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Original Article

Evaluation of VITEK MS, Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card for identification of *Bacteroides fragilis* group isolates and antimicrobial susceptibilities of these isolates in a Chinese university hospital

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Antimicrobial susceptibility

Abstract *Background and purpose: Bacteroides fragilis* group isolates are most frequently isolated anaerobic pathogens. This study aimed to evaluate the accuracy of VITEK MS, Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card on the identification of clinical *B. fragilis* group isolates, as well as to determine their antimicrobial susceptibilities.
Methods: A total of 138 isolates of *B. fragilis* group isolates were identified with the three MALDI-TOF MS systems and VITEK 2 ANC cards. 16S rRNA gene sequencing was used as the reference identification method for comparison. Antimicrobial susceptibilities were determined by agar dilution method to 19 antimicrobial agents recommended by Clinical and Laboratory Standards Institute (CLSI).

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Results: Hundred thirty three isolates of *Bacteroides* spp. and 5 isolates of *Parabacteroides* spp. were identified by 16S rRNA sequencing. The rates of accurate identification at species level of VITEK MS, Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card were 94.2%, 94.2%, 98.6% and 94.9%, respectively, while that at genus level were 99.3%, 100%, 100% and 97.8%, respectively. Metronidazole and chloramphenicol were the most susceptible agents (99.3% and 92.8%, respectively), followed by meropenem, ertapenem, imipenem and piperacillin/tazobactam to which the susceptible rates ranged from 76.8% to 79.0%. The susceptible rates to carbapenems decreased 12.4—15.3% from 2010—2013 to 2014—2017.
Conclusion: All the four systems provided high accurate rate on the identification of *B. fragilis* group isolates. Metronidazole showed highest activity against these isolates. Attention should be paid to the higher resistant rates to carbapenems, clindamycin, moxifloxacin and tigecycline than the other countries.

Introduction

Bacteroides fragilis group isolates are most frequently associated with intra-abdominal, pelvic, complicated skin and soft tissue and blood stream infections.[1—3] The polymicrobial nature of these infections often complicates the isolation and identification of anaerobes. The traditional identification methods for anaerobes are time-consuming, technically complex and expensive, thus challenging in most clinical microbiology laboratories. Antimicrobial susceptibility test is usually not routinely performed, as a result, only limited susceptibility data were available for the subsequent antimicrobial therapy.

The introduction of Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) had made a revolution in the identification of microorganisms.[4,5] The two main commercial MALDI-TOF MS systems available for routine use VITEK MS (bioMérieux, Marcy l'Etoile, France) and MALDI Biotyper systems (Bruker Daltonics, Bremen, Germany) have been evaluated and validated for the identification of anaerobes previously.[6—13] Recently, two additional MALDI-TOF MS based systems Clin-ToF-II MS (Bioyong Technologies, Beijing, China) and Autof MS 1000 (Autobio Diagnostics, Zhengzhou, China), manufactured by Chinese technological companies, which have been employed mainly on the identification of aerobic bacteria and yeasts in many laboratories in China. However, their performance and application on the identification of anaerobes have not been fully evaluated. In addition, limited data is available on the antimicrobial susceptibilities of anaerobes in China.

The purpose of this study was to evaluate the accuracy of VITEK MS, Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card (bioMérieux, Marcy l'Etoile, France) on the identification of *B. fragilis* group isolates using 16S rRNA gene sequencing as reference method, and to identify the antimicrobial susceptibilities of these anaerobic isolates in a Chinese university hospital.

Methods

Bacterial isolates

The study was conducted at the department of clinical laboratory, Peking union medical college hospital in Beijing, China. A total of one hundred and thirty-eight strains of non-duplicated *B. fragilis* group isolates were collected from August 2010 to September 2017. All the isolates were stored at −80 °C. Before identification, frozen isolates were subcultured twice on Brucella blood agar (BBL™, Sparks, MD, USA) supplemented with hemin and vitamin K, in an anaerobic atmosphere produced by GENbags (bioMérieux, Marcy l'Etoile, France) at 35 °C for 48 h.

16S rRNA gene sequencing

All the isolates were identified by 16S rRNA gene sequencing. DNA was extracted by dissolving the isolates in 250 μL of sterile water and heating for 10 min at 100 °C, followed by 1 min of centrifugation at 13,000 rpm. The sample DNA was stored at 4 °C. The primers used for amplification were F27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and R1522 (5′-AAGGAGGTGATCCAGCCGCA-3′). PCR mixtures were amplified by initial holding at 94 °C for 10 min, and then 30 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 90 s. The reaction ended with a final extension at 72 °C for 10 min and a hold at 4 °C. The PCR products were purified and sequenced by the same primers above. The sequences were compared to the GenBank database by nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The criteria for genus- and species-level identifications were assigned as previously described[14]: identification at the species level (≥99% sequence identity with a reference entry), identification at the genus level (95.0—98.9% of sequence identify) and cannot be identified definitively (<95% identity to any reference sequence).

