

## Revised Abstract

**Background:** Tigecycline, a member of a new class of antimicrobials (glycylcyclines), has been shown to have potent expanded broad spectrum activity against most commonly encountered species responsible for community and hospital acquired infections. The T.E.S.T. program determined the *in vitro* activity of tigecycline compared to piperacillin-tazobactam, clindamycin, metronidazole, meropenem, penicillin, and ceftioxin against anaerobic multi-drug resistant (MDR) strains collected from 18 investigational sites in 7 countries in Europe from 2007 through 2008. **Methods:** A total of 2,267 clinical anaerobic pathogens were identified to the species level at each participating site and confirmed by the central laboratory. Minimum Inhibitory Concentrations (MICs) were determined using agar dilution. Antimicrobial resistance was interpreted according to EUCAST breakpoints or CLSI and FDA breakpoints where no EUCAST breakpoints were available. Strains were grouped by resistance to 0, 1, or 2 drug classes. **Results:** The MIC<sub>90</sub>s of tigecycline to MDR groups 0 – 2 are shown in the following table:

	MDR Group MIC <sub>90</sub> (n) *		
	Group 0 (n=1715)	Group 1 (n=521)	Group ≥ 2 (n=31)
<i>Anaerococcus</i> spp.	0.12 (83)	0.12 (10)	-
<i>Anaerovorax</i> spp.	-	≤0.06 (1)	-
<i>Bacteroides</i> spp.	2 (671)	4 (185)	2 (29)
<i>Clostridium</i> spp.	1 (334)	0.5 (235)	-
<i>Eubacterium</i> spp.	0.25 (1)	-	-
<i>Finegoldia</i> spp.	0.12 (92)	≤0.06 (15)	-
<i>Fusobacterium</i> spp.	-	-	≤0.06 (1)
<i>Peptoniphilus</i> spp.	0.12 (49)	0.12 (5)	-
<i>Peptostreptococcus</i> spp.	0.12 (199)	≤0.06 (3)	-
<i>Prevotella</i> spp.	1 (285)	1 (66)	0.12 (1)
<i>Veillonella</i> spp.	≤0.06 (1)	-	-

MDR Group is defined as resistant to 0, 1, or 2 or more separate drug classes.

**Conclusions:** Tigecycline retained activity against 96.8% of anaerobes resistant to 2 or more drug classes. Tigecycline's *in vitro* activity against multi-drug resistant anaerobic pathogens may be useful in the treatment of infections caused by such therapeutically challenging strains.

## Introduction

Increasing bacterial resistance worldwide has stimulated the search for more effective antimicrobial compounds. Wyeth Pharmaceuticals (Wyeth) has developed tigecycline, the first of the glycylcycline antimicrobial agents to reach a major market. This synthetic tetracycline analogue exhibits antibacterial activity that is both bacteriostatic and in certain instances bactericidal with killing activity that is as much as fourfold better than vancomycin and daptomycin [1, 2]. Tigecycline has also been shown to be active *in vitro* against a broad spectrum of gram-negative and gram-positive aerobic and anaerobic organisms [3]. The development of tigecycline is important in that tigecycline is active, as are other glycylcyclines, against bacterial strains carrying either or both of the two major forms of tetracycline resistance: efflux and ribosomal protection.

Tigecycline received EMEA approval for treatment of complicated skin and soft tissue and intra-abdominal infections and community acquired pneumonia [4]. The objective of this study was to determine the *in vitro* activity of tigecycline and comparative agents against a large number of recent anaerobic pathogens from geographically diverse populations throughout Europe.

