Comparison of the Etest and Sensititre methods for anaerobe susceptibility testing

Karin Puechler[1], Katia Scalzo[2], Maira Nicoletti[2], Stefanie Wieser[1], Richard Aschbacher[2], Elisabetta Pagani[2*]

[1]Scuola Provinciale Superiore di Sanità, Claudiana, Bolzano, Italy; [2]Laboratorio Aziendale di Microbiologia e Virologia, Bolzano, Italy

Summary

Background and Aims: Antimicrobial susceptibility testing of anaerobic clinical isolates is of paramount importance for patient therapy and resistance monitoring. In our laboratory the MIC gradient Etest method and broth microdilution with Sensititre trays are used for susceptibility testing of anaerobes and the aim of this study was to compare the two methods on a panel of anaerobes routinely isolated from patients in the province of Bolzano, Italy.

Materials and Methods: Totally, 74 non-repetitive Gram-positive and Gram-negative patient isolates were tested with Etest strips on Fastidious Anaerobe Agar (F.A.A.) and with Sensititre trays, according to manufacturer's instructions. Interpretation of MICs was by EUCAST or CLSI criteria, resistance percentages were calculated and Categorical Agreement (CA) and Essential Agreement (EA) between the two methods were determined.

Results: Of the 74 isolates, 68 (91.9%) grew on both systems and agreement for these was compared in the study. CA for all isolates was ≥90% for all tested antibiotics except moxifloxacin, whereas EA was generally lower. Resistance was generally low, except for clindamycin in all isolates and tigecycline in Gram-negatives. In our study Etest was a superior and more handy method.

Conclusions: To conclude, we believe the Etest method is more suitable for routine diagnostic laboratory usage. Nevertheless, multicenter studies are required to evaluate the two methods for anaerobic susceptibility testing.

Correspondence: Elisabetta Pagani, Laboratorio Aziendale di Microbiologia e Virologia, Bolzano, Italy.
Tel. +39 471439627.
Fax. +39 471435159.
E-mail: elisabetta.pagani@sabes.it

Key words: anaerobes; bacteroides; clostridium; Etest; Sensititre; agreement.

Authors' contributions: all the authors made a substantive intellectual contribution. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no potential conflict of interest.

Availability of data and materials: all data generated or analyzed during this study are included in this published article.

Funding: none.

Received: 3 December 2022.
Accepted:17 February 2023.

Licensee PAGEPress, Italy
Microbiologia Medica 2023; 38:11056
doi:10.4081/mm.2023.11056

Introduction

Antimicrobial susceptibility testing of anaerobes is important for providing individual and cumulative susceptibility results to guide empirical or directed therapy and monitor antimicrobial resistance [4]. It is recommended that susceptibility testing be performed on organisms from sterile sites, those isolated in pure culture, and on isolates that are clinically relevant and have unpredictable susceptibility patterns [2].

Current available methods for susceptibility testing of anaerobes include agar dilution, broth microdilution, MIC gradient diffusion, the spiral gradient endpoint technique and the disc diffusion method [11,12]. Clinical and Laboratory Standards Institute (CLSI) recommends agar dilution as reference "gold standard" and, for *Bacteroides fragilis* group only, gives broth microdilution as an alternative [4], whereas European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends agar dilution as reference method, providing breakpoints also for disc diffusion and for not specified MIC methods, recommending to follow manufacturer's instructions for MIC products/devices [7,8]. The agar dilution reference method to determine MIC values for anaerobes is reserved to specialized centers and is not suitable for routine use [2]. Broth microdilution and gradient diffusion are two attractive alternative methods. In a recent nationwide Italian survey on antimicrobial susceptibility of anaerobic bloodstream isolates, agar gradient tests were used by 15 centers, while four centers used broth microdilution systems [6].

In our laboratory the MIC gradient Etest method (BioMerieux,

Marcy l'Etoile, France) and broth microdilution with Sensititre trays (Thermo Fisher Scientific, Waltham, MA, USA) are used for routine susceptibility testing of anaerobes and the aim of this study was to compare the two methods on a panel of anaerobe pathogens routinely isolated from patients in the province of Bolzano, Italy.