Identification by MALDI-TOF MS

All the isolates were identified by VITEK MS, Clin-ToF-II MS and Autof MS 1000 following the manufacturers' instructions. For all the three MALDI-TOF MS systems, bacterial samples were prepared by direct deposit method. Briefly, a single colony was spotted onto target slide to form a homogeneous smear, and then treated by the ready-to-use matrix solution of each brand, with α-cyano-4-

hydroxycinnamic acid (HCCA) as the main component for all the three systems. After drying at ambient temperature, the target slide was inserted in to the MALDI-TOF MS machine. Microbial identification was performed by comparing the generated spectra from the samples with the reference spectra in the database.

For VITEK MS, disposable target slides were used. The bacterial spots were treated by 1 μl of VITEK MS-CHCA matrix (3.10 g/mL HCCA in 25.57 g/mL ethanol - 25.44 g/mL acetonitrile). The sample spectra were analyzed by VITEK MS IVD database version 3.2. The calibration and quality control of every group of sixteen samples was performed using *Escherichia coli* ATCC 8739.

For Clin-ToF-II MS, a metal reusable target slide with three hundred and eighty-four sample sites was used. The bacterial spots were treated firstly by 1 μl of Bioyong pretreat agent I (acetonitrile and formic acid) and air dried, and then treated by 1 μl of Bioyong pretreat agent II (supersaturated HCCA in acetonitrile and trifluoroacetic acid) and air dried. Data was collected and analyzed by Bioyong Explore software version 3.2. The calibration and quality control was done by *E. coli* ATCC 8739 for every thirty samples. The manufacturer's interpretation criteria were applied, with a score >25 for creditable identification, a score between 20 and 25 for possible identification which need to be validated by complementary tests and a score <20 for unidentified.

For Autof MS 1000, the target slide is also a metal reusable slide with ninety-six sample sites. The bacterial spots were treated by 1 μl of Autobio sample pretreatment reagent (10 mg/mL HCCA in acetonitrile and trifluoroacetic acid). Spectra analysis was collected by software Autof Acquirer version 1.0.123 and analyzed by software Autof Analyaer version 1.0.50. The calibration was done using Autobio calibrating agent consist of nine calibrating proteins for every ninety-six samples. The manufacturer's interpretation criteria were applied, with a score >9.0 for species-level identification, a score between 6.0 and 9.0 for genus-level identification and a score <6.0 for unidentified.

Identification by VITEK 2 ANC card

Biochemical identification of all the isolates was performed by VITEK 2 Compact system using VITEK 2 ANC cards according to manufacturer's instruction. Bacterial colonies were suspended in 0.45% sodium chloride into the turbidity of 2.7–3.3 McFland, inoculated into the ANC cards and incubated in VITEK 2 Compact for about 6 h. Additional information of Gram stain, morphology and aerotolerance tests were input into software for final identification.

By MALDI-TOF MS or VITEK 2 ANC cards, isolates that cannot be identified to the species level or shown different identification results to 16S rRNA sequencing were retested for one more time. Data were categorized as follows: (I) accurate identification at the species level; (II) mixed species, including multiple species choice or low identification within genus; (III) accurate identification at the genus level but misidentification at the species level; (IV) no identification and (V) incorrect identification at genus level.

Statistical analysis

The correlation among various identification platforms were analyzed by the χ^2 and Mantel–Haenszel tests using SPSS v25.0.

Antimicrobial susceptibility testing

In vitro antimicrobial susceptibilities were determined by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) document M11-A8[15] to the following agents: ampicillin, piperacillin, ampicillin/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, cefoperazone, ceftriaxone, cefotaxime, cefmetazole, cefoxitin, ertapenem, imipenem, meropenem, moxifloxacin, clindamycin, metronidazole, chloramphenicol, tetracycline and tigecycline. *B. fragilis* ATCC 25285 and *Clostridioides difficile* ATCC700057 served as quality control strains and were run with each batch of isolates tested. The minimum inhibitory concentrations (MICs) were interpreted based on CLSI M100-28th ed (2018) document,[16] with the exception of tigecycline, which was categorized by U.S. Food and Drug Administration (FDA) breakpoints.[17]

Results

One hundred and thirty-eight isolates of *B. fragilis* group were collected in this study, of which the main source was blood cultures (n = 88, 63.8%). Other categories of clinical samples included soft tissues (n = 25, 18.1%), intra-abdominal samples (n = 22, 15.9%) and non-blood sterile body fluids (n = 3, 2.2%).

