

Resistensbestemmelse av anaerobe bakterier

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Hva er spesielt med anaerobe bakterier?



Iboende R for
metronidazol



Ofte blandingsinfeksjoner

Empirisk behandling

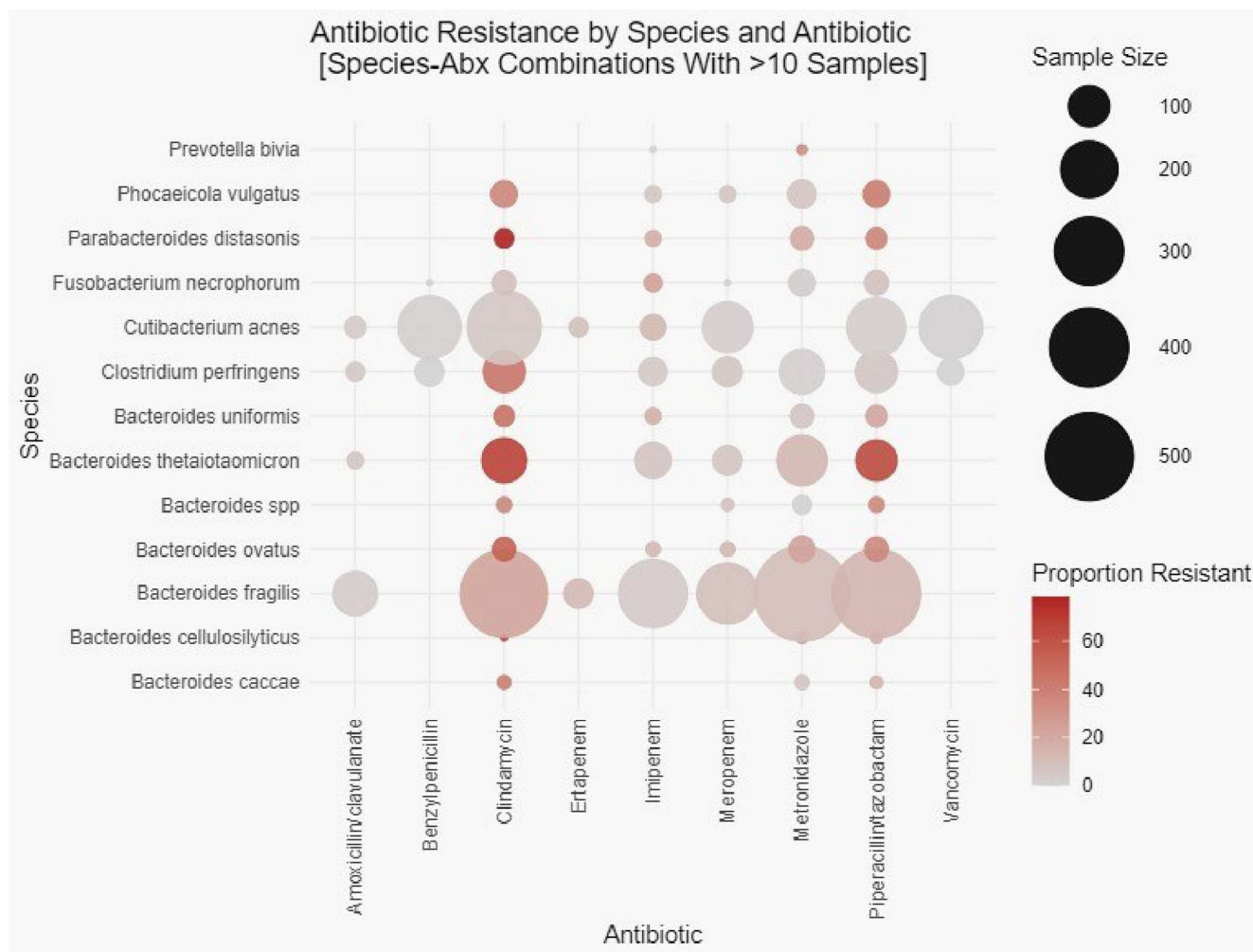
Metronidazol og/eller piperacillin-tazobactam
(ev. meropenem) inngår ofte i norske empiriske
regimer ved anaerobe infeksjoner



Når skal man resistensbestemme anaerobe bakterier?

