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Surveillance

Antimicrobial susceptibility surveillance of obligate anaerobic bacteria in the Kinki area

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ABSTRACT

Obligate anaerobes exist as resident flora in various sites in humans, but they are also emphasized as endogenous causative microorganism of infections. We performed surveillance to understand the trend of drug susceptibility in obligate anaerobic bacteria in the Kinki area of Japan. In the experiment, we used 156 obligate anaerobe isolates collected from 13 institutions that participated in the Study of Bacterial Resistance Kinki Region of Japan. MALDI Biotyper was used to identify the collected strains, and among the 156 test strains, those that could be identified with an accuracy of Score Value 2.0 or more included 6 genera, 30 species, and 144 strains (*Bacteroides* spp. 77 strains, *Parabacteroides* sp. 2 strains, *Prevotella* spp. 29 strains, *Fusobacterium* spp. 14 strains, *Porphyromonas* spp. 2 strains, and *Clostridioides difficile* 20 strains), and they were assigned as subject strains for drug susceptibility testing. The drug susceptibility test was carried out by broth microdilution method using Kyokuto Opt Panel MP ANA (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) and judged according to CLSI criteria. As a result, *Bacteroides* and *Parabacteroides* species showed good sensitivities to tazobactam-piperacillin, imipenem, metronidazole and chloramphenicol, and low sensitivities to ampicillin, cefoperazone and vancomycin. *Prevotella* species showed good sensitivities to sulbactam-ampicillin, tazobactam-piperacillin, cefmetazole, imipenem, doripenem and metronidazole. Susceptibility rates to other drugs were slightly different depending on the bacterial species. Both *Fusobacterium* spp. and *Porphyromonas* spp. showed high sensitivities to many drugs. *C. difficile* was highly sensitive to vancomycin and metronidazole, having MIC_{90}s of 0.5 μg/mL and ≤2 μg/mL, respectively.

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1. Introduction

Obligate anaerobes exist together with facultative anaerobes as resident flora on the skin and in the oral cavity, rhinopharynx and lower digestive tract of the human body. These resident flora have a useful role as barriers to the colonization of various kinds of pathogenic microbes producing toxic substances. However, once the skin or mucosa is breeched by a surgical operation or other procedure, obligate anaerobes invade originally sterile sites to proliferate and provoke infectious diseases. In addition, obligate anaerobes infect various sites in the whole body, such as the central nervous system, thorax, abdomen and the soft tissues, and cause infectious diseases including intraperitoneal infections, pelvic inflammatory disease, peritonsillar abscess and aspiration pneumonitis. Also, it is well-known that *Clostridioides difficile* is associated with antimicrobial-associated diarrhea [1–5]. In the treatment of these infectious diseases caused by obligate anaerobes, surgical management as palliative care (removal of dead tissues, washing and drainage) is performed, but the primary treatment is chemotherapy. Therefore, routine bacteriological testing (isolation and antimicrobial susceptibility testing) is necessary, and additionally, implementation of surveillance to effectively use these accumulated data is required. However, the main target for surveillance in Japan is resistant bacteria such as MRSA, and although there are several reports on obligate anaerobes [1,6–8], the fact is that surveillance is not conducted actively. Here, we investigated the drug susceptibility rates of obligate anaerobes (*Bacteroides* spp., *Parabacteroides* sp., *Prevotella* spp., *Fusobacterium* spp., *Porphyromonas* spp. and *C. difficile*), which were isolated in 13 institutions in the Kinki area, and report our findings.

2. Materials and methods

2.1. Targets and procedures

The collection of isolates was carried out in 13 institutions that participated in the Study of Bacterial Resistance Kinki Region of Japan (Naga Municipal Hospital, Kobe University Hospital, Hospital of Hyogo College of Medicine, Tenri Hospital, JCHO Shiga Hospital, Japanese Red Cross Otsu Hospital, Kindai University Hospital, Osaka University Hospital, Osaka Police Hospital, Sumitomo Hospital, Otemae Hospital, Osaka City University Hospital and Japanese Red Cross Kyoto Daini Hospital) for four months from August 2017 to November 2017. There were no restrictions on specimen types and identification of bacteria responsible for infectious diseases, the methods of examination were left to each institution, and the isolated obligate anaerobes were surveyed. The isolates were suspended in skim milk in each institution and temporarily stored at −20 °C or below. Then, they were gathered in Otemae Hospital and removed from the skim milk all together for use in the experiment. For re-isolation from storage in the skim milk, Brucella HK agar (rabbit) (Kyokuto Pharmaceutical Co., Ltd., Tokyo, Japan) was used, and after anaerobic culture at 35 °C for 48 h, it was confirmed that bacterial growth was under pure culture conditions. The strains were then sub-cultured on Brucella HK agar (RS) (Kyokuto Pharmaceutical Co., Ltd.), and anaerobically incubated at 35 °C for an additional 48 h, after which they were used for identification and drug susceptibility testing.