Identification evaluation

Based on the 16S rRNA sequencing results, 137 isolates were identified at species level. Among the 137 isolates, 133 belonged in 8 species in *Bacteroides* sp., while 4 belonged in 2 species in *Parabacteroides* sp.. The remaining isolate from blood culture was identified to genus level as *Parabacteroides* spp (Table 1). Autof MS 1000 provided the highest rate of accurate identification at species level (98.6%, 136/138). The rates of accurate identification of VITEK MS and Clin-Tof II were both 94.2% (130/138), and that of VITEK 2 ANC card was 94.9%. At species level, there's no statistical difference between the rate of accurate identification of VTIEK MS and those of the other three systems ($p > 0.05$). At genus level, the rates of accurate identification of Clin-ToF-II MS and Autof MS 1000 were both 100%, and those of VITEM MS and VITEK 2 ANC card were 99.3% and 97.8%, respectively. 106 out of 108 *B. fragilis* isolates and 11 *Bacteroides thetaiotaomicron* isolates were correctly identified by all the systems. Two *Bacteroides uniformis* isolates and one *Bacteroides caccae* isolate were also correctly identified at the species level by all the systems.

There were several disagreements among different systems at the level of species identification (Table 2). Four isolates of *Bacteroides ovatus* were reported as *B. ovatus*/

Table 1 Identification of 138 *Bacteroides fragilis* group isolates by VITEK MS, Clin-ToF II MS, Autof MS 1000 and VITEK 2 ANC card.

Species	No.	VITEK MS				Clin-ToF-II MS			Autof MS 1000		VITEK 2 ANC		
		Species level	Mixed species	Genus level	No ID	Species level	Mixed species	Genus level	Species level	Genus level	Species level	Genus level	No ID
Bacteroides fragilis	108	108				108			108		106	2	
Bacteroides thetaiotaomicron	11	11				11			11		11		
Bacteroides vulgatus	5	5				1	4		5		5		
Bacteroides ovatus	4	0	4			2	2		4		4		
Bacteroides uniformis	2	2				2			2		2		
Bacteroides caccae	1	1				1			1		1		
Bacteroides dorei	1	0		1		0	1		0	1			1
Bacteroides intestinalis	1	0	1			1			1			1	
Parabacteroides spp.	1	0			1	0		1	0	1			1
Parabacteroides distasonis	3	3				3			3		2	1	
Parabacteroides gordonii	1	0	1			1			1				1
Total isolates (%)	138	130 (94.2%)	6 (4.3%)	1 (0.7%)	1 (0.7%)	130 (94.2%)	7 (5.1%)	1 (0.7%)	136 (98.6%)	2 (1.4%)	131 (94.9%)	4 (2.9%)	3 (2.2%)

Table 2 Isolates misidentified at species level or reported as mixed species or no identification by VITEK MS, Clin-ToF II MS, Autof MS 1000 and VITEK 2 ANC card.

Identification by 16S rRNA sequencing (no of isolates)	Identification by VITEK MS	Identification by Clin-ToF-II MS	Identification by Autof MS 1000	Identification by VITEK 2 ANC card
Bacteroides dorei (1)	*Bacteroides vulgatus*	*Bacteroides vulgatus/dorei*	*Bacteroides vulgatus*	No identification
Bacteroides fragilis (3)	Correct identification	Correct identification	Correct identification	*Bacteroides stercoris*
Bacteroides intestinalis (1)	*Bacteroides cellulosilyticus*	Correct identification	Correct identification	*Bacteroides ovatus*
Bacteroides ovatus (2)	*Bacteroides ovatus/xylanisolvens*	*Bacteroides ovatus/xylanisolvens*	Correct identification	Correct identification
Bacteroides ovatus (2)	*Bacteroides ovatus/xylanisolvens*	Correct identification	Correct identification	Correct identification
Bacteroides vulgatus (5)	Correct identification	*Bacteroides vulgatus/dorei*	Correct identification	Correct identification
Parabacteroides spp. (1)	No identification	*Parabacteroides goldsteinii*	*Parabacteroides goldsteinii*	*Parabacteroides distasonis*
Parabacteroides distasonis (1)	Correct identification	Correct identification	Correct identification	*Bacteroides uniformis*
Parabacteroides gordonii (1)	*Parabacteroides distasonis*	Correct identification	Correct identification	No identification

Table 3 *In vitro* antimicrobial susceptibilities of 138 isolates of *Bacteroides fragilis* group.