## Materials & Methods

There were 2,267 gram-positive and -negative anaerobes collected from 2007 through 2008 from 7 European countries (19 sites): Belgium (1), Czech Republic (1), France (5), Germany (7), Greece (1), Hungary (3), and the United Kingdom (1). All isolates were tested and identified locally with only one isolate per patient. All isolates were shipped to a central reference lab (IHMA, Schaumburg, IL, USA) where identification and MICs were confirmed by agar dilution using guidelines published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID):

Organism	AST Conditions			
	Test medium	Temp	Time (hrs)	Atmosphere
Anaerobes	Brucella agar; 5 mcg hemin; 1 mcg vitamin K with laked sheep blood	35–37°C	48	Anaerobic: 5% H <sub>2</sub> , 5% CO <sub>2</sub> , 90% N <sub>2</sub>

Drugs and concentrations (mg/L) tested against anaerobes included tigecycline (0.06-32); clindamycin (0.25-8); metronidazole (0.12-16); meropenem (0.06-8); piperacillin-tazobactam (0.06/4-64/4); penicillin (0.25-32, gram-positive only); and ceftioxin (2-32, gram-negative only). MIC interpretive criteria followed published clinical breakpoints established by EUCAST, where applicable [5].

## References

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- Projan, S.J., *Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent*. *Pharmacotherapy*, 2000, 20(9 Pt 2): p. 219S-223S; discussion 224S-228S.
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## Acknowledgements

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## Results

Table 1. Demographic listing of 31 multi-drug resistant anaerobes.