2.2. Identification test

For identification of pure culture of the strains, MALDI Biotyper (Bruker Daltonics) was used, and according to Veloo et al. [9], three methods were performed: cell smear method, formic acid extraction method and ethanol-formic acid extraction method. In the cell smear method, bacteria from the pure culture were applied to a dedicated plate with a 1-μL inoculating loop, and after drying at room temperature, the plate was overlaid with 1 μL of HCCA (a-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid), which was the matrix solution. In the formic acid extraction method, after bacteria from the pure culture were applied to a dedicated plate, 1 μL 70% formic acid was added, and after drying at room temperature, the plate was overlaid with HCCA matrix. In the ethanol-formic acid extraction method, a 1-μL loopful of the pure bacterial culture was suspended in 300 μL purified water and then 900 μL of ethanol was added. After the suspension was centrifuged at 13,000×*g* for 2 min, the supernatant was discarded. A similar centrifugal separation was conducted once again, and the supernatant was completely removed. A 30-μL solution of 70% formic acid and acetonitrile was added to the sediment and centrifuged further. Then, 1 μL of supernatant was dropped onto a dedicated plate, the plate was dried at room temperature, and then measured after the addition of 1 μL of matrix.

The spectra obtained by the measurement were compared and collated with MALDI Biotyper Real Time Classification version 3.1 (Bruker Daltonics). Regarding the obtained results, only strains that were identified with a Score Value ≥ 2.0 were judged to be a match at strain level, those between ≥1.7 and < 2.0 were judged to be a match at genus level, and those with a score <1.7 or with no peak were judged to be unidentifiable.

2.3. Drug susceptibility test

For drug susceptibility measurements, Kyokuto Optopanel MP ANA (Kyokuto Pharmaceutical Co. Ltd.) was used via a broth microdilution method. In the experiment, the following sixteen different antimicrobial agents (measurement range) were used: ampicillin (≤0.25 to >8 μg/mL), piperacillin (≤4 to >128 μg/mL), sulbactam-ampicillin (≤2/1 to >64/32 μg/mL), tazobactam-piperacillin (≤4/4 to >128/4 μg/mL), ceftriaxone (≤2 to >64 μg/mL), cefotaxime (≤2 to >64 μg/mL), cefoperazone (≤2 to >64 μg/mL), cefmetazole (≤2 to >64 μg/mL), imipenem (≤0.5 to >16 μg/mL), doripenem (≤0.5 to >16 μg/mL), vancomycin (≤0.12 to >4 μg/mL), moxifloxacin (≤0.25 to >8 μg/mL), chloramphenicol (≤2 to >32 μg/mL), tetracycline (≤0.5 to >16 μg/mL), metronidazole (≤2 to >64 μg/mL) and clindamycin (≤0.5 to >16 μg/mL). The test strains were first prepared by culturing them anaerobically for 48 h with Brucella HK agar (RS) (Kyokuto Pharmaceutical Co. Ltd.) adjusted to a turbidity of McFarland 1.0 with sterile saline. Then, 50 μL of this bacterial suspension was added to Brucella broth (Kyokuto Pharmaceutical Co. Ltd., Tokyo, Japan) with lysed horse blood, which was mixed by inverting and divided into 100-μL aliquots in each well of the drug plate. Immediately after the inoculation of bacteria, anaerobic culture was performed at 35 °C for 48 h in an anaerobic chamber. In addition, the operations from the adjustment of bacterial suspension to the start of anaerobic culture were carried out within 30 min. The status of bacterial growth (status of growth inhibition) of each culture panel was judged visually, and the judgment was in accordance with the criteria of CLSI M-100 S-27 [10] (Table 1).