Materials and Methods

Anaerobic bacteria were cultured on Schaedler Anaerobe Agar with Sheep Blood (Thermo Fisher Scientific, Waltham, MA, USA). Organisms were subcultured for purity and identified using MALDI-Tof Biotyper Sirius (Bruker Daltonics, Bremen, Germany), according to manufacturer's instructions; this system is also able to identify the cfiA phenotype (encoding an Ambler class B metallo-β-lactamase) of *B. fragilis*. Isolates from previous years were stored in the laboratory culture collection at -80°C (Cryobeads, Thermo Fisher Scientific).

Etest (BioMerieux) was done according to manufacturer's instructions, placing the organism in Mueller-Hinton cation-adjusted broth (Thermo Fisher Scientific, Cleveland, Ohio, USA) and adjusting to a 1.0 McFarland standard. Fastidious Anaerobe Agar (F.A.A.) plates (Thermo Fisher Scientific) were inoculated by using the RETRO C80 semiautomatic inoculator, strips were placed in the centre of each plate with the NEMA 80 system and incubated at 36±1°C for 48 h using the AnaeroGenTM system (Oxoid, Basingstoke, Hampshire, England). Reading of MICs was done according to manufacturer's indications. MIC values between doubling dilutions were rounded up to the nearest doubling dilution.

Broth microdilution with Sensititre FRAM1ANA trays (validated for *Bacteroides spp.*) was done according to manufacturer's instructions, using pre-reduced supplemented Brucella broth tubes for anaerobes for inoculation into the wells. FRAM1ANA plates contain the following antibiotics: amoxicillin, amoxicillin/clavulanic acid (2/1), chloramphenicol, clindamycin, linezolid, metronidazole, moxifloxacin, penicillin G, piperacillin, piperacillin/tazobactam, rifampicin, tigecycline and vancomycin. The panels were inoculated with the Automated Inoculation Delivery System (AIM), incubated anaerobically at 36±1°C for 48 h using the AnaeroGenTM system (Oxoid) and read with the Vizion Digital MIC Viewing System (Thermo Fisher Scientific). All antibiotics tested with Sensititre, except amoxicillin, chloramphenicol and piperacillin, were also tested with the Etest method.

Quality control for anaerobic growth and MIC determination withEtest and Sensititre was performed with *B. fragilis* ATCC 25285 and measured MICs were within the expected ranges.

The Carbapenem Inactivation Method (CIM) was used for evaluation of carbapenemase activity in a cfiA positive *B. fragilis* isolate (identified by MALDI-Tof), using a 4 h incubation time [13].

For the interpretation of susceptibility results EUCAST breakpoints were used for antibiotics with defined EUCAST breakpoints for anaerobes (piperacillin/tazobactam, clindamycin, metronidazole, penicillin G, vancomycin), whereas for antibiotics without defined EUCAST breakpoints for anaerobes CLSI interpretative criteria were used (amoxicillin/clavulanic acid, chloramphenicol, imipenem, moxifloxacin) [5,7,8]. Amoxicillin for Gram-positive isolates was interpreted using CLSI breakpoints for amoxicillin/clavulanic acid, whereas interpretation was not done for Gram-negatives because amoxicillin is not recommended for primary testing and reporting and *Bacteroides spp.* is considered intrinsically resistant [5]. For antibiotics without approved EUCAST or CLSI breakpoints for anaerobes S/I/R interpretation (and without approved method for MIC determination), MIC interpretation was done applying EUCAST PK/PD (pharmacokinetic/pharmacodynamic) breakpoints (linezolid, tigecycline) and for antibiotics without PK/PD breakpoints MICs were not interpreted (rifampicin).

Essential Agreement (EA) and Categorical Agreement (CA) were evaluated as established by the International Organization for Standardization (ISO) [10]. EA was calculated comprising MIC values due to truncations in the tested concentration range for the Sensititre method. Acceptable correlation performance, ≥90% for EA and CA, was evaluated according to the ISO criteria [10].

Statistical significance for agreement and antibiotic resistance comparisons, defined as $p \leq 0.05$, was calculated with MedCalc® software Version 17.4.4, and 95% confidence intervals were determined by the web-based graphpad software for confidence intervals for proportions (https://www.graphpad.com/quickcalcs/ConfInterval1.cfm).

Results

Seventy-four non-repetitive routine anaerobic bacterial isolates, 62 of them collected in 2022 and 12 in previous years, all from patients in the Province of Bolzano, were included in the study. Species distribution of the isolates is shown in Table 1. Specimens were from wounds/pus/ulcera (37 isolates), blood (14 isolates), fluids (3 isolates) and various other sample types (46 isolates).