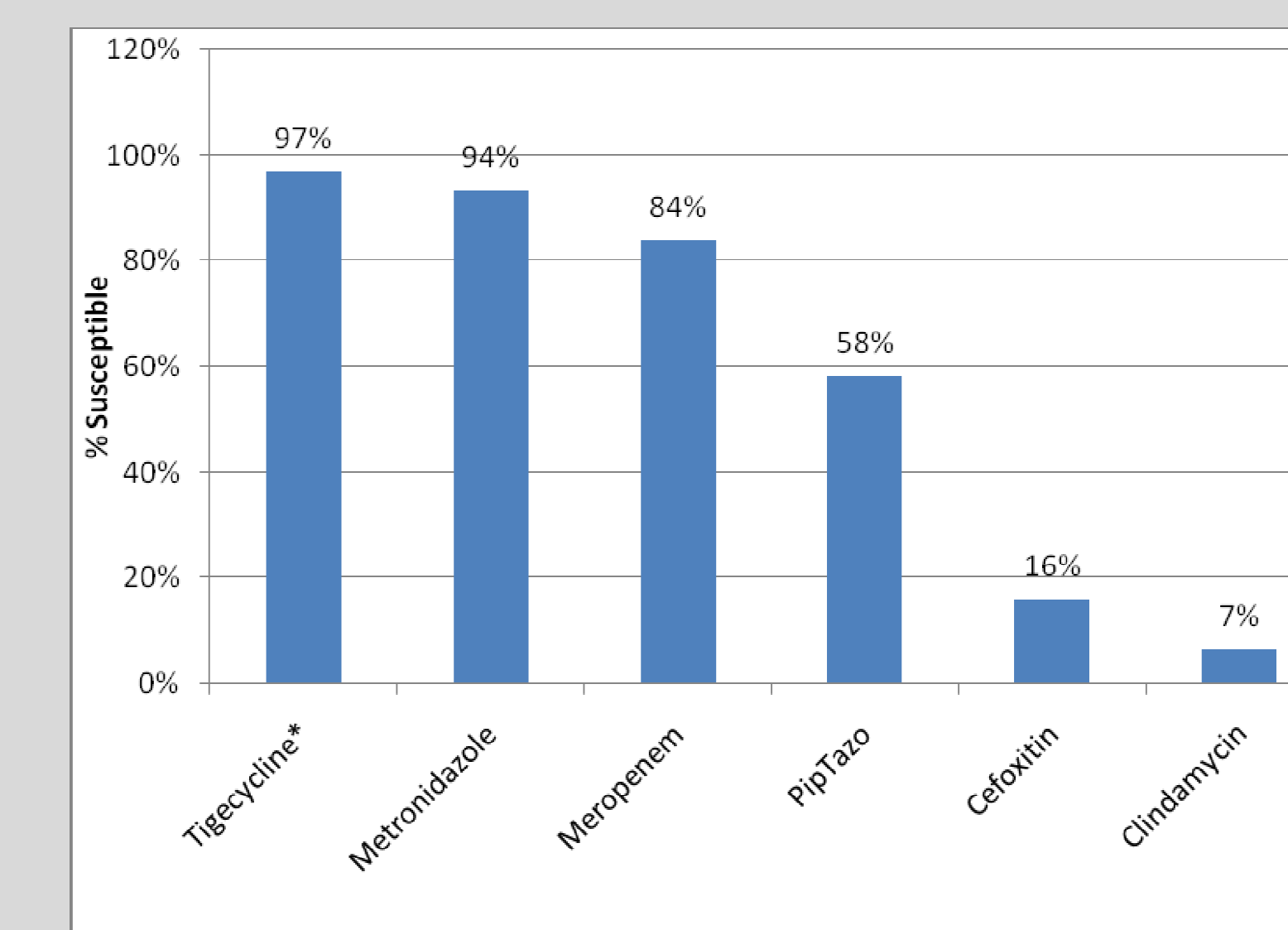
SiteID	Country	Organism	Source	No. of Resistant Drug Classes
6455	Hungary	<i>B. fragilis</i>	Gastrointestinal	2
6455	Hungary	<i>B. fragilis</i>	Unknown	2
6455	Hungary	<i>B. fragilis</i>	Blood	5
6455	Hungary	<i>B. fragilis</i>	Unknown	2
6492	Germany	<i>B. fragilis</i>	Unknown	4
6492	Germany	<i>B. uniformis</i>	Unknown	2
6492	Germany	<i>B. vulgatus</i>	Unknown	2
6492	Germany	<i>Fusobacterium nucleatum</i>	Unknown	2
15653	France	<i>B. thetaiotaomicron</i>	Blood	2
15716	Germany	<i>B. thetaiotaomicron</i>	Unknown	2
15716	Germany	<i>Prevotella oralis</i>	Unknown	2
15842	France	<i>B. distasonis</i>	Abscess	2
15856	Germany	<i>B. eggerthii</i>	Ovary	2
15856	Germany	<i>B. uniformis</i>	Gastrointestinal	2
15856	Germany	<i>B. vulgatus</i>	Gastrointestinal	2
16444	Germany	<i>B. fragilis</i>	Unknown	2
16444	Germany	<i>B. fragilis</i>	Gastrointestinal	2
16955	France	<i>B. fragilis</i>	Unknown	2
16955	France	<i>B. stercoris</i>	Unknown	2
17568	France	<i>B. fragilis</i>	Skin/Skin Structures	2
17568	France	<i>B. fragilis</i>	Respiratory	2
17568	France	<i>B. fragilis</i>	Peritoneal fluid	2
17584	Germany	<i>B. uniformis</i>	Genital/Urinary	2
17631	Hungary	<i>B. fragilis</i>	Unknown	2
17631	Hungary	<i>B. fragilis</i>	Unknown	2
17631	Hungary	<i>B. thetaiotaomicron</i>	Unknown	2
17631	Hungary	<i>B. uniformis</i>	Unknown	2
17868	Czech Republic	<i>B. fragilis</i>	Unknown	2
17868	Czech Republic	<i>B. vulgatus</i>	Abscess	2
17928	Hungary	<i>B. fragilis</i>	Unknown	2
17928	Hungary	<i>B. uniformis</i>	Unknown	2

Table 2. Listing of 31 multi-drug resistant anaerobes with *in vitro* activity for tigecycline and comparators.

