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Antimicrobial susceptibility profile of clinically relevant *Bacteroides*, *Phocaeicola*, *Parabacteroides* and *Prevotella* species, isolated by eight laboratories in the Netherlands

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Objectives: Recently, reports on antimicrobial-resistant *Bacteroides* and *Prevotella* isolates have increased in the Netherlands. This urged the need for a surveillance study on the antimicrobial susceptibility profile of *Bacteroides*, *Phocaeicola*, *Parabacteroides* and *Prevotella* isolates consecutively isolated from human clinical specimens at eight different Dutch laboratories.

Methods: Each laboratory collected 20–25 *Bacteroides* (including *Phocaeicola* and *Parabacteroides*) and 10–15 *Prevotella* isolates for 3 months. At the national reference laboratory, the MICs of amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem, imipenem, metronidazole, clindamycin, tetracycline and moxifloxacin were determined using agar dilution. Isolates with a high MIC of metronidazole or a carbapenem, or harbouring *cfiA*, were subjected to WGS.

Results: *Bacteroides thetaiotaomicron/faecis* isolates had the highest MIC₉₀ values, whereas *Bacteroides fragilis* had the lowest MIC₉₀ values for amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem, imipenem and moxifloxacin. The antimicrobial profiles of the different *Prevotella* species were similar, except for amoxicillin, for which the MIC₅₀ ranged from 0.125 to 16 mg/L for *Prevotella bivia* and *Prevotella buccae*, respectively. Three isolates with high metronidazole MICs were sequenced, of which one *Bacteroides thetaiotaomicron* isolate harboured a plasmid-located *nimE* gene and a *Prevotella melaninogenica* isolate harboured a *nimA* gene chromosomally.

Five *Bacteroides* isolates harboured a *cfiA* gene and three had an IS element upstream, resulting in high MICs of carbapenems. The other two isolates harboured no IS element upstream of the *cfiA* gene and had low MICs of carbapenems.

Conclusions: Variations in resistance between species were observed. To combat emerging resistance in anaerobes, monitoring resistance and conducting surveillance are essential.

Introduction

Anaerobic bacteria are a major part of the human microbiota and important pathogens in human infections.¹ They are believed to be susceptible to common antimicrobial agents used to treat polymicrobial infections with anaerobic bacteria, such as metronidazole and carbapenems. However, antimicrobial resistance among anaerobes is steadily increasing.² *Bacteroides* and *Prevotella* isolates are important anaerobic clinical pathogens that can be cultured from a wide range of clinical samples.³ Resistance among *Bacteroides* spp. to important antimicrobial agents has been reported, including metronidazole and meropenem, and *Bacteroides* spp. can carry many antimicrobial resistance genes (ARGs).⁴ Taxonomic changes have been made among the species of the genus *Bacteroides*. In 2006, *Bacteroides distasonis*, *Bacteroides merdae* and *Bacteroides goldsteinii* were accommodated in the genus *Parabacteroides*.⁵ More recently, several other species, including *Bacteroides vulgatus*, *Bacteroides dorei* and *Bacteroides massiliensis* have been reclassified as *Phocaeicola vulgatus*, *Phocaeicola dorei* and *Phocaeicola massiliensis*.⁶

Prevotella spp. are often described as an important part of the human oral and urogenital microbiota. They can cause serious infections in the head/neck region, but are also isolated from other sites, including the abdomen.^{7,8} In recent years, resistance within this genus has increased, and metronidazole-resistant isolates have been described, as well as β -lactamase-producing isolates.^{9,10} *Prevotella* isolates can also carry multiple ARGs, but to a lesser extent compared with *Bacteroides*.¹¹

In 2019, five MDR *Bacteroides fragilis* isolates harbouring several mobile genetic elements (MGEs) carrying ARGs were reported in five hospitals in the Netherlands.¹² The finding of MDR *B. fragilis* isolates in different hospitals in the Netherlands is worrisome and underscores the need to perform an antibiotic susceptibility surveillance of Bacteroidales isolates (specifically *Bacteroides*, *Parabacteroides*, *Phocaeicola* and *Prevotella*) from hospitals located throughout the Netherlands. This prospective study describes the antimicrobial susceptibility profile of *Bacteroides*, *Phocaeicola*, *Parabacteroides* and *Prevotella* spp. isolated from clinical specimens in eight laboratories in the Netherlands in 2021.