Of the 74 isolates, 6 (8.1%) grew on the F.A.A. agar for Etest but they repeatedly did not grow in Sensititre plates within 48 h (2 *Fusobacterium necrophorum*, 1 *Veillonella atypica*, 1 *Veillonella dispar*, 2 *Clostridium paraputrificum*).

Essential Agreement (EA) and Categorical Agreement (CA) for all of the 68 isolates (Gram-positives + Gram-negatives) grown on Etest plates and in Sensititre panels is shown in Figure 1; CA for all isolates was >90% for all tested antibiotics except moxifloxacin (CA=83.8%). For Gram-positive isolates moxifloxacin, piperacillin/tazobactam, penicillin and clindamycin and for Gram-negatives moxifloxacin had CA values <90% (Figure 2). CA values for Gram-positive and Gram-negative isolates were not significantly different. EA values for all isolates (Figure 1) and for Gram-positive

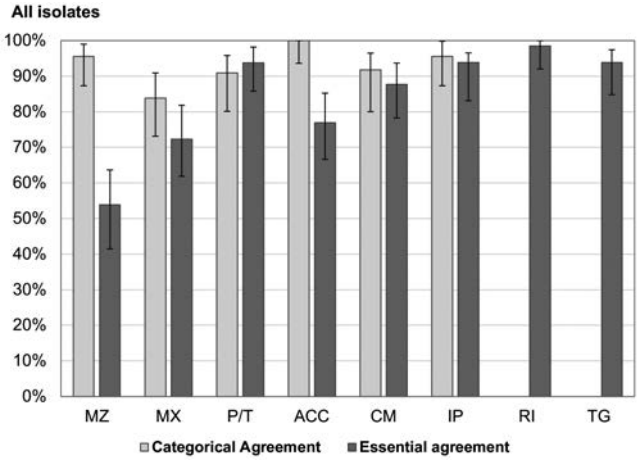

Figure 1. Categorical agreement and essential agreement for 68 Gram-positive and Gram-negative isolates. For piperacillin/tazobactam (P/T) comparison has been done for 64 isolates. CA has not been determined for rifampicin (RI) and tigecycline (TG). MZ, metronidazole; MX, moxifloxacin; ACC, amoxicillin/clavulanic acid; CM, clindamycin; IP, imipenem. Error bars indicate 95% confidence intervals.

and Gram-negative isolates (Figure 2) were generally lower, but the differences were statistically significant only for metronidazole (for all isolates and Gram-negatives p<0.001, for Gram-positives p=0.004) and amoxicillin/clavulanic acid (for all isolates and Gram-negatives p<0.001). EA values for Gram-positive isolates were generally higher compared with values for Gram-negative isolates (Figure 2), but the differences were statistically significant only for metronidazole (p=0.001), amoxicillin/clavulanic acid (p=0.005) and moxifloxacin (p=0.009).

For *Bacteroides spp.* and *Prevotella spp.* (9 isolates) CA between Etest and Sensititre was ≥90% for all antibiotics except moxifloxacin. On the other hand, the lowest EA value of all generaantibiotic combinations was found for *Bacteroides spp*. isolates tested with metronidazole (11%), followed by *Prevotella spp.* tested with amoxicillin/clavulanic acid (22%), moxifloxacin (44%) and metronidazole (56%) (Table 2).

For *Clostridium spp.* (10 isolates) CA was ≥90% for all antibiotics except metronidazole, piperacillin/tazobactam, imipenem and linezolid, whereas EA values were ≥90% for all antibiotics except moxifloxacin, penicillin, amoxicillin/clavulanic acid, imipenem and linezolid. Intrinsic vancomycin resistance in the single *Clostridium innoquum* isolate was confirmed with both methods. CA and EA of the four tested *Clostridioides difficile* isolates was 100% for metronidazole and vancomycin (Table 2).

For *Actinomyces spp.* / *Actinotignum schaalii* (13 isolates) all antibiotics except clindamycin showed CA values ≥90%, whereas EA was ≥90% for all antibiotics except moxifloxacin, penicillin and clindamycin (Table 2).

Resistance percentages for all isolates were below 20%, except for clindamycin and tigecycline and differences between Etest and

Table 1. Genus and species distribution of the 74 isolates included in the study.

