

Anaerobe bakterier og resistens

Ulrik Stenz Justesen

Klinisk Mikrobiologisk Afdeling

Odense Universitetshospital

Odense, Denmark

Programme – anaerobic bacteria

Carbapenem and metronidazole resistance

New methods for detecting resistance, including MALDI-TOF (and WGS)

... supplemented with a few cases

→ the first "mentometerfråga"

The Danish situation

High rates of reduced susceptibility in the *Bacteroides fragilis* group isolated from blood cultures – The first national survey in Denmark

Table 1

Percentage of *Bacteroides fragilis* group isolates from the study with reduced susceptibility (I/R) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical minimum inhibitory concentration (MIC) breakpoints.^a

<i>Bacteroides</i> sp. (n)	TZP (S ≤ 8 mg/L; R > 16 mg/L)	MER (S ≤ 2 mg/L; R > 8 mg/L)	CLI (S ≤ 4 mg/L; R > 4 mg/L) ^b
<i>B. caccae</i> (2)	–	–	–
<i>B. cellulosilyticus</i> (5)	–	20% (1/0)	40% (–/2)
<i>B. dorei</i> (4)	–	–	–
<i>B. fragilis</i> (76)	2.6% (1/1)	3.9% (2/1)	19.7% (–/15)
<i>B. ovatus</i> (4)	–	–	–
<i>B. thetaiotaomicron</i> (21)	38.1% (8/0)	–	47.6% (–/10)
<i>B. vulgatus</i> (2)	–	–	50% (–/1)
<i>Parabacteroides distasonis</i> (4)	–	–	25% (–/1)
Total (118)	8.5% (9/1)	3.4% (3/1)	24.6% (–/29)

I, intermediate-susceptible; R, resistant; S, susceptible; TZP, piperacillin/tazobactam; MER, meropenem; CLI, clindamycin.

^a Numbers of I/R are given in parentheses.

^b There is no intermediate-susceptible category for clindamycin.

Letters to the Editor / International Journal of Antimicrobial Agents 42 (2013) 187–193

Carbapenem-resistance

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

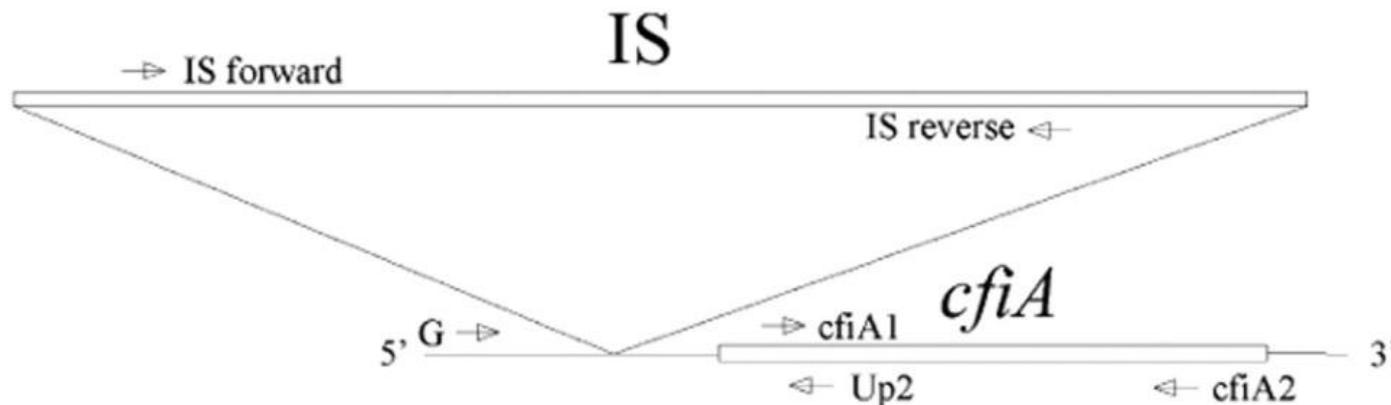
Metallo-beta-lactamases hydrolyses (break down) almost all beta-lactam antibiotics – penicillins, cephalosporins and carbapenems

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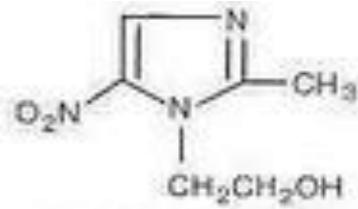
Carbapenem-resistance and the *cfiA* gene

cfiA* gene (chromosomal) encodes a metallo-beta-lactamase (MBL) in *Bacteroides fragilis