Organism (no. of isolates) and antimicrobial agent	Breakpoint (μg/mL)			MIC (μg/mL)			%		
	S	I	R	50%	90%	Range	S	I	R
B. fragilis group (138)									
Ampicillin	≤0.5	1	≥2	>128	>128	1 to >128	0	0.7	99.3
Piperacillin	≤32	64	≥128	128	>256	2 to >256	35.5	7.2	57.2
Ampicillin/Sulbactam	≤8/4	16/8	≥32/16	4	64	0.5 to >128	65.2	9.4	25.4
Ticarcillin/Clavulanic acid	≤32/2	64/2	≥128/2	8	>256	≤4 to >256	67.4	3.6	29
Piperacillin/Tazobactam	≤16/4	32/4–64/4	≥128/4	1	256	≤0.064 to >256	76.8	2.2	21
Cefoperazone	≤16	32	≥64	128	>256	4 to >256	12.3	14.5	73.2
Ceftriaxone	≤16	32	≥64	256	>256	≤4 to >256	24.6	8	67.4
Cefotaxime	≤16	32	≥64	128	>256	≤4 to >256	34.8	9.4	55.8
Cefmetazole	≤16	32	≥64	32	128	8 to 128	41.3	12.3	46.4
Cefoxitin	≤16	32	≥64	16	64	2 to 128	66.7	14.5	18.8
Ertapenem	≤4	8	≥16	1	128	0.064 to >256	78.3	2.2	19.6
Imipenem	≤4	8	≥16	0.5	>256	0.032 to >256	77.5	0	22.5
Meropenem	≤4	8	≥16	0.25	256	0.064 to >256	79	0	21
Moxifloxacin	≤2	4	≥8	2	32	0.032 to >32	53.6	9.4	37
Clindamycin	≤2	4	≥8	64	64	1 to >32	8	2.2	89.9
Metronidazole	≤8	16	≥32	1	2	0.25 to 32	99.3	0	0.7
Chloramphenicol	≤8	16	≥32	8	8	4 to 64	92.8	5.1	2.2
Tetracycline	≤4	8	≥16	64	64	0.25 to >32	4.3	0.7	94.9
Tigecycline	≤4	8	≥16	4	16	0.125 to 32	63.8	13.8	22.5
B. fragilis (108)									
Ampicillin	≤0.5	1	≥2	>128	>128	2 to >128	0	0	100
Piperacillin	≤32	64	≥128	128	>256	2 to >256	34.3	8.3	57.4
Ampicillin/Sulbactam	≤8/4	16/8	≥32/16	4	128	0.5 to >128	64.8	6.5	28.7
Ticarcillin/Clavulanic acid	≤32/2	64/2	≥128/2	8	>256	≤4 to >256	67.6	1.9	30.6
Piperacillin/Tazobactam	≤16/4	32/4–64/4	≥128/4	0.5	256	≤0.064 to >256	75	0.9	24.1
Cefoperazone	≤16	32	≥64	128	>256	4 to >256	13	15.7	71.3
Ceftriaxone	≤16	32	≥64	256	>256	≤4 to >256	28.7	6.5	64.8
Cefotaxime	≤16	32	≥64	128	>256	≤4 to >256	36.1	6.5	57.4
Cefmetazole	≤16	32	≥64	32	128	8 to 128	47.2	10.2	42.6
Cefoxitin	≤16	32	≥64	8	64	4 to 128	69.4	9.3	21.3
Ertapenem	≤4	8	≥16	1	128	0.064 to >256	73.1	1.9	25
Imipenem	≤4	8	≥16	0.5	>256	0.064 to >256	73.1	0	26.9
Meropenem	≤4	8	≥16	0.25	256	0.064 to >256	73.1	0	26.9
Moxifloxacin	≤2	4	≥8	2	32	0.125 to >32	53.7	9.3	37
Clindamycin	≤2	4	≥8	64	64	1 to >32	10.2	0.9	88.9
Metronidazole	≤8	16	≥32	1	1	0.25 to 32	99.1	0	0.9
Chloramphenicol	≤8	16	≥32	8	8	4 to 64	97.2	0	2.8
Tetracycline	≤4	8	≥16	64	64	0.25 to >32	3.7	0.9	95.4
Tigecycline	≤4	8	≥16	4	16	0.125 to 32	63	14.8	22.2
B. thetaiotaomicron (11)									
Ampicillin	≤0.5	1	≥2	>128	>128	32 to >128	0	0	100
Piperacillin	≤32	64	≥128	>256	>256	32 to >256	18.2	9.1	72.7
Ampicillin/Sulbactam	≤8/4	16/8	≥32/16	8	16	1 to 32	54.5	36.4	9.1
Ticarcillin/Clavulanic acid	≤32/2	64/2	≥128/2	16	128	≤4 to 128	72.7	9.1	18.2
Piperacillin/Tazobactam	≤16/4	32/4–64/4	≥128/4	16	32	4 to 128	81.8	9.1	9.1
Cefoperazone	≤16	32	≥64	256	>256	32 to >256	0	9.1	90.9
Ceftriaxone	≤16	32	≥64	256	>256	64 to >256	0	0	100
Cefotaxime	≤16	32	≥64	128	>256	16 to >256	9.1	18.2	72.7
Cefmetazole	≤16	32	≥64	64	128	16 to 128	18.2	27.3	54.5
Cefoxitin	≤16	32	≥64	32	64	8 to 64	36.4	45.5	18.2
Ertapenem	≤4	8	≥16	1	4	0.5 to 4	100	0	0
Imipenem	≤4	8	≥16	1	4	0.25 to 4	100	0	0
Meropenem	≤4	8	≥16	0.5	1	0.125 to 4	100	0	0
Moxifloxacin	≤2	4	≥8	2	16	0.125 to >32	63.6	9.1	27.3
Clindamycin	≤2	4	≥8	64	64	4 to >32	0	9.1	90.9