Genus and species (number of isolates)
Bacteroides spp. (18)
B. fragilis (11)
B. thetaiotaomicron (5)
B. vulgatus (1)
B. nordii (1)
Clostridium spp. (12)
C. putrificum (3)
C. paraputrificum (2)
C. perfringens (2)
C. clostridioforme (1)
C. glycolicum (1)
C. innocuum (1)
C. septicum (1)
Clostridium spp. (1)
Prevotella spp. (11)
P. bivia (3)
P. buccae (2)
P. disiens (2)
P. melaninogenica (2)
P. nigrescens (1)
P. bergensis (1)
Actinomyces spp./Actinotignum spp. (10)
A. schaalii (4)
A. turicensis (1)
A. europeus (1)
A. naeslundii (1)
A. neuii (1)
A. odontolyticus (1)
A. funkei (1)
Fusobacterium spp. (6)
F. necrophorum (3)
F. nucleatum (2)
F. periodonticum (1)
Clostridioides difficile (4)
Parvimonas micra (3)
Finegoldia magna (3)
Veillonella spp. (2)
V. atypica (1)
V. dispar (1)
Peptostreptococcus anaerobius (1)
Anaerococcus vaginalis (1)
Leptotrichia trevisanii (1)
Cutibacterium acnes (1)
Propionibacterium granulosum (1)

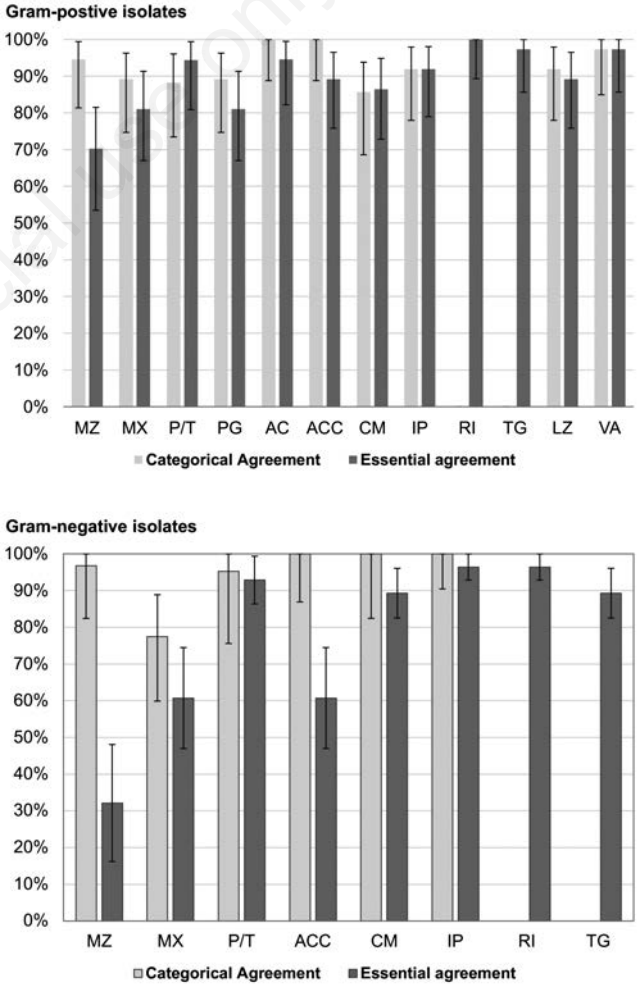

Figure 2. Essential agreement and categorical agreement for 37 Gram-positive (top) and 31 Gram-negative (bottom) isolates. Penicillin (PG), amoxicillin (AC), linezolid (LZ) and vancomycin (VA) have not been tested for Gram-negative isolates. CA has not been determined for rifampicin (RI) and tigecycline (TG). MZ, metronidazole; MX, moxifloxacin; ACC, amoxicillin/clavulanic acid; CM, clindamycin; IP, imipenem. Error bars indicate 95% confidence intervals.

Sensititre were not statistically significant (Figure 3). Differences in resistance between Gram-positive and Gram-negative isolates, tested with Etest, were significant only for tigecycline (7.7% for Gram-positives and 54.3% for Gram-negatives, p<0,001) (Figure 4).

One *B. fragilis* blood isolate, resistant to imipenem and meropenem by Etest (showing a heteroresistance phenotype) and resistant to imipenem by Sensititre was identified as cfiA positive by MALDI-Tof and carbapenemase activity was confirmed by the CIM test; the isolate was resistant to piperacillin/tazobactam and intermediate to amoxicillin/clavulanate (MIC = 8 mg/l, CLSI breakpoint).

