



Discrepancies in antimicrobial susceptibility testing of metallo- β -lactamase-producing *Bacteroides fragilis*

Case report

NordicAST Workshop 2017

Tore Taksdal Stubhaug

Dept. of Microbiology, Vestfold Hospital Trust

Carbapenem resistance in *B. fragilis*

- Usually mediated by the *cfiA* metallo- β -lactamase¹. Other mechanisms (e.g. modified PBPs) exist, but are rare²
- Broad spectrum beta-lactamase, degrades penicillins, cephalosporins and carbapenems
- Poorly inhibited by tazobactam³
- Chromosomally present in division II of *B. fragilis*, but with a varying degree of expression

1. Edwards R, Read PN. J Antimicrob Chemother. 2000;46:1009-12.

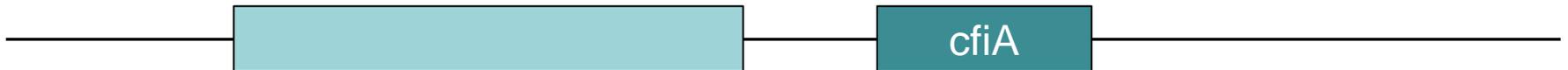
2. Yamazoe K et al. Antimicrob Agents Chemother. 1999;43:2808-10.

3. Bush K et al. Antimicrob Agents Chemother. 1993;37:851-8.

Carbapenem resistance in *B. fragilis*

Expression of the *cfiA* gene depends on the promoter strength of the region upstream of the *cfiA* gene.

Strong promoters are usually carried by IS elements, but may also occur without the presence of an IS element¹

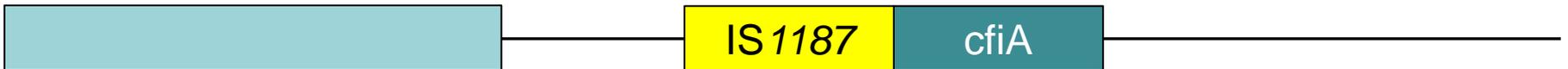


1. Soki J et al. J Med Microbiol. 2004;53:413-9

Carbapenem resistance in *B. fragilis*

Expression of the *cfiA* gene depends on the promoter strength of the region upstream of the *cfiA* gene.

Strong promoters are usually carried by IS elements, but may also occur without the presence of an IS element¹

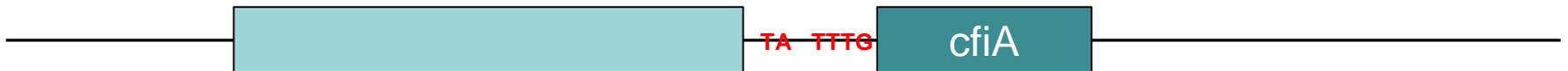


1. Soki J et al. J Med Microbiol. 2004;53:413-9

Carbapenem resistance in *B. fragilis*

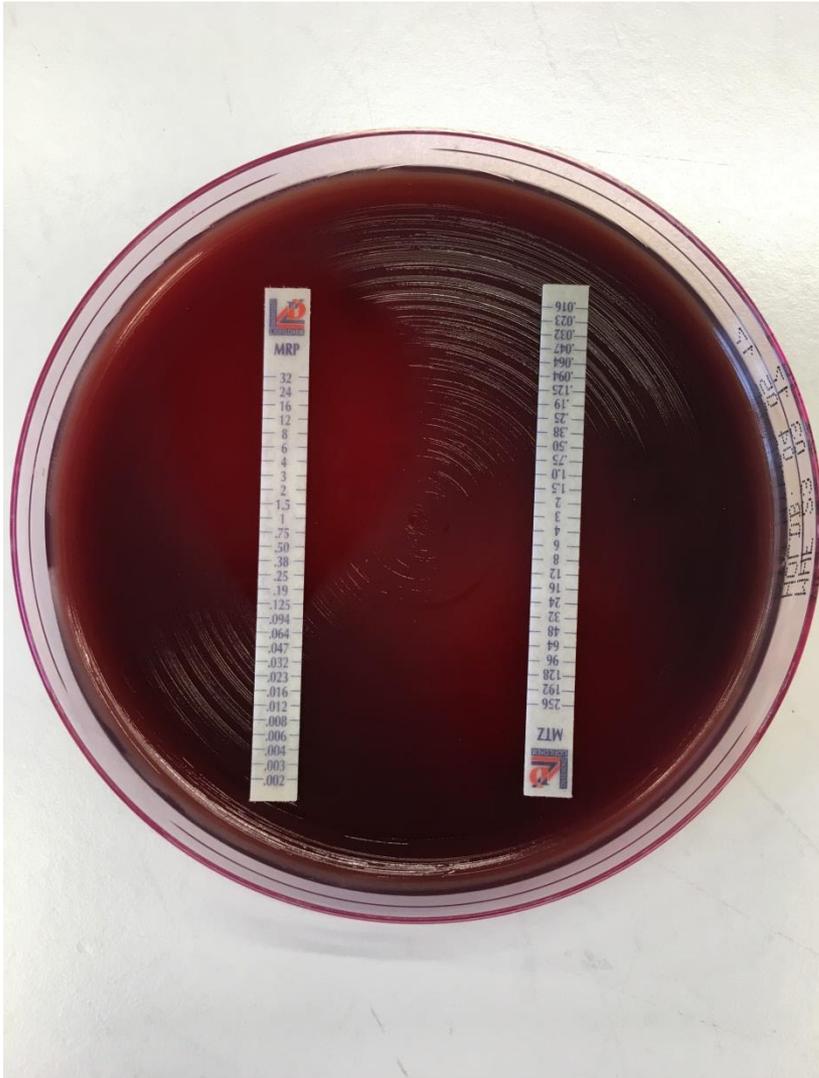
Expression of the *cfiA* gene depends on the promoter strength of the region upstream of the *cfiA* gene.