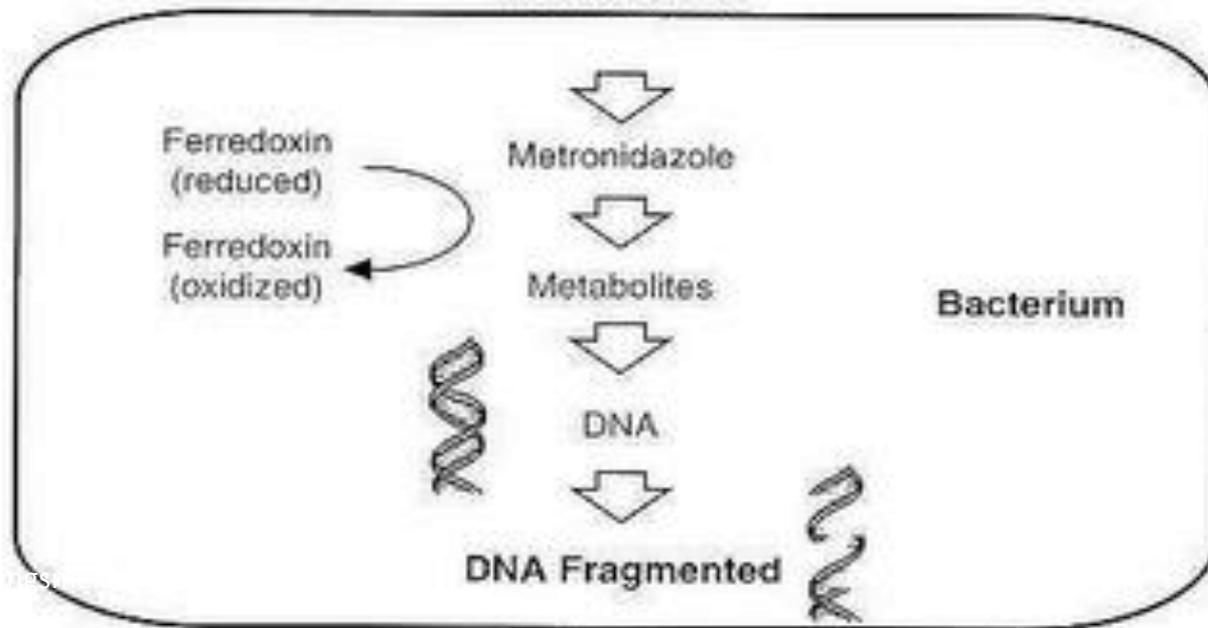
The degree of resistance is determined by IS (insertion sequences) elements, which serves as promoters for the *cfiA* gene



Metronidazole mechanism of action



Metronidazole

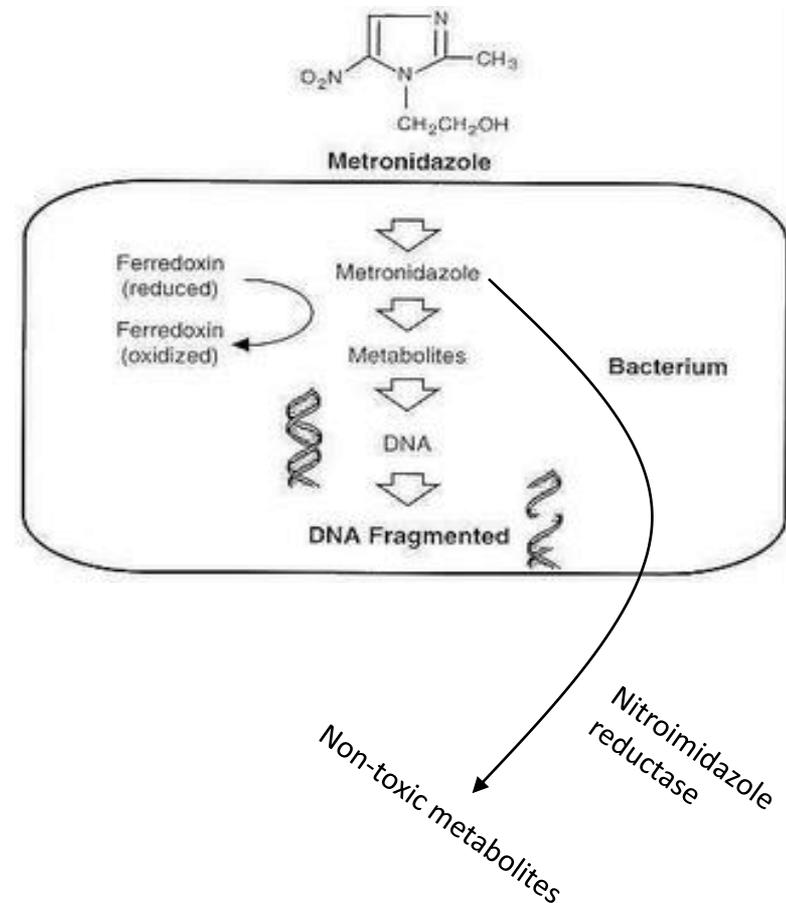


Metronidazole is reduced by ferredoxin to toxic metabolites (only in anaerobic cells)

The toxic metabolites disrupt the DNA

Metronidazole-resistance

- Rare, in otherwise susceptible species (*Bacteroides*, *Prevotella*, *Clostridium*)
- Resistance mechanism: *nim* genes (A-J)
 - encodes a nitroimidazole reductase
 - chromosomal or on a plasmid
- The nitroimidazole reductase converts metronidazole to non-toxic metabolites
- The presence of a *nim* gene is NOT equal to resistance and resistance is NOT equal to the presence of a *nim* gene