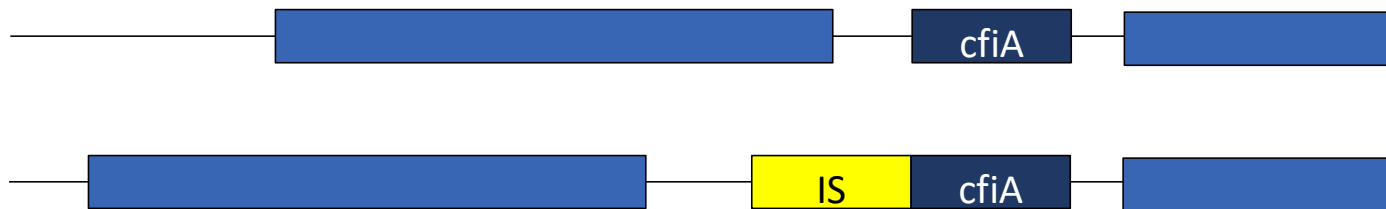
Noen forslag:

- Ved alvorlige/livstruende infeksjoner
- Spesielt virulente arter
- Arter med uforutsigbart eller ukjent resistensmønster
- Ved manglende effekt av empirisk behandling
- Ved behov for langvarig antibiotikabehandling
- Når arten isoleres i renkultur eller fra et normalt sterilt område



Boattini M et al. Diagnostic and epidemiological landscape of anaerobic bacteria in Europe, 2020-2023 (ANAEuROBE). Int J Antimicrob Agents. 2025;65(6):107478. doi: 10.1016/j.ijantimicag.2025.107478. Gjengitt med tillatelse iht. [Creative Commons CC-BY](https://creativecommons.org/licenses/by/4.0/)

Antibiotikum	Hyppigste resistensmekanisme
Penicillin	Betalaktamaser
Pip/tazo	Betalaktamaser, (PBP-forandringer)
Karbapenemer	Kromosomale karbapenemaser <i>Ikke</i> det samme som ESBL _{CARBA} hos Gram-negative aerobes
Klindamycin	23S rRNA metylase (MLS _B -resistens, samme som hos stafylokokker og streptokokker)
Metronidazol	Metronidazolreducerende enzymer (<i>nim</i> -gener) Andre mekanismer finnes også



Flere resistensgener hos anaerober har variabelt uttrykk. Fenotypisk profil (MIC) kan variere betydelig avhengig av hvor kraftig genet uttrykkes.

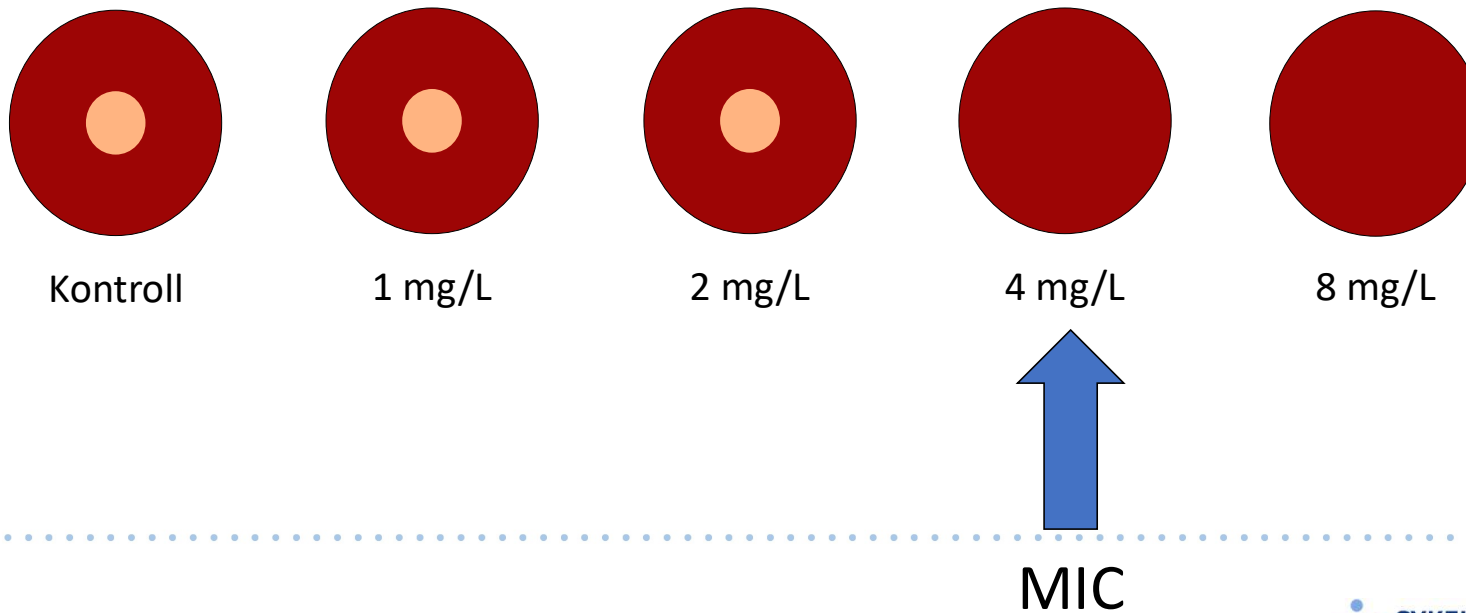
Tilstedeværelse av insersjonssekvenser oppstrøms for genet gir kraftig oppregulering.

