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## Revised Abstract

**Background:** *Bacteroides fragilis* is an important anaerobic co-pathogen in many polymicrobial infections. *B. fragilis* reduced susceptibility to carbapenems is due primarily to the metallo-β-lactamase CfiA gene (meropenem MICs 1-4) with high-level resistance secondary to acquired upstream insertion sequences (IS) causing expression of CfiA (MICs >16). **Methods:** T.E.S.T. evaluated 36/542 (6.6%) *B. fragilis* with reduced susceptibility to carbapenems (Bf-RSC phenotype) from a collection of anaerobes spanning two years, 2007 - 2008. The isolates were identified to the species level at the participating sites and confirmed by a central laboratory. MICs were determined by the central laboratory using agar dilution according to CLSI guidelines. **Results:** *In vitro* activities against Bf-RSC phenotype (meropenem):

Drug	MIC <sub>50</sub>	MIC <sub>90</sub>	%Sus*
Tigecycline	1	4	100
Cefoxitin	16	>32	61.1
Clindamycin	2	>8	66.7
Meropenem	2	8	61.1
Metronidazole	0.5	2	100
Piperacillin Tazobactam	1	8	91.7

\*EUCAST breakpoints used where available (May, 2009); FDA breakpoints used for tigecycline (Tygacil®, 2005).

**Conclusions:** All Bf-RSC isolates were susceptible to tigecycline at its FDA breakpoint of 4 mcg/ml. The *in vitro* activity of tigecycline was similar to metronidazole and piperacillin-tazobactam. No strains with high-level resistance to meropenem were identified.

## Introduction

*Bacteroides fragilis* remains one of the most important anaerobic pathogens responsible for a variety of significant clinical infections including intra-abdominal, complicated skin and skin structure, bacteremia and deep abscesses. Antibiotic resistance in this important pathogen has been noted and is increasing in many parts of the world [1,2]. Resistance to beta-lactam antibiotics is typically due to beta-lactamase production and carbapenem resistance due to production of metallo-beta-lactamases [3].

The preferred agents of choice for *B. fragilis* infections include broad spectrum penicillins, cephalosporins, metronidazole, clindamycin, piperacillin-tazobactam, carbapenems and recently tigecycline. Today the majority of anaerobic infections are treated empirically and minimal antimicrobial susceptibility testing is performed. With the knowledge that antimicrobial resistance is increasing to many classes of agents used to treat anaerobic infections active ongoing surveillance of resistance patterns of anaerobes is warranted. The use of carbapenems as one of the preferred agents of choice has led to the recognition of carbapenem resistance and the recognition of the metallo-beta-lactamase gene CfiA and its expression.

The Tigecycline European Surveillance Trial (TEST) began monitoring the susceptibility of anaerobes to tigecycline and six other drugs in Europe in 2007. This report documents the *in vitro* activity of tigecycline and comparators to 542 *B. fragilis* isolates collected in Europe during 2007-2008 and further documents the activity of tigecycline and comparators to isolates with reduced susceptibility to meropenem.

## Materials & Methods

- All isolates were derived from blood, wounds, fluids, gastrointestinal, and complicated intra-abdominal infections. Isolates were identified to genus and species by the local laboratory. Only one isolate per patient was accepted.
- 542 clinical isolates of *B. fragilis* were collected in 2007 to 2008 from 31 cumulative laboratory sites in six European countries (Belgium, Czech Republic, France, Germany, United Kingdom, and Hungary).
- All isolates were sent to a central laboratory in the USA (Laboratories International for Microbiology Studies, a subsidiary of International Health Management Associates, Inc.) for confirmation of identification and susceptibility testing.
- Minimum inhibitory concentrations (MICs) were determined by agar dilution as specified by the Clinical and Laboratory Standards Institute (CLSI) [5]. 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 cefoxitin (2-32).
- MIC interpretive criteria followed published breakpoints established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) where applicable [4]. Since no EUCAST guidelines were available for a cefoxitin, CLSI breakpoints [5] were used. FDA anaerobe breakpoints were used for tigecycline [6].
- Quality control testing was done following CLSI guidelines [5].

## References

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## Results

The *in vitro* activity of tigecycline and comparators versus *B. fragilis* and *B. fragilis* with reduced susceptibility to meropenem is shown in the following Tables and Figures.

Table 1. *In vitro* activity of tigecycline and comparators versus 542 European *B. fragilis*.

Drug	Breakpoints (SII/R) <sup>a</sup>	MIC <sub>50</sub>	MIC <sub>90</sub>	%Sus	%Int	%Res	Range
Tigecycline	≤ 4   8   ≥ 16	0.5	2	98.0	1.7	0.4	0.06 - 32
Cefoxitin	≤ 16   32   ≥ 64	4	16	90.0	6.5	3.5	≤ 2 - > 256
Clindamycin	≤ 4   --   ≥ 8	1	> 8	81.0	0	19	0.06 - > 256
Meropenem	≤ 2   4-8   ≥ 16	0.12	0.5	96.3	3.1	0.6	≤ 0.06 - > 32
Metronidazole	≤ 4   --   ≥ 8	0.5	1	99.6	0	0.4	0.03 - > 256
Pip-Tazo	≤ 8   16   ≥ 32	0.25	1	97.8	1.5	0.7	≤ 0.06 - > 256

<sup>a</sup> Breakpoints are defined by EUCAST (May 2009), where available; cefoxitin breakpoints defined by CLSI (document M100-S19, 2009); and tigecycline breakpoints defined by FDA (Tygacil®, 2005).