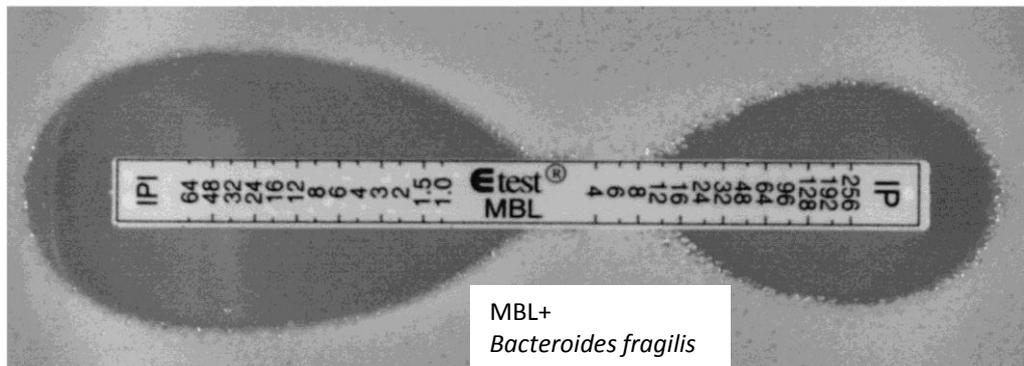
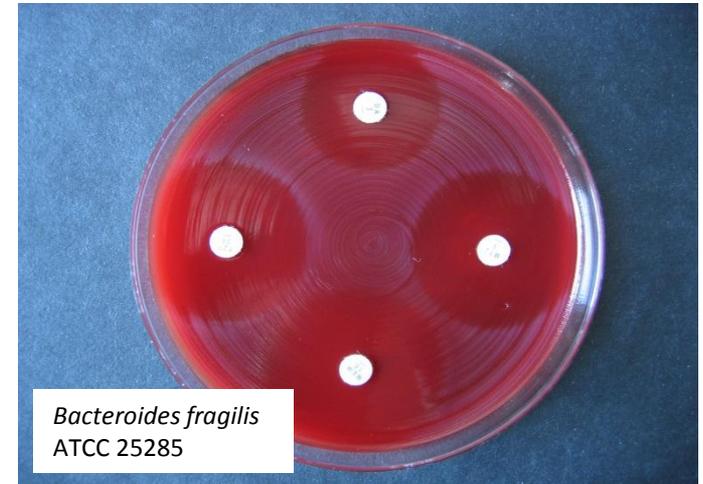
Phenotypic tests – the Gold standard

- Agar dilution for MICs
- Impossible in a routine laboratory



Phenotypic tests

- Screening with disk diffusion (some crazy labs)!!!
- Gradient strips (breakpoints according to EUCAST)
- Meropenem-EDTA or imipenem-EDTA double-Etest – demonstrate MBL (*cfiA* mediated?)



Pseudo metronidazole-resistance

Clostridium perfringens DSM 25589

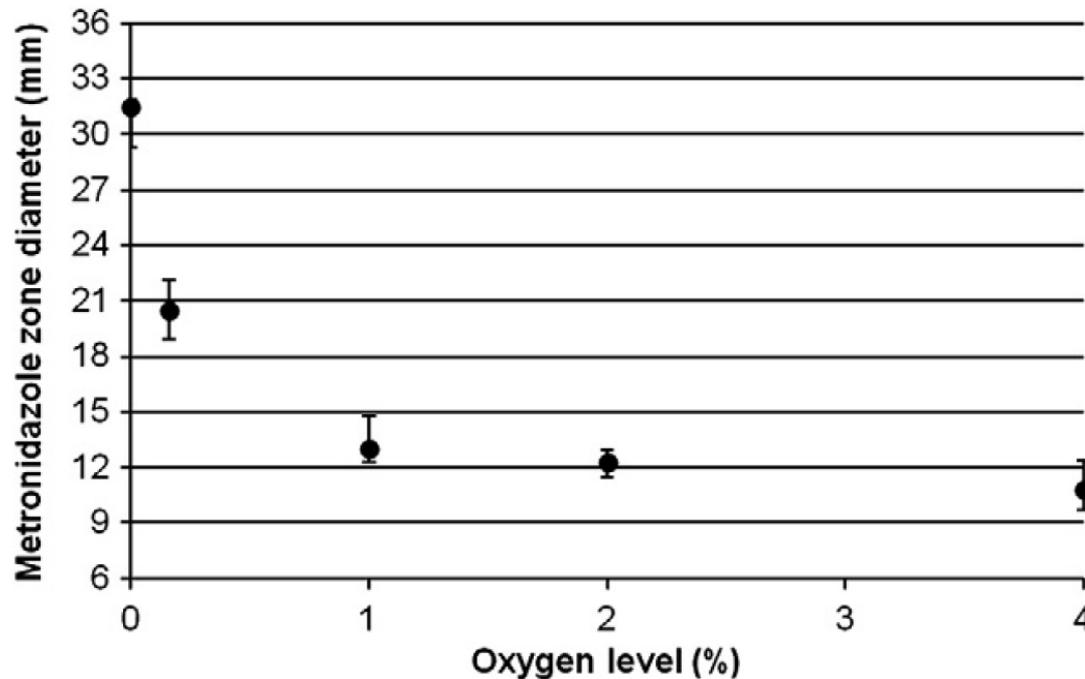


Fig. 2. Results from Part I of the study. The metronidazole zone diameter versus the oxygen level in the Anoxomat jar system. The median with range is shown (n = 12 at each oxygen level).

Metronidazole resistance in the laboratory is very often caused by insufficient anaerobic conditions

Oxygen in the "anaerobic" atmosphere

Metronidazole is NOT reduced to toxic metabolites

The new methods for detecting resistance

- MALDI-TOF
- Whole genome sequencing (WGS)

Methods available for antimicrobial susceptibility testing with MALDI-TOF

- ~~1) Demonstrating a genotype = resistance
(e.g. the mecA gene)~~
- 2) Demonstrating break down of an antibiotic
(e.g. hydrolysis by a beta-lactamase)
- 3) Demonstrating bacterial growth
- 4) Demonstrating bacterial kill

2) Demonstrating break down of an antibiotic

MALDI-TOF hydrolysis assay

Beta-lactam antibiotics, in this case carbapenems, are hydrolysed by the metallo-beta-lactamase encoded by the *cfiA* gene in *B. fragilis*

Hydrolysis results in mass changes of beta-lactams, which can be measured by MALDI-TOF

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MALDI-TOF – hydrolysis assay