Materials and methods

Collection of isolates

From February to July 2021, seven university hospitals and one regional laboratory providing microbiological services to regional hospitals and GPs, scattered across the Netherlands, consecutively collected 20–25 *Bacteroides*, *Phocaeicola* and *Parabacteroides* isolates and 10–15 *Prevotella* isolates during a maximum period of 3 months from a selection of clinically relevant materials using selective culture media provided by the national reference centre at the University Medical Center Groningen (UMCG), Groningen, the Netherlands. Sinus fluids, and pus/drain fluids from the abdominal region, thorax region, and head/neck region were cultured on Brucella laked blood agar supplemented with 80 mg/L kanamycin and 8 mg/L vancomycin (BBKV; Mediaproducts, Groningen, the Netherlands) and *Bacteroides* bile esculin agar (BBE; Mediaproducts, Groningen, the Netherlands) in addition to the standard isolation protocols of the laboratories. Plates were incubated at 37°C in an anaerobic atmosphere according to the standard protocol of the

participating laboratories. After 48 h, plates were checked for bacterial growth. All morphologically different colonies were identified using MALDI-TOF MS, according to the standard protocol of the participating laboratories. Per patient, one isolate per species was included and stored in duplicate at –80°C using Microbank vials (Pro-Lab diagnostics, Birkenhead, UK). Positive blood culture bottles and other clinical materials were also included when containing *Bacteroides*, *Phocaeicola*, *Parabacteroides* or *Prevotella* spp. After the inclusion period, Microbank vials were sent by courier to the national reference centre. Upon arrival, all isolates were cultured on Brucella blood agar (BBA; Mediaproducts, Groningen, the Netherlands), supplemented with 10 mg/L haemin and 20 mg/L vitamin K1. Isolates were checked for purity and re-identified using MALDI-TOF MS (Bruker Microflex LT/SH, Bruker Daltonics, Bremen, Germany), with the version 11 database, including the MALDI Biotyper Subtyping Module, which was used to detect the *cfiA* gene in *B. fragilis* isolates.¹³ MALDI-TOF MS cannot differentiate between *Bacteroides thetaiotaomicron* and *Bacteroides faecis*; *Bacteroides ovatus* and *Bacteroides xylanisolvens*; and between *P. vulgatus* and *P. dorei*. Therefore, these species were combined and reported as *B. thetaiotaomicron/faecis*, *B. ovatus/xylanisolvens* and *P. vulgatus/dorei* complex, respectively.¹⁴

Antimicrobial susceptibility testing

Prior to antimicrobial susceptibility testing, isolates were cultured on BBA for 48 h at 37°C in an anaerobic atmosphere (80% N₂, 10% H₂, 10% CO₂). Antimicrobial susceptibility testing for amoxicillin (Duchefa Farma BV, Haarlem, the Netherlands), amoxicillin/clavulanic acid (fixed ratio of 2:1) (Sigma-Aldrich, St. Louis, MO, USA), piperacillin/tazobactam (fixed concentration of 4 mg/L tazobactam; Fresenius Kabi, Bad Homburg vor den Höhe, Germany), meropenem (Fresenius Kabi), imipenem (Thermo Scientific, Waltham, MA, USA), metronidazole (B. Braun, Melsungen, Germany), moxifloxacin (Thermo Scientific) and tetracycline (Duchefa Farma BV) was performed using the agar dilution method, which is the recommended reference method for antimicrobial susceptibility testing of anaerobic organisms, using the EUCAST- and CLSI-recommended medium fastidious anaerobic agar supplemented with 5% defibrinated horse blood (FAA-HB; Mediaproducts) supplemented with the antimicrobial agent in increasing concentrations of a 2-fold serial dilution with a range of 0.008–256 mg/L.¹⁵ Of each bacterium, 2 μ L of a 0.5 McFarland suspension was pipetted on the agar plates containing a dilution of the antibiotic tested at that moment and left to dry for about 10 min in an ambient atmosphere. Plates were incubated at 37°C for 48 h in an anaerobic atmosphere. Quality control was performed on every agar dilution plate using *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741, and for each antimicrobial agent, a control plate without antimicrobial agent was incubated under aerobic and anaerobic conditions. The MIC was the antibiotic concentration at which a significant reduction in growth was observed, as advised by the CLSI guidelines.¹⁵

