



News from EUCAST Development Laboratory (EDL)

Erika Matuschek

Jenny Åhman

EUCAST Development Laboratory

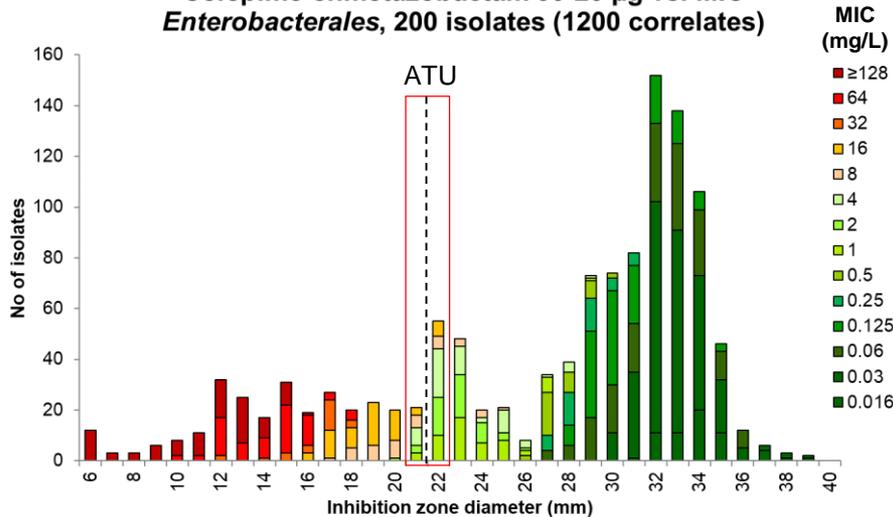
NordicAST WS 28-29 May 2024

Revised zone diameter breakpoints

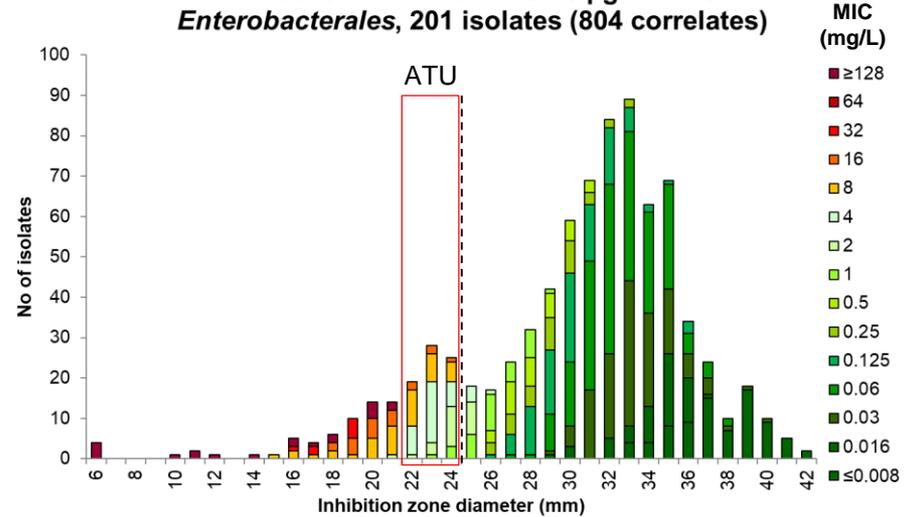
- Revised zone diameter breakpoints in EUCAST Breakpoint Tables v 14.0
 - *Enterobacteriales*
 - Cefiderocol
 - Ertapenem
 - Fosfomicin iv (MIC and zone diameter breakpoints revised)
 - *Staphylococcus* spp.
 - Ciprofloxacin (MIC and zone diameter breakpoints revised)
 - *Corynebacterium* spp. other than *C. diphtheriae* and *C. ulcerans*
 - Benzylpenicillin (MIC and zone diameter breakpoints revised)
- Breakpoints for new antimicrobial agents
 - Cefepime-enmetazobactam and aztreonam-avibactam

Zone diameter breakpoints for new antimicrobial agents

Cefepime-enmetazobactam 30-20 µg vs. MIC *Enterobacterales*, 200 isolates (1200 correlates)



Aztreonam-avibactam 30-20 µg vs. MIC *Enterobacterales*, 201 isolates (804 correlates)



Breakpoints for *Enterobacterales* and cefepime-enmetazobactam (30-20 µg disk):
 $S \leq 4$, $R > 4$ mg/L
 $S \geq 22$, $R < 22$ mm, ATU 21-22 mm

Quality control: *E. coli* ATCC 25922 and *E. coli* NCTC 13353

Breakpoints for *Enterobacterales* and aztreonam-avibactam (30-20 µg disk):
 $S \leq 4$, $R > 4$ mg/L
 $S \geq 25$, $R < 25$ mm, ATU 22-24 mm

Quality control: *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603

Cefiderocol AST

- MIC determination of cefiderocol is difficult
 - Iron-depleted broth (ID-CAMHB)
 - Endpoints difficult to read for some isolates
- The warning on the EUCAST website is still valid
 - Problems with accuracy of commercial MIC products
 - Disk diffusion can be trusted when mean results from ≥ 5 tests are on QC target ± 1 mm for both *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853

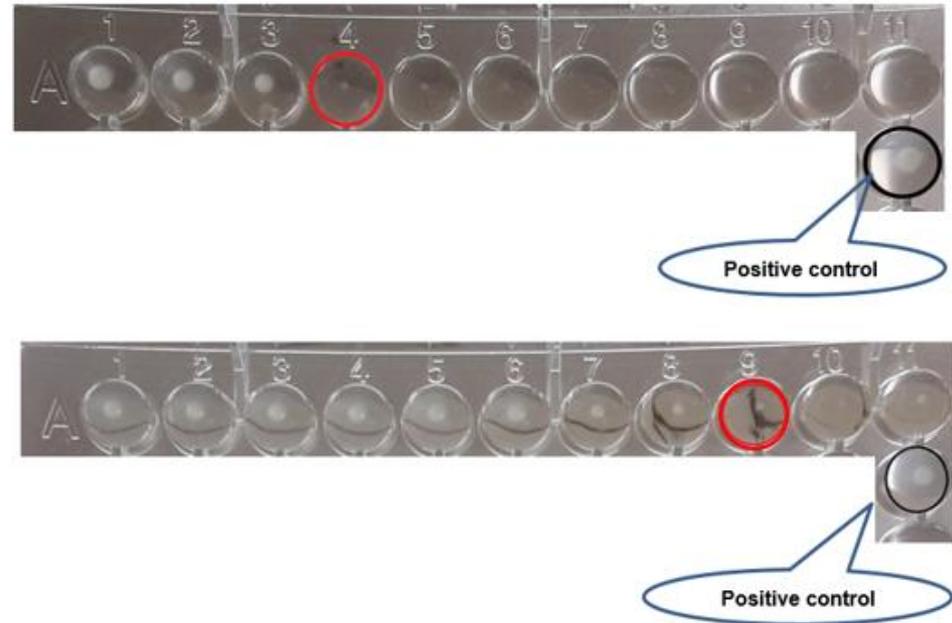
<https://www.eucast.org/ast-of-bacteria/warnings>

Reading cefiderocol endpoints

The MIC is read as the first well in which the reduction of growth corresponds to a button of <1 mm or is replaced by the presence of light haze/faint turbidity ($\geq 80\%$ reduction in haze as compared to the growth control).