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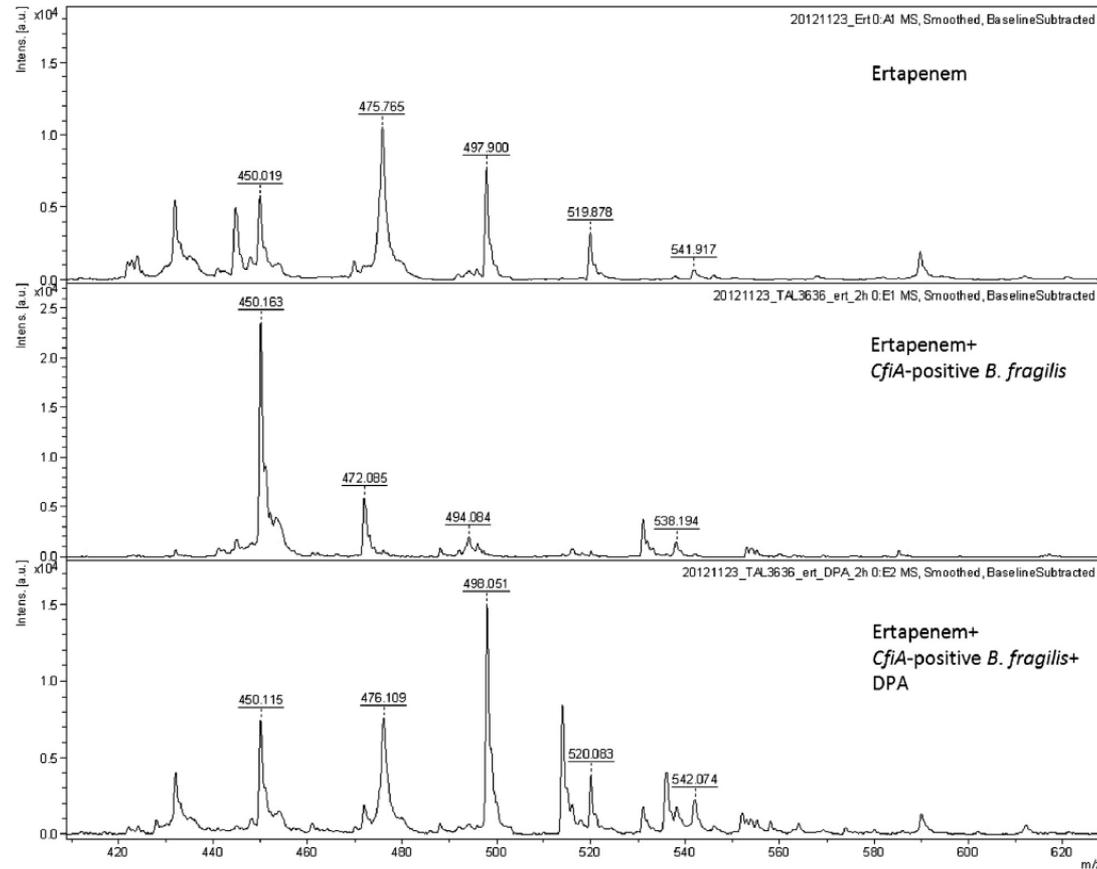


Fig. 1. Intact ertapenem display the following peak pattern: 476.5, 498.5, 520.5 and 542.5 Da, and is shown in the upper part of the figure. Hydrolyzed ertapenem display the following peak pattern: 450.5, 472.5, 494.5 and 538.5 Da. The hydrolysis of ertapenem by a *cfiA*-positive *B. fragilis* strain is shown in the middle segment. The lower part of the figure shows the pattern of intact ertapenem when a *cfiA*-positive *B. fragilis* strains has been incubated with DPA, which acts as an inhibitor for metallo- β -lactamases.

MALDI-TOF – hydrolyse assay



Table 1
 Description of strains used in the study and the results in the ertapenem hydrolysis assays measured by MALDI-TOF MS.

<i>B. fragilis</i>	IS element	PCR detection of <i>cfiA</i> gene	Imipenem MIC (mg/L)	Ertapenem MIC (mg/L)	Ertapenem hydrolysis	Inhibition DPA	Reference
TAL3636	IS942	+	>256	>32	+	+	[14]
1672	IS1186, (IS4351) ^b	+	>32	>32	+	+	[15]
2944	IS614B	+	>32	>32	+	+	[15]
1776	IS614B	+	>32	>32	+	+	[15]
2685	IS614B	+	>32	>32	+	+	[15]
4729	IS1187	+	>32	>32	+	+	[15]
6712	IS612B	+	>32	>32	+	+	[15]
16997	–	+	16	16	+	+	[15]
388	–	+	4	2	+	+	[15]
BF8 (BFR81)	–	+	1	4	+	+	[16]
72	–	+	0.25	2	+	–	[7]
55474/1	–	–	0.125	2	+	–	This study
22	–	–	0.064	2	+	–	[7]
16827	–	+	0.064	2	+	+	This study
AA-137-TH	–	+	2	32	+	+	This study
12-538566	–	+	1	8	+	+	This study
638R	–	–	0.064	0.125	–	NT ^a	[17]
43	–	–	0.125	0.125	–	NT	This study
363	–	–	0.125	0.125	–	NT	This study
51981/1	–	–	0.032	0.25	–	NT	This study
38860/1	–	–	0.25	0.5	–	NT	This study
56001/2	–	–	0.5	0.125	–	NT	This study
38360/2	–	–	0.25	0.5	–	NT	This study
664	–	–	0.125	0.25	–	NT	This study
480	–	–	0.064	0.125	–	NT	This study
461	–	–	0.125	0.125	–	NT	This study
76240	–	–	0.5	0.5	–	NT	This study

Imipenem R > 8 mg/L

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Ertapenem R > 1 mg/L

^a Not tested.