Drug Resistant Groups <sup>a</sup>	Organism	Tigecycline		Meropenem		Metronidazole		PipTazo		Ceftioxin		Clindamycin		
		N	%S <sup>b</sup>	Range	%S	Range	%S	Range	%S	Range	%S	Range		
Group 2 (resistant to 2 drug classes)	<i>B. fragilis</i>	13	100	0.25-2	76.9	0.25-8	92.3	0.25-8	69.2	0.25->64	7.7	8->32	15.4	1->8
	<i>B. uniformis</i>	5	100	≤0.06-2	100	0.25-2	100	0.5-4	40	0.25->64	20	≤2->32	0	>8->8
	<i>B. thetaiotaomicron</i>	3	100	≤0.06-2	100	0.25-0.25	100	1-1	33.3	4-16	0	32->32	0	>8->8
	<i>B. vulgatus</i>	3	100	0.5-1	100	0.25-0.5	100	0.25-1	66.7	0.5->64	33.3	≤2->32	0	>8->8
	<i>B. distasonis</i>	1	100	0.5	100	1	100	4	100	8	0	32	0	>8
	<i>B. eggerthii</i>	1	100	0.5	100	0.25	100	4	0	>64	100	≤2	0	>8
	<i>B. stercoris</i>	1	100	0.12	100	0.5	100	1	100	0.5	0	32	0	>8
	<i>F. nucleatum</i>	1	100	≤0.06	100	2	100	0.5	100	4	100	≤2	0	>8
	<i>P. oralis</i>	1	100	0.12	100	1	100	1	100	0.5	0	>32	0	>8
Group 4	<i>B. fragilis</i>	1	100	0.5	0	>8->8	100	0.5	0	>64	0	>32	0	>8
Group 5	<i>B. fragilis</i>	1	0	32	0	8	0	8	0	>644	0	>32	0	>8
<b>Totals</b>	<b>All MDR Anaerobes</b>	<b>31</b>	<b>96.8</b>	<b>≤0.06-2</b>	<b>83.9</b>	<b>0.25-8</b>	<b>93.5</b>	<b>0.25-8</b>	<b>58.1</b>	<b>0.25-&gt;64</b>	<b>16.1</b>	<b>≤2-&gt;32</b>	<b>6.5</b>	<b>1-&gt;8</b>

<sup>a</sup> Multi-drug resistant is resistance to 2 or more drug classes as defined by CLSI (M100-S19, 2009). Each "Group" represents how many drug classes are resistant. There was no Group 3 (no isolates resistant to 3 drug classes).  
<sup>b</sup> Susceptibility of each drug is defined by EUCAST Clinical Breakpoints (March 2008), where available; Tigecycline susceptibility defined by FDA (Tygacil®, 2005).

Figure 1 Percent susceptible (%) for 31 multi-drug resistant anaerobes – European data.



\* Susceptibility of each drug is defined by EUCAST Clinical Breakpoints (March 2008), where available; Tigecycline susceptibility defined by FDA (Tygacil®, 2005).

## Conclusions

- MDR resistant anaerobes are not common as is demonstrated in this study. MDR isolates comprised just 1.4% (31/2267) of the study species. All MDR anaerobes were gram-negative, none gram-positive.
- Tigecycline demonstrated excellent *in vitro* activity against all anaerobic species collected in this European population. Tigecycline MIC<sub>90</sub> values were ≤2 mg/L for all MDR isolates (resistant to 2 or more drug classes) with the exception of 1 isolate that was resistant to all 5 study drugs (confirmed, *B. fragilis*). Using the FDA breakpoint of ≤4 mg/L as susceptible, this gives tigecycline the highest percentage susceptible rate, 96.8% (30/31 isolates), against all MDR anaerobes in this study.
- Tigecycline MICs were equal to or lower than all study drugs including drugs that are commonly used to treat anaerobic infections such as clindamycin, metronidazole, piperacillin-tazobactam, meropenem and ceftioxin. However, meropenem and metronidazole continue to have good activity against MDR with overall percents susceptible of 83.9% and 93.5%, respectively.
- Anaerobes, especially gram-negative anaerobes, are co-pathogens in many complicated intra-abdominal infections (cIAI). Many cIAI pathogens, particularly gram-negative aerobes, harbor drug-resistant determinants (eg., ESBLs and AmpC) making them difficult to treat. Tigecycline may be useful in the treatment of such polymicrobial infections with known effectiveness against both gram-negative aerobes and demonstrated inhibition *in vitro* against anaerobes in this study. Additional studies are warranted.