The positive control should show strong growth in the form of a button of >2 mm or heavy turbidity.

Red text = new



EUCAST BMD Reading Guide, v 5.0, 2024

https://www.eucast.org/ast_of_bacteria/mic_determination

New evaluation of EUCAST breakpoints and methods for cefiderocol

- The same isolates used to establish EUCAST zone diameter breakpoints in 2020 were tested (246 *Enterobacterales* and 99 *P. aeruginosa*), plus 53 additional carbapenemase-producing *Enterobacterales*
- Broth microdilution
 - ID-CAMHB prepared from broth from two manufacturers (BBL and Difco)
 - MICs were read by two technicians
 - For isolates with difficult-to-read endpoints, a consensus MIC based on readings by at least three technicians was used
- Disk diffusion on MH agar from two manufacturers (BBL and Oxoid)

Quality control data

E. coli ATCC 25922

ID-CAMHB Difco	ID-CAMHB BBL
0.25	0.25
0.5	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.25
0.25	0.125
0.25	0.25

0.125-0.25
0.06-0.5

P. aeruginosa ATCC 27853

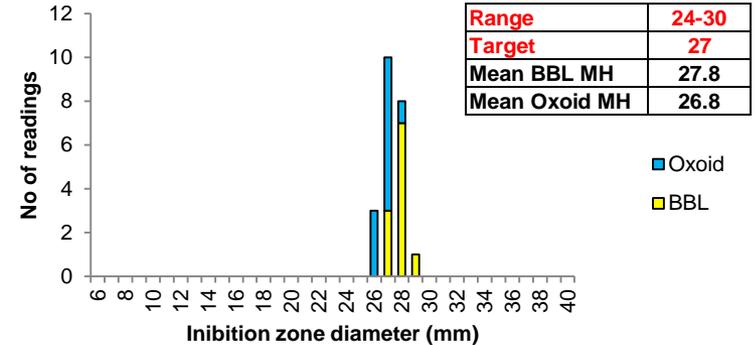
ID-CAMHB Difco	ID-CAMHB BBL
0.25	0.06
0.25	0.125
0.5	0.06
0.25	0.125
0.25	-
0.5	0.06
0.25	0.125
0.25	0.125
0.25	0.06
0.25	0.06
0.25	0.125

0.125-0.25
0.06-0.5

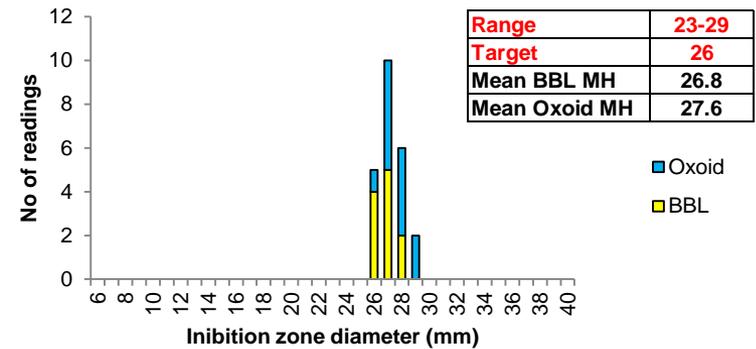
Target
Range

On target
Upper limit
Lower limit
Out of range

Cefiderocol 30 µg vs. MHA
E. coli ATCC 25922, 22 results

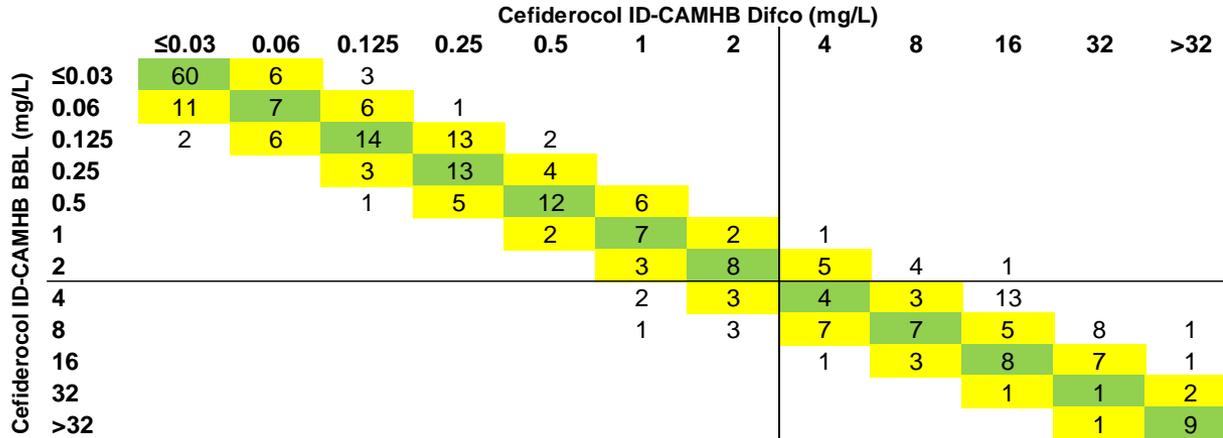


Cefiderocol 30 µg vs. MHA
P. aeruginosa ATCC 27853, 22 results



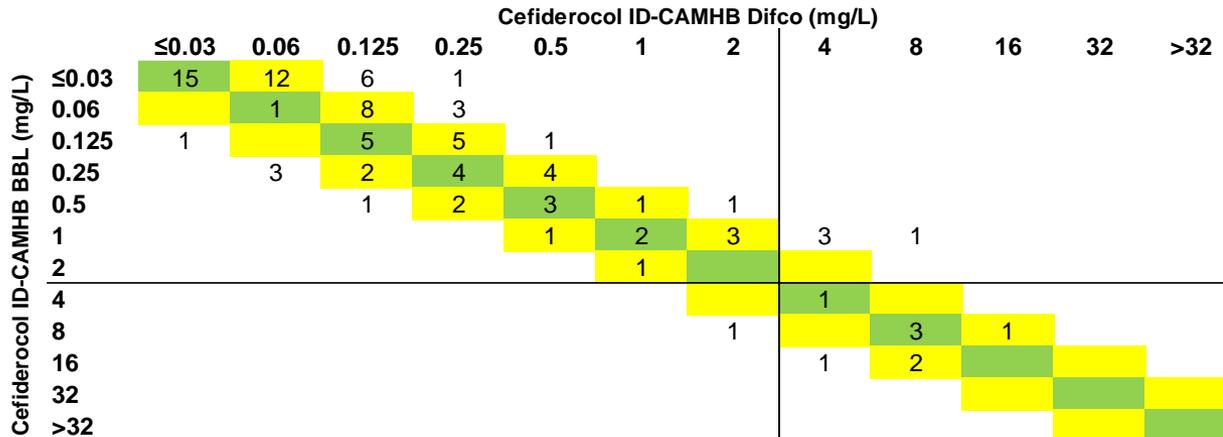
Comparison of cefiderocol MIC determination in ID-CAMHB BBL and Difco

a) *Enterobacterales* (n=299)