Table 3 (*continued*)

Organism (no. of isolates) and antimicrobial agent	Breakpoint (μg/mL)			MIC (μg/mL)			%		
	S	I	R	50%	90%	Range	S	I	R
Metronidazole	≤8	16	≥32	1	2	1 to 8	100	0	0
Chloramphenicol	≤8	16	≥32	8	16	4 to 16	63.6	36.4	0
Tetracycline	≤4	8	≥16	32	64	16 to >32	0	0	100
Tigecycline	≤4	8	≥16	4	16	0.25 to 32	72.7	9.1	18.2

xylanisolvens by VITEK MS, while 2 of them were identified as *B. ovatus/xylanisolvens* by Clin-ToF-II MS. All 5 isolates of *Bacteroides vulgatus* were identified as mixed *Bacteroides vulgatus/dorei* by Clin-ToF-II MS. One isolate of *Bacteroides intestinalis* was detected as *Bacteroides cellulosilyticus* and *B. ovatus* by VITEK MS and VITEK 2 ANC card, respectively; however, this species was not included in the database of the two systems. *Parabacteroides gordonii*, which was not covered by the database of the two systems, was detected as *Parabacteroides distasonis* by VITEK MS, and reported as no identification by VITEK 2 ANC card. Two isolates of *B. fragilis* were detected as *Bacteroides stercoris* and 1 isolate of *P. distasonis* was misidentified as *B. uniformis* by VITEK 2 ANC card, but they were all correctly identified at the species level by the other three MALDI-TOF MS systems. *Bacteroides dorei* was poorly identified in this study. VITEK MS, the only system had this species in the database, reported it as *B. vulgatus*. Clin-ToF-II MS reported it as *B. vulgatus/dorei*. Without *B. dorei* in their databases, Autof MS 1000 reported it as *B. vulgatus*, and VITKE 2 ANC card reported no identification. For the one isolate of *Parabacteroides* spp. identified based on 16S rRNA sequencing, VITEK MS reported no identification, both Clin-ToF-II MS and Autof MS 1000 reported it as *Parabacteroides goldsteinii*, in contrast, VITEK 2 ANC card reported it as *P. distasonis*.

Antimicrobial susceptibilities

Of the nineteen antimicrobial agents tested against *B. fragilis* group isolates (Table 3), metronidazole and chloramphenicol were the most active agents with susceptible rates of 99.3% and 92.8%, respectively, followed by meropenem, ertapenem, imipenem and piperacillin/tazobactam, to which susceptible rates ranged from 76.8% to 79.0%. Thirty-three isolates including 30 isolates of *B. fragilis*, 2 isolates of *P. distasonis* and 1 isolate of *Parabacteroides* spp. were non-susceptible to carbapenems. The susceptible rates of carbapenem-non-susceptible isolates against all the β-lactams were no more than 12.1% (Fig. 1). All the isolates of *B. thetaiotaomicron* were carbapenem susceptible. Ticarcillin/clavulanic acid, ampicillin/sulbactam, cefoxitin, tigecycline and moxifloxacin showed only mild antimicrobial activities (53.6%—67.2% susceptible). Of all the isolates tested, only one isolate was resistant to metronidazole; it was also resistant to carbapenems, but susceptible to clindamycin and chloramphenicol. Ampicillin, tetracycline and clindamycin had the highest resistant rates, 99.3%, 94.9% and 89.9%, respectively.

