

Phenotypic confirmation of carbapenemases in *Bacteroides fragilis*

MBL+
Bacteroides fragilis

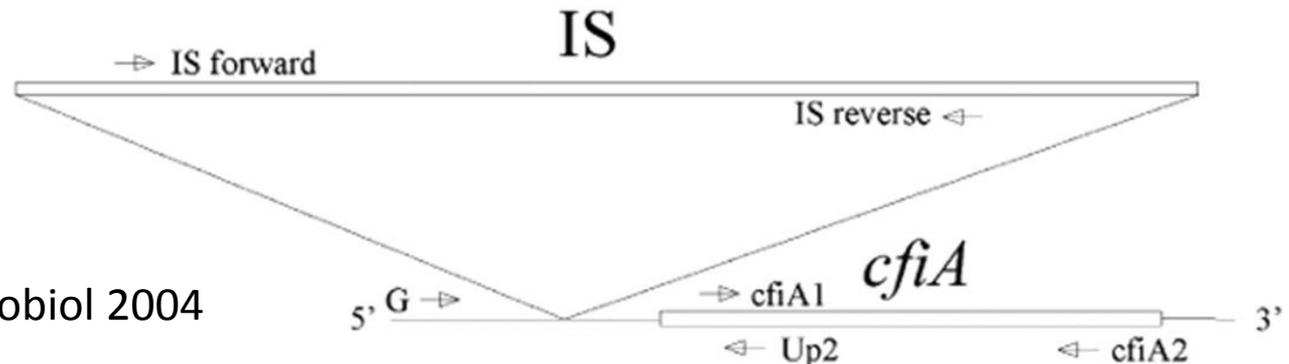
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Carbapenem resistance and the *cfiA* gene

The most common type of carbapenem resistance in anaerobes is metallo-beta-lactamase production in *Bacteroides fragilis*

The *cfiA* gene (chromosomal) “usually” encodes the metallo-beta-lactamase in *B. fragilis*

The degree of resistance is determined by IS (insertion sequences) elements, which serves as promoters for the *cfiA* gene. Isolates with the *cfiA* gene can be S to meropenem.



Level of the *cfiA* gene?

As high as 27% has been reported in clinical *B. fragilis* group isolates

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Actually it was 43% in *B. fragilis*!

Usually it is less than 10%

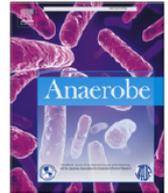
Increasing?



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Note

Susceptibility profiles and resistance genes for carbapenems (*cfiA*) and metronidazole (*nim*) among *Bacteroides* species in a Turkish University Hospital[☆]

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ABSTRACT

Sixty-six nonduplicate *Bacteroides* clinical isolates collected at Marmara University Hospital were tested to investigate carbapenem and metronidazole resistance profiles and to detect the resistance genes (*cfiA* and *nim*) and related insertion sequence (IS) elements. The study found that there were no strains resistant to metronidazole and *nim* genes were not detected in any of the strains. Five *Bacteroides fragilis* strains were resistant to meropenem, one of which was also resistant to imipenem. The *cfiA* gene was detected in 27% of strains, 32% of strains had the IS1187 element, and five strains harbored both gene *cfiA* and IS1187. These results indicate higher rates of carriage of the *cfiA* gene and IS1187 insertion elements than have been reported in other countries.

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Problem or not?

Well; should genes conferring carbapenem resistance in our dominating gut bacteria be considered a potential problem?

If they are S, who cares?

Low level going to high level?

- *In vitro* and *in vivo* results indicates that this is sometimes the case

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A silent carbapenemase gene in strains () can be expressed after a one-st

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Simultaneous resistance to metronidazole, co-amoxiclav, and imipenem in clinical isolate of *Bacteroides fragilis*

Paul Turner, Richard Edwards, Vivienne Weston, Anastasios Gazis, Purvin Ispahani, David Greenwood

A blood-culture isolate of *Bacteroides fragilis*, taken from a woman after elective laparotomy, was resistant to metronidazole and had reduced susceptibility to imipenem and co-amoxiclav. After treatment with imipenem, pus drained from the pleural cavity yielded an identical isolate that had become highly resistant to imipenem and co-amoxiclav. Emergence of full resistance to the β -lactam antibiotics was accompanied by a tenfold rise in the specific activity of a metallo- β -lactamase. Clinicians need to be alert to simultaneous resistance to metronidazole, co-amoxiclav, and imipenem and development of high-level resistance to imipenem in *B fragilis* during treatment with this agent.