3. Results

3.1. Identification results and isolate origins

As a result of analyzing all 156 strains isolated in the 13 institutions collectively with MALDI Biotyper (Bruker Daltonics), 144 strains were obtained with an accuracy of Score Value 2.0 or higher, and therefore these 144 strains were designated as subject strains for the drug susceptibility test. Their breakdown was as follows:

Table 1
Interpretive categories and MIC breakpoints.

Antimicrobial agent	CLSI breakpoint		
	S	I	R
Sulbactam-ampicillin	≦8/4	16/8	≧32/16
Ampicillin	≦0.5	1	≧2
Tazobactam-piperacillin	≦32/4	64/4	≧128/4
Piperacillin	≦32	64	≧128
Ceftriaxone	≦16	32	≧64
Cefmetazole	≦16	32	≧64
Cefotaxime	≦16	32	≧64
Cefoperazone	≦16	32	≧64
Imipenem	≦4	8	≧16
Doripenem	≦2	4	≧8
Tetracycline	≦4	8	≧16
Vancomycin	≦2		≧4
Metronidazole	≦8	16	≧32
Moxifloxacin	≦2	4	≧8
Clindamycin	≦2	4	≧8
Chloramphenicol	≦8	16	≧32

Bacteroides spp. 77 strains, *Parabacteroides* sp. 2 strains, *Prevotella* spp. 29 strains, *Fusobacterium* spp. 14 strains, *Porphyromonas* spp. 2 strains, and *C. difficile* 20 strains (Table 2).

In addition, 57 of the 144 strains (39.6%) came from specimens obtained in abscesses including pus, wound areas and drainage fluid, which were the most common, followed by 27 strains (18.7%) obtained from the hematologic system, 27 strains (18.7%) from the digestive system, 20 strains (13.9%) from puncture fluid, 7 strains (4.9%) from the urinary system and 6 strains (4.2%) from the upper respiratory tract (Table 3). Although the clinical backgrounds of these isolates could not be accurately determined, that is, whether the isolates were the bacteria responsible for the infectious diseases, almost all of the isolates came from originally sterile materials except for the stool samples, and they were strongly suspected of being pathogenic. Also, 19 strains isolated from stool and one strain isolated from the intestinal mucosa were all *C. difficile*, and these 20 strains were presumed to be the responsible pathogenic bacteria.

Table 2
Results of mass spectrometry identification of the isolates.

Bacterial strain	Number of strains
Bacteroides spp. (n = 77)	
Bacteroides fragilis	42
Bacteroides thetaiotaomicron	15
Bacteroides ovatus	8
Bacteroides vulgatus	6
Bacteroides uniformis	3
Bacteroides cellulosilyticus	1
Bacteroides nordii	1
Bacteroides stercoris	1
Parabacteroides sp. (n = 2)	
Parabacteroides distasonis	2
Prevotella spp. (n = *29)*	
Prevotella bivia	6
Prevotella disiens	4
Prevotella melaninogenica	4
Prevotella timonensis	4
Prevotella intermedia	3
Prevotella baroniae	1
Prevotella buccae	1
Prevotella buccalis	1
Prevotella denticola	1
Prevotella histicola	1
Prevotella nigrescens	1
Prevotella oris	1
Prevotella pallens	1
Fusobacterium spp. (n = *14)*	
Fusobacterium nucleatum	7
Fusobacterium necrophorum	3
Fusobacterium varium	2
Fusobacterium mortiferum	1
Fusobacterium ulcerans	1
Porphyromonas spp. (n = *2)*	
Porphyromonas gingivalis	1
Porphyromonas uenonis	1
Clostridioides sp. (n = 20)	
Clostridioides difficile	20
Total	144

3.2. Results of drug susceptibility

The drug susceptibility test results of the 144 test strains are summarized below for every genus and shown in Table 4 and Supplemental Data 1 to 5.

3.2.1. Results of 77 strains of 8 bacterial species of Bacteroides spp. (Table 4 and Supplemental Data 1)

For *B. fragilis*, satisfactory sensitivities were shown to tazobactam-piperacillin, imipenem, doripenem, metronidazole and chloramphenicol, whereas low susceptibility rates were shown to ampicillin, cefoperazone and vancomycin. This tendency was also similar in the other 7 strains, but rates for cefmetazole and clindamycin differed depending on the bacterial species.

3.2.2. Results of 2 strains of Parabacteroides distasonis (Supplemental Data 2)

Similar to *Bacteroides* spp., in the 2 strains of *P. distasonis*, satisfactory sensitivities were shown to piperacillin-tazobactam, imipenem, doripenem, metronidazole and chloramphenicol, and low susceptibility rates were shown to ampicillin, tetracycline, vancomycin and clindamycin.