Table 2. *In vitro* activity of tigecycline and comparators versus 36 European *B. fragilis* with reduced susceptibility to meropenem.

Drug	Breakpoints (SII/R) <sup>a</sup>	MIC <sub>50</sub>	MIC <sub>90</sub>	%Sus	%Int	%Res	Range
Tigecycline	≤ 4   8   ≥ 16	1	4	100	0	0	≤ 0.06 - 4
Cefoxitin	≤ 16   32   ≥ 64	16	> 32	61.1	13.9	25	4 - > 32
Clindamycin	≤ 4   --   ≥ 8	2	> 8	66.7	0	33.3	≤ 0.25 - > 8
Meropenem	≤ 2   4-8   ≥ 16	2	4	61.1	38.9	0	1 - 4
Metronidazole	≤ 4   --   ≥ 8	0.5	2	100	0	0	0.25 - 2
Pip-Tazo	≤ 8   16   ≥ 32	0.5	8	91.7	8.3	0	≤ 0.06 - 16

<sup>a</sup> Breakpoints are defined by EUCAST (May 2009), where available; cefoxitin breakpoints defined by CLSI (document M100-S19, 2009); and tigecycline breakpoints defined by FDA (Tygacil®, 2005).

Figure 1. *B. fragilis* (542) cumulative % inhibited – European data.