^b IS element in parenthesis was detected in the genome, but not upstream of the *cfiA* gene [15].

Not quite there with MALDI-TOF – yet!

- The presence of beta-lactam hydrolysis is not always equal to beta-lactam resistance

... in the presented examples with *B. fragilis*

→ We need more ...

3) & 4) Demonstrating bacterial growth/kill

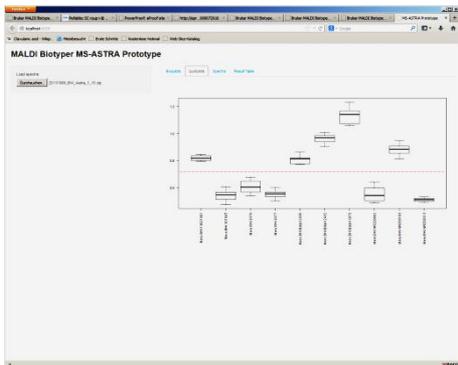
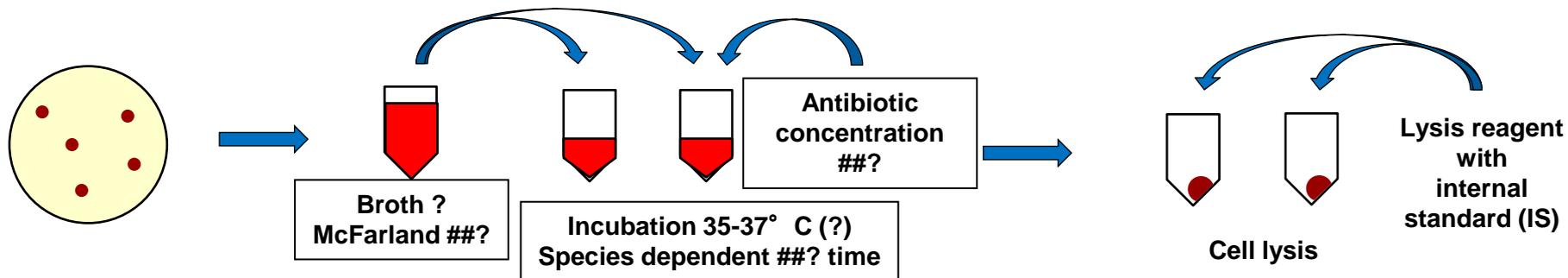
- with the