Essential agreement	85%
Bias	-22%
Categorical agreement	93%

b) *Pseudomonas aeruginosa* (n=99)

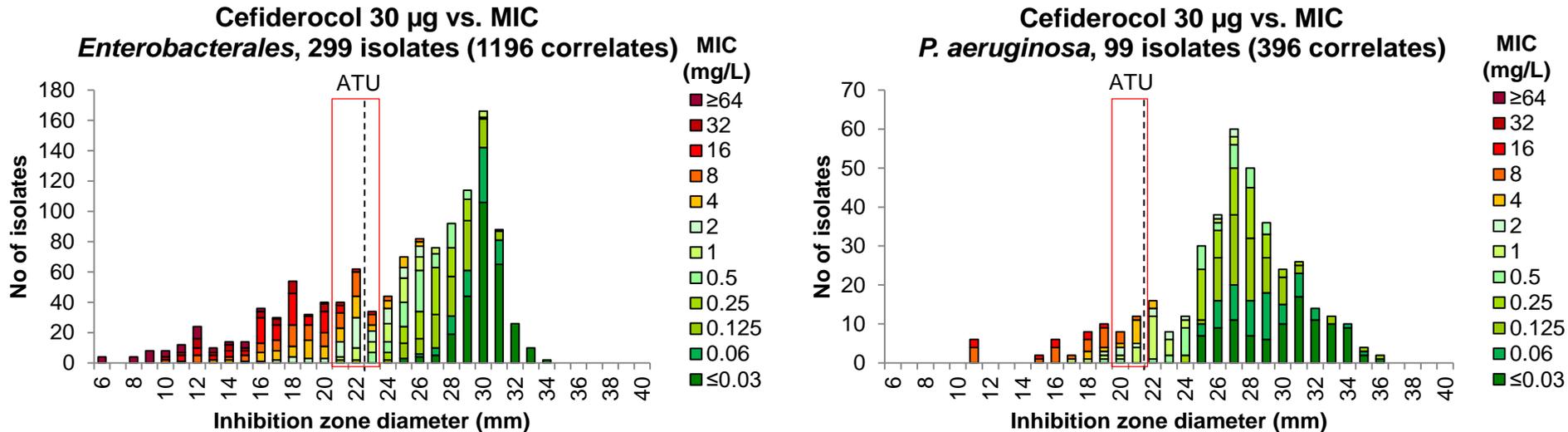


Essential agreement	77%
Bias	-45%
Categorical agreement	95%

ISO 20776-2:
EA ≥90%
Bias <±30%

MIC-zone diameter correlation data

Aggregated data from BMD in ID-CAMHB BBL and Difco and disk diffusion on BBL and Oxoid MH



EUCAST Breakpoint Tables v 14.0, 2024

- Revised zone diameter breakpoints for *Enterobacterales*
- Revised (more narrow) ATUs for *Enterobacterales* and *P. aeruginosa*
- Valid in combination with the EUCAST warning (QC results close to target values)

Enterococcus spp. other than
E. faecalis and *E. faecium*

Background

- EUCAST breakpoints for *Enterococcus* spp. are valid for all species of enterococci, but were mainly developed using data for *E. faecalis* and *E. faecium*.
- The applicability of these breakpoints to other species of enterococci is less certain as data for these species have been lacking.
- Other enterococcal species are less frequently isolated but can also cause severe infections such as endocarditis and bacteremia, especially in immunocompromised or chronically ill patients.
- Enterococci are known to cause nosocomial infections.

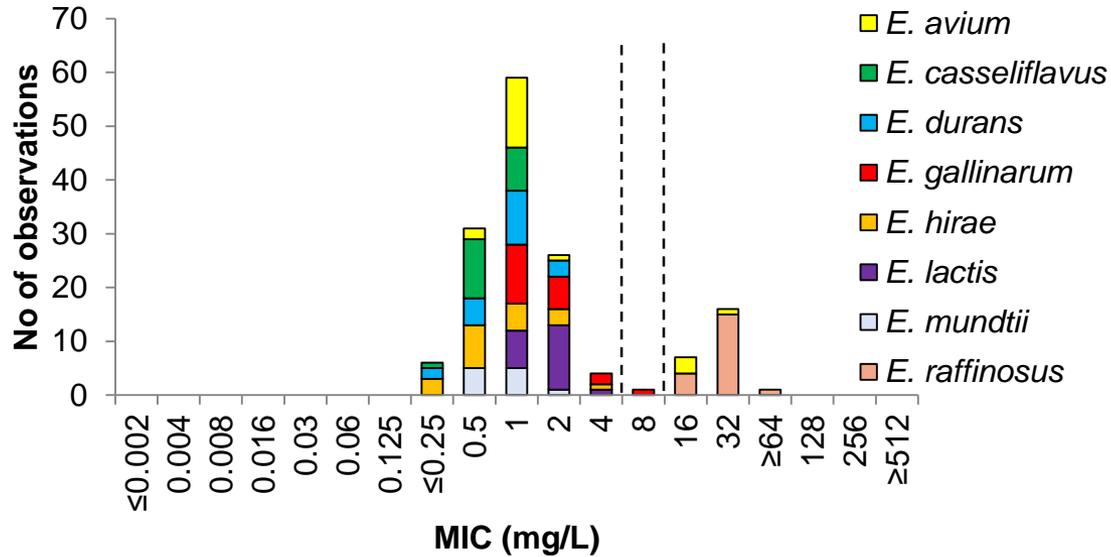
Methods and isolates

- Broth microdilution and disk diffusion (3 MH media) in parallel against agents listed in EUCAST Breakpoint Tables.
- 151 isolates of different international origin:

Species	Number
<i>E. avium</i>	20
<i>E. casseliflavus</i>	20
<i>E. durans</i>	20
<i>E. gallinarum</i>	20
<i>E. hirae</i>	20
<i>E. lactis</i>	20
<i>E. raffinosus</i>	20
<i>E. mundtii</i>	11

Ampicillin

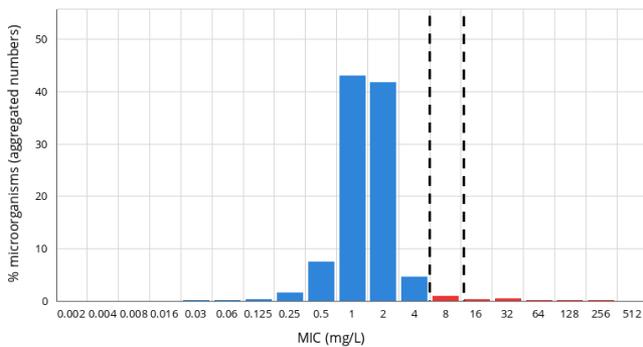
Enterococcus spp.