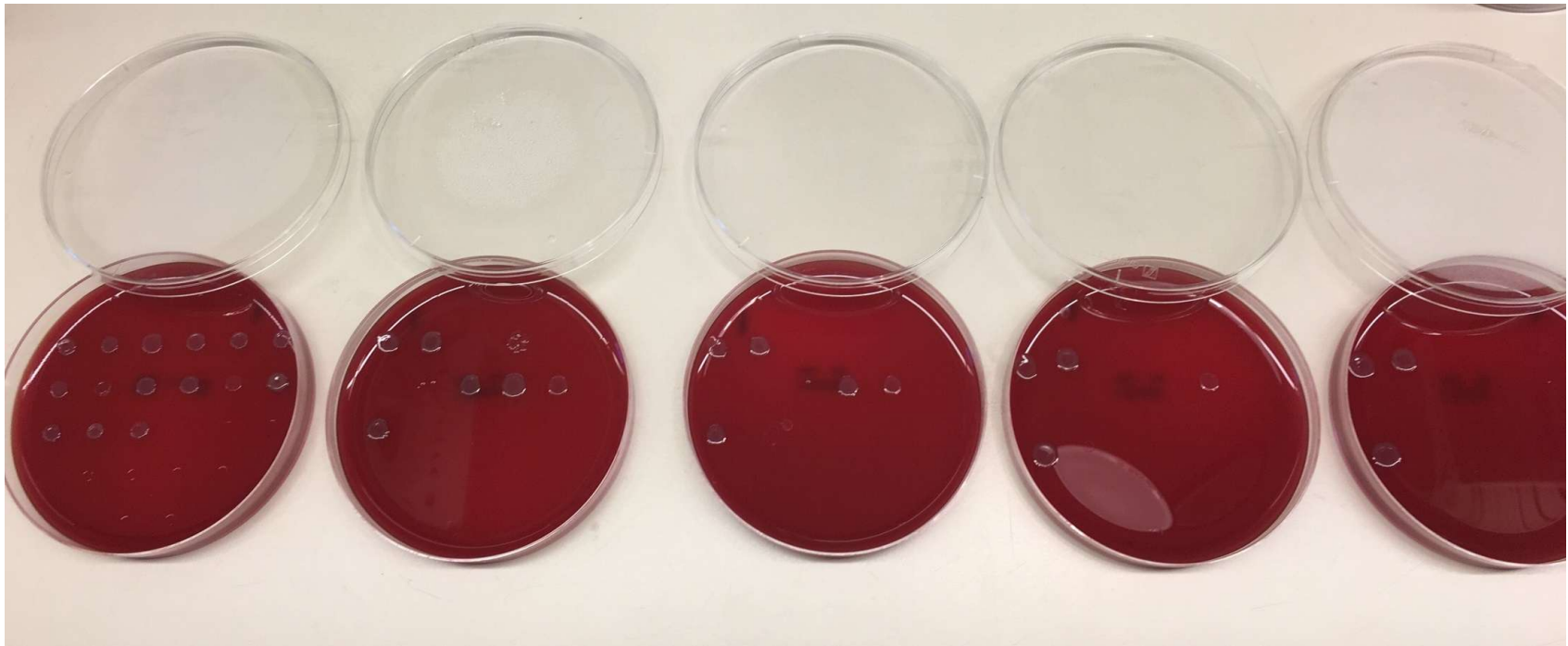
Gjelder blant annet

- karbapenemasegen *cfiA*
- metronidazolresistensgener (*nim*)
- klindamycinresistensgener (*erm*)