The quality control strains for clindamycin resulted in a higher MIC value than the CLSI's range.¹⁶ Due to these quality control issues, the clindamycin MIC was assessed using ETEST (bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's recommendations. The MIC₅₀ and MIC₉₀ were determined per species when at least 10 isolates were tested for each antimicrobial agent. Species with fewer than 10 isolates were grouped as species (e.g. *Bacteroides* species). The use of different breakpoints between studies hampers the interpretation of differences in resistance between studies based on the percentage resistance. Therefore, the results of this study will be discussed using the MIC₅₀ and MIC₉₀. A complete overview of the percentage resistance using the different breakpoints is provided in Table S1 (available as [Supplementary data](#) at JAC Online). Furthermore, the results of the *Phocaeicola* isolates were joined with *Bacteroides* to facilitate comparison with other studies.

WGS

Isolates with MICs higher than 4, 8 and 4 mg/L to metronidazole, meropenem and imipenem, respectively, or carrying the *cfiA* gene, as determined by MALDI-TOF MS, were subjected to WGS. DNA extraction was performed using the DNeasy Ultraclean Microbial kit (MO BIO Laboratories, Carlsbad, CA, USA). Library preparation was performed using the Nextera XT v2 kit (Illumina, San Diego, CA, USA), followed by short-read sequencing on an Illumina MiSeq (Illumina) generating 250–300 bp paired-end reads using the MiSeq reagent kit v2 or v3, respectively.¹⁷ Using CLC Genomics workbench v12 (QIAGEN, Hilden, Germany), *de novo* assembly was performed.¹⁷ Analysis of WGS data, including detection of ARGs and MGEs, was performed as described previously.¹² The identity of the isolates was confirmed by comparing the 16S rRNA gene with the NCBI database using BLASTn (<https://blast.ncbi.nlm.nih.gov/>).

Results

Clinical isolates

In total, 298 isolates were sent to the national reference centre, varying from 31 to 45 per participating centre. A total of 280 isolates were included in the study: 184 *Bacteroides*, *Phocaeicola* and *Parabacteroides* isolates and 96 *Prevotella* isolates. Eighteen isolates were not included in the study: six were identified as a species belonging to a different genus, 12 could not be resuscitated. Most isolates were cultured from abdominal samples (43%), followed by head/neck region samples (11%) and positive blood cultures (11%). An overview of isolates and associated clinical specimens is shown in Table 1.

Antimicrobial susceptibility profile of *Bacteroides*, *Phocaeicola* and *Parabacteroides* isolates

Table 2 shows the differences in antimicrobial profile observed among the *Bacteroides*, *Phocaeicola*, *Parabacteroides* and *Prevotella* isolates per species. An overview of percentages resistance using EUCAST v11.0, EUCAST v13.1 and CLSI 2023 breakpoints is given in Table S1. For some isolates, susceptibility testing could not be performed due to issues in the growth and purity of the culture.

The MIC₉₀ of amoxicillin/clavulanic acid was similar for *B. thetaiotaomicron/faecis*, *B. ovatus/xylanisolvens*, *P. vulgatus/dorei* and *P. distasonis*, with MIC₉₀ values between 16 and 32 mg/L. However, *B. fragilis* and the *Bacteroides* ‘other species’ group had much lower MIC₉₀ values of 4 mg/L. A similar tendency was seen for piperacillin/tazobactam. The MIC₉₀ of meropenem and imipenem ranged from 1 to 4 mg/L. The highest MIC₉₀, for both antibiotics, was 4 mg/L for *B. thetaiotaomicron/faecis* and *B. ovatus/xylanisolvens* isolates, and the imipenem MIC₉₀ of *P. vulgatus/dorei* was 4 mg/L. Similar to amoxicillin/clavulanic acid and piperacillin/tazobactam, the meropenem and imipenem MIC₉₀ of *B. fragilis* and the *Bacteroides* ‘other species’ group had the lowest values for both antibiotics. Four of the 62 *B. fragilis* isolates harboured the *cfiA* gene as determined by MALDI-TOF MS MBT Subtyping Module. Two isolates had high MICs of meropenem and imipenem, where one isolate had MICs of 256 and 128 mg/L, and the other had MICs of 16 and 8 mg/L, respectively. The other two isolates had lower MIC values; one isolate had MICs of 4 and 2 mg/L and the other had MICs of 0.5 and 0.25 mg/L for meropenem and imipenem, respectively.

