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Antibiotic susceptibility profiles of anaerobic pathogens in The Netherlands

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ABSTRACT

The antibiotic susceptibility profile of the *Bacteroides fragilis* group, Gram-positive anaerobic cocci (GPAC), *Fusobacterium* spp., *Prevotella* spp., *Veillonella* spp. and *Bilophila wadsworthia* for amoxicillin, amoxicillin–clavulanic acid, clindamycin and metronidazole was determined. Human clinical isolates were isolated between 2011 and 2013 at the Microbiological Diagnostic Laboratory of the University Medical Center Groningen, The Netherlands and subjected to MALDI-TOF MS identification and susceptibility testing using E-test for MIC determination.

Differences in clindamycin susceptibility between species of the *B. fragilis* group and GPAC were observed, with *Bacteroides ovatus* and *Peptoniphilus harei* having the highest resistance rates. Compared to other European countries, in The Netherlands the MIC_{90} for clindamycin of fusobacteria is low. Metronidazole resistance was first encountered in the genus *Prevotella* in 2013, but not in species of GPAC as reported in Belgium and Bulgaria. The differences in clindamycin resistance between the different European countries and reports of metronidazole resistance within the genera *Prevotella* and GPAC warrant more extensive susceptibility studies on anaerobic pathogens.

1. Introduction

Anaerobic bacteria play an important role in infections consisting of aerobic and anaerobic bacteria. Most of the anaerobic pathogens originate from the commensal microbiota [1]. Many laboratories perform limited anaerobic culture and antibiotic susceptibility testing and rely on antibiotic susceptibility surveys, thus antibiotic treatment is often empirical. It has been recommended to perform susceptibility testing of virulent anaerobic pathogens of which the susceptibility cannot be predicted. These include *Bacteroides* spp., *Prevotella* spp., *Fusobacterium* spp., *Clostridium* spp., *Bilophila wadsworthia* and *Sutterella wadsworthensis* [2].

Resistance rates are determined by factors such as species or ribotype, geography, antibiotic consumption and the type of specimen [3]. In the last years an increase in antibiotic resistance has been reported in *Bacteroides*/*Parabacteroides* spp. towards amoxicillin–clavulanic acid, ampicillin-sulbactam and clindamycin. These changes vary among different countries [3].

It has been shown that the emerge of antibiotic resistance differs between species from the same genus. The antimicrobial resistance among non-*Bacteroides fragilis* species has been shown to be greater than among *B. fragilis* [4]. Also among the Gram-positive anaerobic cocci (GPAC) the antibiotic resistance differs between *Finegoldia magna*, *Parvimonas micra* and *Peptoniphilus harei* [5]. The identification of anaerobic strains was, until recently, time consuming and not always unambiguous. With the introduction of the Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) technique, identification of anaerobes at the species level has become feasible for many laboratories [6].

Only a limited amount of data is available concerning the antibiotic resistance of anaerobic bacteria in Europe. In this study we describe the antibiotic susceptibility profiles of the most prevalent species of the *B. fragilis* group and of GPAC as well as for the genera *Clostridium* (excluding *Clostridium difficile*), *Fusobacterium*, *Veillonella* and *Prevotella*, and the species *B. wadsworthia* against amoxicillin, amoxicillin–clavulanic acid, clindamycin and metronidazole.

2. Material and methods

2.1. Bacterial strains

Bacterial strains belonging to the *B. fragilis* group, GPAC, *Fusobacterium*, *Prevotella*, *Veillonella*, *Clostridium* (excluding *C. difficile*)

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and the species *B. wadsworthia* isolated from human clinical specimens at the University Medical Center Groningen (UMCG), The Netherlands, between 2011 and 2013 were subjected to antibiotic susceptibility testing. Since the UMCG is a university hospital, the majority of the patients originate from the northern part of The Netherlands. Only one strain was included in the study in case of multiple isolation of the same species from different specimen of a given patient. Isolates with incomplete antibiotic profiles, species represented by a small (<7) number of isolates and isolates not identified were excluded from the study.

2.2. Identification

Strains were identified on the MALDI-TOF MS (Bruker Daltonik, Gmbh, Germany) as described previously [7]. Briefly, strains were spotted twice on a stainless target using a tooth pick. After drying one spot was overlaid with 1 μl HCCA matrix (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoro-acetic acid) and left to dry at ambient temperature. The second spot was overlaid with 1 μl 70% formic acid. After drying at ambient temperature the spot was overlaid with 1 μl matrix. Spectra were obtained using the standard settings advised by the manufacturer. The unknown spectra were compared with the reference spectra using the Biotyper software. Log score ≥2 were interpreted as reliable species identification.