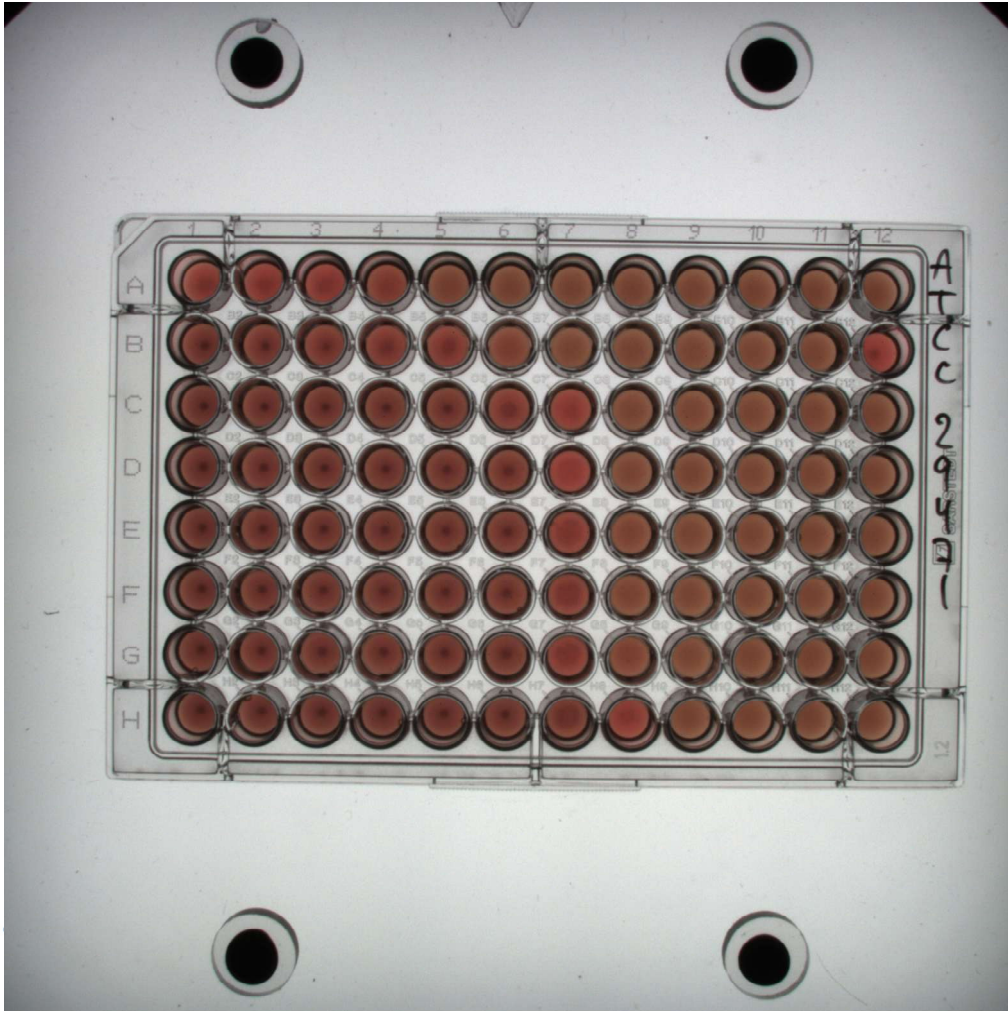
Referansemetode: Agarfortynning

BBA (CLSI) / FAA (EUCAST) tilsatt antibiotika i tofolds fortynninger
Punktformet inokulasjon av bakterieoppslemming
42-48 timer anaerob inkubasjon





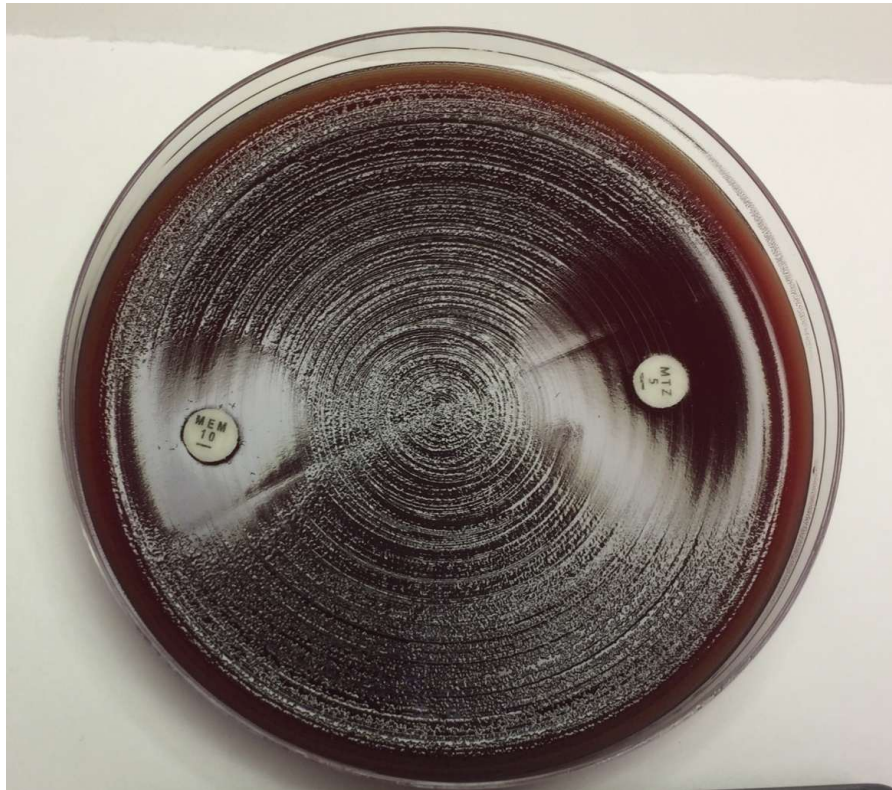
Buljongfortynning



Kun for *Bacteroides*

Kommersielle paneler finnes

EUCAST lappediffusjon



Fastidious Anaerobe Agar med
5% hesteblood (FAA-HB)