The MIC₉₀ of metronidazole was similar for all species, ranging from 0.75 to 1 mg/L. The MIC₉₀ of clindamycin was >256 mg/L for *B. fragilis*, *B. thetaiotaomicron/faecis* and *B. ovatus/xylanisolvens*. For all other *Bacteroides*, *Phocaeicola* and *Parabacteroides* species, the MIC₉₀ was lower, ranging from 0.75 mg/L for *P. vulgatus/dorei* to 4 mg/L for *P. distasonis*. The MIC₉₀ of tetracycline was mostly similar for all species, with MIC₉₀ values between 64 and 128 mg/L. For moxifloxacin, *Parabacteroides* isolates had the lowest MIC₉₀ value of 1 mg/L, and *Phocaeicola* isolates had the highest values of 128 mg/L.

Antimicrobial susceptibility profile of *Prevotella* isolates

Of 96 *Prevotella* isolates, most species were represented by <10 isolates, with only *Prevotella bivia* and *Prevotella buccae* represented by ≥10 isolates. Therefore, most *Prevotella* species were grouped, and only these two species could be compared with the average *Prevotella* data (Table 2). The MIC₅₀ and MIC₉₀ values for most antimicrobial agents were similar for *P. bivia*, *P. buccae* and the other species. The biggest difference was visible for amoxicillin, where the MIC₅₀ was only 0.125 mg/L for *P. bivia* but 16 mg/L for *P. buccae*, with an average for all other species of 4 mg/L.

WGS of metronidazole-resistant isolates

The assembly output of the sequenced isolates is shown in Table S2. Three isolates with an MIC value of 8 mg/L for metronidazole (resistant according to EUCAST guidelines) were subjected to WGS, of which two harboured a *nim* gene encoding metronidazole resistance in their genome. The *B. thetaiotaomicron* isolate carried a *nimE* gene on a pBFS01_2 plasmid, and the *Prevotella melaninogenica* isolate harboured a *nimA* gene in its chromosome. The latter *nim* gene was not located on an MGE. The *Prevotella nanceiensis* isolate did not harbour a *nim* gene. Furthermore, the *cfxA* gene (*n*=3), the *tet(Q)* gene (*n*=1) and the *mef(En2)* gene (*n*=1) were encountered in these isolates.

WGS of carbapenem-resistant isolates

A total of 12 isolates were subjected to WGS to detect carbapenem resistance determinants. Of six *B. fragilis* isolates, four harboured the *cfiA* gene. Two had either IS1186 or IS614 upstream and had high MICs of meropenem and imipenem (meropenem MIC: 16 and 256 mg/L; imipenem MIC: 8 and 128 mg/L, respectively). The other two *cfiA*-harbouring *B. fragilis* isolates did not have an IS element upstream and had low MICs of meropenem and imipenem (meropenem MIC: 4 and 0.5 mg/L; imipenem MIC: 2 and 0.25 mg/L, respectively). Two *B. fragilis* isolates sequenced due to high MICs of imipenem (MIC: 8 and 8 mg/L), but with low MICs of meropenem (MIC: 2 and 0.25 mg/L), did not harbour a *cfiA* gene. Of the other six sequenced isolates [i.e. *B. thetaiotaomicron* (*n*=1), *B. xylanisolvens* (*n*=1), *Bacteroides* spp. (*n*=1) and *P. vulgatus* (*n*=3)] the *Bacteroides* spp. isolate harboured the *cfiA* gene with an IS614 upstream. Other genes encountered in the 12 sequenced isolates were the *cepA* gene (*n*=2), the *cfxA* gene (*n*=2), the *mef(A)* gene (*n*=1), *erm(F)* gene (*n*=2), the *mef(En2)* gene (*n*=1), the *tet(X)* gene (*n*=1) and the *tet(Q)* gene (*n*=4).

