

# *Enterococcus* spp.

## MIC and zone diameter distributions

MIC data from JMI

DD data from Nordic labs

# Enterococcus species

Species included in the genus *Enterococcus* (list of prokaryotic species with standing in nomenclature-LPSN; last full update of the related Website [www.bacterio.net](http://www.bacterio.net): July 22, 2019)

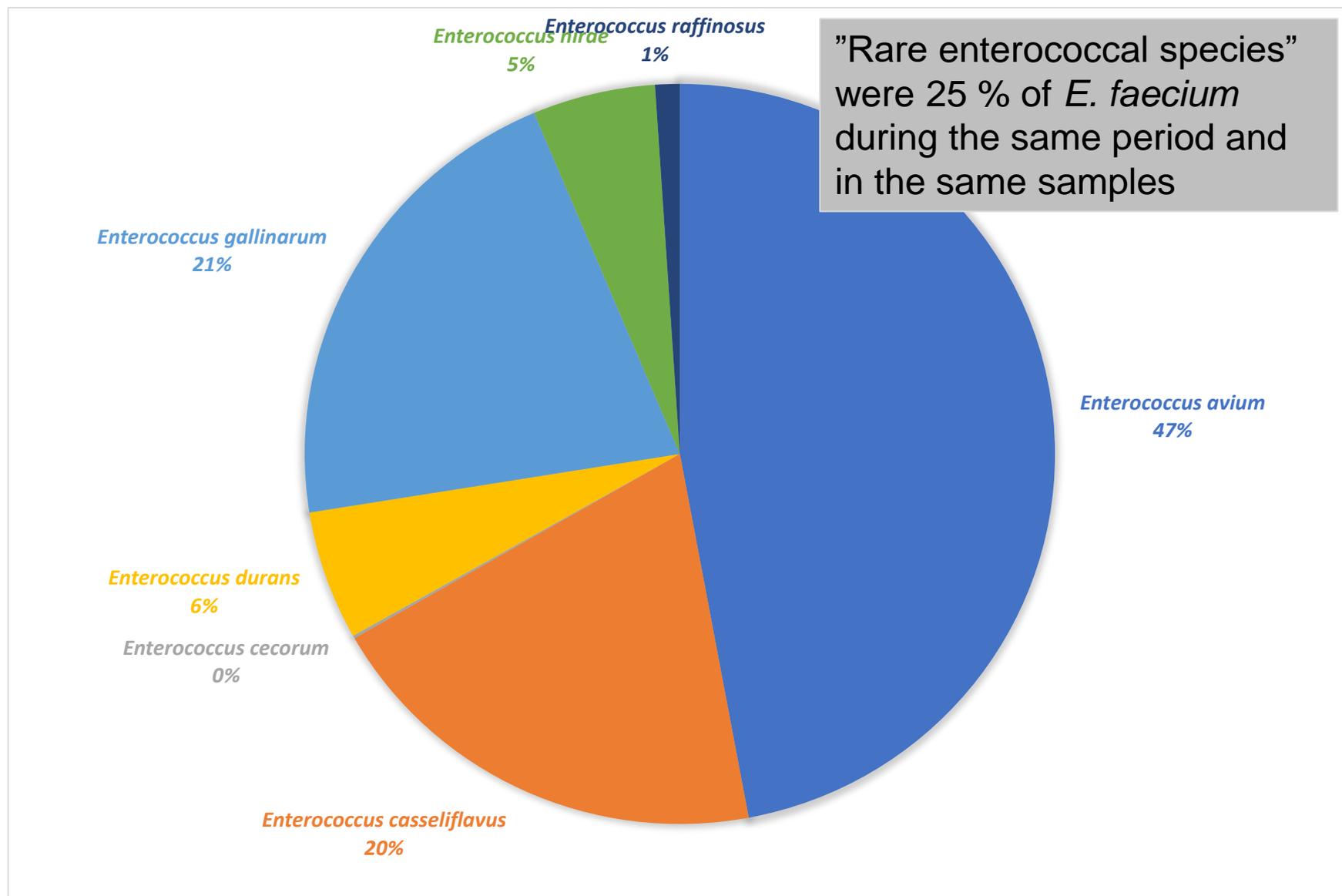
Species	Habitat/Isolation source
<i>Enterococcus alcedinis</i>	Fish
<i>Enterococcus aquimarinus</i>	Water, seawater
<i>Enterococcus asini</i>	Donkey intestine
<i>Enterococcus avium</i>	Poultry (rare) and mammalian intestines
<i>Enterococcus bulliens</i>	Camel milk
<i>Enterococcus caccae</i>	Human stools
<i>Enterococcus camelliae</i>	Fermented tea leaves
<i>Enterococcus canintestini</i>	Dog feces
<i>Enterococcus canis</i>	Dog anal swabs
<i>Enterococcus casseliflavus</i>	Grass, silage, plants, soil
<i>Enterococcus cecorum</i>	Clinical origin, animals
<i>Enterococcus columbae</i>	Pigeon intestine
<i>Enterococcus crotali</i>	Reptile feces
<i>Enterococcus devriesei</i>	Animal sources
<i>Enterococcus diestrammenae</i>	Insect gut
<i>Enterococcus dispar</i>	Human origin
<i>Enterococcus durans</i>	Clinical isolate
<i>Enterococcus eurekensis</i>	Swine-manure
<i>Enterococcus faecalis</i>	Human and other animal intestines
<i>Enterococcus faecium</i>	Human and other animal intestines
<i>Enterococcus gallinarum</i>	Poultry intestine
<i>Enterococcus gilvus</i>	Human clinical specimens
<i>Enterococcus haemoperoxidus</i>	Water/surface waters
<i>Enterococcus hermanniensis</i>	Packaged broiler meat; canine tonsils
<i>Enterococcus hirae</i>	Animal intestines; chicken pathogen
<i>Enterococcus xiangfangensis</i>	Pickle