Table 3
Origins of the isolates (specimen type) and the number of specimens.

Specimen type	Number of strains	
	Number per item	Total number according to the strain
Abscess		57
Pus	31	
Wound	16	
Drainage fluid	9	
Lung tissues	1	
Hematologic system		27
Blood cultures	26	
Thrombus	1	
Digestive system		27
Stool	19	
Bile	6	
Intestinal mucosa	1	
Gastric mucus	1	
Puncture fluid system		20
Ascites	13	
Pleural effusion	6	
Effusion	1	
Urinary system		7
Vaginal/uterus contents	5	
Urethral discharge	2	
Upper airway system		6
Oral abscess	3	
Tonsillar abscess	2	
Paranasal sinuses	1	
Total	144	144

Table 4
Distribution of MICs for each antimicrobial agents.

Bacteroides fragilis (n=42)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					25	7	4	3	2		1		≦2/1	16/8	85.7
Ampicillin			1			1	5	35					>8	>8	2.4
Γazobactam-piperacillin						40		1				1	≦4/4	≦4/4	97.6
Piperacillin						8	13	7		3	1	10	8	>128	66.7
Ceftriaxone						2	6	3	7	11	13		64	>64	26.2
Cefmetazole					1	2	24	7	3	4	1		8	64	81.0
Cefotaxime						4	4	6	12	3	13		32	>64	33.3
Cefoperazone							1	3	9	12	17		64	>64	9.5
Imipenem			32	7		2			1				≦0.5	1	97.6
Doripenem			32	4			2	2	2				≦0.5	8	85.7
Tetracycline			10			1		11	20				16	>16	26.2
Vancomycin							42						> 4	> 4	0.0
Metronidazole					25	10	7						≦2	8	100
Moxifloxacin		2	15	6	8	3	5	3					1	8	73.8
Clindamycin			13	8	3				18				1	>16	57.1
Chloramphenicol					1	34	5	1	1				4	8	95.2

Bacteroides thetaiotaomicron (n=15)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					8	3	1	2			1		≦2/1	16/8	80.0
Ampicillin		1						14					>8	>8	6.7
Γazobactam-piperacillin						1	7	5	1			1	8/4	32/4	93.3
Piperacillin						1		1	4	2	2	5	64	>128	40.0
Ceftriaxone					1						14		>64	>64	6.7
Cefmetazole					1				1	10	3		64	>64	6.7
Cefotaxime					1					5	9		>64	>64	6.7
Cefoperazone					1					5	9		>64	>64	6.7
Imipenem			11	2	1				1				≦0.5	2	93.3
Doripenem			11	1	2	1							≦0.5	2	93.3
Tetracycline			4	1	1			5	4				16	>16	40.0
Vancomycin						1	14						> 4	> 4	0.0
Metronidazole					10	4	1						≦2	4	100
Moxifloxacin		1		2	7	2	1	2					2	>8	66.7
Clindamycin			1	2	3	2			7				4	>16	40.0
Chloramphenicol					2	5	8						8	8	100

other *Bacteroides fragilis* group (n=22)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					10	1	5	4	1		1		4/2	16/8	72.7
Ampicillin					1		1	20					> 8	> 8	0.0
Γazobactam-piperacillin						18	3					1	≦ 4/4	8/4	95.5
Piperacillin						3	3	3	1	1		11	64	> 128	45.5
Ceftriaxone					1	1	1		1	4	14		> 64	> 64	13.6
Cefmetazole					1		4	7	3	4	3		16	> 64	54.5
Cefotaxime					1	1	2		7	2	9		32	> 64	18.2
Cefoperazone						1			3	6	12		> 64	> 64	4.5
Imipenem			15	5	1				1				≦ 0.5	1	95.5
Doripenem			14	3	3	1	1						≦ 0.5	2	90.9
Tetracycline			3				4	6	9				16	> 16	13.6
Vancomycin							22						> 4	> 4	0.0
Metronidazole					16	4	2						≦ 2	4	100
Moxifloxacin			3	2	7	2	2	6					2	> 8	54.5
Clindamycin			3		4	2			13				> 16	> 16	31.8
Chloramphenicol					1	14	7						4	8	100

