

Revised Abstract

Background: The isolation of anaerobic pathogens has been increasing with the emergence of more resistant strains. It is recommended that clinical laboratories do antimicrobial testing of anaerobic bacteria from clinically significant sites. The established method of susceptibility testing has been agar dilution, but this methodology is usually performed only in reference laboratories. In this study we evaluated Etest agar gradient strips (BioMerieux) as a possible alternative for antimicrobial testing in the routine clinical laboratory.

Methods: Antibiotic susceptibilities of 300 anaerobic clinical isolates from North America, Europe and South America were evaluated using agar dilution and Etest strips. Agar dilution plates were prepared in house following CLSI guidelines. Etest strips were set up according to manufacturer's instructions. The following antimicrobials were tested: ampicillin/clavulanate, ampicillin/sulbactam, clindamycin, metronidazole, piperacillin/tazobactam, and tigecycline. Isolates tested consisted of *Bacteroides* spp., *Clostridium* spp., *Prevotella* spp., and anaerobic cocci. **Results:** Of 1800 individual results, 489 (27%) had a 0 dilution difference, 785 (44%) had a 1 doubling dilution (DD) difference, 296 (17%) had a 2 DD difference and 108 (6%) had a 3 DD difference. The remaining 122 results (7%) differed by ≥ 4 doubling dilutions. Of the doubling dilutions that had a difference of four or more, there were 46 significant errors(2.5%) where the difference resulted in a change of susceptible versus resistant. Of these 46 significant errors, 29 were with metronidazole, 7 with clindamycin, 5 piperacillin/tazobactam, 2 ampicillin/sulbactam, 2 ampicillin/clavulanate, and 1 tigecycline

Conclusions: Etest strips may provide an accurate alternative to agar dilution testing of anaerobes for the clinical laboratory, with the possible exception of metronidazole. Metronidazole is the most sensitive of the antimicrobics to changes in aerobic atmosphere and extreme caution should be taken to ensure that testing of metronidazole is done under strict anaerobic conditions.

Introduction

The isolation of anaerobic pathogens has been increasing with the emergence of more resistant strains. It is recommended that clinical laboratories do antimicrobial testing of anaerobic bacteria from clinically significant sites. The established method of susceptibility testing has been agar dilution, but this methodology is usually performed only in reference laboratories. In this study we evaluated Etest agar gradient strips (BioMerieux) as a possible alternative for antimicrobial testing in the routine clinical laboratory.

Materials & Methods

Antibiotic susceptibilities of 300 anaerobic clinical isolates from North America, Europe and South America were evaluated. Minimum inhibitory concentrations (MICs) were determined using Etest (bioMérieux) following manufacturer's guidelines and agar dilution following the Clinical Laboratory Standards Institute (CLSI) recommended protocols [1]. The following antimicrobial agents were tested: ampicillin/sulbactam, amoxicillin/clavulanate, clindamycin, piperacillin/tazobactam, metronidazole, and tigecycline. Isolates tested consisted of *Bacteroides* spp., *Clostridium* spp., *Prevotella* spp., *Fusobacterium* spp. and anaerobic cocci. Quality control followed CLSI guidelines using the following ATCC strains: *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 [2].

References

1. CLSI, Methods for Antimicrobial Susceptibility Tests of Anaerobic Bacteria; Approved Standard—Sixth Edition 2004: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
2. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S18. Wayne, PA, 2008.

Results

Results are shown in the following tables.

Table 1. List of organisms tested.

Organism	N
Anaerobic cocci	102
<i>Bacteroides</i> spp.	92
<i>Prevotella</i> spp.	77
<i>Clostridium</i> spp.	22
<i>Fusobacterium</i> spp.	7
Total	300

Table 2. Comparison of agar dilution and agar gradient (Etest) results for all antibiotics.