MALDI BioTyper Antibiotic Suseptibility Test Rapid Assay

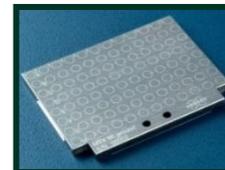
→ **MBT-ASTRA**

Workflow MBT-ASTRA

The boxes = variables



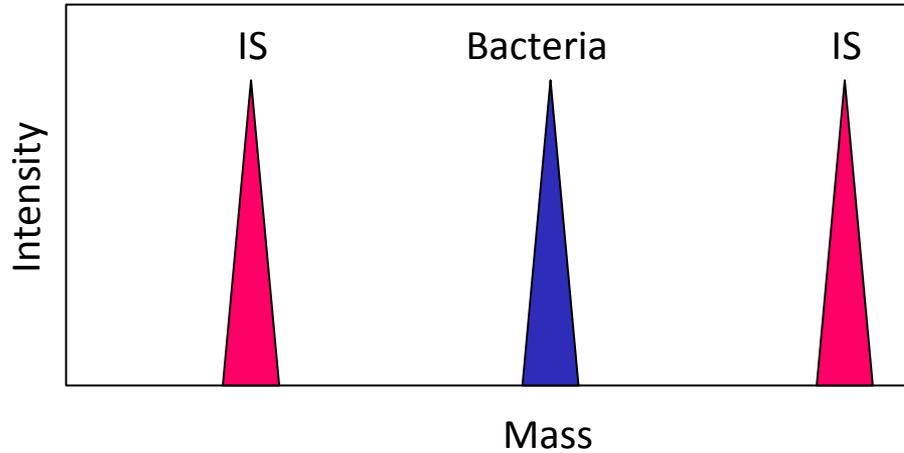
Acquisition of MS profile spectra



Target preparation

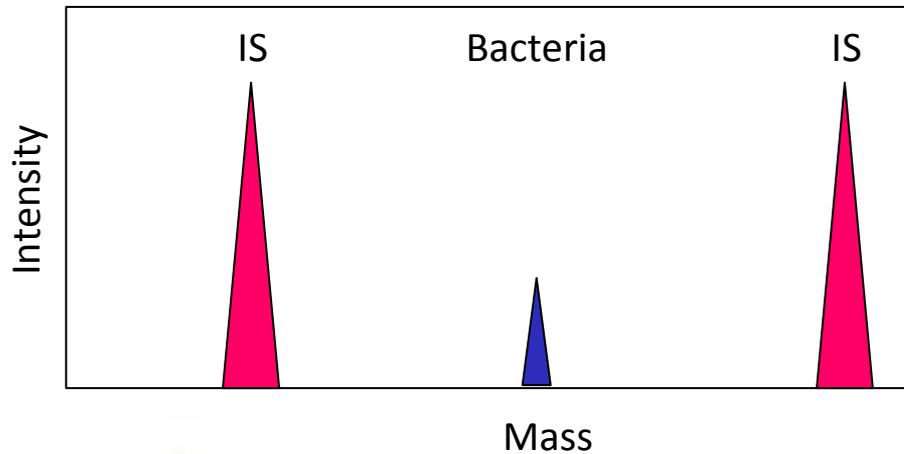
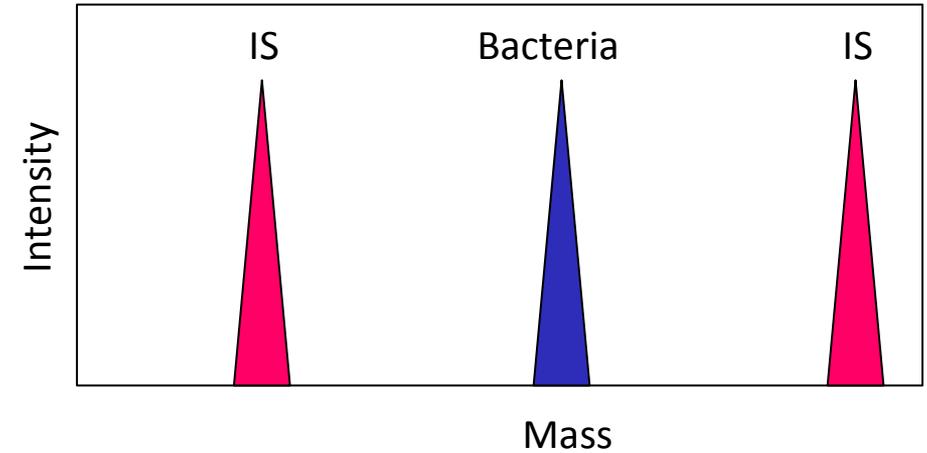
MBT-ASTRA – measuring the AUC