Prevotella spp. (n=29)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					25	4							≦ 2/1	4/2	100
Ampicillin		10	2	1	1		2	13					8	> 8	41.4
Γazobactam-piperacillin						29							≦ 4/4	≦ 4/4	100
Piperacillin						14	1	5	5	4			8	64	86.2
Ceftriaxone					13	1	2	4	5	2	2		8	64	69.0
Cefmetazole					26	1	2						≦ 2	4	100
Cefotaxime					13	4	3	4	3	2			4	32	82.8
Cefoperazone					13	5	4	4	3				4	32	89.7
Imipenem			29										≦ 0.5	≦ 0.5	100
Doripenem			29										≦ 0.5	≦ 0.5	100
Tetracycline			15	1		1	4	2	6				≦ 0.5	> 16	58.6
Vancomycin	1	1	1			4	22						> 4	> 4	10.3
Metronidazole					25	3	1						≦ 2	4	100
Moxifloxacin		4	6	1	2	3	3	10					4	> 8	44.8
Clindamycin			11				1		17				> 16	> 16	37.9
Chloramphenicol					24	3	1			1			≦ 2	4	96.6

Fusobacterium spp. (n=14)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					12	2							≦ 2/1	4/2	100
Ampicillin		11		1		2							≦ 0.25	4	78.6
Γazobactam-piperacillin						13	1						≦ 4/4	≦ 4/4	100
Piperacillin						13	1						≦ 4	≦ 4	100
Ceftriaxone					14								≦ 2	≦ 2	100
Cefmetazole					12	1	1						≦ 2	4	100
Cefotaxime					13	1							≦ 2	≦ 2	100
Cefoperazone					11		3						≦ 2	8	100
Imipenem			12		2								≦ 0.5	2	100
Doripenem			14										≦ 0.5	≦ 0.5	100
Tetracycline			12	1		1							≦ 0.5	1	100
Vancomycin	3						11						> 4	> 4	21.4
Metronidazole					14								≦ 2	≦ 2	100
Moxifloxacin		7		2	2	2		1					≦ 0.25	4	78.6
Clindamycin			11			2	1						≦ 0.5	4	78.6
Chloramphenicol					14								≦ 2	≦ 2	100

Porphyromonas spp. (n=2)

	MIC(μg/mL)											
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128
Sulbactam-ampicillin					2							
Ampicillin		1				1						
Γazobactam-piperacillin						2						
Piperacillin						2						
Ceftriaxone					1	1						
Cefmetazole					2							
Cefotaxime					1	1						
Cefoperazone					2							
Imipenem			2									
Doripenem			2									
Tetracycline			2									
Vancomycin						2						
Metronidazole					2							
Moxifloxacin		1	1									
Clindamycin			1						1			
Chloramphenicol					2							

Gray columns are columns outside the MIC measurement range.

3.2.3. Results of 29 strains of 13 bacterial species of Prevotella spp. (Table 4 and Supplemental Data 3)

In the *Prevotella* spp., satisfactory sensitivities were shown to ampicillin-sulbactam, piperacillin-tazobactam, cefmetazole, imipenem, metronidazole and chloramphenicol, and although some differences existed depending on the bacterial species, the sensitivities to the other drugs were low. Also, the MIC of ampicillin exhibited bimodal distribution, and it was frequently found that the strains with high MICs also showed high MICs to cephalosporin antimicrobial agents.

3.2.4. Results of 14 strains of 5 bacterial species of Fusobacterium spp. (Table 4 and Supplemental Data 4)

In the *Fusobacterium* spp., all 5 bacterial species showed similar results and exhibited satisfactory sensitivities to many drugs. In contrast, sensitivities to ampicillin and moxifloxacin differed depending on the bacterial species.

3.2.5. Results of 2 strains of 2 bacterial species of Porphyromonas spp. (Table 4 and Supplemental Data 5)

Although it was difficult to obtain an overall picture of the analysis results from only 2 strains of 2 bacterial species of *Porphyromonas* spp., they showed high susceptibility rates to many drugs. However, based on the MIC values of ampicillin and clindamycin, sensitivities were revealed in *P. gingivalis* but resistance was shown in *P. uenonis*, indicating different results.

3.2.6. Results of 20 strains of C. difficile (Table 5)

In the 20 strains of *C. difficile*, satisfactory sensitivities were found to sulbactam-ampicillin, tazobactam-piperacillin, piperacillin, ceftriaxone, vancomycin, metronidazole and chloramphenicol. However, sensitivities were low to ampicillin, cefotaxime, cefoperazone and imipenem.