	N	%
0 dilution difference	489	27.2
1 dilution difference	785	43.6
2 dilution difference	296	16.4
3 dilution difference	108	6.0
4 dilution difference	40	2.2
5 dilution difference	19	1.1
6 dilution difference	27	1.5
7 dilution difference	28	1.6
8 or more dilution difference	8	0.4

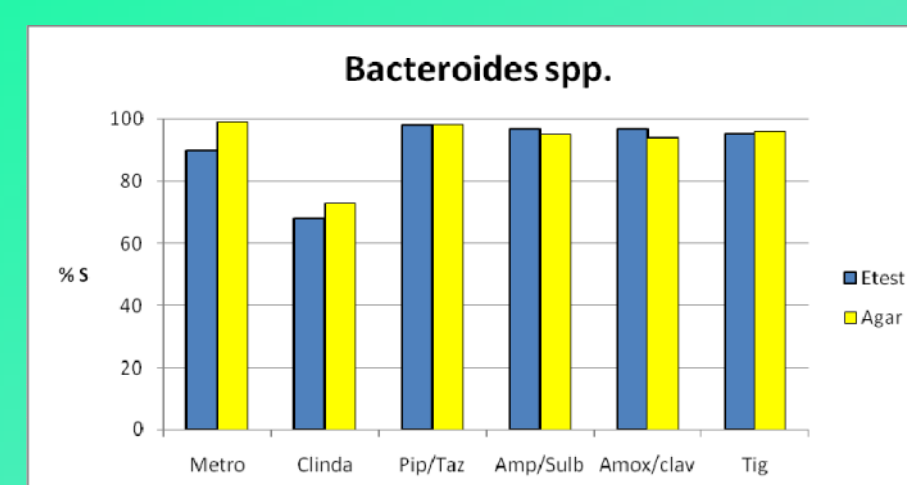
Table 3. Significant errors resulting in a change of interpretation from susceptible to resistant by antibiotic.

Antibiotic	Number of significant errors
Metronidazole	29
Clindamycin	7
Piperacillin/tazobactam	5
Ampicillin/sulbactam	2
Ampicillin/clavulanate	2
Tigecycline	1

Conclusions

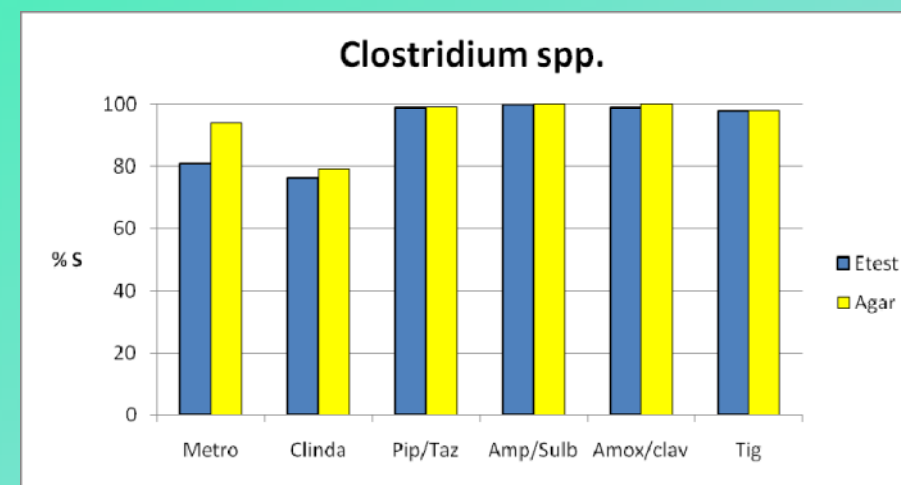
- ❑ 87.2% of the results from agar dilution and Etest strips were within two dilutions.
- ❑ An additional 8.2% were within four dilutions. There were a total of forty six significant errors (2.5%) where the difference resulted in a change of susceptible to resistant.
- ❑ Despite inherent difficulties often experienced with performing anaerobe susceptibility tests, in this study Etest results yielded susceptible results comparable to those obtained using agar dilution.
- ❑ Of the significant errors obtained, the majority were with metronidazole.
- ❑ Metronidazole testing is the most susceptible to being affected by changes in anaerobic environment. Every precaution should be taken ensure that the testing of metronidazole is done under strict anaerobic conditions.