Lancet 1995; **345**: 1275–77

Problem or not?

If we don't look for it
- we will never know!

How to look for it?



NordicAST Förklarande metoddokument (OLD)

Anaerobe bakterier og resistens:

”Karbapenemer: Til påvisning af MBL hos isolater med nedsat sensitivitet for karbapenemer kan anvendes meropenem-EDTA eller imipenem-EDTA dobbelt-Etest.”



Imipenem-EDTA double-ended Etest

Figure 1.

Bacteroides fragilis strain 48091.

Imipenem gradient strip +/-
EDTA (MBL double Etest).

MIC ratio can not
be calculated.



IP: imipenem.

IPI: imipenem + EDTA.

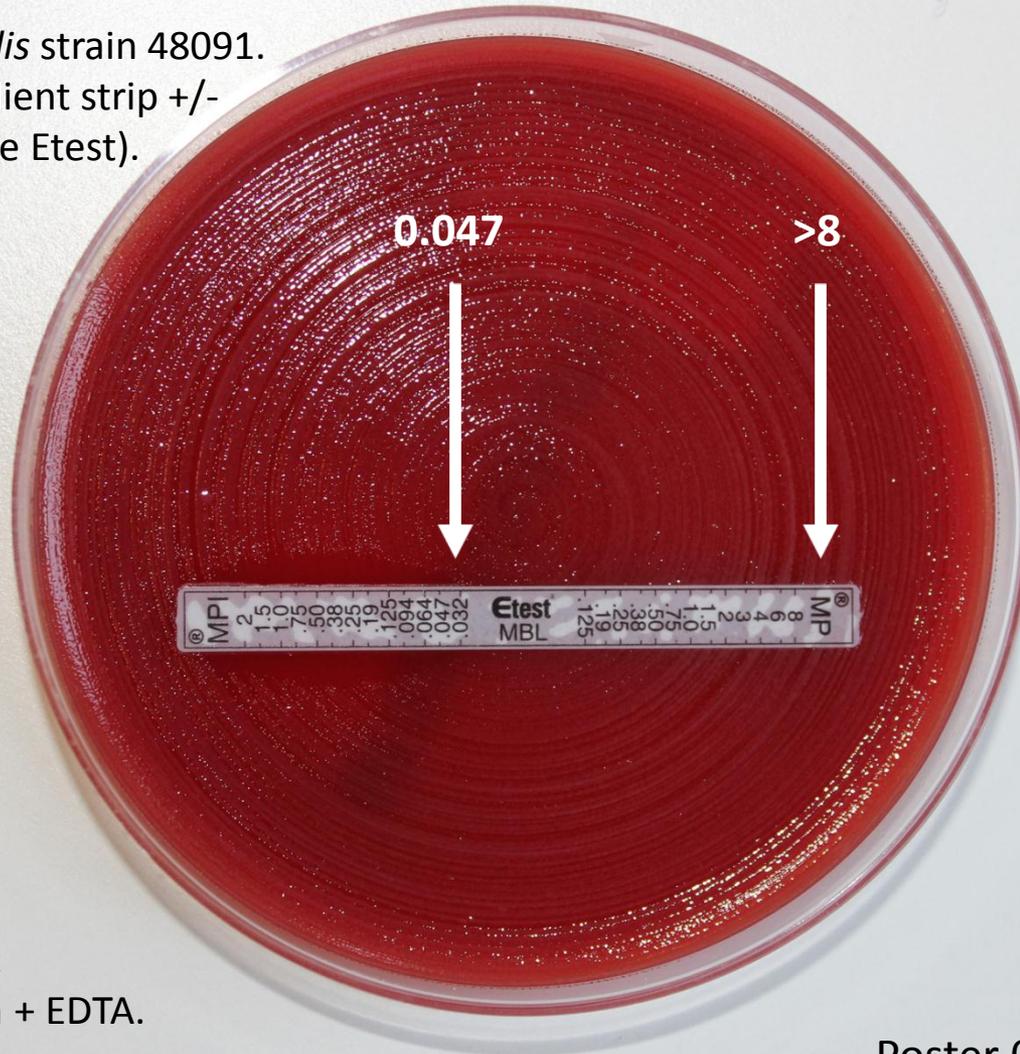
Meropenem-EDTA double-ended Etest

Figure 2.

