

## Revised Abstract

**Background:** The role of toxin-producing *Clostridium difficile* in antibiotic-associated diarrhea is well established. The incidence of this difficult to treat disease has increased dramatically since the 1980's. 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, including gram-positive anaerobes. The T.E.S.T. program determined the *in vitro* activity of tigecycline and five comparators against *Clostridium difficile* strains collected from 18 investigational sites in 7 countries in Europe throughout 2008. **Methods:** A total of 256 clinical isolates of *C. difficile* 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. **Results:** Results are shown in the following table:

	<i>Clostridium difficile</i> (n = 256)			
	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	Range
Tigecycline	≤0.06	0.25	na	≤0.06 - 2
Clindamycin	4	>8	53.9	≤0.25 - >8
Meropenem	1	2	98.4	≤0.06 - 8
Metronidazole	0.5	2	100	≤0.12 - 4
Penicillin	1	4	9.0	≤0.25 - >32
Pip/tazo	8	16	89.8	≤0.06 - >64

**Conclusions:** Tigecycline showed excellent *in vitro* activity against European clinical isolates of *Clostridium difficile*, with MIC<sub>50</sub>/MIC<sub>90</sub> values of ≤0.06 and 0.25mg/L. Tigecycline's low MIC<sub>50</sub>/MIC<sub>90</sub> values suggest that it may be an option against this difficult to treat pathogen.

## Introduction

Tigecycline is a novel antimicrobial with expanded broad-spectrum activity from a new class of compounds, the glycylcyclines. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, it has shown some bactericidal activity against key targeted pathogens [1,2]. Tigecycline has demonstrated broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative microorganisms [2-4].

Tigecycline resistance is very infrequent and is also difficult to induce in the laboratory [5, 6] with a selection frequency observed at less than 10<sup>-9</sup> [3, 5, 7]. Tetracycline-resistant bacteria (with the exception of *P. aeruginosa*), with either tetracycline efflux pumps or ribosomal protective features are sensitive to tigecycline [2-4, 7-11]. Tigecycline has shown to be active against multi-resistant *Acinetobacter* spp., particularly *A. baumannii* that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum β-lactamase (ESBL) and AmpC producing strains [10]. Tigecycline has demonstrated MIC<sub>90</sub> values of ≤0.5 mg/L against methicillin-resistant *Staphylococcus aureus* (MRSA) and other gram-positive organisms [2, 4-6]. Tigecycline has shown excellent *in vitro* activity against both gram-positive and gram-negative anaerobes [12].

Tigecycline was approved for use in Europe in 2006 for complicated skin and soft tissue and intra-abdominal infections (cIAI) (<http://www.emea.europa.eu/humandocs/Humans/EPAR/tygacil/tygacil.htm>). This study, a part of the ongoing global Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) program, was designed to evaluate the *in vitro* activity of tigecycline compared to five comparator antimicrobials against *Clostridium difficile* isolates collected from 18 hospitals in Europe in 2008.

## Materials & Methods

- For the T.E.S.T program all isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids, and other defined sources. Isolates were identified to genus and species by the local laboratory. Only one isolate per patient was accepted.
- For this study 256 clinical isolates were collected in 2008 from 18 hospitals in seven European countries (Belgium, Czech Republic, France, Germany, United Kingdom, Greece, Hungary).
- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended agar dilution testing 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); and penicillin (0.25-32).
- MIC interpretive criteria followed published breakpoints established by EUCAST [15]
- Quality control followed CLSI guidelines using the following ATCC strains: *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741.
- 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).

## References

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## Results

Table 1. *In vitro* activity of tigecycline and comparative agents against 256 *Clostridium difficile* from Europe.

