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An Update to a Comparison of Etest and Agar Dilution MICs for European Anaerobes – A Multi-year Analysis

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Revised Abstract

Background: The Tigecycline Evaluation and Surveillance Trial (TEST) program is a global surveillance study designed to monitor activity of tigecycline (TIG) and comparators [clindamycin, penicillin (P), cefoxitin (CFX), metronidazole, pip/tazo (PT), meropenem (MER)]. This report compares Etest results from each site to agar dilution at a central lab. **Methods**: 17 labs in Europe collected 1,346 anaerobic isolates between 2007-2008. MICs were determined locally by Etest, and at a central lab by agar dilution. %S by each method was compared using EUCAST breakpoints. **Results**: The following table lists the four drug/organism combinations tested that had >10% difference in % Susceptible (S) between agar dilution and Etest. All drugs not listed had %S by Etest and agar dilution within 10% of each other.

Org. Group	N	Drug	Agar Dil. %S	Etest %S
Anaerococcus spp.	57	P	68.6	88.2
Bacteroides spp.	566	CFX	85.0	33.6
Clostridium spp.	315	MER	98.4	85.4
Prevotella spp.	243	CFX	99.0	86.5

Among individual species with n≥10, however, there were 24/119 drug/organism combinations where %S by Etest differed by ≥10% from the agar dilution result. In 21 of those cases Etest %S was lower than agar dilution.

Conclusions: Despite inherent difficulties often experienced with performing anaerobe susceptibility tests, in this study Etest results from 17 diverse labs across Europe yielded %S values broadly comparable to those obtained by a single reference lab using agar dilution. However, use of Etest in some specific drug/bug combinations such as *Bacteroides* spp. vs. cefoxitin, and *Clostridium* spp. vs. meropenem, may under-report susceptibility. Tigecycline Etest and agar dilution % S were comparable for all organisms tested.

Introduction

Tigecycline is a novel glyclycycline antibiotic which has demonstrated broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [1-4].

Tigecycline was approved for use in Europe in 2006 for complicated skin and soft tissue and complicated intra-abdominal infections (cIAI) (http://www.emea europa.eu/humandocs/Humans/EPAR/tygacil/tygacil.htm).
Anaerobes are present in 80 to 90% of cIAI, with *Bacteroides* spp. making up two-thirds of these organisms [5]. The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) was designed to evaluate the *in vitro* activity of tigecycline against both aerobic and anaerobic cIAI pathogens. It is expected that laboratories testing tigecycline against anaerobes will often use Etest strips to do so. This analysis compares the susceptibility levels determined by Etest and agar dilution of tigecycline and five comparator antimicrobials against anaerobic gram-positive and gram-negative isolates collected from 17 hospitals in six European countries during 2007 to 2008.

Materials & Methods

- For the T.E.S.T program all isolates were derived from blood, wounds, fluids, gastrointestinal, and cIAI. Isolates were identified to genus and species by the local laboratory. Only one isolate per patient was accepted.
- For this study 1,346 clinical isolates were collected in 2007 to 2008 from 17 hospitals in six European countries (Belgium, Czech Republic, France, Germany, Greece, and Hungary).
- Minimum inhibitory concentrations (MICs) were determined using Etest methodology at each participating laboratory. Thereafter, all isolates were sent to the central lab for re-identification and susceptibility testing by the CLSI recommended agar dilution method [14]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). The following antimicrobial agents were tested with their dilution ranges (expressed in mg/L): tigecycline (0.06-32); clindamycin (0.25-8); metronidazole (0.12-16); piperacillin/tazobactam (0.06/4-64/4); meropenem (0.06-8); penicillin (0.25-32, gram-positive only); and cefoxitin (2-32, gram-negative only).
- MIC interpretive criteria followed published breakpoints established by EUCAST where applicable [7]. If no EUCAST guidelines were available for a given antimicrobial, CLSI breakpoints [8] were used.

			Breakpoints (<=S|I|>=R)		
GramType	Drug	EUCAST	CLSI	FDA
AnNeg	Meropenem	2|4-8|16	4|8|16	--
AnNeg	PipTazo	8|16|32	32|64|128	--
AnNeg	Cefoxitin	NBP	16|32|64	--
AnNeg	Clindamycin	4|-|8	2|4|8	--
AnNeg	Metronidazole	4|-|8	8|16|32	--
AnNeg	Tigecycline	NBP	NBP	4|8|16
AnPos	Meropenem	2|4-8|16	4|8|16	--
AnPos	PipTazo	8|16|32	32|64|128	--
AnPos	Penicillin	0.25|.5|1	0.5|1|2	--
AnPos	Clindamycin	4|-|8	2|4|8	--
AnPos	Metronidazole	4|-|8	8|16|32	--
AnPos	Tigecycline	NBP	NBP	4|8|16

- Quality control followed CLSI guidelines using the following ATCC strains: *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741.
- Fisher's Exact Test was used to determine statistical significance.
- The collection and transportation of organisms, confirmation of identification, and construction and management of a centralized database were conducted and coordinated by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

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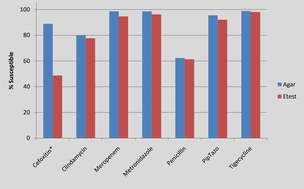
Results

Table 1 shows the number of strains of each anaerobic species included in the study. Figures 1-8 and Table 2 compare susceptibility levels of isolates determined using Etests at each laboratory to those determined at the central laboratory using agar dilution.