All isolates tested with Sensititre had chloramphenicol MICs ≤8 mg/l, interpreted as susceptible according to CLSI breakpoints. Rifampicin MICs tested with Etest were generally low, with MIC_{50} ≤0.06 mg/l and MIC_{90} = 0.5 mg/l (Sensititre MIC range: 1-64 mg/l), but EUCAST or CLSI breakpoints for anaerobes have not been defined.

Discussion

In this study by comparing two commercially available methods, Etest strips on F.A.A. agar plates and broth microdilution Sensititre trays, we determined MICs of 74 anaerobes for various antibiotics (12 for Gram-positives, 9 for Gram-negatives), isolated from patients in the province of Bolzano, Italy. For 8.1% of isolates growth was evident only on Etest plates. Limited growth of anaerobes in Sensititre trays has been noted by other authors [9].

In our study CA between Etest and Sensititre for all isolates was >90% for all tested antibiotics except moxifloxacin, whereas EA values were generally lower. Similar results to our study for the comparison of Etest and Sensititre, testing various Gram-negative clinical anaerobic isolates, have been found by other authors, with CA ≥90% for clindamycin, metronidazole, piperacillin/tazobactam,

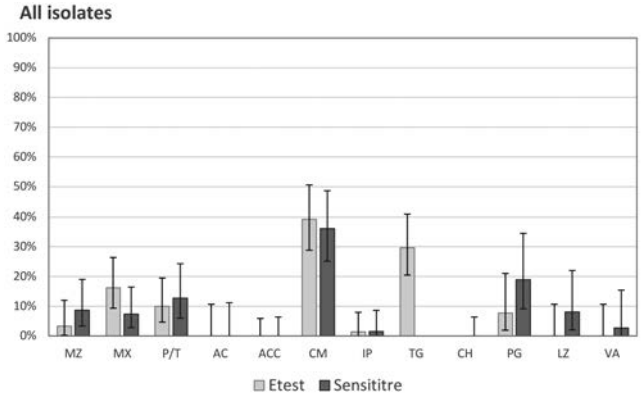

Figure 3. Resistance percentages for all isolates tested by Etest and Sensititre. 74 isolates were tested with Etest and 68 with Sensititre. Chloramphenicol (CH) was tested only with Sensititre. Tigecycline (TG) was interpreted only for Etest and piperacillin/tazobactam (P/T) was interpreted for 72 isolates with Etest and for 64 isolates with Sensititre. Actinomyces spp., *Actinotignum schaalii*, *Propionibacterium spp.* e *Cutibacterium spp.*, naturally resistant to metronidazole (MZ) were excluded from the analysis of metronidazole. *Clostridium innoquum*, naturally resistant to vancomycin, was excluded from analysis of vancomycin. Penicillin (PG), amoxicillin (AC), linezolid (LZ) and vancomycin (VA) have been tested only for Gram-positive isolates. MX, moxifloxacin; ACC, amoxicillin/clavulanic acid; CM, clindamycin; IP, imipenem. Error bars indicate 95% confidence intervals.

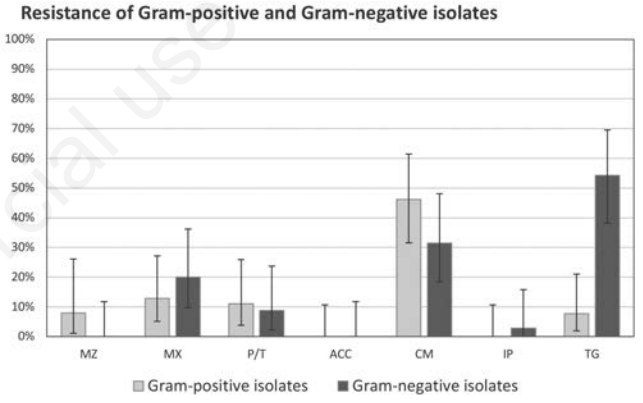

Figure 4. Resistance percentages with Etest for Gram-positive and Gram-negative isolates. *Actinomyces spp.*, *Actinotignum schaalii*, *Propionibacterium spp.* e *Cutibacterium spp.*, naturally resistant to metronidazole (MZ) were excluded from the analysis of metronidazole. MX, moxifloxacin; P/T, piperacillin/tazobactam; ACC, amoxicillin/clavulanic acid; CM, clindamycin; IP, imipenem; TG, tigecycline. Error bars indicate 95% confidence intervals.

