]		**32**	**8**[c]		**8**
F. magna (31)	28	3 (9.7 %)		28		3 (9.7 %)
Pa. micra (27)	27			27		
Pn. harei (16)	16			16		
A. vaginalis (8)	8			8		
Pn. ivorii (5)	5			5		
P. anaerobius (4)	4			4		
Pn. lacrimalis (4)	4			4		
Pn. gorbachii (4)	4			4		
A. murdochii (3)	3			3		
At. parvulum (4)	2	2		2		2
A. tetradius (2)	2			2		
Pn. octavius (1)	1			1		
R. gnavus (1)	1			1		
A. lactolyticus (1)	1			1		
GPAC (4)	4			4		

Table 4. Continued.

	CLSI			EUCAST		
	≤S (n)	I (n [%][a])	≥R (n [%])	≤S (n)	I (n [%])	>R (n [%])
Tigecycline						
(interpretive criteria)	**4**[b]		**16**	**n.a.**		**n.a.**
F. magna (31)	31					
Pa. micra (27)	27					
Pn. harei (16)	16					
A. vaginalis (8)	8					
Pn. ivorii (5)	5					
P. anaerobius (4)	4					
Pn. lacrimalis (4)	4					
Pn. gorbachii (4)	4					
A. murdochii (3)	3					
At. parvulum (4)	4					
A. tetradius (2)	2					
Pn. octavius (1)	1					
R. gnavus (1)	1					
A. lactolyticus (1)	1					
GPAC (4)	4					

[a] The percentage of intermediair/resistent strains is only given for species of which more than 10 strains were present.

[b] Breakpoints for anaerobic bacteria.

[c] Breakpoints for gram-positive anaerobic bacteria.

[d] n.a. = not applicable

The clinically most important GPAC in our study are *F. magna*, *Pa. micra*, and *Pn. harei*. Especially the latter can be difficult to identify phenotypically, since its biochemical features resemble those of *Pn. asaccharolyticus* [10]. In the past *Pn. harei* was probably often misidentified as *Pn. asaccharolyticus*, resulting in limited susceptibility data on this species. Brazier et al. [4] included 44 clinical isolates of *Pn. harei* in a European study, all phenotypically identified. No resistance was reported. In a susceptibility study in England and Wales [5] 4 clinical isolates of *Pn. harei* were included, also phenotypically identified. Resistance (MIC > 256) was reported to clindamycin. In our study, the MIC_{50} and MIC_{90} for clindamycin were 0.25 and 1.5, respectively. The latter being the highest MIC found for *Pn. harei*.

Our study is the first to include *Pn. gorbachii* and *A. murdochii*, although the numbers are low. It is worth to mention that one strain of *A. murdochii* had high

MIC-values for 4 of the 14 antibiotics; doxycycline, ertapenem, levofloxacin, and penicillin G.

Differences in susceptibility to antibiotics were described for *P. anaerobius* and *P. stomatis* [12]. *P. anaerobius* has higher MIC values for amoxicillin, amoxicillin-clavulanic acid, cefoxitin, ertapenem, azithromycin, clindamycin, metronidazole and moxifloxacin than *P. stomatis*, only the MIC_{90} of azithromycin and moxifloxacin was not two dilution steps higher. Brazier et al. [5], also suggests that some GPAC species are more resistant to antibiotics than others. For example, *P. anaerobius* had a higher MIC_{50} for tetracycline, but had lower MIC values for erythromycine as *F. magna*. Roberts et al. [17] described that *P. anaerobius* has higher MIC_{50} and MIC_{90} values for amoxicillin-clavulanic acid, piperacillin-tazobactam, cefoxitin, cefotetan and meropenem when compared to *F. magna*, *Pa. micra* and *Pn. asaccharolyticus*. Koeth et al. [11] showed that *F. magna* has a higher MIC_{50} for clindamycin as *Pa. micra* and *P. anaerobius*, while *P. anaerobius* has the highest MIC_{90} for amoxicillin-clavulanic acid.

Metronidazole is often the drug used for empiric treatment of anaerobic infections. However, GPAC strains are described which are resistant to this drug [11, 13, 16]. We encountered one strain of *Pa. micra* which was resistant to metronidazole (MIC > 256). Microbiologists should be aware of this possibility. It is remarkable to notice the difference in susceptibility to the different antibiotics between the three most clinically important GPAC; *F. magna*, *Pa. micra*, and *Pn. harei*. Therefore, it is important to identify clinical isolates of GPAC. *F. magna* and *Pa. micra* can be reliable phenotypically identified using a commercially available enzymatic kit like Rapid ID 32A [20]. However, *Pn. harei* cannot be phenotypically distinguished from *Pn. asaccharolyticus* [10, 20]. The combination of diminished antimicrobial susceptibility, its prevalence and the described virulence factors [8], gives *F. magna* a special position among the GPAC.

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References

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** Basic local alignment search tool. J. Mol. Biol. 1990; 215:403-410.
2. **Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim- van Dillen PM, van der Noordaa J.** Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 1990; 28:495-503.
3. **Bowker KE, Wootton M, Holt HA, Reeves DS, MacGowan AP.** The in-vitro activity of trovafloxacin and nine other antimicrobials against 413 anaerobic bacteria. J. Antimicrob. Chemother. 1996; 38:271-281.