Bacteroides fragilis strain 48091.

Meropenem gradient strip +/-
EDTA (MBL double Etest).

MIC ratio > 256.



MP: meropenem.

MPI: meropenem + EDTA.

Meropenem and imipenem-EDTA double-ended Etest

Strains	cfiA	MP and IP susceptibility	MP Etest	IP Etest	MBL Etest MP/MPI	≈Ratio	MBL Etest IP/IPI	≈Ratio
ATCC 25285	-	MP S and IP S	0.12	0.094	<0.125 / <0.032	ID	<4 / <1	ID
ATCC 29741	-	-	0.12	0.125	0.19 / 0.032	6	<4 / <1	ID
n=9	+	MP S and IP S	0.5-2	0.064-2	0.75->8 / 0.032-0.064	24->128	<4 / <1	ID
n=6	+	MP I and IP S	3-8	0.125-0.75	4->8 / <0.032-0.125	>64->512	<4 / <1	ID
Isolate A	+	MP R and IP S	>32	1	>8 / 0.047	>128	<4 / <1	ID
Isolate B	+	-	>32	1.5	>8 / 0.047	>128	<4 / <1	ID
n=3	+	MP R and IP R	>32	32->32	>8 / 0.032-0.047	>128->256	<4 / <1	ID
Isolate C	+	-	>32	>32	>8 / 0.032	>256	256 / <1	>256

MP: meropenem. MPI: meropenem + EDTA. IP: imipenem. IPI: imipenem + EDTA. MBL: metallo-β-lactamase. ID: indeterminate.

ROSCO KPC/MBL confirm kit



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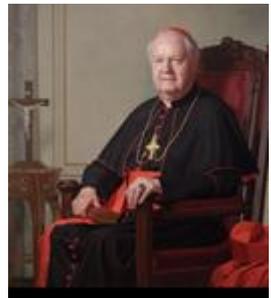
ROSCO KPC/MBL confirm kit

Strains	cfiA	MP and IP susceptibility	MP Etest	IP Etest	Zone diameter median and range (mm)		Difference MRP10 and MRPDP (mm)
					MRP10	MRPDP	
ATCC 25285	-	MP S and IP S	0.12	0.094	44	40	-4
ATCC 29741	-	-	0.12	0.125	38	39	1
n=22	-	-	NA	NA	38 (34-42)	37 (34-45)	-1 (-2-3)
n=9	+	MP S and IP S	0.5-2	0.064-2	28 (26-33)	34 (32-37)	5 (4-8)
n=6	+	MP I and IP S	3-8	0.125-0.75	24.5 (21-30)	31.5 (27-38)	7 (6-8)
Isolate A	+	MP R and IP S	>32	1	19	30	11
Isolate B	+	-	>32	1.5	10	30	20
n=3	+	MP R and IP R	>32	32->32	10 (10)	29 (28-30)	19 (18-20)
Isolate C	+	-	>32	>32	10	30	20

MP: meropenem. IP: imipenem. NA: not available. MRP10: meropenem 10 µg. MRPDP: meropenem 10 µg + dipicolinic acid.