Comparison of susceptible rates of *B. fragilis* group isolates in 2010—2013 and 2014—2017 showed that the antimicrobial susceptibilities to β-lactams, clindamycin, chloramphenicol and tetracycline decreased over time (Fig. 2). The susceptible rates to carbapenems decreased from 85.3% in 2010—2013 to 70.0—72.9% in 2014—2017, and those to β-lactam/β-lactamase inhibitors declined from 75.0 to 85.3% in 2010—2013 to 55.7—68.6% in 2014—2017. The susceptible rate of cefoxitin showed the largest decline in the antibiotics tested for 31.0%. In contrast, susceptible rates of moxifloxacin and tigecycline increased 10.1% and 15.5%, respectively.

Discussion

Accurate identification of pathogen is important to correct disease diagnosis and proper patient treatment. In this study, we evaluated the performance of the VITEK MS v3.2,

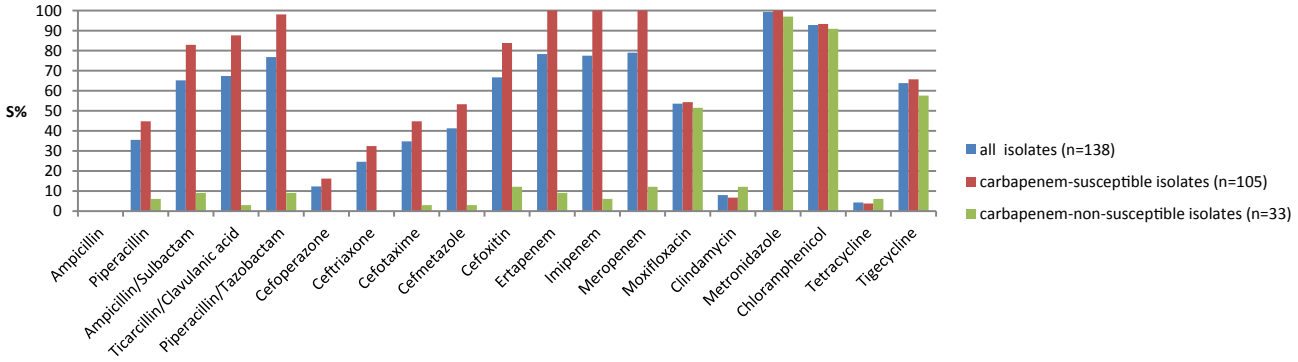

Figure 1. Comparison of susceptible rates between carbapenem-susceptible isolates (n = 105), carbapenem-non-susceptible isolates (n = 33) and all the isolates (n = 138) of *Bacteroides fragilis* group.