SUSCEPTIBLE STRAIN

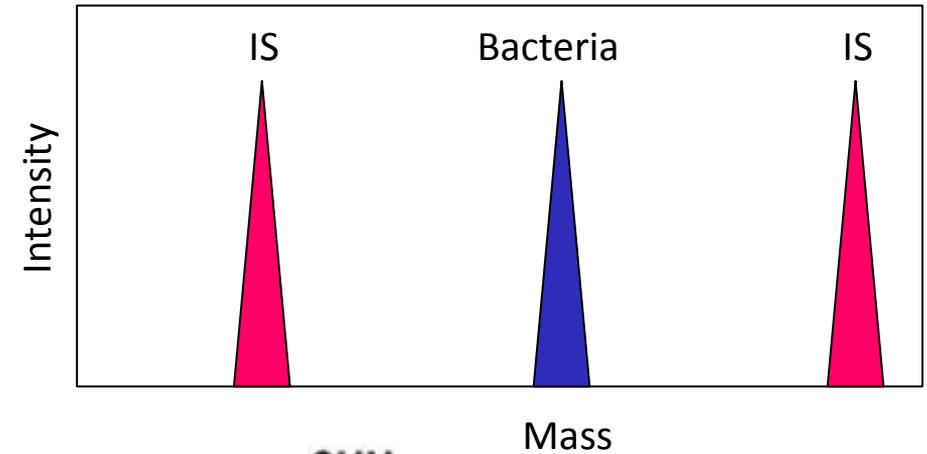


Broth only

RESISTANT STRAIN

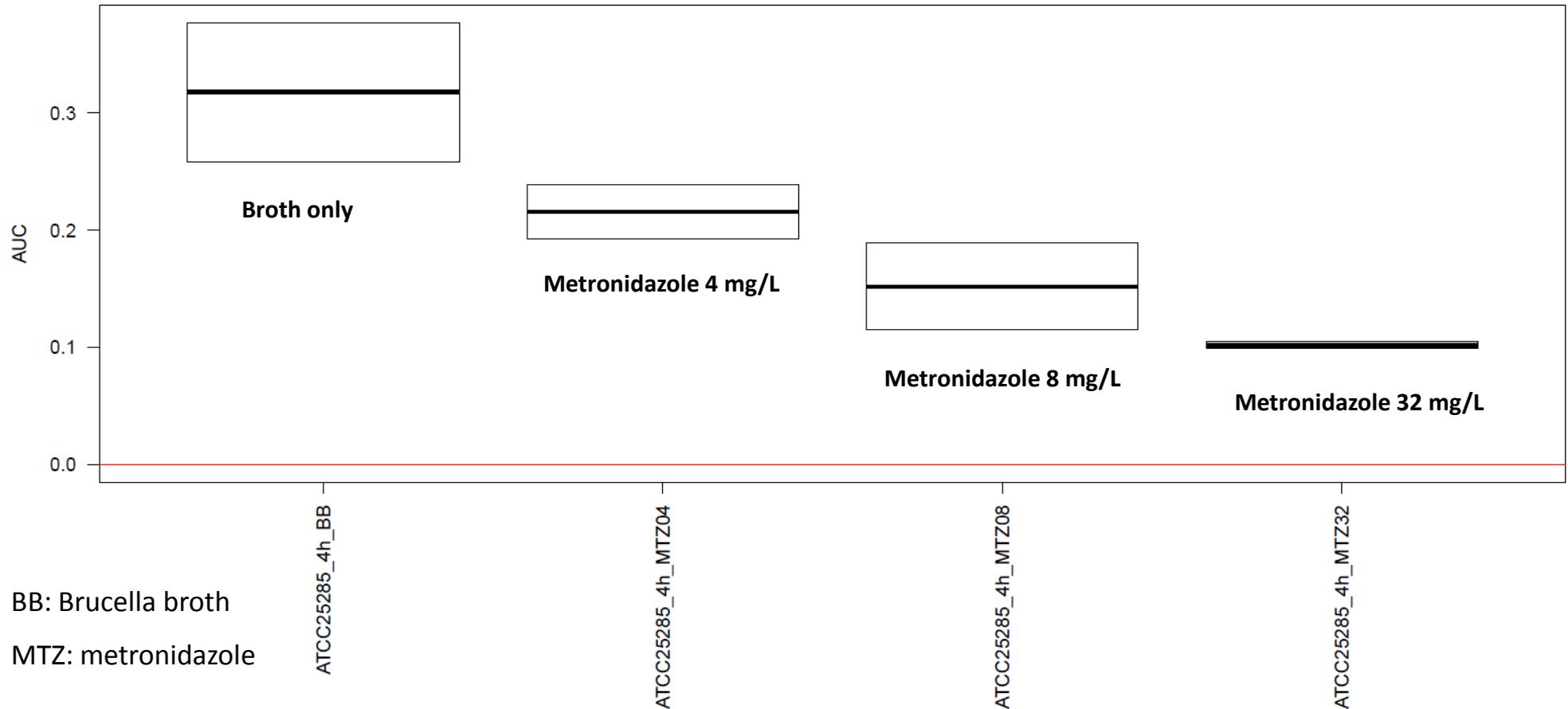


Broth + antibiotic



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MBT-ASTRA and *Bacteroides fragilis* ATCC 25285*



BB: Brucella broth
MTZ: metronidazole

ATCC25285_4h_BB

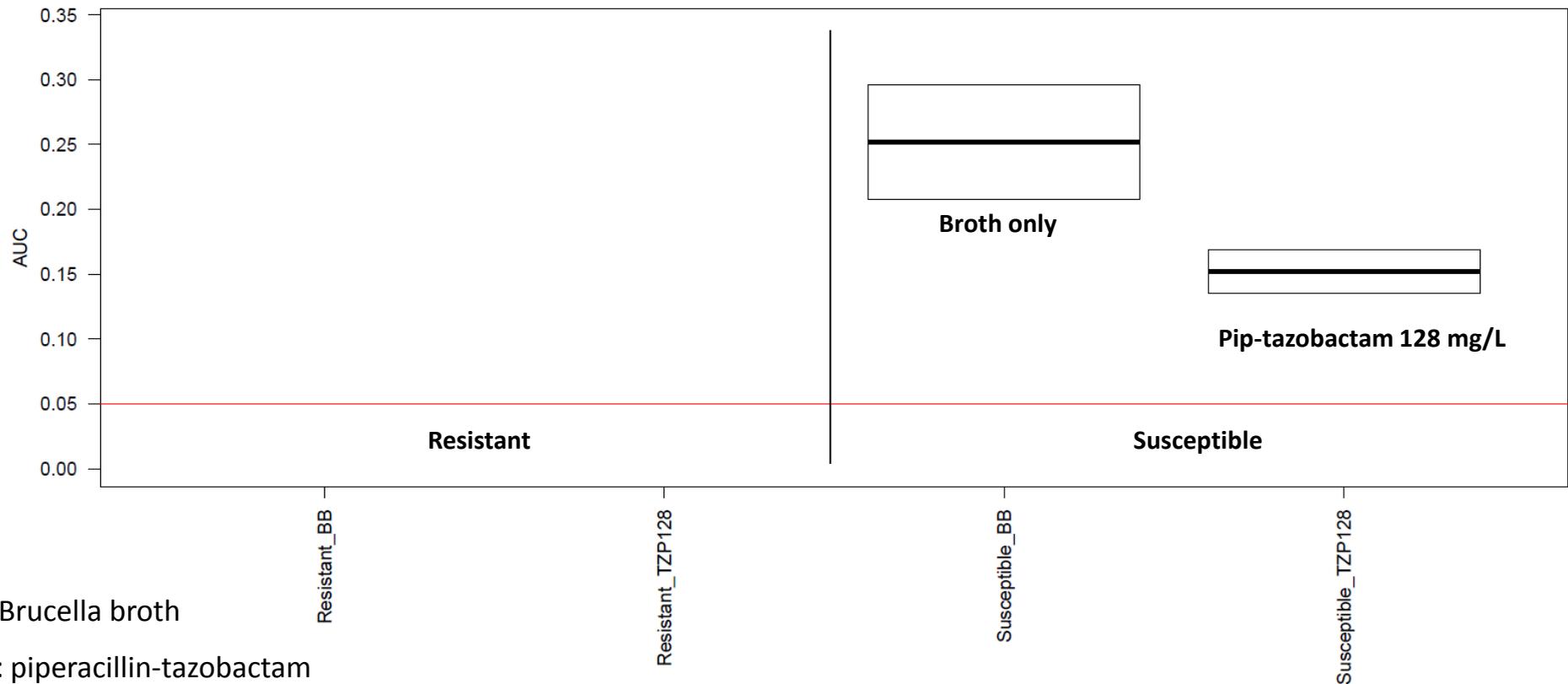
ATCC25285_4h_MTZ04

ATCC25285_4h_MTZ08

ATCC25285_4h_MTZ32

MBT-ASTRA and *Bacteroides fragilis*

Resistant and susceptible – can we detect a difference?



BB: Brucella broth

TZP: piperacillin-tazobactam

Still some work ahead

- Quantifying the level of resistance from the AUC
- Isolates close to the breakpoint will probably be a challenge!