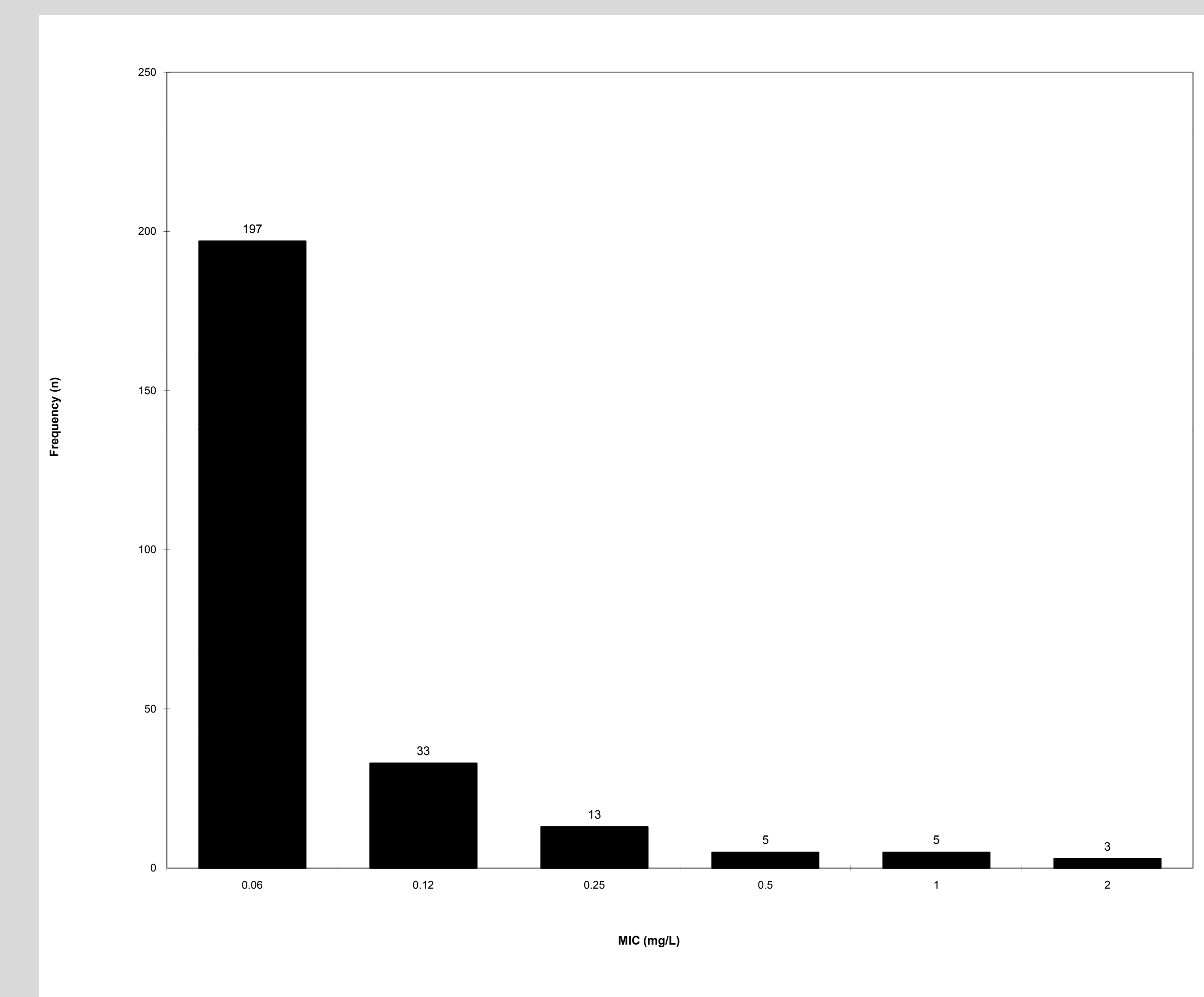
Organism/Drug	N	MIC (mg/L)			%S <sup>a</sup>	%I	%R
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>			
Tigecycline	256	≤0.06 - 2	≤0.06	0.25	na	na	na
Clindamycin	256	≤0.25 - >8	4	>8	53.9	0	46.1
Meropenem	256	≤0.06 - 8	1	2	98.4	1.6	0
Metronidazole	256	≤0.12 - 4	0.5	2	100	0	0
Penicillin	256	≤0.25 - >32	1	4	9.0	7.8	83.2
PipTazo	256	≤0.06 - >64	8	16	89.8	9.4	1

<sup>a</sup> Interpretive criteria as defined by EUCAST.

Table 2. Frequency distribution of tigecycline and comparator MICs (mg/L) against 256 isolates of *Clostridium difficile* from Europe.

MIC (mg/L)	N	Cumulative%
0.06	197	77.0
0.12	33	89.8
0.25	13	94.9
0.5	5	96.9
1	5	98.8
2	3	99.8
4	1	100
8	1	100
16	1	100
32	1	100
64	1	100
128	1	100

Figure 1 Frequency distribution graph of tigecycline MICs (mg/L) against 256 isolates of *Clostridium difficile*.



## Conclusions

- Tigecycline has excellent *in vitro* activity against *Clostridium difficile* with the lowest MIC<sub>50/90</sub> values (0.06/0.25 mg/L) of all compounds tested.
- With many clinical laboratories and physicians relying on empirical data to determine therapy choices when faced with anaerobes, tigecycline's demonstrated *in vitro* activity against *Clostridium difficile* isolates may be a useful addition to hospital formularies.