Species	Habitat/Isolation source
<i>Enterococcus alcedinis</i>	Fish
<i>Enterococcus aquimarinus</i>	Water, seawater
<i>Enterococcus asini</i>	Donkey intestine
<i>Enterococcus italicus</i>	Italian cheeses
<i>Enterococcus lactis</i>	Raw milk cheeses
<i>Enterococcus lemanii</i>	Swine-manure
<i>Enterococcus malodoratus</i>	Gouda cheese
<i>Enterococcus moraviensis</i>	Water
<i>Enterococcus mundtii</i>	Grass, silage, plants, soil
<i>Enterococcus olivae</i>	Green olive fermentation
<i>Enterococcus pallens</i>	Human clinical specimens
<i>Enterococcus phoeniculicola</i>	Uropygial gland of the red-billed woodhoopoe
<i>Enterococcus plantarum</i>	Plant material
<i>Enterococcus pseudoavium</i>	Bovine skin
<i>Enterococcus quebecensis</i>	Water
<i>Enterococcus raffinosus</i>	Clinical origin
<i>Enterococcus ratti</i>	Animal enteric disorders
<i>Enterococcus rivorum</i>	Water/surface water
<i>Enterococcus rotai</i>	Water, plants, mosquitoes
<i>Enterococcus saccharolyticus</i>	Bedding and skin of cattle
<i>Enterococcus saigonensis</i>	Chicken
<i>Enterococcus silesiacus</i>	Drinking water
<i>Enterococcus sulfureus</i>	Plant material
<i>Enterococcus termitis</i>	Termite gut
<i>Enterococcus thailandicus</i>	Fermented sausage
<i>Enterococcus ureasiticus</i>	Water
<i>Enterococcus ureilyticus</i>	Water, plants
<i>Enterococcus viikkiensis</i>	Broiler
<i>Enterococcus villorum</i>	Animal enteric disorders
<i>Enterococcus wangshanyuanii</i>	Yak feces
<i>Enterococcus xiangfangensis</i>	Pickle