1.0 McFarland

18 ± 2 timer

Valgfritt system for anaerob
inkubering

Artsspesifikke brytningspunkter

Egen guide for avlesning av
sonestørrelser

Reading of inhibition zones

- Read FAA plates from the **front** with the lid removed and with **reflected light**.
- Hold the plate about **30 cm from the eye at a 45-degree angle** to the work bench.
- Measure zone diameters with a **calliper or ruler** at the point of complete inhibition as judged by the naked eye.
 - In case of double zones, read the inner zone edge.
 - **If faint haze within the zone occurs, disregard haze and read the most obvious zone edge. Tilt the plate towards you to better define the obvious zone edge.**
 - Ignore haemolysis and swarming when reading zones.
- Isolated colonies within the inhibition zone should be taken into account when reading. **For clindamycin, it is particularly important to examine zones carefully for colonies growing within the zone.**

- *Bacteroides* spp. (inkl. *Parabacteroides*, *Phocaeicola*)
- *Prevotella* spp.
- *Fusobacterium necrophorum*
- *Clostridium perfringens*
- *Cutibacterium acnes*
- *Clostridioides difficile*

Øvrige arter:



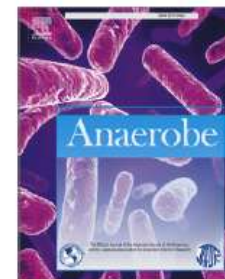
EUCAST guidance on
When there are no breakpoints in breakpoint tables?



Contents lists available at ScienceDirect

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Antimicrobial susceptibility testing of *Bacteroides* species by disk diffusion: The NordicAST *Bacteroides* study