4. Discussion

Of particular concern in obligate anaerobe infections are highly virulent *C. botulinum*, *C. tetani* and *C. perfringens* of the *Clostridium* spp. However, the *Clostridium* spp. bacteria often cause diseases based on their habitat due to infectious opportunities and are rarely targeted in routine microbiological testing. In contrast, although obligate anaerobic Gram-negative bacilli such as the *Bacteroides* spp. are encountered frequently in microbial examination, they are also widely known to be causative pathogens of bacteremia, sepsis, meningitis, peritonitis and respiratory infection, and their importance is stressed in infectious disease testing. Isolation rates of Gram-negative bacilli were high in the present surveillance, and the bacteria of *Bacteroides* spp. in particular comprised slightly more than half of the strains (77 of 144 strains [53.5%]). The microdilution method with dry plate used in this study is a modified method of the CLSI [10]. It is thus possible that the data obtained are not equal to those obtained with reference methods such as the agar dilution method, especially on species belonging to genera other than *Bacteroides*. However, the drug susceptibility test results accumulated for these isolates is thought to be of therapeutic importance.

The susceptibility rates of *Bacteroides* spp. and *Parabacteroides* sp. to ampicillin and piperacillin were low. It is thought that *cepA* and *cfxA* are involved in MICs of penicillin antimicrobial agents. *cepA* is a gene that is involved in resistance to cephalosporin and aminopenicillins but not to piperacillin and β-lactam derivative/β-lactamase inhibitor (BLBLIs). Meanwhile, *cfxA* is a gene involved in high-level resistance of other β-lactam derivatives [11]. The results of the present study showed the susceptibility rate of penicillin antimicrobial agents was low whereas the susceptibility rate of piperacillin-tazobactam was good, which was similar to the results of other previous reports [1,7,8,12–15].

Based on these results, most of the susceptibility rates were thought to be due to the *cepA* gene encoded on the chromosome. However, among those sensitive to piperacillin-tazobactam, there existed strains with a piperacillin MIC of >128 μg/mL, and some kind of resistance factor except for β-lactamase may be involved. There were three strains whose MICs of piperacillin and piperacillin-tazobactam were high and in which involvement of the *cfxA* gene was suggested. MICs of cephalosporins were high, but at the same time, the MIC of imipenem was also high at >16 μg/mL, suggesting the potential presence of carbapenemase. Imipenem and doripenem, the carbapenem antibiotics, showed different susceptibility rates of 97.6% and 85.7%, respectively, against *B. fragilis*, but other than those antibiotics, there was no difference in the *Bacteroides* spp., and the *Parabacteroides* sp., with susceptibility rates of 94.6% and 91.9%, respectively.

Takesue et al. [1] reported that no difference was found in the MIC_{50} and MIC_{90} of imipenem and doripenem between *Bacteroides* genera, but Karlowsky et al. [15] reported that the susceptibility rate is lower in doripenem than in imipenem. In the present report, the only bacterial species to which doripenem was less sensitive than imipenem were *B. fragilis* and *B. uniformis*, and it may be necessary to continue investigating in the future whether the susceptibility rate of doripenem decreases even in other bacterial

Table 5
Distribution of MICs for each antimicrobial agents.

Clostridioides difficile (n=20)

	MIC(μg/mL)														CLSI
	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	MIC_{50}	MIC_{90}	%S
Sulbactam-ampicillin					19	1							≦2/1	≦2/1	100
Ampicillin				5	14	1							2	2	0.0
Tazobactam-piperacillin						3	12	4	1				8/4	16/4	100
Piperacillin						2	13	4	1				8	16	100
Ceftriaxone								2	4	3	11		>64	>64	10.0
Cefmetazole								5	5	6	4		32	>64	25.0
Cefotaxime										4	16		>64	>64	0.0
Cefoperazone									5	1	14		>64	>64	0.0
Imipenem							8	7	5				16	>16	0.0
Doripenem				1	10	7	2						2	4	55.0
Tetracycline			12			2	2	3	1				≦0.5	16	70.0
Vancomycin		11	8	1									0.25	0.5	100
Metronidazole					20								≦2	≦2	100
Moxifloxacin				1	6		6	7					8	>8	35.0
Clindamycin				3	2	4	1		10				8	>16	25.0
Chloramphenicol					7	12	1						4	4	100