# Svebar

Enterococci from positive blood cultures 2019 – 2022 in Sweden (source: Svebar)					
Species	2019	2020	2021	2022	Total per species
ENTEROCOCCUS FAECALIS	10536	10773	12581	12659	46549
ENTEROCOCCUS FAECIUM	5941	5596	7571	7880	26988
ENTEROCOCCUS CASSELI FLAVUS	371	341	383	390	1485
ENTEROCOCCUS GALLINARUM	306	259	294	357	1216
ENTEROCOCCUS, not ID:ed	185	122	114	155	576
ENTEROCOCCUS HIRAE	43	109	32	74	258
ENTEROCOCCUS DURANS	44	22	49	34	149
ENTEROCOCCUS RAFFINOSUS	32	42	16	24	114
ENTEROCOCCUS MUNDTII	7		8	8	23
ENTEROCOCCUS MALODORATUS			9		9
ENTEROCOCCUS CECORUM	7				7
ENTEROCOCCUS THAILANDICUS	6				6
Totalsumma årligen	17478	17264	21057	21581	

# Rare enterococcal species at Karolinska University Hospital in blood and deep cultures, excluding *E. faecalis* and *E. faecium* (2015 – 2022, n=850)



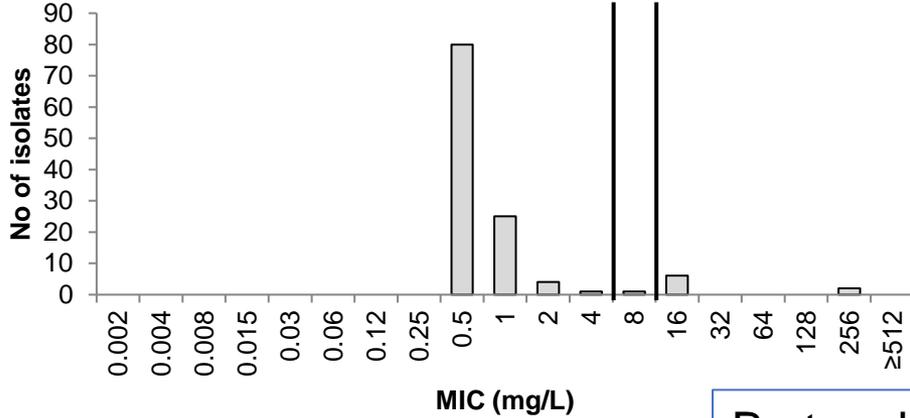
# Are all of these of clinical importance?

- No, most of them are commensals
- Some appear in mixed cultures where most microbiologists would ignore them.
- Any of them could appear as a single finding in a prosthetic device or an endocarditis, and if the finding is reproducible will need to be taken seriously.
- For most of them we lack data – please generate data or save isolates for later retrieval.
- As far as we can analyse the data we have, the current breakpoints (MIC and disk diffusion) for enterococci will do a reasonable job.

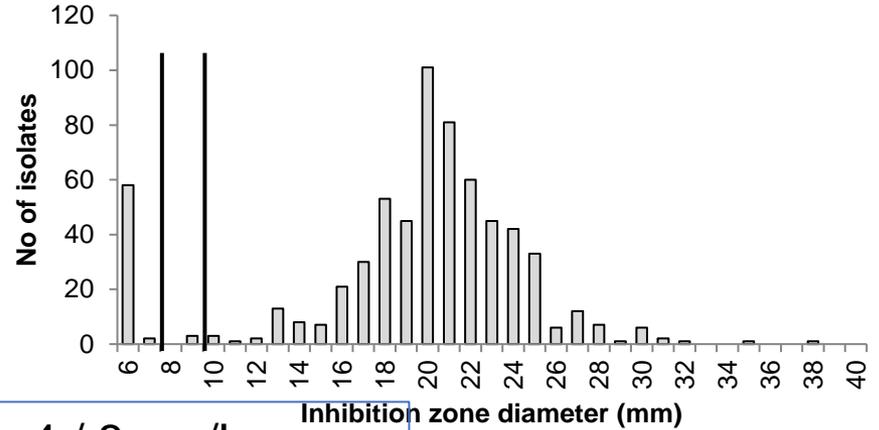
# Breakpoints were determined for *E. faecalis* and *E. faecium*