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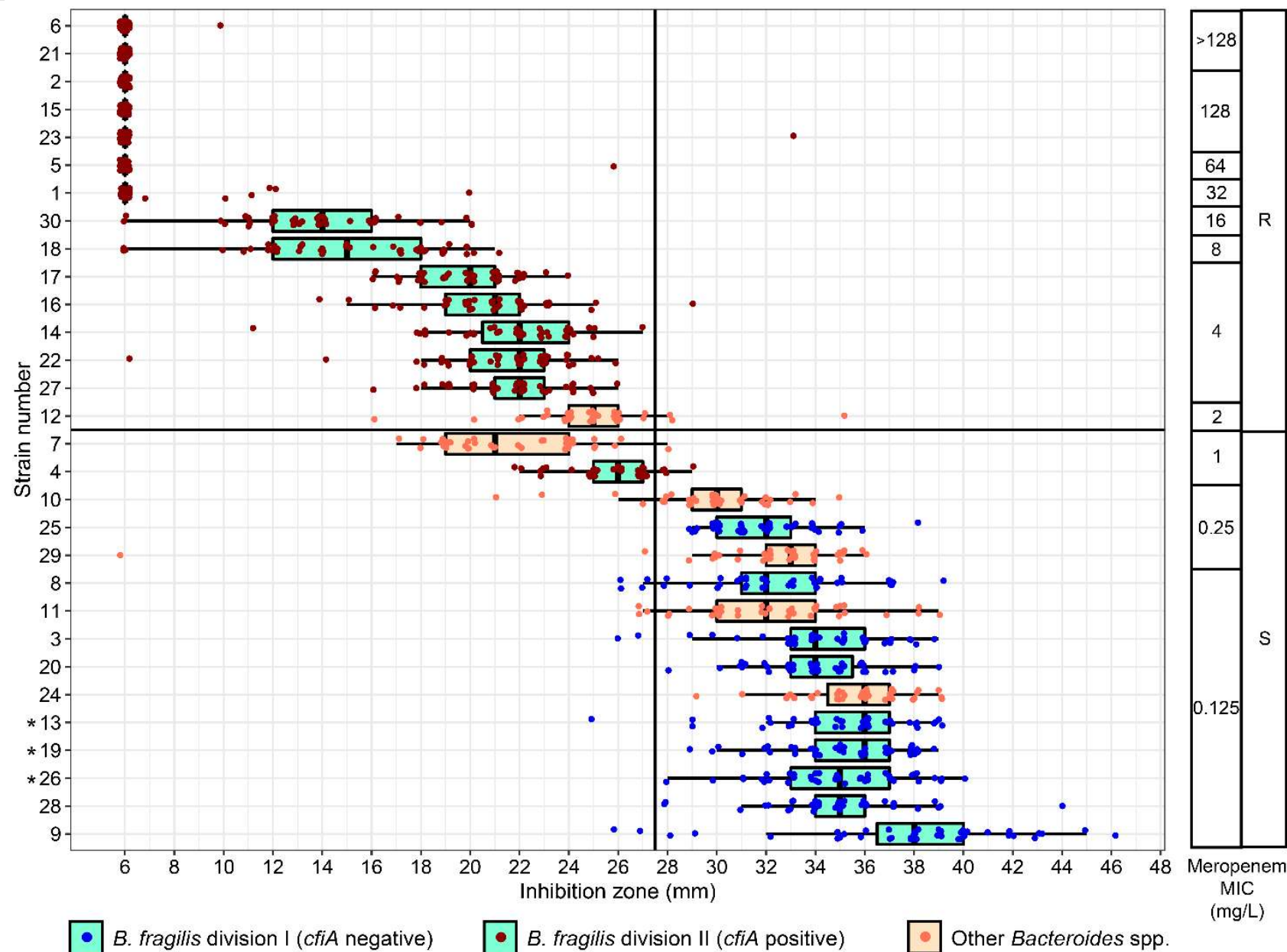
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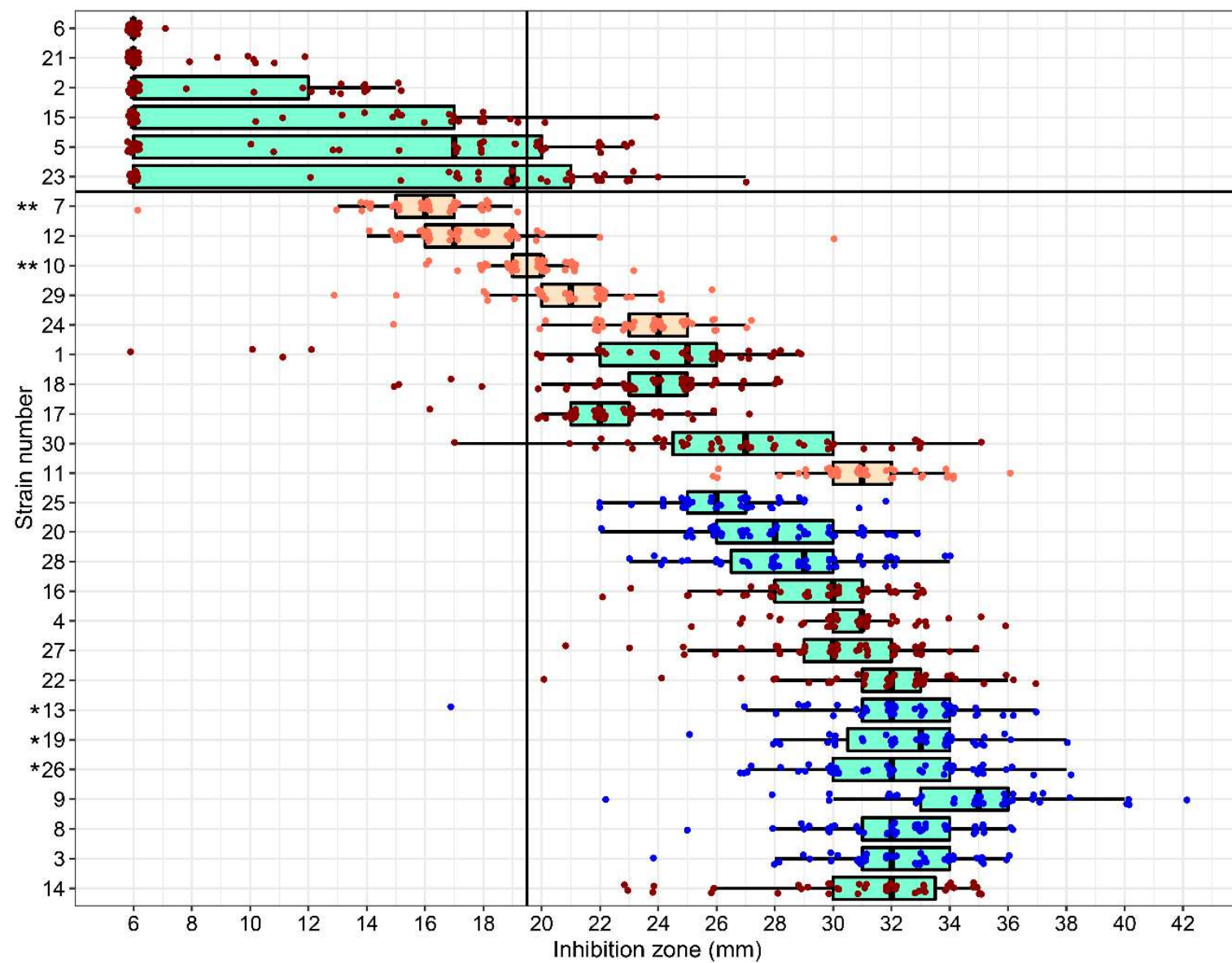
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VESTFOLD