Table 1. List of Study Organisms with matched paired Agar Dilution and Etest MICs

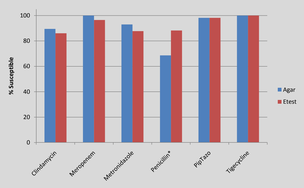
Organism	Total N	Organism	Total N
Anaerococcus prevotii	12	*Clostridium ramosum*	4
Anaerococcus tetradius	1	*Clostridium septicum*	5
Anaerococcus, non-speciated	44	*Clostridium sordellii*	7
Bacteroides bivius	1	*Clostridium sphenoides*	1
Bacteroides caccae	5	*Clostridium sporogenes*	14
Bacteroides capillosus	2	*Clostridium subterminale*	1
Bacteroides distasonis	20	*Clostridium tertium*	4
Bacteroides eggerthii	5	*Clostridium, non-speciated*	5
Bacteroides fragilis	393	*Finegoldia magna*	65
Bacteroides fragilis Group 2	5	*Peptoniphilus asaccharolyticus*	29
Bacteroides melaninogenicus	1	*Peptoniphilus indolicus*	2
Bacteroides merdae	5	*Peptostreptococcus anaerobius*	44
Bacteroides nordii	1	*Peptostreptococcus lactolyticus*	6
Bacteroides ovatus	13	*Peptostreptococcus magnus*	8
Bacteroides stercoris	4	*Peptostreptococcus micros*	59
B. thetaiotaomicron	57	*Peptostreptococcus porvulus*	1
Bacteroides uniformis	27	*Peptostreptococcus, non-speciated*	6
Bacteroides, non-speciated	3	*Prevotella bivia*	87
Clostridium bifermentans	2	*Prevotella buccae*	25
Clostridium butyricum	10	*Prevotella buccalis*	4
Clostridium cadaveris	2	*Prevotella corporis*	2
Clostridium clostridioforme	14	*Prevotella denticola*	7
Clostridium difficile	126	*Prevotella disiens*	9
Clostridium glycolicum	1	*Prevotella intermedia*	25
Clostridium hastiforme	1	*Prevotella loescheii*	12
Clostridium histolyticum	4	*Prevotella melaninogenica*	16
Clostridium innocuum	6	*Prevotella oralis*	28
Clostridium novyi	1	*Prevotella oris*	7
Clostridium paraputrificum	4	*Prevotella tannerae*	1
Clostridium perfringens	103	*Prevotella, non-speciated*	12
Total			1346

Figure 1. Comparison of susceptibility of 1,346 anaerobes as determined by agar dilution vs. Etest



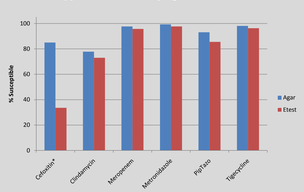
*Difference is statistically significant ($p<.05$).

Figure 2. Comparison of susceptibility of 57 strains of *Anaerococcus* spp. as determined by agar dilution vs. Etest.



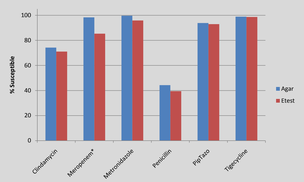
*Difference is statistically significant ($p<.05$).

Figure 3. Comparison of susceptibility of 566 strains of *Bacteroides* spp. as determined by agar dilution vs. Etest



*Difference is statistically significant ($p<.05$).

Figure 4. Comparison of susceptibility of 315 strains of *Clostridium* spp. as determined by agar dilution vs. Etest.



*Difference is statistically significant ($p<.05$).

Figure 5. Comparison of susceptibility of 65 strains of *Finegoldia magna* as determined by agar dilution vs. Etest.

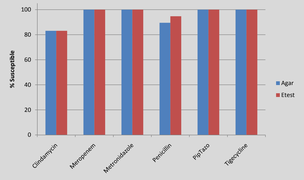


Figure 6. Comparison of susceptibility of 31 strains of *Peptoniphilus* spp. as determined by agar dilution vs. Etest.

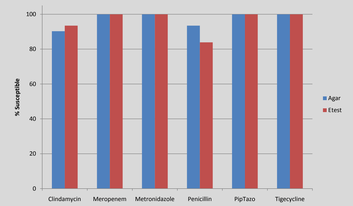


Figure 7. Comparison of susceptibility of 128 strains of *Peptostreptococcus* spp. as determined by agar dilution vs. Etest.