A few cases with a little WGS

Case: Abscess with *B. fragilis*

Akershus universitetssykehus HF

	MIC (mg/L)
Meropenem:	>256
Piperacillin-tazobactam	>256
Clindamycin	>32
Metronidazol	>32

imipenem-EDTA og meropenem-EDTA double-Etest **POSITIVE (Ratio ≥ 8)**

Mechanisms?

WGS workflow for dummies



Results from big computer (*B. fragilis*)

ujustesen - Outlook Web x Center for Genomic Epide x
 https://cge.cbs.dtu.dk/cgi-bin/webface.fcgi?jobid=554CA0F800001350B3E9F7D1

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ResFinder-2.1 Server - Results

Aminoglycoside
 No resistance genes found.

Beta-lactam

Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
<i>cfxA</i>	99.90	966 / 966	NODE_126_length_10901_cov_18.0291_ID_253	10..975	Beta-lactam resistance	U38243
<i>cfiA4</i>	100.00	750 / 750	NODE_22_length_58417_cov_14.519_ID_43	57553..58302	Beta-lactam resistance	AB087229

Fluoroquinolone
 No resistance genes found.

Fosfomycin
 No resistance genes found.

Fusidic Acid
 No resistance genes found.

MLS - Macrolide, Lincosamide and Streptogramin B

Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
<i>erm(F)</i>	99.88	801 / 801	NODE_153_length_6395_cov_9.73963_ID_305	95..895	Macrolide resistance	M17808

Nitroimidazole

Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
<i>nimE</i>	100.00	435 / 435	NODE_4_length_99023_cov_117.479_ID_7	34247..34681	metronidazole (5-nitroimidazole)	AM042593

Oxazolidinone
 No resistance genes found.

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Case: Blood culture with *B. fragilis*

Mikrobiologen, Sunderby sjukhus, Luleå

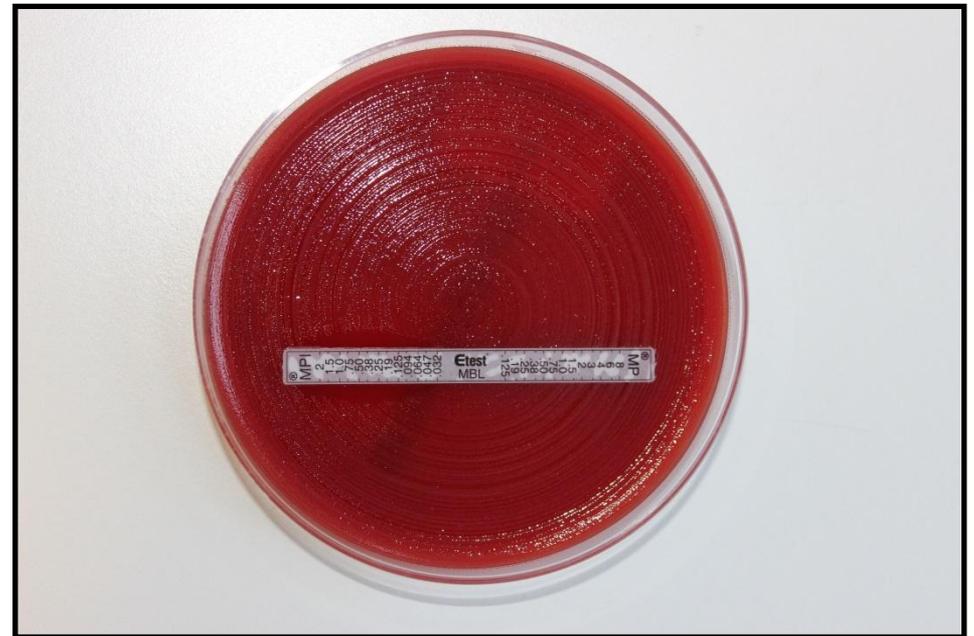
	MIC (mg/L)
Imipenem:	12
Meropenem:	>256
Piperacillin-tazobactam	>256
Clindamycin	2
Metronidazol	0.25

imipenem-EDTA double-Etest **NEGATIVE!?**

Mechanism?

Phenotypic tests

Meropenem-EDTA or imipenem-EDTA double-Etest to demonstrate MBL – which one?



MALDI-TOF with the right peaks → *cfiA*+

Case: Blood culture with *Parabacteroides goldsteinii*

Odense Universitetshospital

	MIC (mg/L)
Meropenem:	>256
Piperacillin-tazobactam	>256
Clindamycin	>32
Metronidazol	0.25

imipenem-EDTA og meropenem-EDTA double-Etest **POSITIVE (Ratio ≥ 8)**

Mechanisms?

WGS workflow for dummies



Results from big computer (*P. goldsteinii*)

Center for Genomic Epidemiology

Home Services Instructions Output Overview of genes Article abstract

ResFinder-2.1 Server - Results

Aminoglycoside
No resistance genes found.

Beta-lactam
No resistance genes found.

Fluoroquinolone
No resistance genes found.

Fosfomycin
No resistance genes found.

Fusidic Acid
No resistance genes found.

MLS - Macrolide, Lincosamide and Streptogramin B						
Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
<i>erm(F)</i>	100.00	801 / 801	NODE_64_length_38914_cov_5.52669_ID_127	3188..3988	Macrolide resistance	M17808

Nitroimidazole
No resistance genes found.

Oxazolidinone
No resistance genes found.

Phenicol
No resistance genes found.

Rifampicin
No resistance genes found.

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Conclusion

- **No single completely perfect method for carbapenemase characterization**
- **Use a mixture of methods if possible**
- **Whole genome sequencing is definitely an excellent confirmatory tool**

Thank you for your attention!

→ the second "mentometerfråga"