Table 1. Overview of clinical specimens

Isolates	Blood culture	(Intra-) abdominal ^a _b	Hepato-pancreato-biliary system ^a	Groin region ^a	Head/neck region ^a	Thorax region ^a	Vascular prosthesis ^a	Orthopaedic material ^a	Bone tissue	Urine	Not specified ^a
<i>Bacteroides</i> total (n=139)	22	71	8	12	2	1	2	2	1	0	18
<i>B. fragilis</i> (n=62)	17	29	2	4	0	0	1	0	0	0	9
<i>B. ovatus/xyloisolvans</i> (n=25)	0	16	4	3	0	0	0	1	0	0	1
<i>B. thetaioaomicron/faecis</i> (n=31)	4	11	2	3	2	1	1	1	0	0	6
<i>Bacteroides</i> spp. ^c (n=22)	1	15	0	2	0	0	0	0	1	0	2
<i>Phocaeicola</i> total (n=26)	1	16	5	2	0	0	0	0	0	0	2
<i>P. massiliensis</i> (n=1)	0	1	0	0	0	0	0	0	0	0	0
<i>P. vulgatus/dorei</i> (n=25)	1	15	5	2	0	0	0	0	0	0	2
<i>Parabacteroides</i> total (n=19)	2	15	2	0	0	0	0	0	0	0	0
<i>Parabacteroides distasonis</i> (n=14)	2	11	1	0	0	0	0	0	0	0	0
<i>Parabacteroides</i> spp. ^d (n=5)	0	4	1	0	0	0	0	0	0	0	0
<i>Prevotella</i> total (n=96)	6	18	6	9	30	7	2	2	0	2	13
<i>Prevotella bivia</i> (n=13)	0	5	0	2	1	3	0	1	0	0	1
<i>Prevotella buccae</i> (n=15)	3	3	1	1	4	0	1	1	0	0	1
<i>Prevotella</i> spp. ^e (n=68)	4	10	5	6	25	4	1	0	0	2	11
Total all isolates (n=280)	32	120	21	23	32	8	4	4	1	2	33

^aIncluding abscess/fluid/pus/tissue.

^bIncluding ascites, perianal and peritoneal samples.

^c*Bacteroides* spp. includes *Bacteroides caccae* (n=6), *Bacteroides cellulosilyticus* (n=2), *Bacteroides clarus* (n=1), *Bacteroides coagulans* (n=1), *Bacteroides nordii* (n=1), *Bacteroides salyersiae* (n=3), *Bacteroides stercoris* (n=2), *Bacteroides uniformis* (n=5).

^d*Parabacteroides* spp. includes *Parabacteroides goldsteinii* (n=1), *Parabacteroides merdae* (n=4).

^e*Prevotella* spp. includes *Prevotella baroniae* (n=3), *Prevotella bergensis* (n=3), *Prevotella dentalis* (n=1), *Prevotella denticola* (n=8), *Prevotella disiens* (n=7), *Prevotella histicola* (n=1), *Prevotella intermedia* (n=6), *Prevotella jejuni* (n=1), *Prevotella loescheii* (n=1), *Prevotella maculosa* (n=1), *Prevotella melaninogenica* (n=7), *Prevotella nanceiensis* (n=1), *Prevotella nigrescens* (n=9), *Prevotella oralis* (n=2), *Prevotella oris* (n=8), *Prevotella pallens* (n=3), *Prevotella salivae* (n=3), *Prevotella timonensis* (n=2), *Prevotella veroralis* (n=1).