Ongoing consultation:
Proposed BP
4/4 mg/L

E. faecalis

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

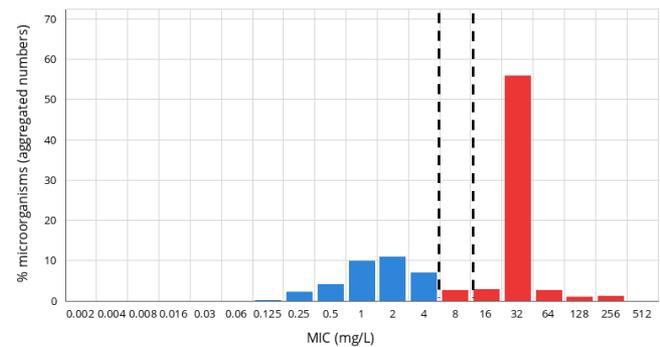


MIC Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L

Confidence interval: 1 - 4
13643 observations (26 data sources)

E. faecium

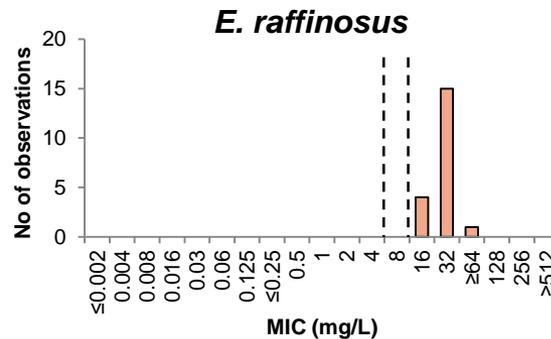
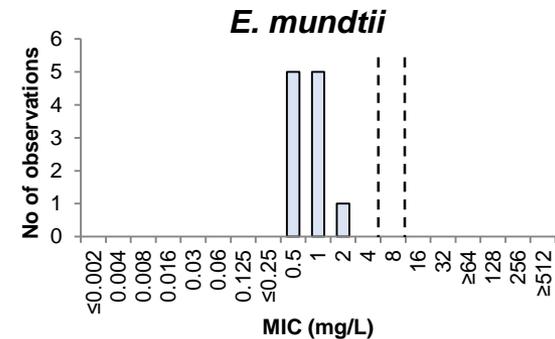
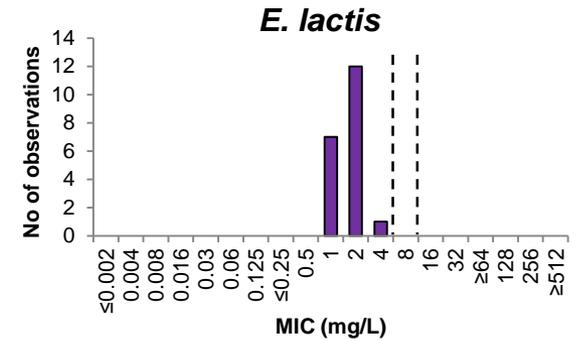
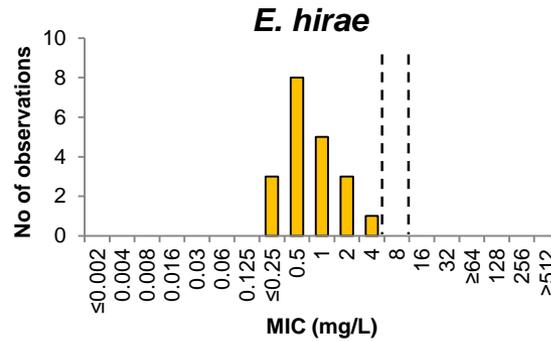
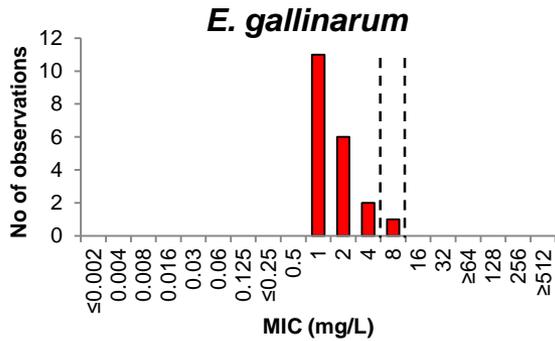
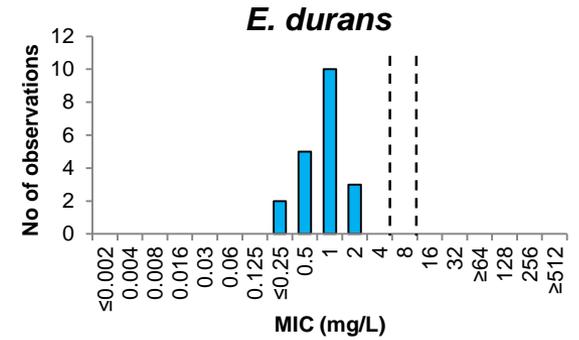
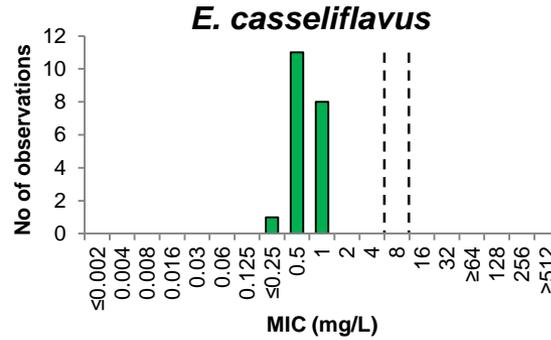
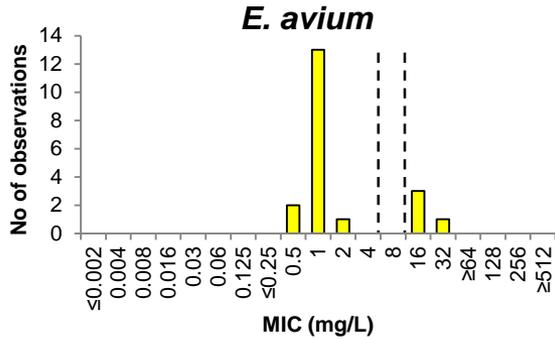
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L