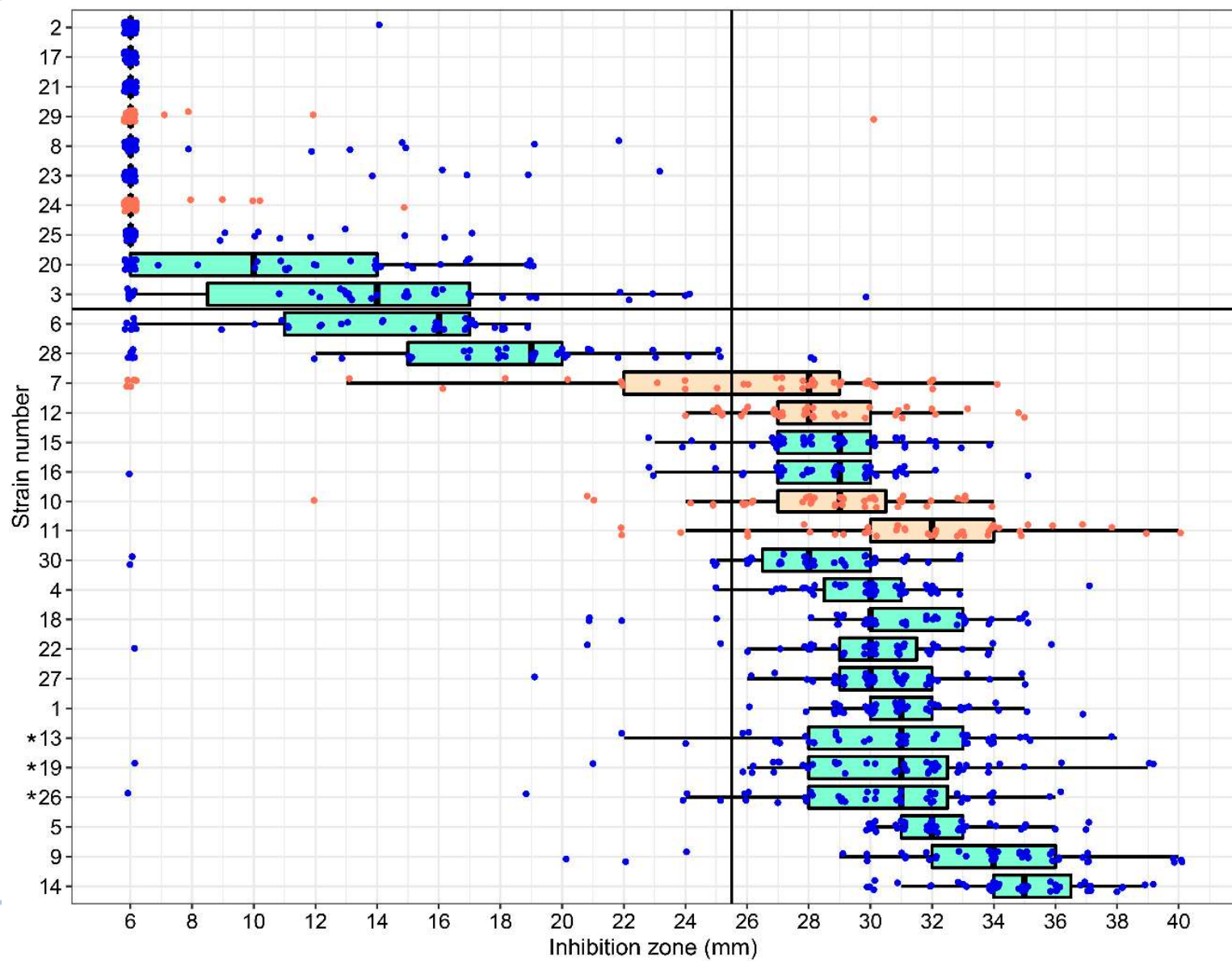
>128	R
64	
8	S
4	
2	
1	
0.5	
0.25	
0.125	
0.06	

Piperacillin/
tazobactam
MIC
(mg/L)

VESTFOLD

	General	Piperacillin-tazobactam	Meropenem	Metronidazole
Difficulty identifying the zone edge	5	6	5	5
Irregular zone shape	0	0	0	1
Double zone	1	19	10	2
Haze	1	12	1	4
Colonies in zone	0	14	2	1
Poor growth	2	0	0	0

Table S1. Number of free-form comments mentioning specific issues out of the total 80 comments received. The numbers do not add up to 80 because each comment could mention multiple issues and/or antimicrobials.