Table 2. MIC₅₀ and MIC₉₀ (mg/L) of all isolates for the different antibiotics

Isolates (n = 280)	Amoxicillin		Amoxicillin/ clavulanic acid		Piperacillin/ tazobactam		Meropenem		Imipenem		Metronidazole		Clindamycin		Tetracycline		Moxifloxacin	
	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
Bacteroides total (138–139) ^a	32	>256	2	16	4	32	0.5	4	1	2	0.5	1	1.5	>256	64	128	1	16
<i>B. fragilis</i> (62)	32	256	1	4	2	8	0.25	1	0.25	2	0.5	1	0.25	>256	64	128	0.5	16
<i>B. thetaiotaomicron/faecis</i> (31)	64	256	2	32	16	32	0.5	4	1	4	0.5	1	4	>256	32	128	2	16
<i>B. ovatus/xylanisolvers</i> (25)	64	>256	2	16	8	32	0.5	4	1	4	0.5	1	3	>256	64	128	2	16
Bacteroides spp. (20–21) ^{a,b}	32	>256	2	4	4	8	0.25	1	1	1	0.5	0.75	1	4	32	64	2	4
Phocaeicola total (25–26) ^{a,c}	128	>256	2	16	8	32	0.5	2	1	4	0.5	1	0.023	0.75	32	64	1	128
<i>P. vulgatus/dorei</i> (24–25) ^a	128	>256	2	16	8	32	0.5	2	1	4	0.5	1	0.023	0.75	32	64	1	128
Bacteroides and Phocaeicola total (163–165) ^a	32	>256	2	16	4	32	0.5	2	1	4	0.5	1	0.75	>256	32	128	1	16
Parabacteroides total (19) ^d	>256	>256	8	32	4	16	1	2	2	2	1	1	1	3	64	64	0.5	1
Parabacteroides distasonis (14)	>256	>256	8	32	4	16	0.5	2	2	2	1	1	1.5	4	64	64	0.5	2
Prevotella total (87–96) ^a	4	128	0.5	4	2	4	0.125	0.25	0.125	0.5	0.5	1	0.016	>256	1	64	1	4
<i>P. bivia</i> (12–13) ^a	16	64	2	4	1	4	0.125	0.125	0.125	0.125	1	1	0.023	>256	1	64	2	4
<i>P. buccae</i> (14–15) ^a	0.125	256	0.25	8	2	8	0.125	0.5	0.25	0.5	0.5	1	0.016	>256	0.5	32	0.5	1
Prevotella spp. (60–68) ^{a,e}	4	128	0.5	4	2	4	0.0625	0.25	0.125	0.25	0.25	1	<0.016	>256	1	64	0.5	2

^aNot all strains were tested for all antibiotics.

^bBacteroides spp. includes *Bacteroides caecae* (n = 6), *Bacteroides cellulosilyticus* (n = 2), *Bacteroides clarus* (n = 1), *Bacteroides coagulans* (n = 1), *Bacteroides nordii* (n = 1), *Bacteroides salyersiae* (n = 3), *Bacteroides stercoris* (n = 2), *Bacteroides uniformis* (n = 5).

^cPhocaeicola total includes *Phocaeicola vulgatus/dorei* (n = 25), *Phocaeicola massiliensis* (n = 1).

^dParabacteroides total includes *Parabacteroides distasonis* (n = 14), *Parabacteroides goldsteinii* (n = 1), *Parabacteroides merdae* (n = 4).
^ePrevotella spp. includes *Prevotella baroniae* (n = 3), *Prevotella bergensis* (n = 3), *Prevotella dentalis* (n = 1), *Prevotella denticola* (n = 8), *Prevotella disiens* (n = 7), *Prevotella histocola* (n = 1), *Prevotella intermedia* (n = 6), *Prevotella jejuni* (n = 1), *Prevotella loeschii* (n = 1), *Prevotella maculosa* (n = 1), *Prevotella melaninogenica* (n = 7), *Prevotella nancaiensis* (n = 1), *Prevotella nigrescens* (n = 9), *Prevotella oralis* (n = 2), *Prevotella oris* (n = 8), *Prevotella pallens* (n = 3), *Prevotella salivae* (n = 3), *Prevotella timonensis* (n = 2), *Prevotella veroralis* (n = 1).