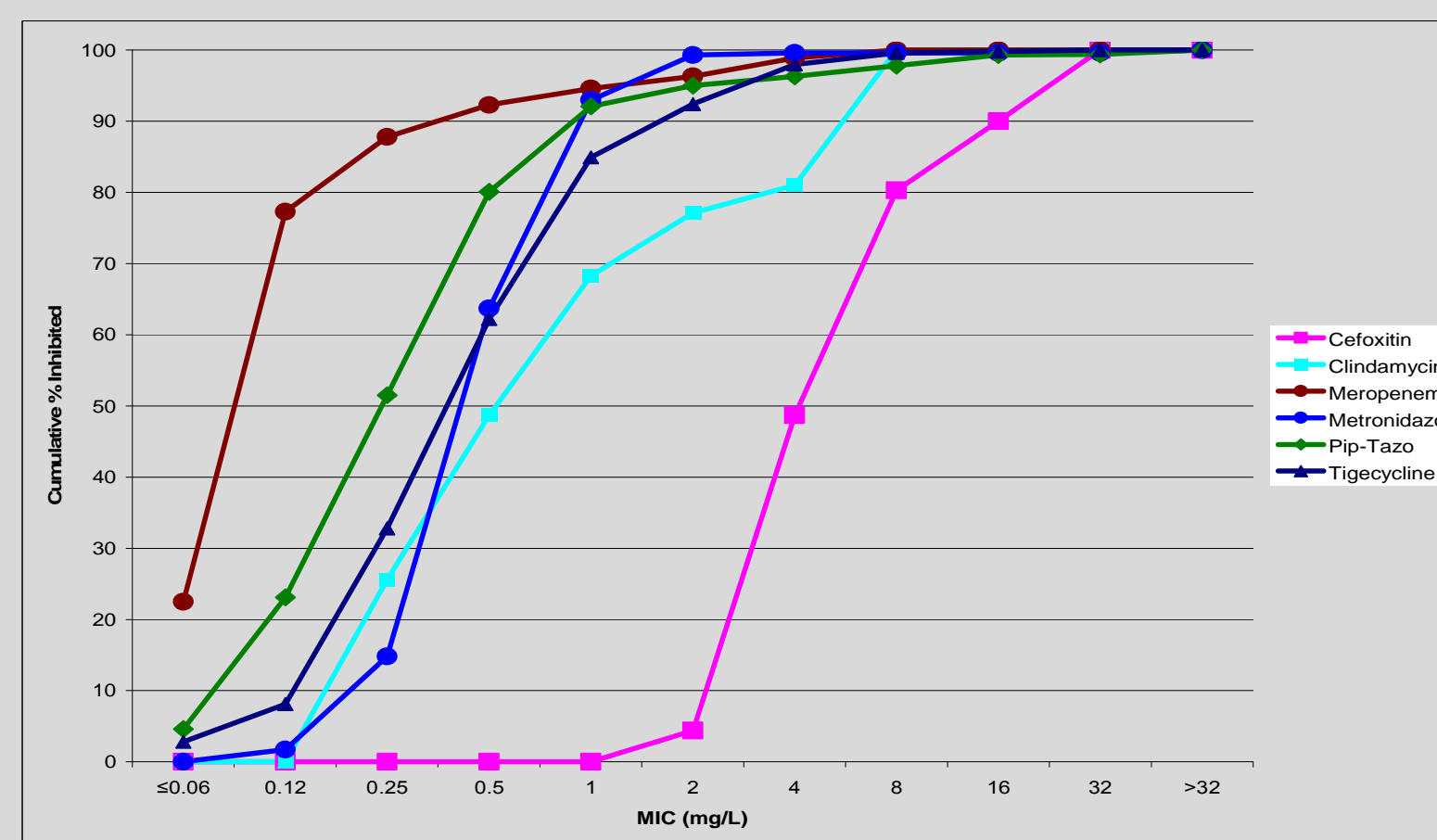
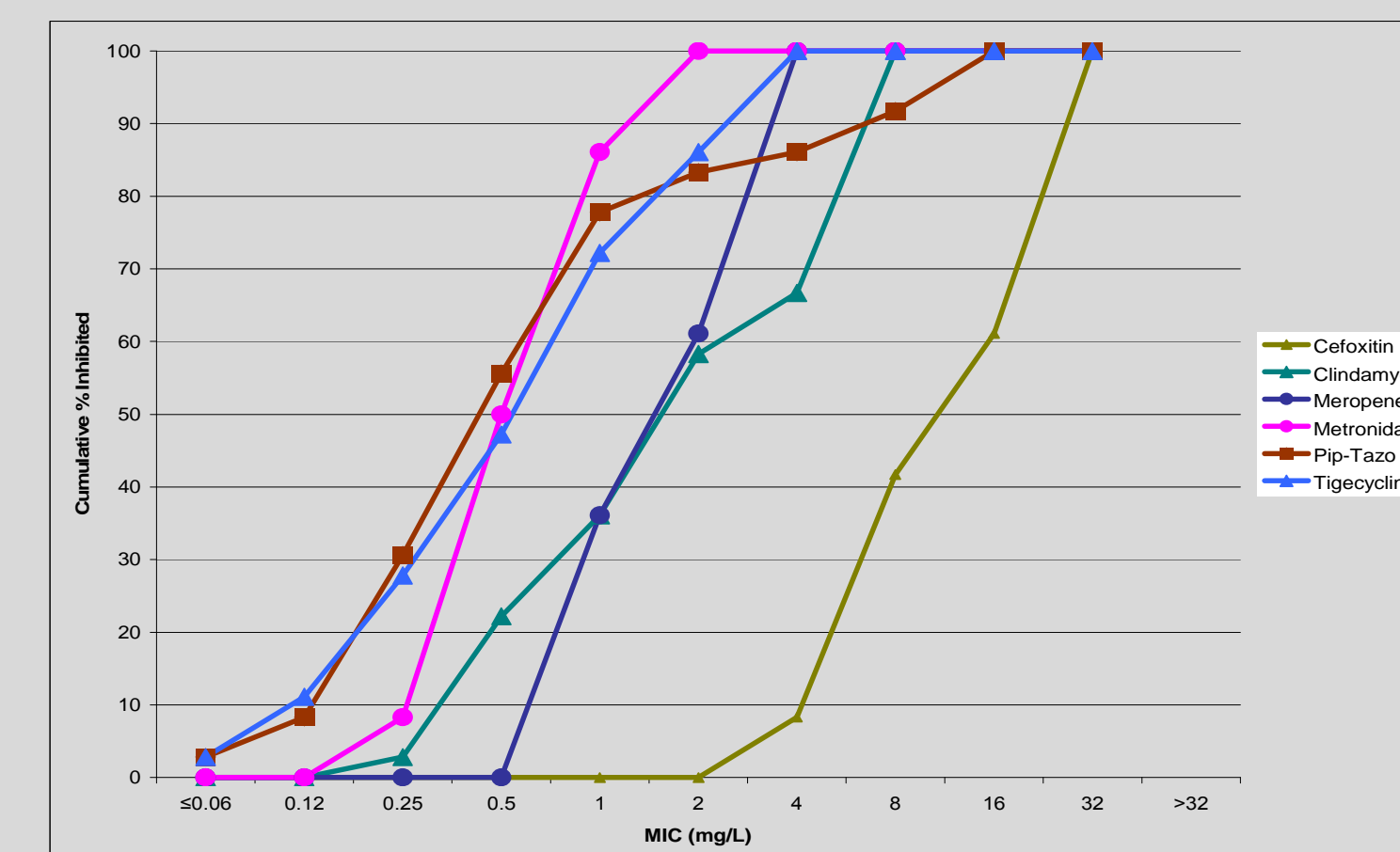


Figure 2. *B. fragilis* (36) with reduced meropenem susceptibility. Cumulative % inhibited – European data.



## Conclusions

- Tigecycline, piperacillin-tazobactam, meropenem, and metronidazole all inhibited >90% of isolates in all 6 European countries.
- Tigecycline and metronidazole inhibited 100% of all isolates with reduced susceptibility to meropenem.
- No one country stood out as having lower or higher overall susceptibility levels than other European countries participating in this study.
- With increasing carbapenem resistance appearing in *B. fragilis*, continued global surveillance is warranted.