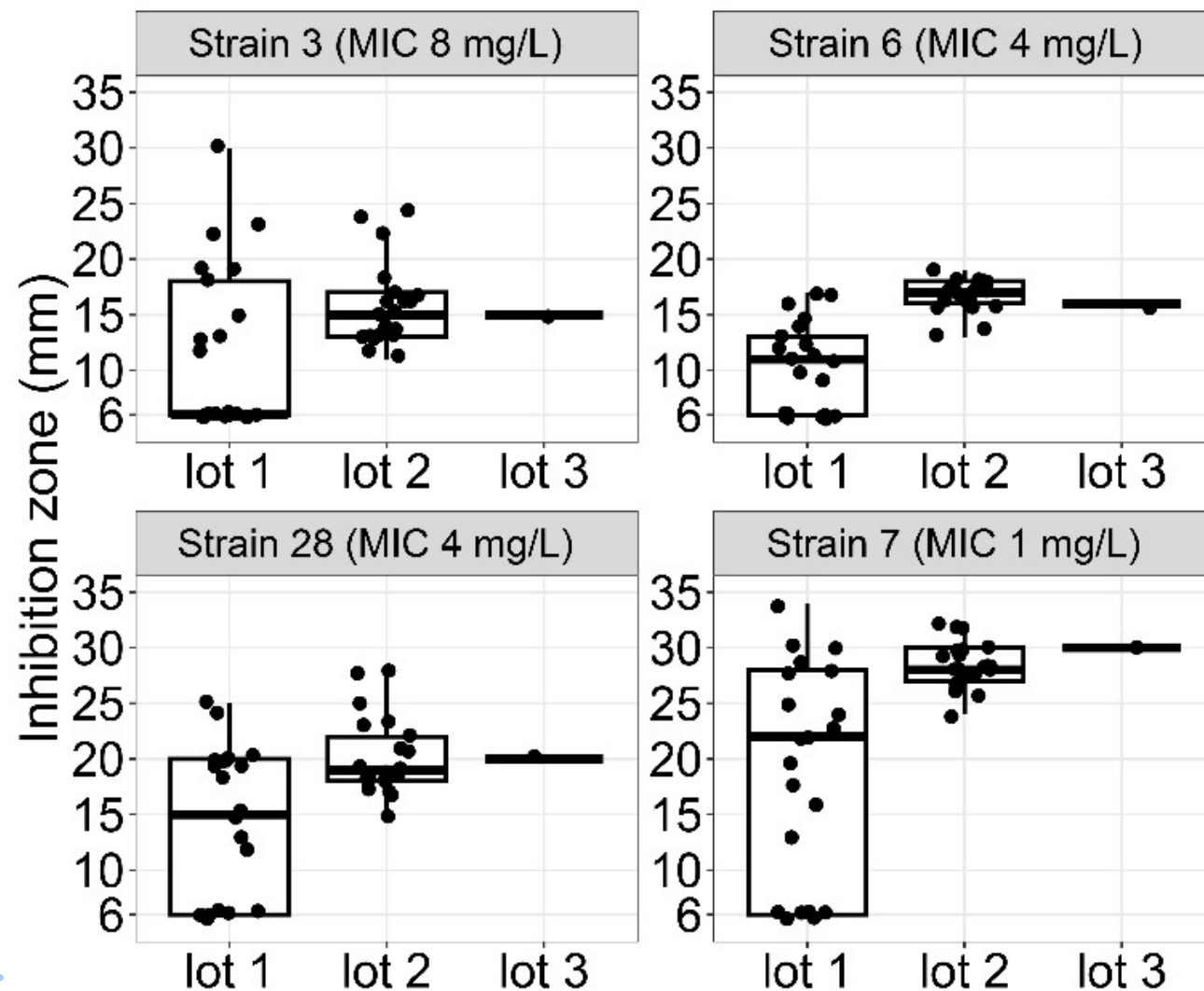
• *B. fragilis*

• Other *Bacteroides* spp.

64	R
32	
16	
8	
4	S
1	
0.5	

Metronidazole
MIC
(mg/L)

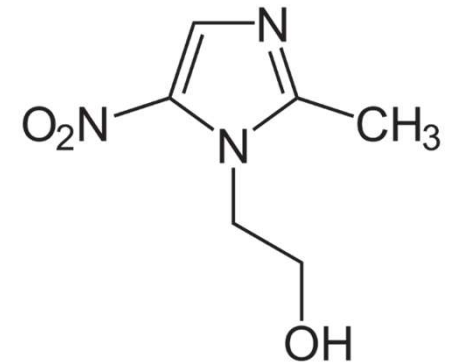
VESTFOLD



Kvalitetskontroll!

Metronidazol

- Metronidazol omdannes til aktiv metabolitt kun under anaerobe forhold
- Selv beskjeden svikt i anaerobe forhold eller feil ved mediet kan gi falsk resistens
- Uren resistensbestemmelse (forurensning med aerobe bakterier) kan også gi tilsynelatende falsk R for metronidazol



Control of the anaerobic environment when performing antimicrobial susceptibility testing for anaerobic bacteria using EUCAST methods

Test according to EUCAST disk diffusion methodology for anaerobic bacteria (FAA-HB medium). See EUCAST Breakpoint Tables for short description of disk diffusion methodology.

Clostridium perfringens DSM 25589
(NCTC 14679, CCUG 75076)

Antimicrobial agent	Disk content (µg)	Cut-off value ¹ (mm)
Metronidazole	5	<25

1. A zone diameter of <25 mm indicates an insufficient anaerobicity. This may affect growth and susceptibility test results of anaerobic bacteria.

Hva med gradienttester?

Vi observerte økt mengde feil (spesielt falsk S for pip-tazo og metronidazol) når man brukte andre medier enn det produsenten anbefaler



Oppsummering

- EUCAST lappediffusjonsmetode med artsspesifikke brytningspunkter er på plass
- Piperacillin-tazobactam kan være vanskelig å lese av hos *Bacteroides*
- Metronidazol er spesielt utsatt for feil som skyldes dårlig anaerob atmosfære eller feil ved mediet
- Ved bruk av gradienttester er det økt feilrate når man bruker andre medier enn det produsenten anbefaler

Takk for oppmerksomheten