Table 2. Categorical agreement and essential agreement for various genera of anaerobes.

		MZ	MX	P/T	PG	AC	ACC	CM	IP	RI	TG	LZ	VA
Bacteroides spp. (18)	CA	100%	83%	94%	/	/	100%	100%	100%	N.I.	N.I.	/	/
	EA	11%	78%	94%	/	/	72%	89%	94%	100%	78%	/	/
Prevotella spp. (9)	CA	100%	56%	N.I.	/	/	100%	N.I.	100%	N.I.	N.I.	/	/
	EA	56%	44%	100%	/	/	22%	89%	100%	100%	100%	/	/
Clostridium spp. (10)	CA	80%	90%	78% (9)	90%	100%	100%	90%	80%	N.I.	N.I.	80%	90%
	EA	100%	80%	90%	80%	90%	80%	90%	80%	100%	90%	70%	100%
Clostridioides difficile (4)	CA	100%	/	/	/	/	/	/	/	/	/	/	100%
	EA	100%	/	/	/	/	/	/	/	/	/	/	100%
Actinomyces spp./Actinotignum spp. (12)	CA	100%	100%	100%	100%	100%	100%	67%	100%	N.I.	N.I.	100%	100%
	EA	100%	83%	100%	75%	92%	100%	67%	100%	100%	100%	100%	92%

CA, Categorical Agreement; EA, Essential Agreement; MZ, metronidazole; MX, moxifloxacin; P/T, piperacillin/tazobactam; PG, penicillin; AC, amoxicillin; ACC, amoxicillin/clavulanic acid; CM, clindamycin; IP, imipenem; RI, rifampicin; TG, tigecycline; LZ, linezolid; VA, vancomycin; N.I., not interpretable (Sensititre MIC range out of EUCAST breakpoints). Numbers in brackets indicate numbers of tested isolates.

amoxicillin/clavulanate and meropenem and EA ≥90% only for meropenem [9].

For metronidazole we found low EA values and significant differences between CA and EA for all Gram-positive and Gram-negative isolates. Other authors found major discordance for metronidazole between Sensititre and Etest; an explanation could be a possibly different speed of antibiotic activation for the two methods [9].

We found variable CA and EA results for *Clostridium spp*. Other authors compared *Clostridium spp*. clinical isolates between the reference agar dilution method and gradient diffusion strips (Liofilchem, Waverley, MA, USA) or Sensititre trays. For gradient dilution strips they found CA values ≥90% for metronidazole, piperacillin/tazobactam and vancomycin, but <90% for clindamycin and penicillin and for Sensititre trays EA was ≥90% for piperacillin/tazobactam, metronidazole and vancomycin, but <90% for penicillin and clindamycin; EA values were generally lower [1].

In our study, for *Actinomyces spp.* / *Actinotignum schaalii* nearly all antibiotics showed CA and EA values ≥90%. Other authors also found good EA for *Actinomyces spp*. between the Etest and agar dilution reference method for various antibiotics [14].

Resistance percentages were generally low, except for clindamycin in all isolates and tigecycline in Gram-negatives. Our overall antibiotic resistance percentages were mostly in keeping with recently published Italian data [6]. Nevertheless, care must be taken comparing data from different studies, as methodology and interpretation have changed over time and differ significantly between EUCAST [7,8] and CLSI [5]. One imipenem (Etest and Sensititre) and meropenem (Etest) resistant cfiA metallo-β-lactamase producing *B. fragilis* isolate was found. This isolate showed a clear heteroresistance phenotype for β-lactam antibiotics with the Etest method.

Both tests were relatively simple to perform, however the Sensititre method was more time consuming and sometimes more difficult to interpret due to trailing endpoints (defined as gradual fading of growth over 2 to 3 wells), though also for the Etest method trailing endpoints rarely made interpretation difficult [9]. A significant number of isolates failed to grow in Sensititre trays, despite care with rapid anaerobiosis and broth pre-reduction before plate inoculation. Moreover, the Etest may be a more reliable method to detect resistant subpopulations in heteroresistant isolates [3].