2.3. Susceptibility testing

The susceptibility against amoxicillin, amoxicillin–clavulanic acid, clindamycin and metronidazole was determined using E-test (bioMérieux, Marcy l'Etoile, France). Bacterial suspensions of 1 McFarland were prepared in saline and confluently applied on pre-reduced Brucella Blood Agar plates (BBA, Media products, Groningen, The Netherlands), supplemented with hemin (5 mg/L) and vitamin K_1 (1 mg/L). All handlings were performed in the anaerobic cabinet. Cultures were incubated in an anaerobic atmosphere (80% N_2, 10% CO_2, 10% H_2) at 37 °C for 24–72 h before reading the minimal inhibitory concentrations (MIC). For clindamycin the MIC was determined after 48 h of incubation.

The range, MIC_{50} and MIC_{90} were determined for each antibiotic. Percentage resistance was determined using the breakpoints advised by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for all antibiotics tested.

3. Results

3.1. Bacterial strains

The *B. fragilis* group, *Prevotella*, *Clostridium*, *Fusobacterium*, *Veillonella*, GPAC and *B. wadsworthia* were most prevalent in all the anaerobic strains isolated between 2011 and 2013 (Table 1). The most encountered species within the *B. fragilis* group were *B. fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus* and *Bacteroides ovatus*, with 47%, 22%, 9% and 7% respectively of the total number of all strains. This distribution was similar each separate year (Table 1). For the GPAC the most prevalent species were *P. micra*, *F. magna* and *P. harei*, with 25%, 28% and 16% respectively of all GPAC strains isolated. The susceptibility of these different species was also determined, expressed as the range, MIC_{50}, MIC_{90} and percentage resistance.

3.2. Susceptibility

The susceptibility profiles of the test species from each year are presented in Table 2. A summary of all three years is presented in Table 3. Almost all species within the *B. fragilis* group appeared resistant to amoxicillin but susceptible to amoxicillin–clavulanic acid, except for one strain of *B. fragilis* that was resistant to amoxicillin–clavulanic acid. No difference between the different species was observed. Also most strains of *B. wadsworthia* (92%) were resistant to amoxicillin, but susceptible to amoxicillin–clavulanic acid. The resistance of *Prevotella* spp. to amoxicillin increased from 30% in 2011 to 60% in 2013. No remarkable difference between the three years in resistance to amoxicillin was observed for the fusobacteria. Among the *Fusobacterium* strains resistant to amoxicillin, two strains appeared resistant to amoxicillin–clavulanic acid. All GPAC strains and *Veillonella* spp. were sensitive to amoxicillin. In 2011 no resistance was encountered among the clostridia. However, in 2012 and 2013 the resistance for amoxicillin was 10%.

No clindamycin resistance was encountered in *B. wadsworthia*, fusobacteria and *Veillonella* spp. An increase in the percentage of resistance to clindamycin in the *B. fragilis* group was observed from 14% in 2011 to 27% and 21% in 2012 and 2013. The number of clindamycin resistant *Prevotella* spp. remained the same in the three years, with an average of 11%. The genus *Clostridium* showed a higher amount of resistant strains in 2012, compared to the percentage resistant strains in 2011 and 2012. The percentage resistance was 64% in 2012, but 19% and 20% in 2011 and 2013,

Table 1
The prevalence of anaerobic species and genera in The Netherlands isolated from human clinical specimens in 2011–2013.

	2011[a]		2012[a]		2013[a]		Total	
	No. of strains	%	No. of strains	%	No. of strains	%	No. of strains	%
B. fragilis group spp.	80		88		115		283	
B. fragilis	35	43.8	38	43.2	60	52.2	133	47.0
B. thetaiotaomicron	14	17.5	19	21.6	28	24.3	61	21.6
B. ovatus	6	7.5	7	8.0	6	5.2	19	6.7
B. vulgatus	7	8.8	10	11.4	9	7.8	26	9.2
GPAC	54		97		98		249	
F. magna	14	25.9	22	22.7	25	25.5	61	24.5
P. micra	16	29.6	23	23.7	30	30.6	69	27.7
P. harei	10	18.5	12	12.4	17	17.3	39	15.7
Prevotella spp.	24		47		52		123	
B. wadsworthia	13		9		2		24	
Clostridium spp.	21		21		20		62	
Fusobacterium spp.	9		11		19		39	
Veillonella spp.			9		10		19	

[a] Other anaerobic bacteria recovered from human clinical specimens were *Propionibacterium acnes*, other *Propionibacterium* species, *Actinomyces* spp., *Clostridium difficile*, unidentified Gram-negative rods and unidentified Gram-positive rods. Rare species and species recovered in small numbers are not mentioned.