Figure 1. Comparison of susceptibility* of 92 strains of *Bacteroides* spp. as determined by agar dilution vs. Etest.



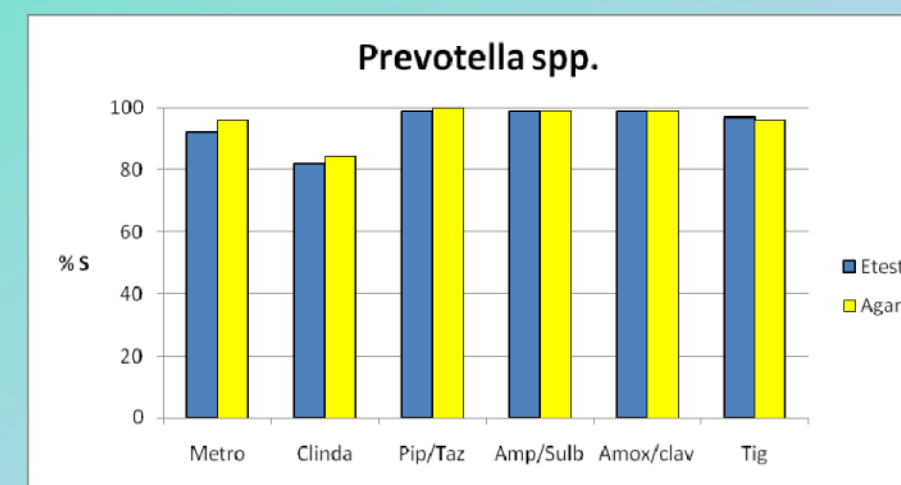
*Tigecycline susceptibility defined by FDA, all others by CLSI.

Figure 2. Comparison of susceptibility* of 22 strains of *Clostridium* spp. as determined by agar dilution vs. Etest.



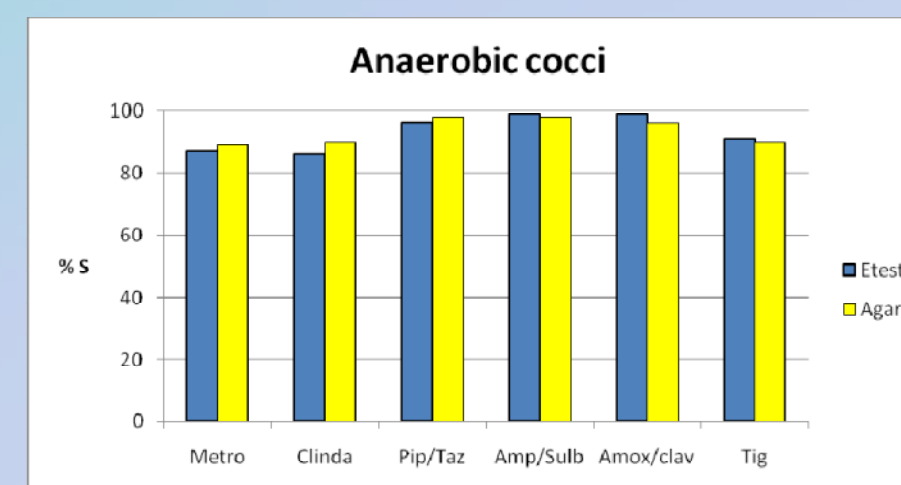
*Tigecycline susceptibility defined by FDA, all others by CLSI.

Figure 3. Comparison of susceptibility* of 77 strains of *Prevotella* spp. as determined by agar dilution vs. Etest.



*Tigecycline susceptibility defined by FDA, all others by CLSI.

Figure 4. Comparison of susceptibility* of 102 strains of anaerobic cocci as determined by agar dilution vs. Etest.



*Tigecycline susceptibility defined by FDA, all others by CLSI.