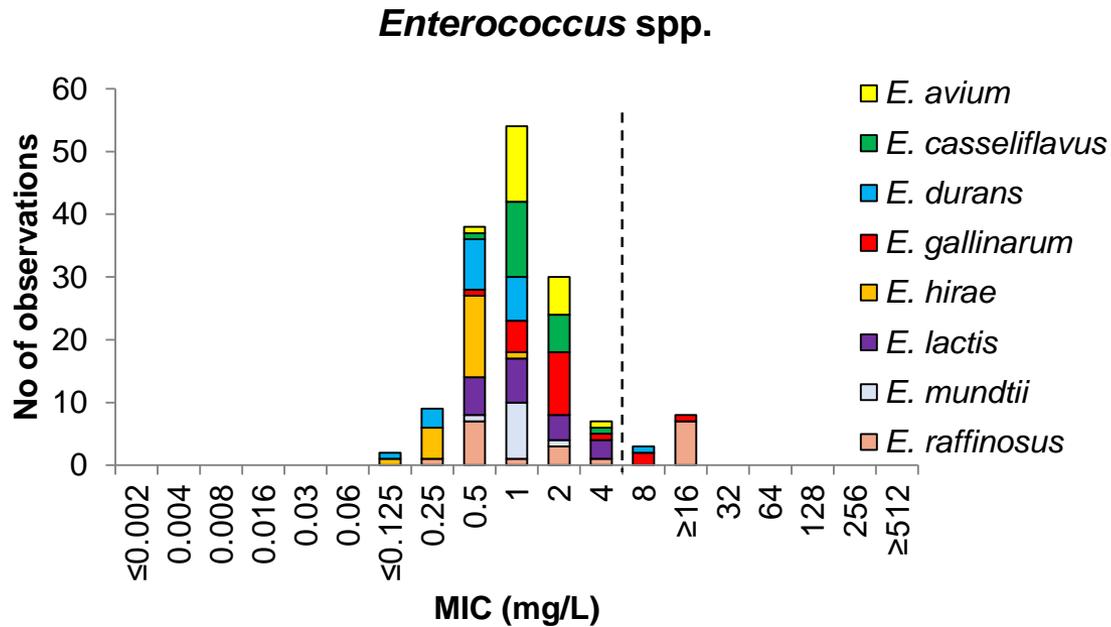
Confidence interval: 4 - 16
6164 observations (20 data sources)

Ampicillin



Ciprofloxacin

Breakpoints
(uncomplicated UTI only)
4/4 mg/L

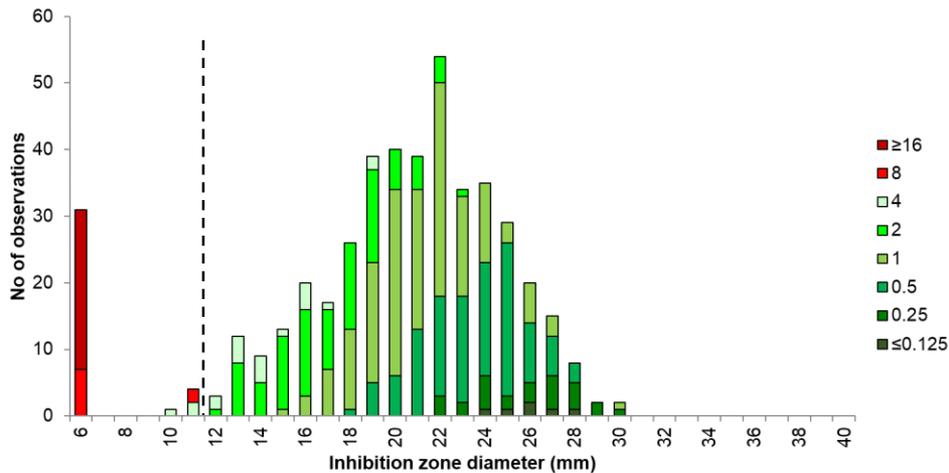


The norfloxacin disk can be used to screen for fluoroquinolone resistance: Susceptibility to ciprofloxacin and levofloxacin can be inferred from the norfloxacin disk diffusion screening test.