Discussion

This study presents the first national surveillance of antimicrobial resistance in *Bacteroides*, *Phocaeicola*, *Parabacteroides* and *Prevotella* within the Netherlands. Through a collaboration involving eight clinical laboratories across the country, we achieved a comprehensive understanding of the nationwide susceptibility patterns among clinical isolates of these genera. Thus far, the only comparable study on antimicrobial susceptibility patterns in the Netherlands was from the national reference laboratory in 2011–13.¹⁸ For *Bacteroides* and *Phocaeicola* isolates, an increase in the MIC₉₀ of amoxicillin/clavulanic acid is observed, from an MIC₉₀ of 1.5 mg/L in 2011–13 to 16 mg/L in this study. Among *Prevotella* isolates, an increase in MIC values was observed for amoxicillin/clavulanic acid, clindamycin and metronidazole. The MIC₅₀ and MIC₉₀ of amoxicillin/clavulanic acid went from 0.094 and 1 mg/L in 2011–13 to 0.5 and 4 mg/L in 2021. For clindamycin, the MIC₅₀ was identical, at 0.016 mg/L; however, the MIC₉₀ went from 32 mg/L in 2011–13 to >256 mg/L in 2021. For amoxicillin and metronidazole, similar values were found, with MIC₉₀ values of 128 and 0.75 mg/L, respectively, in 2011–13, to 128 and 1 mg/L, respectively, in 2021.

Three isolates of different species (i.e. *B. thetaiotaomicron*, *P. melaninogenica* and *P. nanceiensis*) had a metronidazole MIC of 8 mg/L. Two of these isolates harboured a *nim* gene, which plays a role in metronidazole resistance.¹⁹ In the *B. thetaiotaomicron* isolate, the *nimE* gene was present on a pBFS01_2 plasmid, together with an ISBf6 insertion sequence element, which has been previously described by Sydenham *et al.*²⁰ from an MDR *B. fragilis* isolate. This plasmid was also detected in 4/5 MDR *B. fragilis* isolates from the Netherlands and 2/23 *cfiA*-harbouring *B. fragilis* isolates from Hong Kong.^{12,21} The *P. melaninogenica* isolate resistant to metronidazole harboured a *nimA* gene in its chromosome. The *nimA* gene can be present on plasmids and in the chromosome. It is often associated with an IS1168 element, which was not detected in this isolate.¹⁹ The *nimA* gene has been observed in *Bacteroides* spp., but is less prevalent in *Prevotella* isolates.²² The third isolate resistant to metronidazole, *P. nanceiensis*, did not harbour any *nim* gene in its genome. Either this isolate harbours a not-yet-described *nim* gene or another resistance mechanism.²³

WGS of isolates with high MICs of a carbapenem antibiotic, or harbouring the *cfiA* gene as determined by MALDI-TOF MS, showed that in five strains the *cfiA* gene was present, of which three were accompanied by an IS element. These IS elements were located upstream of the gene and were assigned to the IS1186 and IS614 family, which has been described previously by Soki *et al.*²⁴ These IS elements have been reported to activate the *cfiA* gene, resulting in higher MIC values, as was seen in the carbapenem resistance in these three isolates. In this study, one *Bacteroides non-fragilis* isolate was found harbouring the *cfiA* gene with an IS614 upstream, with meropenem MICs of >256 mg/L and imipenem MICs of 64 mg/L. WGS results showed that an identical *cfiA* element was present in a *B. fragilis* isolate retrieved from the same clinical sample, indicating that horizontal gene transfer (HGT) occurred between the two isolates.²⁵

Differences in antimicrobial susceptibility profiles can occur between different hospitals in the same country due to factors such as disparities in patient populations (e.g. patient origins),

specialties of the associated university hospitals, and previous antibiotic treatments, among others. Therefore, surveillance of a single laboratory cannot fully represent the antimicrobial susceptibility patterns of a species across an entire country.²⁶

With antimicrobial resistance rising among clinical *Bacteroidales* isolates, performing antimicrobial resistance testing is essential to guide appropriate treatment decisions.

This study represents the first national surveillance in the Netherlands involving isolates collected nationwide. By conducting regular surveillance of antimicrobial susceptibility profiles in clinically significant anaerobic bacteria, empirical treatments can be optimized, and trends in antimicrobial resistance can be closely monitored.

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Author contributions

Study conceptualization: K.E.B., T.J.H.S., E.J.K., A.C.M.V.; study design: all authors; data collection: K.E.B., A.C.M.V.; data interpretation: K.E.B., A.C.M.V.; manuscript writing: K.E.B.; manuscript reviewing: all authors; project supervision: J.W.A.R., E.J.K., A.C.M.V.

Supplementary data

Tables S1 and S2 are available as [Supplementary data](#) at JAC Online.

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