Gray columns are columns outside the MIC measurement range.

species. Similar to the reports of Takesue et al. [1] and Yunoki et al. [8], cefmetazole, a cephamycin antibiotic, showed a difference in susceptibility rate between *B. fragilis* and other *Bacteroides* spp., and the susceptibility rates against *B. fragilis* were 81.0% and 35.1% against the *Bacteroides* spp. including the *Parabacteroides* sp. except for *B. fragilis*. Among species of the *Bacteroides* spp. except for *B. fragilis*, there are strains with a dominantly low susceptibility rate of 6.7% such as *B. thetaiotaomicron*, and although there are only a few such strains, those with no resistance, such as *B. uniformis* or *B. vulgatus*, do exist. At present, it is unclear whether there is any resistance mechanism in these strains.

Clindamycin also had low susceptibility rates in previous surveillances, and in the present report, it had low susceptibility rates of 57.1% against *B. fragilis* and 35.1% against *Bacteroides* spp. including *Parabacteroides* sp. except for *B. fragilis*, which were similar to or lower than those in previous reports [1,7,8,12–15]. The *erm* gene involved in the resistance of clindamycin is also reported to be involved in the resistance of tetracycline at the same time [16]. In the present study, among 37 strains with a MIC >16 μg/mL of clindamycin, 19 strains (51.4%) were confirmed to have a MIC >16 μg/mL of tetracycline. Susceptibility rates of moxifloxacin against *B. fragilis* were 73.8% and 59.5% against *Bacteroides* spp. including *Parabacteroides* sp. except for *B. fragilis*, and as compared to the reports of Yunoki et al. [7,8], a remarkable decrease was found in the same Kinki area. To reveal whether the reduction in the susceptibility rate of moxifloxacin is characteristic of the Kinki area, or whether a similar reduction in susceptibility rate is found even in areas other than the Kinki area, additional accumulation of surveillance data is required in Japan. The results of metronidazole susceptibility were good against all of the bacterial species. The MIC_{50} and MIC_{90} of metronidazole against *B. fragilis* were ≤2 μg/mL and 8 μg/mL, respectively, and ≤2 μg/mL and 4 μg/mL, respectively, against other *Bacteroides* spp. The results in the present study showed 100% susceptibility rates, but it is reported that strains are present in which an increase in the MIC is not found even though the strain carries the *nim* gene, which is involved in the resistance of metronidazole [17]. Accordingly, to examine the resistance to metronidazole, it is thought that not only determination of the MIC but also genetic screening is desirable.

Prevotella spp. showed low susceptibility rates to penicillin and cephalosporin including ampicillin and ceftriaxone but 100% susceptibility rates to ampicillin-sulbactam and piperacillin-tazobactam, which are BLBLIs. *Prevotella* also showed 100% susceptibility rates to carbapenems such as imipenem and doripenem, and metronidazole, and these results were similar to those of other reports [7,8,12,18]. However, increases in MICs of cephalosporin antimicrobial agents have been reported even in *Prevotella* spp. [19,20], and as with *Bacteroides* spp., *Prevotella* spp. is also reported to be resistant to metronidazole [7,8]. Therefore, it will be necessary to note any increases in the number of isolates of resistant strains in the future.

C. difficile showed 100% susceptibility rates to both metronidazole and vancomycin. However, because a vancomycin-resistant strain has been detected in the environment [21], and metronidazole for injection has been authorized and approved in Japan, a decrease in the susceptibility rate may occur in the future and thus, continued susceptibility measurements will be necessary.

As a limitation of this study, the patient background is unknown for the strains collected this study, and strains collected after antibiotic administration may be included. Therefore, the MICs obtained may originally be higher than the MIC values of the strain. Isolates with a small number of strains, such as 10 or less strains, are strongly affected by this trend, so we believe that it will be necessary to continue collecting strains and analyzing drug susceptibility tests to accumulate additional data.

5. Conclusion

The surveillance in this study showed that β-lactam/β-lactamase inhibitors, carbapenem and metronidazole, which are used in the treatment of anaerobic infectious diseases, have maintained satisfactory rates of susceptibility. It will be necessary to conduct chemosensitive measurements and to investigate trends in the isolation rates of local resistant bacteria on a continuing basis, and, at the same time, to examine the prevalence of resistant genes in the Kinki area.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Authorship statement

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2019.07.018.

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