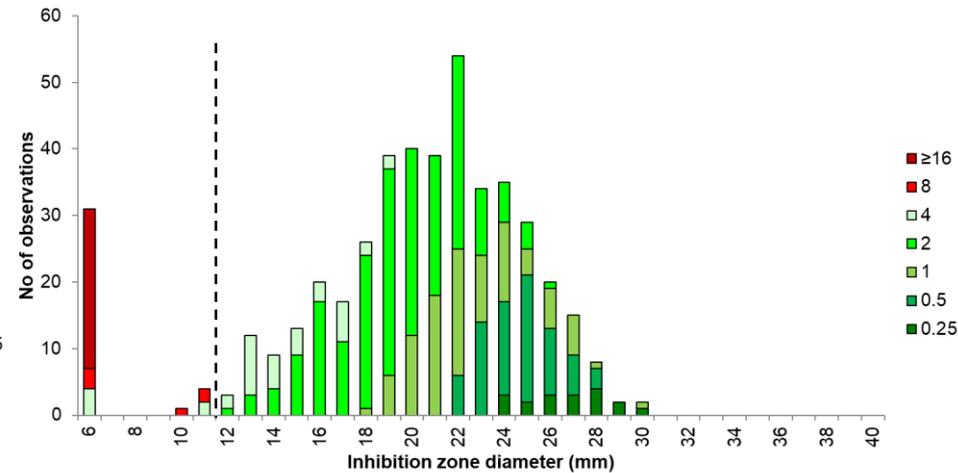
Norfloxacin screen

Norfloxacin
screen:
12/12 mm

Norfloxacin 10 µg vs. Ciprofloxacin MIC
Enterococcus spp., 151 isolates

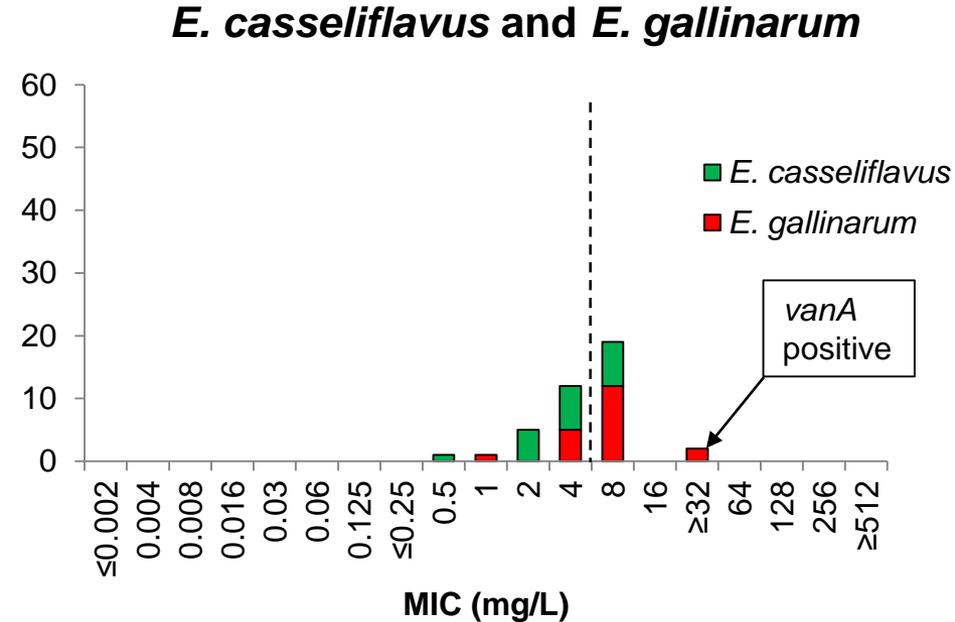
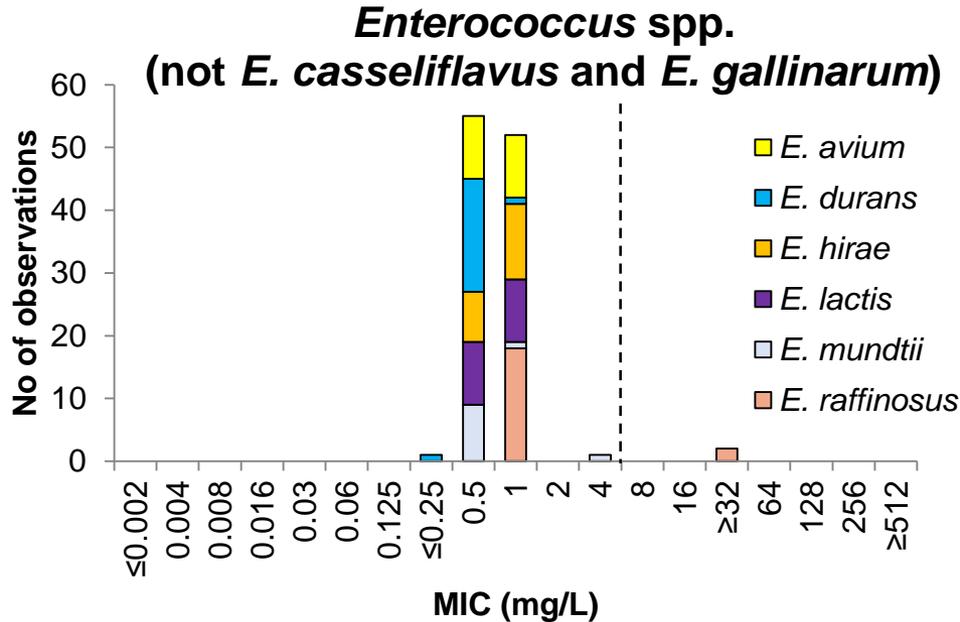


Norfloxacin 10 µg vs. Levofloxacin MIC
Enterococcus spp., 151 isolates



- All isolates susceptible to norfloxacin are susceptible to ciprofloxacin and levofloxacin.
- All isolates resistant to norfloxacin are resistant to ciprofloxacin and/or levofloxacin.

Vancomycin



E. casseliflavus and *E. gallinarum* harbour the *vanC* gene. Breakpoints for vancomycin are not valid for these two species!

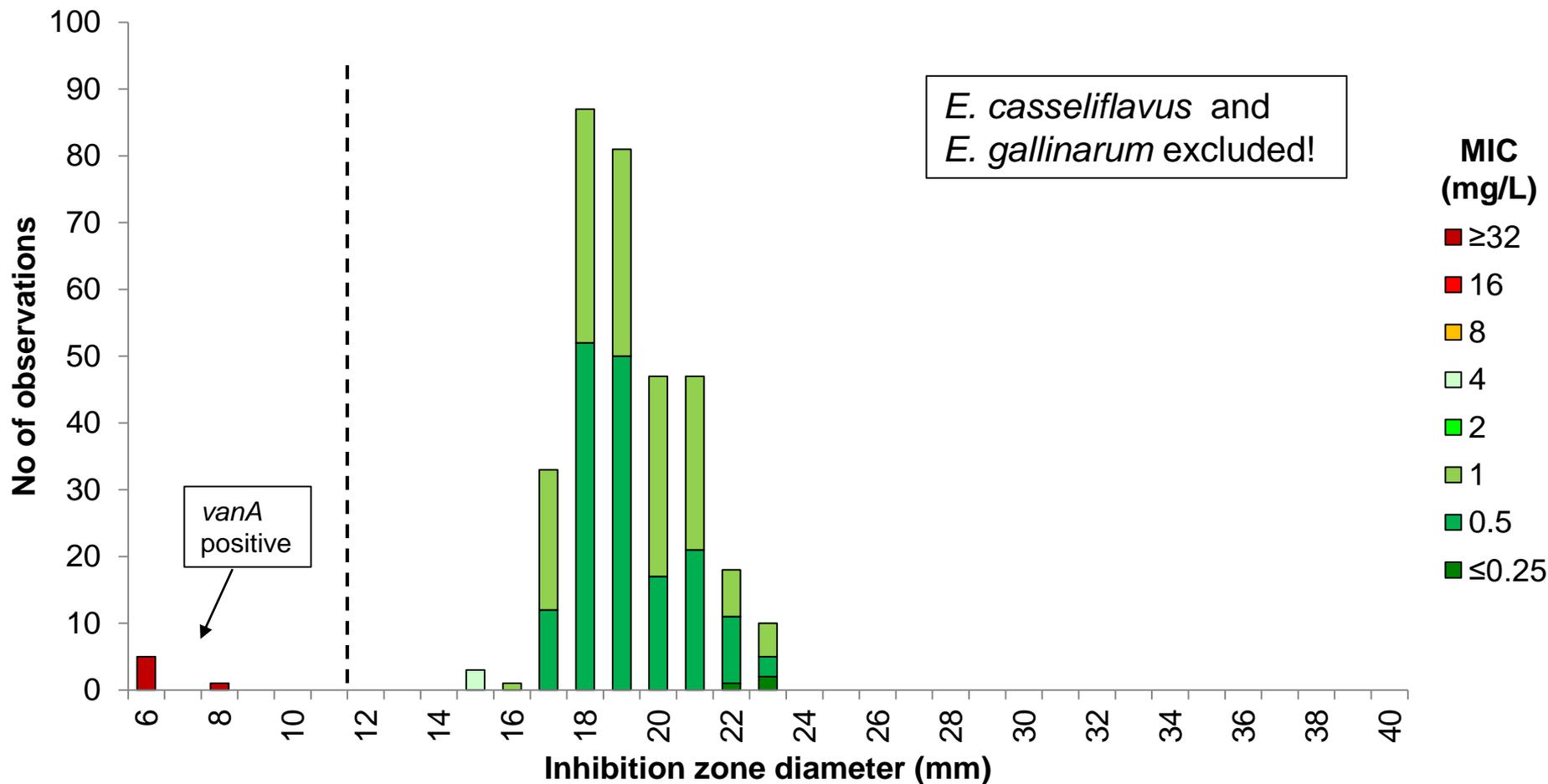
EUCAST Clinical Breakpoint Tables v. 14.0, valid from 2024-01-01

Glycopeptides and lipoglycopeptides	MIC breakpoints		Disk content	Zone diameter		Notes
	S ≤	R >		S ≥	R <	
Vancomycin, enterococci other than <i>E. casseliflavus</i> and <i>E. gallinarum</i>	4 ¹	4 ¹	5	12 ^{A,B}	12 ^{A,B}	Numbered notes relate to general comments and/or MIC breakpoints. 1/A. Vancomycin resistance is the expected phenotype for <i>E. casseliflavus</i> and <i>E. gallinarum</i> and therefore susceptibility testing should not be performed.