Strong promoters are usually carried by IS elements, but may also occur without the presence of an IS element¹



1. Soki J et al. J Med Microbiol. 2004;53:413-9

Current practice at SiV



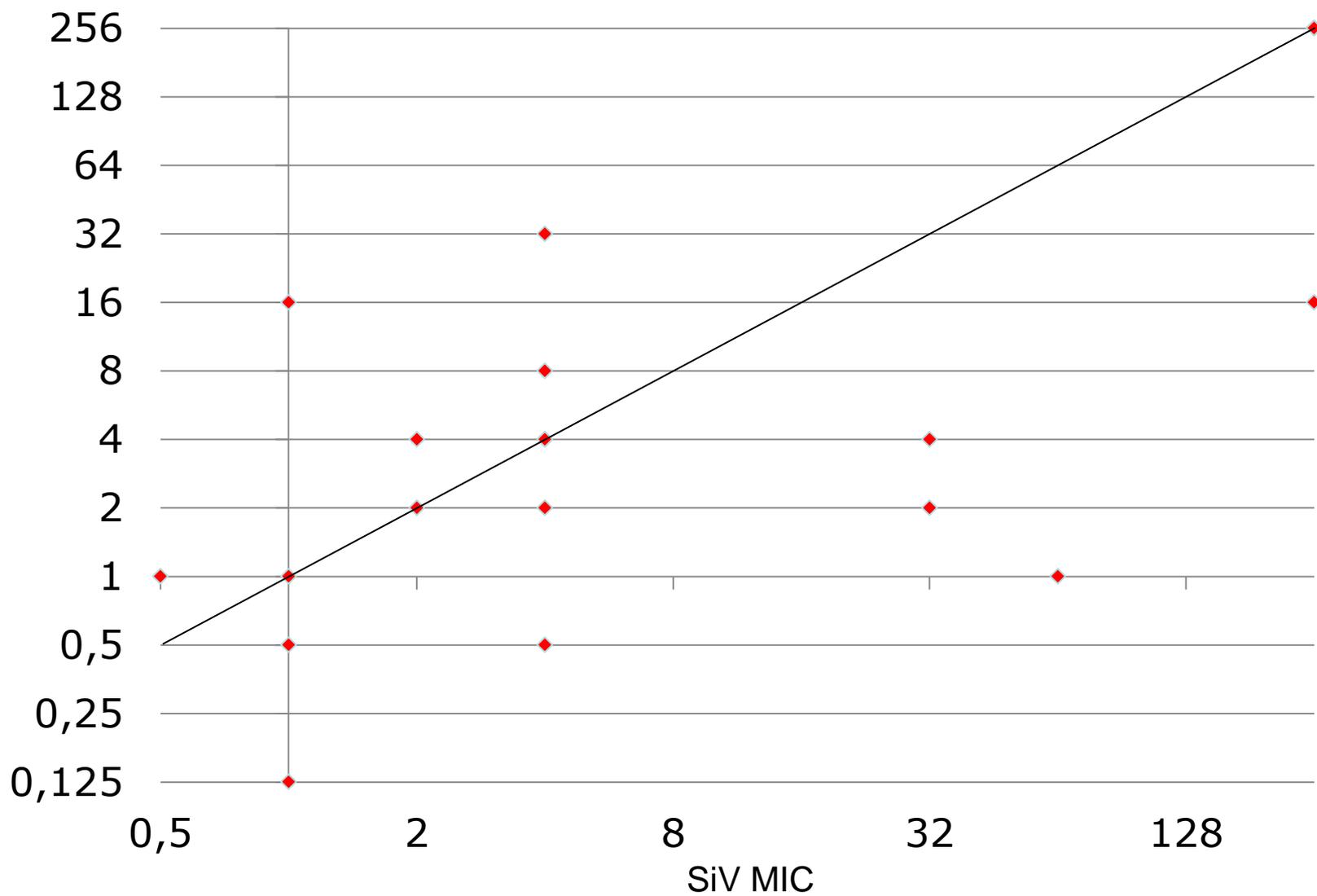
- MIC gradient strips on MH-F agar (Brucella blood agar being implemented)
- All isolates I/R for meropenem are submitted to the national AST reference lab (K-res)
 - 2013 – 2016: 24 isolates
- Testing carried out at K-res:
 - AST using MIC strips on Brucella blood agar
 - EDTA synergy test (double-ended MIC strip)
 - PCR for the *cfiA* gene