This study has a number of limitations. Firstly, we did not compare Sensititre and Etest results with the agar dilution "gold standard". Nevertheless, Etest followed by broth microdilution are the most common methods in use in Italian clinical microbiology laboratories [6]. Secondly, Fastidious Anaerobic Agar plates (F.A.A.) used in this study for the Etest method are recommended by EUCAST for the agar dilution and disc diffusion methods, but not in the manufacturer's instructions for Etest; nevertheless, manufacturer's instructions have been released before the validity date (01.01.2022) of the new EUCAST recommendations [8]. Thirdly, the Sensititre trays come with predefined antibiotics and limited concentration ranges without ability for variation, limiting the MIC and interpretation comparison for various antibiotics. Moreover, a limited number of isolates was included in our study (leading also to broad 95% confidence intervals), isolates came from a single geographic region (province of Bolzano, Italy) and not every genus of anaerobic bacteria was represented. Also, acquired resistances were rare, possibly introducing a bias in comparing agreement values; nevertheless, tested isolates are representative for the local antibiotic susceptibility situation. Finally, no further molecular characterization to determine resistance mechanisms was attempted in this study, but carbapenemase activity in the cfiA positive *B. fragilis* isolate was confirmed by the CIM method.

Conclusions

To conclude, comparing the Etest and Sensititre systems for anaerobe susceptibility testing, methods showed high CA values, but lower EA values. Nevertheless, in spite of higher costs we believe the Etest method to be superior to the Sensititre trays, because of lower failure rates, the ability to select the antibiotics required and broader MIC ranges tested, that make it suitable for routine diagnostic laboratory usage, and given its ability to detect more easily a heteroresistance phenotype. Multicenter studies are needed to further validate our results.

References

1. Baquer F, Sawan AA, Auzou M, et al. Broth microdilution and gradient diffusion strips vs. reference agar dilution method: first evaluation for *Clostridiales* species antimicrobial susceptibility testing. Antibiotics 2021;10:975.
2. Brook I, Wexler HM, Goldstein EJ. Antianaerobic antimicrobials: spectrum and susceptibility testing. Clin Microbiol Rev 2013;26:526-46.
3. Cherkaoui A, Fischer A, Azam N, et al. A comparison of Sentititre™ Anaerobe MIC plate with ATB ANA® test for the routine susceptibility testing of common anaerobe pathogens. European Journal of Clin Microbiol & Infect Dis 2018;37: 2279-84.
4. Clinical and Laboratory Standards Institute (CLSI). Methods for antimicrobial susceptibility testing of anaerobic bacteria: approved standard – eighth edition. M11-A8, Vol. 32 No. 5. Wayne, PA, USA: Clinical and Laboratory Standards Institute. 2012. Available from: https://clsi.org/media/1468/m11a8_sample.pdf
5. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, M100-Ed. 32, 2022. Wayne, PA, USA. Available from: https://clsi.org/standards/products/microbiology/documents/m100/
6. Di Bella S, Antonello RM, Sanson G, et al. Anaerobic bloodstream infections in Italy (ITANAEROBY): a 5-year retrospective nationwide survey. Anaerobe 2022;75:102583.
7. European Committee on Antimicrobial Susceptibility Testing (EUCAST, https://www.eucast.org). Antimicrobial susceptibility tests on groups of organisms or agents for which there are no EUCAST breakpoints.
8. European Committee on Antimicrobial Susceptibility Testing (EUCAST, https://www.eucast.org). Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0.
9. Hughes C, Ashhurst-Smith C, Ferguson JK. Gram negative anaerobe susceptibility testing in clinical isolates using Sensititre and Etest methods. Pathology 2018;50:437-41.
10. ISO 20776-2. Clinical laboratory testing and in vitro diagnostic test systems – susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – Part 2: evaluation of performance of antimicrobial susceptibility test devices. 2007. Available from: http://www.iso.org/standard/41631-html.

11. Nagy E, Boyanova L, Justesen US, on behalf of ESCMID Study group of anaerobic infections. Clin Microbiol Infect 2018;24: 1139-48.
12. Schuetz AN. Antimicrobial resistance and susceptibility testing of anaerobic bacteria. Clin Infect Dis 2014;59:698-705.
13. Van der Zwaluw K, de Haan A, Pluister GN, et al. The carbapenem inactivation method (CIM), a simple and low cost alternative for the carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. PLOSone 2015;10:e0123690.
14. Wolff A, Rodloff AC, Vielkind P, et al. Antimicrobial Susceptibility of Clinical Oral Isolates of *Actinomyces spp*. Microorganisms 2022;10:125.