Vancomycin

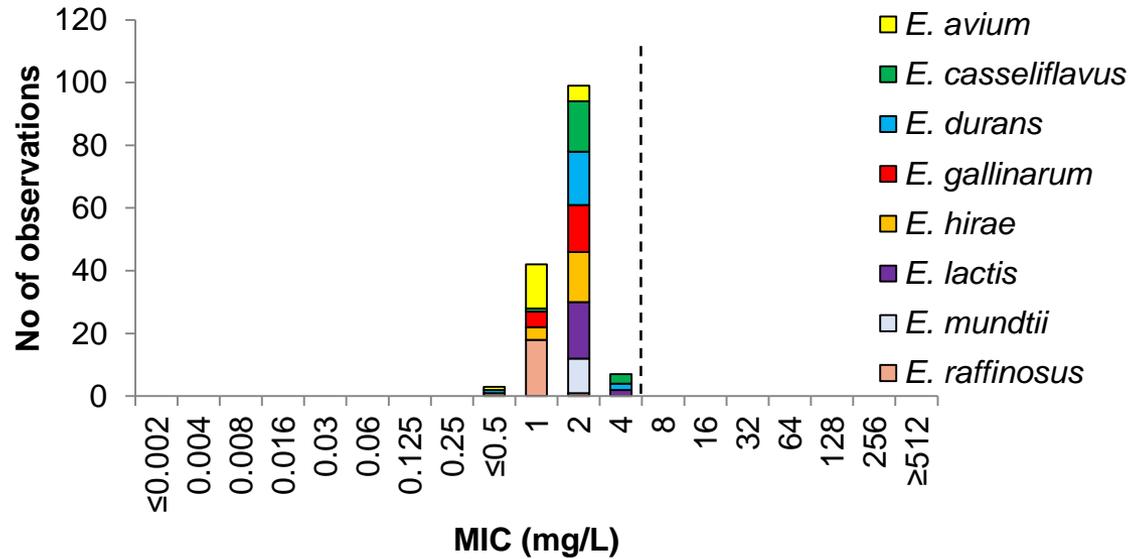
BPs
4/4 mg/L
12/12 mm

Vancomycin 5 μ g vs. MIC *Enterococcus* spp.



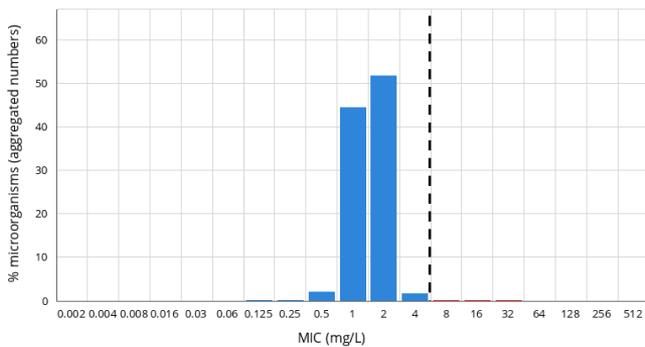
Linezolid

Enterococcus spp.



E. faecalis

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

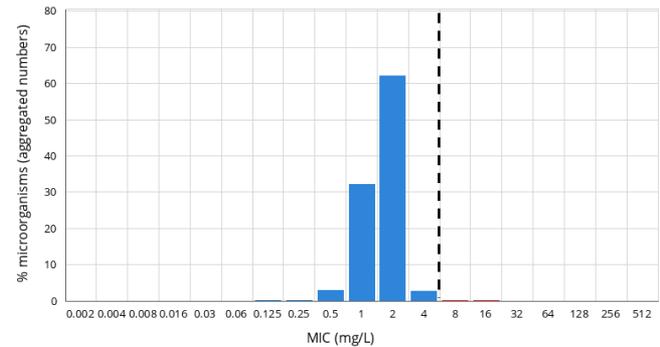


MIC Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L

Confidence interval: -
31441 observations (31 data sources)

E. faecium

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



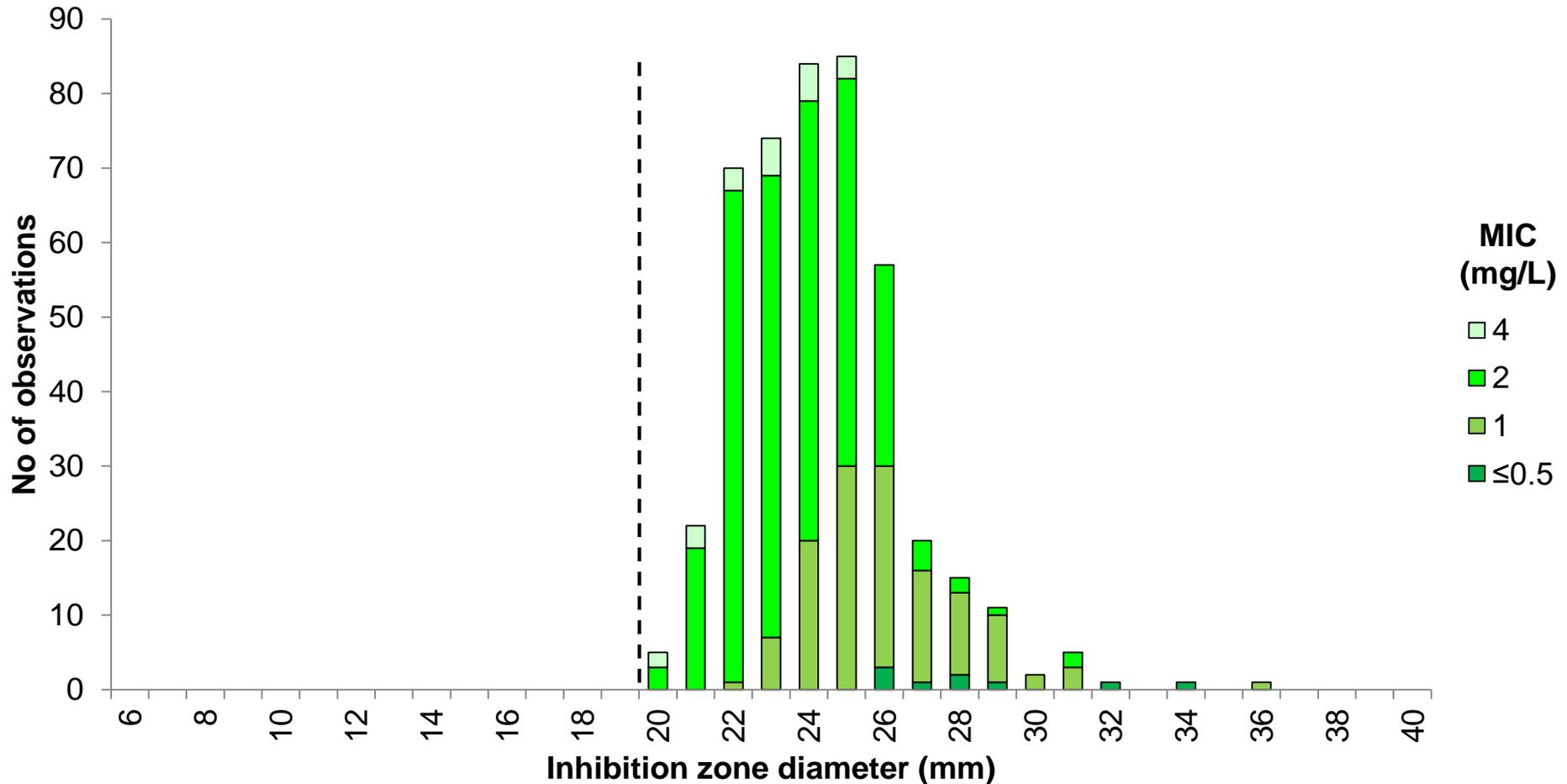
MIC Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L