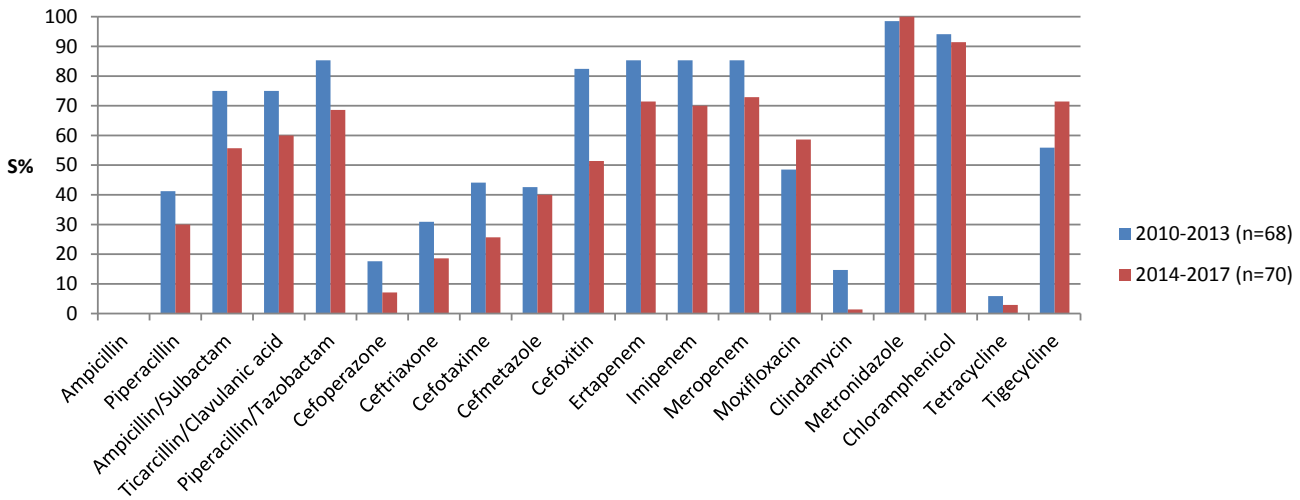

Figure 2. Comparison of susceptibility rates of *Bacteroides fragilis* group isolates from 2010 to 2013 (n = 68) and 2014–2017 (n = 70).

Clin-ToF-II MS, Autof MS 1000 and VITEK 2 ANC card in the identification of clinical *B. fragilis* group isolates, using 16S rRNA gene sequencing as the reference method. Overall, more than 94% of isolates could be correctly identified by the four systems.

In an earlier multicenter evaluation of VITEK MS v2.0 with 263 isolates of *Bacteroides* spp., in which *B. caccae* (n = 30), *B. fragilis* (n = 71), *Bacteroides thetaiotamicron* (n = 51) and *B. vulgatus* (n = 41) showed high rates of species level identification (>93%). In contrast, *B. ovatus* (n = 40) was 85% correct and *B. uniformis* (n = 30) was only 73.3% correct at species level.[18] In another evaluation of VITEK MS v1.1 with 142 isolates of *Bacteroides* spp. and *Parabacteroides* spp., all the isolates of *B. fragilis* (n = 92), *B. thetaiotaomicron* (n = 26) and *P. distasonis* (n = 3) tested were correctly identified at the species level.[8] In our study, three systems of MALDI-TOF MS also provided accurate identification at species level for all the isolates of *B. fragilis* (n = 108) and *B. thetaiotaomicron* (n = 11). VITEK MS and Autof MS 1000 gave accurate identification at species level for all the isolates of *B. vulgatus* (n = 5). Although there are only 2 isolates of *B. uniformis* and 1 isolate of *B. caccae*, all the three MALDI-TOF MS systems identified them correctly at species level. Comparing to database v2.0, Whether VITEK MS have improved the identification accuracy for *B. uniformis* in database v3.2 or not still need further evaluation with more isolates. Similar to our results, Zheng X et al.[19] also reported the misidentification of *B. dorei* by VITEK MS. In the same study, VITEK MS correctly identified more *Bacteroides* spp. isolates compared with VITEK 2 ANC card at the species level (32 vs. 23 in 37 isolates, respectively). But another report[20] and our study showed both VITEK MS and ANC card had similar high rates of correct identification at species level for *B. fragilis* group (91.1% vs. 91.1%, 94.2% vs. 94.9%, respectively).

Comparing to VITEK MS, Clin-ToF II MS achieved the same accuracy at species level identification (94.2%). Autof MS 1000 gave the highest rate of accurate identification (98.6%) at species level. Both Clin-ToF II MS and Autof MS 1000 correctly identified all the isolates at genus level. The number of species in the database of VITEK MS, Clin-ToF II MS and Autof MS 1000 were 13, 30 and 19, respectively. So Clin-ToF II MS and Autof MS 1000 could identify more species compared to VITEK MS v3.2. All the species of *Bacteroides* spp. and *Parabacteroides* spp. in Autof MS 1000 database were listed as single species. But Clin-ToF II MS might report mixed species results for *B. caccae/capillosus/fragilis*, *Bacteroides capillosus/fragilis*, *Bacteroides finegoldii/nordii/salyersiae* and *B. ovatus/xylanisolvens*. According to the manufacture's instruction, Bioyong Explore 3.2 database of Clin-ToF II MS covered 370 genera, 2200 species and spectra of 8100 strains in total, and Autof Analyaer 1.0.50 database of Autof MS 1000 covered 474 genera, 2613 species and spectra of 9531 strains. Their abilities of identification of other taxa of anaerobes require further evaluation.