In conclusion

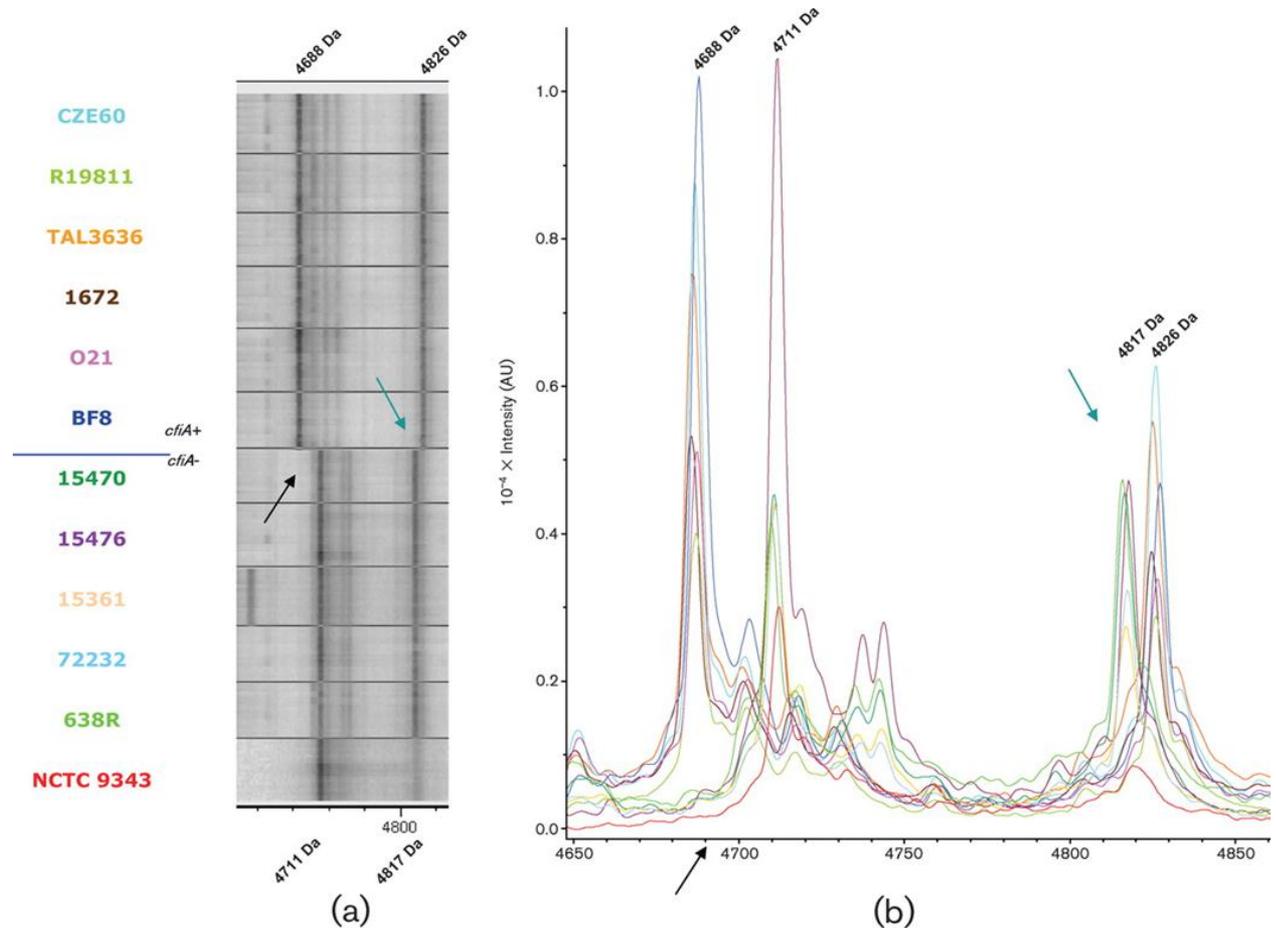
- The meropenem double Etest should be used for phenotypic confirmation of metallo-beta-lactamase production in *B. fragilis*
- The ROSCO MBL/KPC is a less expensive alternative (I + R cases)
- The clinical implications of low level metallo-beta-lactamase production in *B. fragilis* are unknown – how low must you go? (*B. fragilis* meropenem ECOFF = 0.25 mg/L)
- MALDI-TOF can be used to indirectly demonstrate the *cfiA* gene



Indirect detection of the *cfiA* gene

Differentiation between **division I** and **division II** of *B. fragilis* with MALDI-TOF

Corresponds to ***cfiA*-negative** and ***cfiA*-positive** *B. fragilis*



NordicAST Förklarande metoddokument (NEW)

Anaerobe bakterier og resistens:

”Karbapenemer: Til påvisning af MBL hos isolater med nedsat sensitivitet for karbapenemer kan anvendes meropenem-EDTA dobbelt-Etest. Imipenem-EDTA dobbelt-Etesten er mindre lämpligt/egnet på grund af risiko for falsk negativ test”