Confidence interval: 2 - 4
14404 observations (25 data sources)

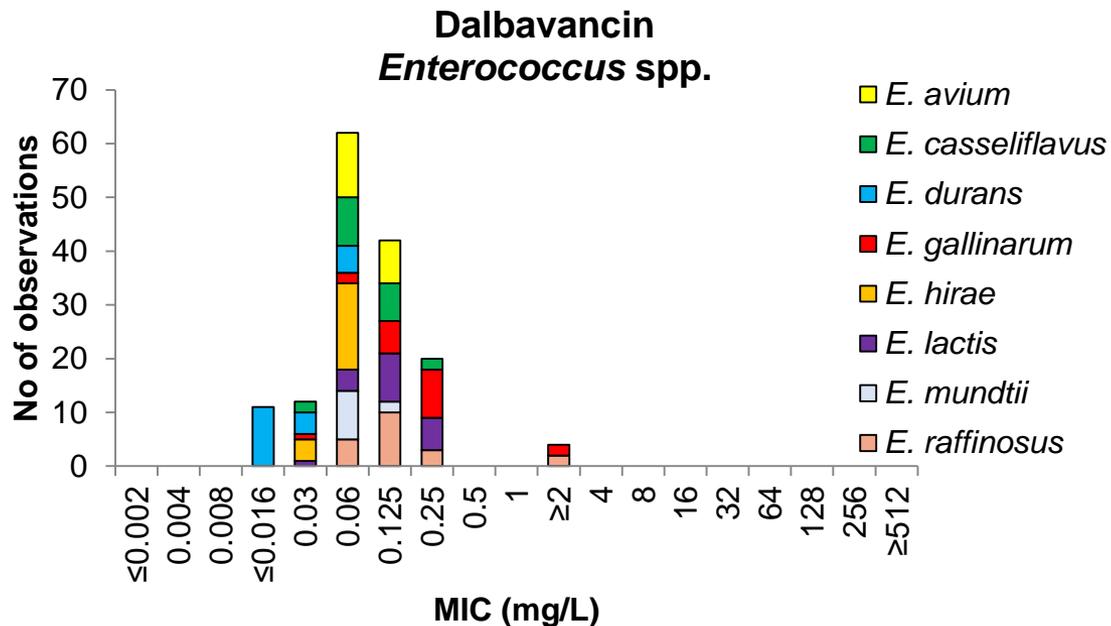
Linezolid

BPs
4/4 mg/L
20/20 mm

Linezolid 10 µg vs. MIC
Enterococcus spp.

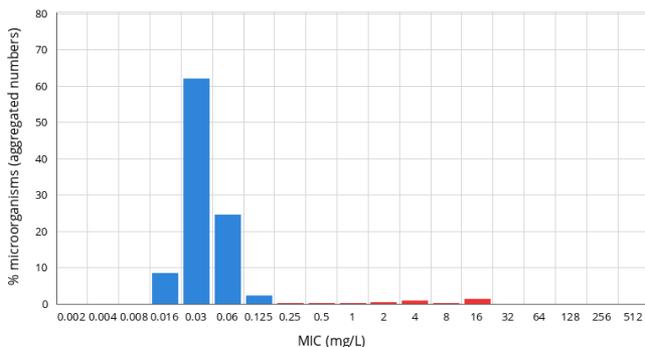


Dalbavancin



E. faecalis

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

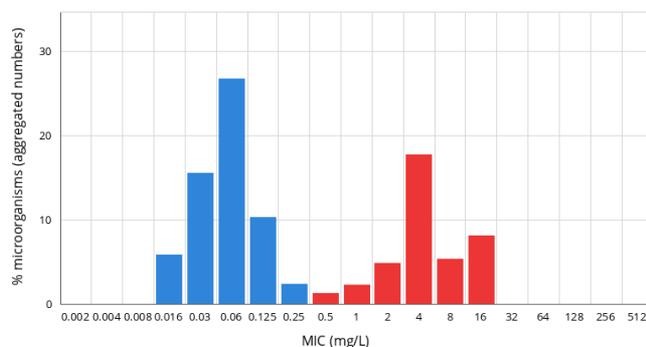


MIC Epidemiological cut-off (ECOFF): 0.125 mg/L
Wildtype (WT) organisms: ≤ 0.125 mg/L

Confidence interval: 0.03 - 0.25
4373 observations (5 data sources)

E. faecium

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC Epidemiological cut-off (ECOFF): (0.25) mg/L
Wildtype (WT) organisms: ≤ 0.25 mg/L

Confidence interval: 0.125 - 0.5
2272 observations (4 data sources)

Conclusions

- This study presents susceptibility data for 8 species of enterococci other than *E. faecalis* and *E. faecium*.
- For most agents the results indicate that current EUCAST breakpoints for *Enterococcus* spp. are also suitable for other species than *E. faecalis* and *E. faecium*.
- EUCAST will decide if current breakpoints for *Enterococcus* spp. need minor revision for Breakpoint Tables v. 15.0 (2025).
- Until then, use current breakpoints for *Enterococcus* spp. for all species of enterococci.

Other ongoing work

- Development of disk diffusion criteria for additional anaerobic species
- Disk diffusion criteria for *S. pneumoniae* and benzylpenicillin
- RAST breakpoints for *Salmonella enterica*
- Investigation of alternative media in addition to MH-F agar for disk diffusion of fastidious organisms
- Development of a disk diffusion method for *Neisseria gonorrhoeae*
- Evaluation of suitable media for antimicrobial susceptibility testing of *Neisseria meningitidis*
- New antimicrobial agents
 - Contezolid, Zoliflodacin (*N. gonorrhoeae*), Zosurabalpin (*Acinetobacter*)

Thanks!

- The EDL staff: Amra Basic, Emma Jonasson, Onur Karatuna, Lucia Ortega, Lized Rodriguez and Gunnar Kahlmeter
- **All clinical laboratories participating in studies, and contributing with data and/or isolates!**

Questions and/or problem?

Contact us!

erika.matuschek@eucast.org

jenny.ahman@eucast.org