Results SiV/K-res

- MIC values for meropenem are largely concordant
- A wide range of MICs are observed for piperacillin/tazobactam, even for isolates that are highly resistant to meropenem
 - The *cfiA* MBL would usually be expected also to hydrolyse piperacillin
- A curiously high number of isolates where piperacillin/tazobactam MICs obtained at SiV and K-res differ
 - Categorical disagreement in 6/24 (25 %)
 - Median 4 dilution steps difference (range 3 – 6)

K-res MIC



Reason?

- Unreliable MIC strips?
- Methodological differences between labs?
- An *in vitro* phenomenon in MBL-producing *B. fragilis*?

1. Problems with piperacillin-tazobactam gradient tests from two manufacturers.

This warning, issued 2015, will be removed when problem is solved.

Following reports of problems related to piperacillin-tazobactam gradient tests, several batches of tests from bioMérieux and Liofilchem (from both single packs and multipacks) were evaluated at the EUCAST Development Laboratory, Växjö, Sweden (January 2016). Enterobacteriaceae and *Pseudomonas aeruginosa* (type strains and strains with resistance mechanisms) and broth micro dilution were used.

Gradient tests from both manufacturers gave variable and unreliable results. The difference between gradient tests and broth micro dilution was not systematic, some values were two dilutions too high, others too low. There was also considerable variation between batches from the same manufacturer.

We urge manufacturers to seriously consider these problems and users to introduce internal quality control of each procedure. We have detected no reason not to trust the disk diffusion (30+6 µg disk) results but currently you can not confirm your disk diffusion results with a gradient test.

Vedrørende innsendt isolat til K-res

Vekst - **BACTEROIDES FRAGILIS (1)**

Innsendt stamme er identifisert med Maldi-TOF.

cfiA-PCR (Sóki J et al. J Med Microbiol 2004;53(Pt 5):413-9):
positiv.

Resistenstabell	1
Klindamycin-	S 2
MBL MP	>8
MBL MPI	0.032
Meropenem-	R >32
Metronidazole-	S 2
Penicillin G high-	R 32
Pip/Tazo-	S 2

S=Sensitiv I=Intermediær R=Resistent,
Tall angir MIC (minste hemmende konsentrasjon).

Kommentar:

Svarskommentar:

Bacteroides fragilis stammen inneholder cfiA-genet som koder for en MBL-karbapenemase. Men MBL-genet kommer sannsynligvis ikke til uttrykk fordi stammen er følsom for piperacillin-tazobaktam. Vi har således ingen sikker forklaring på stammens nedsatte følsomhet for meropenem.

MBL-gradientesten er riktignok positiv, men det kan skyldes EDTAets egenhemmende effekt.

«The *Bacteroides fragilis* isolate possesses the cfiA gene coding for a metallo-β-lactamase. However, the gene is probably not expressed because the isolate is susceptible to piperacillin-tazobactam. Therefore, there is no certain explanation for the isolate's reduced susceptibility to meropenem. The MBL gradient test is positive, but this can be due to the inherent inhibitory activity of EDTA.»

However, testing at the primary lab showed a pip/tazo MIC of 32 mg/L...



Currently no gold standard method in Norway to conclude on whether a *B. fragilis* produces metallo- β -lactamase or not.

Can a pip/tazo S result be trusted if the isolate is meropenem R?

Maybe an expert rule is needed? In that case, a simple method for detection of MBL activity is necessary



Planned NordicAST study

- Approx. 30 *B. fragilis* isolates with different degrees of resistance to meropenem
- Reference examination
 - Reference MIC determination (agar dilution)
 - *cfiA* gene expression analysis (mRNA quantification)
 - Carbapenem hydrolysis assay
- Phenotypic examination by labs in the NordicAST network
 - AST using a novel disk diffusion method
 - Phenotypic tests for MBL production
 - acidimetric tests
 - synergy tests



Thank you for your attention