In this study, the antimicrobial resistance rates to most tested antibiotics are much higher than recent reports in other countries, especially to carbapenems (19.6–22.5% resistance), which may be a great concern in clinical practice. Even worse, the susceptible rates to carbapenems decreased rapidly (12.4–15.3%) from 2010 to 2013 to 2014–2017. The rate of resistance in *Bacteroides* spp. and *Parabacteroides* spp. isolated from US to ertapenem, imipenem and meropenem were 1.8%, 0.8% and 1.5%, respectively during 2010–2012.[21] CANWORD surveillance in Canada during 2010–2011 had similar results, with 0.5% resistant to ertapenem and 0.8% resistant to imipenem.[17] The resistant rate to meropenem of *Bacteroides* spp. and *P. distasonis* isolated from 2010 to 2016 in Europe ranged from 0% to 1.7%.[22] In a study from a Russia cancer research center during 2004–2014, 95.5% isolates of *B. fragilis* group were susceptible to imipenem.[23] A Japan survey in 2014 showed the susceptible rates of *B. fragilis* to imipenem and meropenem were 96.3% and 92.5%, respectively, and that of *Bacteroides* spp. other than *B. fragilis* and *Parabacteroides* spp. were 96.4% and 95.5%, respectively.[24] Resistant rates to imipenem and meropenem of *Bacteroides* spp. from Korean in 2012 were also very low at

0—6%.[25] Our study showed that the carbapenem-non-susceptible isolates were highly resistant to all the other β-lactams, which make the resistance rates of the total isolates to other β-lactams and β-lactam/β-lactamase inhibitors also much higher than the above areas.[17,21—25] In accordance with our data, a study in Belgium showed 23% of *B. fragilis* isolates were non-susceptible to meropenem, with 6% of decline in susceptible rate from 2004 to 2011—2012.[26]

Metronidazole-resistant rate of *B. fragilis* group isolates in the past studies ranged from 0% to 1.6%.[17,21—26] Only one isolate of *B. fragilis* in our study was resistant to metronidazole and carbapenems, but it was susceptible to clindamycin and chloramphenicol. It was isolated in abdominal fluid from a biliary tract infection patient. Metronidazole-resistant *B. fragilis* have been reported all over the world, in which the existence of *nim* genes worked as the resistance mechanism for majority cases.[27]

89.9% of isolate were resistant to clindamycin in this study, which was much higher than the data from other countries. The resistant rates of clindamycin in US,[21] Canada,[17] Europe,[22,26,28] Japan[24] and Korean[25] were 22.1—52%, and that in Russia was 77.6%.[23] The resistant rate of moxifloxacin (37.0%) was somewhat similar but still higher that the reports above, which were 28.5%, 33.8%, 12—25% and 20.6—25.5% resistant in US,[21] Canada,[17] Japan[24] and Korean,[25] respectively. In a recent report from T.E.S.T. program in Europe during 2010—2016, 50% of gram negative anaerobes gave tigecycline MICs between 0.06 and 1 μg/mL.[22] In our study, the MIC_{50} and MIC_{90} are 4 μg/mL and 16 μg/mL, respectively. According to US FDA breakpoint, isolates with MICs $\geq$ 16 μg/mL for tigecycline were supposed to be resistant, which accounted for 22.5% of our collection. But in US,[21] Canada[17] and Korean,[25] the resistant rate to tigecycline were only 2.4%, 8.0% and 7%, respectively.

B. fragilis group isolates (>20 species) have been the most frequently isolated anaerobic pathogens and supposed to be the most virulent anaerobes.[20,29,30] In anaerobic bacteremia, *B. fragilis* group isolates accounted for 38.1—61.0% of the anaerobes,[31—33] so that the improvement of accuracy in identification of them will significantly improve the pathogen diagnosis of anaerobe infections. API 20A (bioMérieux, Marcy l'Etoile, France), VITEK 2 ANC card and VITEK MS are the main kits/systems used to identify anaerobes in China. This is the first report of comparison of two Chinese local brand MALDI-TOF MS systems, Clin-ToF II MS and Autof MS 1000, to VITEK MS and VITEK 2 ANC card. Our study had some limitations, because *B. fragilis* accounted for 73.8% of our collection. Only one isolate of *B. caccae, B. dorei, B. intestinalis* and *P. gordonii* was available in this investigation. Additional anaerobic species and isolates should be included as part of validation for species identification by commercial systems, especially for Clin-ToF II MS and Autof MS 1000. The antimicrobial susceptibilities should also be evaluated for more isolates from China to meet the demand of clinicians and microbiologists.

In conclusion, all the four systems provided accurate identification at species level (>94% of isolates). Metronidazole is still the most susceptible agent to *B. fragilis* group in China, but the higher resistance to carbapenems, clindamycin, moxifloxacin and tigecycline in our collection compared to other countries, especially the rapid decline of antimicrobial susceptibilities to most β-lactams, could be a great public health concern on empirical antimicrobial therapy choice.

Conflicts of interest statement

All contributing authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2018.12.009.