4. **Brazier J, Chmelar D, Dubreuil L, Feierl G, Hedberg M, Kalenic S, Könönen E, Lundgren B, Malamou-Ladas H, Nagy E, Sullivan Å, Nord CE.** European surveillance study on antimicrobial susceptibility of gram-positive anaerobic cocci. Int. J. Antimicrob. Agents 2008; 31:316-320.
5. **Brazier JS, Hall W, Morris TE, Gal M, Duerden BI.** Antibiotic susceptibilities of gram-positive anaerobic cocci: results of a sentinel study in England and Wales. J. Antimicrob. Chemother. 2003; 52:224-228.
6. **Downes J, Wade WG.** *Peptostreptococcus stomatis* sp. nov., isolated from the human oral cavity. Int. J. Syst. Evol. Microbiol. 2006; 56:751-754.
7. **Ezaki T, Kawamura Y, Li N, Li ZY, Zhao L, Shu S.** Proposal of the genera *Anaerococcus* gen. nov., *Peptoniphilus* gen. nov. and *Gallicola* gen. nov. for members of the genus *Peptostreptococcus*. Int. J. Syst. Evol. Microbiol. 2001; 51:1521-1528.
8. **Goto T, Yamashita A, Hirakawa H, Matsutani M, Todo K, Ohshima K, Toh H, Miyamoto K, Kuhara S, Hattori M, Shimizu T, Akimoto S.** Complete genome sequence of *Finegoldia magna*, an anaerobic opportunistic pathogen. DNA Res. 2008; 15:39-47.
9. **Hiraishi A.** Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. Lett. Appl. Microbiol. 1992; 15:210-213.
10. **Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM.** Wadsworth-KTL anaerobic bacteriology manual, 6th ed. Star Publishing, Belmont, CA. 2002.
11. **Koeth LM, Good CE, Appelbaum PC, Goldstein EJC, Rodloff AC, Claros M, Dubreuil L.** Surveillance of susceptibility patterns in 1297 European and US anaerobic and capnophilic isolates to co-amoxiclav and five other antimicrobial agents. J. Antimicrob. Chemother. 2004; 53:1039-1044.
12. **Könönen E, Bryk A, Niemi P, Kanervo-Nordström A.** Antimicrobial susceptibilities of *Peptococcus anaerobius* and the newly described *Peptostreptococcus stomatis* isolated from various human sources. Antimicrob. Agents Chemother. 2007; 51:2205-2207.
13. **Liu CY, Huang YT, Liao CH, Yen LC, Lin HY, Hsueh PR.** Increasing trends in antimicrobial resistance among clinically important anaerobes and *Bacteroides fragilis* isolates causing nosocomial infections: emerging resistance to carbapenems. Antimicrob. Agents Chemother. 2008; 52:3161-3168.
14. **Murdoch DA, Mitchelmore IJ, Tabaqchali S.** The clinical importance of gram-positive anaerobic cocci isolated at St Bartholomew's hospital, London, in 1987. J. Med. Microbiol. 1994; 41:36-43.
15. **Murdoch DA, Shah HN.** Reclassification of *Peptostreptococcus magnus* (Prevot 1933) Holdeman and Moore 1972 as *Finegoldia magna* comb. nov. and *Peptostreptococcus micros* (Prevot 1933) Smith 1957 as *Micromonas micros* comb. nov. Anaerobe 1999; 5:555-59.
16. **Pankuch GA, Jacobs MR, Appelbaum PC.** Susceptibilities of 428 gram-positive and –negative anaerobic bacteria to Bay y3118 compared with their susceptibilities to ciprofloxacin, clindamycin, metronidazole, piperacillin, piperacillin-tazobactam, and cefoxitin. Antimicrob. Agents Chemother. 1993; 37:1649-1654.
17. **Roberts SA, Shore KP, Paviour SD, Holland D, Morris AJ.** Antimicrobial susceptibility of anaerobic bacteria in New Zealand: 1999-2003. J. Antimicrob. Chemother. 2006; 57:992-998.
18. **Song Y, Liu C, Finegold SM.** *Peptoniphilus gorbachii* sp. nov., *Peptoniphilus olsenii* sp. nov., and *Anaerococcus murdochii* sp. nov. isolated from clinical specimens of human origin. J. Clin. Microbiol. 2007; 45:1746-1752.
19. **Tindall BJ, Euzéby JP.** Proposal of *Parvimonas* gen. nov. and *Quatrionicoccus* gen. nov. as replacements for the illegitimate, prokaryotic, generic names *Micromonas* Murdoch and Shah 2000

and *Quadricoccus* Maszenan *et al*. 2002, respectively. Int. J. Syst. Evol. Microbiol. 2006; 56:2711-2713.

20. **Wildeboer-Veloo ACM, Harmsen HJM, Welling GW, Degener JE.** Development of 16S rRNA-based probes for the identification of gram-positive anaerobic cocci isolated from human clinical specimens. Clin. Microbiol. Infect. 2007; 13:985-992.
21. **Wren MWD.** Anaerobic cocci of clinical importance. Br. J. Biomed. Sci. 1996; 53:294-301.