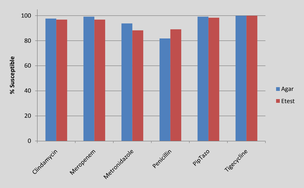


Figure 8. Comparison of susceptibility of 243 strains of *Prevotella* spp. as determined by agar dilution vs. Etest

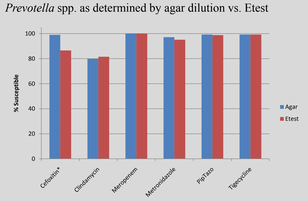


Table 2. Individual species/antimicrobial combinations showing >=10% difference in % susceptible between agar dilution and Etest

Organism	Drug	N	Agar %Sus	Etest %Sus	Difference	P-Value
All Anaerobes	Cefoxitin	704	88.9	48.7	40.2	<0.001
Combined	Clindamycin	1346	79.9	77.6	2.3	NSS
	Meropenem	1343	98.6	94.6	4	<0.05
	Metronidazole	1342	98.5	96.2	2.3	NSS
	Penicillin	480	62.3	61.3	1	<0.05
	PipTazo	1344	95.5	92	3.5	<0.05
	Tigecycline	1346	98.8	98	0.8	NSS
Anaerococcus spp.	Clindamycin	57	89.5	86	3.5	NSS
	Meropenem	57	100	96.5	3.5	NSS
	Metronidazole	57	93	87.7	5.3	NSS
	Penicillin	51	68.6	88.2	-19.6	<0.05
	PipTazo	57	98.2	98.2	0	NSS
	Tigecycline	57	100	100	0	NSS
Bacteroides spp.	Cefoxitin	506	85	33.6	51.4	<0.001
	Clindamycin	567	77.8	73	4.8	NSS
	Meropenem	564	97.7	95.7	2	NSS
	Metronidazole	564	99.3	97.7	1.6	NSS
	PipTazo	566	93.1	85.5	7.6	NSS
	Tigecycline	566	98.1	96.3	1.8	NSS
Clostridium spp.	Clindamycin	315	74.3	71.1	3.2	NSS
	Meropenem	314	98.4	85.4	13	<0.01
	Metronidazole	314	99.7	95.9	3.8	NSS
	Penicillin	271	44.3	39.5	4.8	NSS
	PipTazo	314	93.9	93	0.9	NSS
	Tigecycline	315	99	98.7	0.3	NSS
Finegoldia spp.	Clindamycin	65	83.1	83.1	0	NSS
	Meropenem	65	100	100	0	NSS
	Metronidazole	65	100	100	0	NSS
	Penicillin	57	89.5	94.7	-5.2	NSS
	PipTazo	65	100	100	0	NSS
	Tigecycline	65	100	100	0	NSS
Peptoniphilus spp.	Clindamycin	31	90.3	93.5	-3.2	NSS
	Meropenem	31	100	100	0	NSS
	Metronidazole	31	100	100	0	NSS
	Penicillin	31	93.5	83.9	9.6	NSS
	PipTazo	31	100	100	0	NSS
	Tigecycline	31	100	100	0	NSS
Peptostreptococcus spp.	Clindamycin	128	97.7	96.9	0.8	NSS
	Meropenem	128	99.2	96.9	2.3	NSS
	Metronidazole	128	93.8	88.3	5.5	NSS
	Penicillin	110	81.8	89.1	-7.3	NSS
	PipTazo	128	99.2	98.4	0.8	NSS
	Tigecycline	128	100	100	0	NSS
Prevotella spp.	Cefoxitin	208	99	86.5	12.5	<0.05
	Clindamycin	243	79.8	81.5	-1.7	NSS
	Meropenem	243	100	100	0	NSS
	Metronidazole	243	97.1	95.1	2	NSS
	PipTazo	243	99.2	98.8	0.4	NSS
	Tigecycline	244	99.2	99.2	0	NSS

NSS=Not statistically significant

Conclusions

- Despite inherent difficulties often experienced with performing anaerobe susceptibility tests, in this study Etest results from 17 different hospital laboratories across Europe yielded %S values broadly comparable to those obtained by a single reference lab using agar dilution.
- However, use of Etest in some specific drug/organism combinations such as *Bacteroides* spp. vs. cefoxitin and piperacillin/tazobactam, and *Clostridium* spp. vs. meropenem, may under-report susceptibility.
- Considering that the Etest and agar dilution susceptibility tests in this study were done by different labs at different times, the relatively small number of significant differences in reported susceptibility levels suggests that use of Etest for anaerobic susceptibility testing, at least for the antimicrobials in this study, yields reliable results.
- Tigecycline E-test and agar dilution % S were comparable for all organisms tested.