- In the following slides the breakpoints for *E. faecalis* and *E. faecium* have been inserted in distributions of other Enterococcal species

**Ampicillin**  
***E. avium*, 119 isolates**



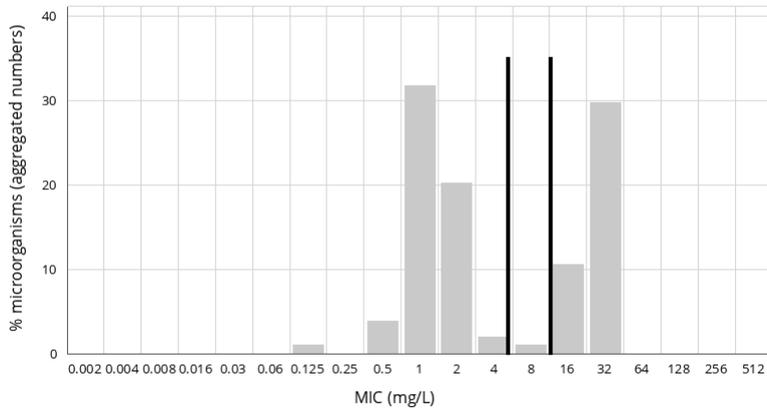
**Ampicillin 2 µg**  
***E. avium*, 645 isolates**



**Brytpunkter 4 / 8 mg/L**  
**Disk diffusion 10 / 8 mm**

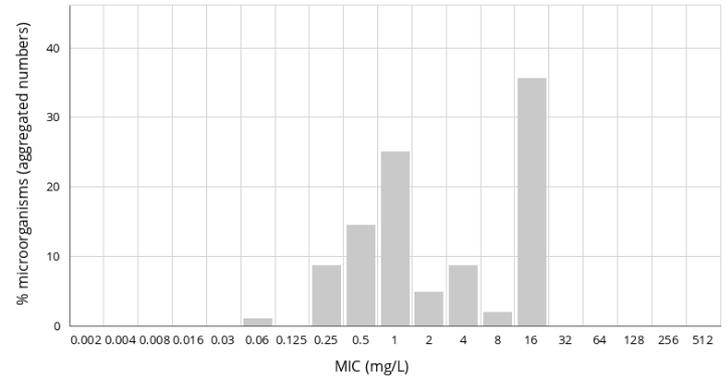
Ampicillin / *Enterococcus avium*  
International MIC distribution - Reference database 2023-02-03  
**Based on single distribution**

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



Imipenem / *Enterococcus avium*  
International MIC distribution - Reference database 2023-05-01  
**Based on single distribution**

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



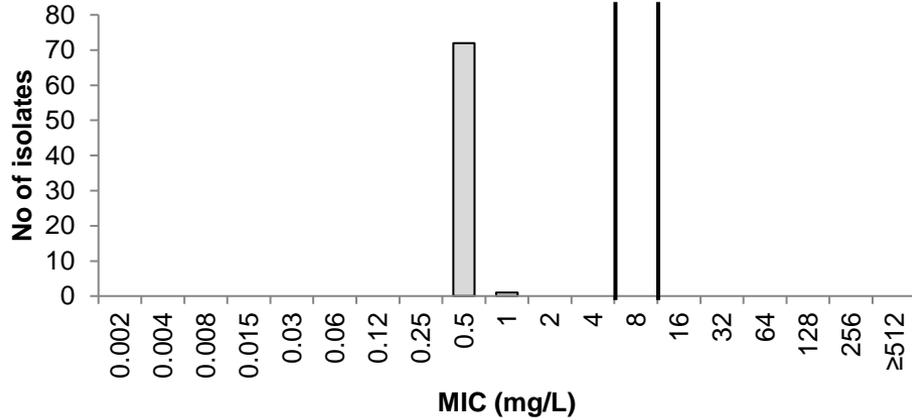
MIC  
Epidemiological cut-off (ECOFF): ID  
Wildtype (WT) organisms: -

Confidence interval: -  
104 observations

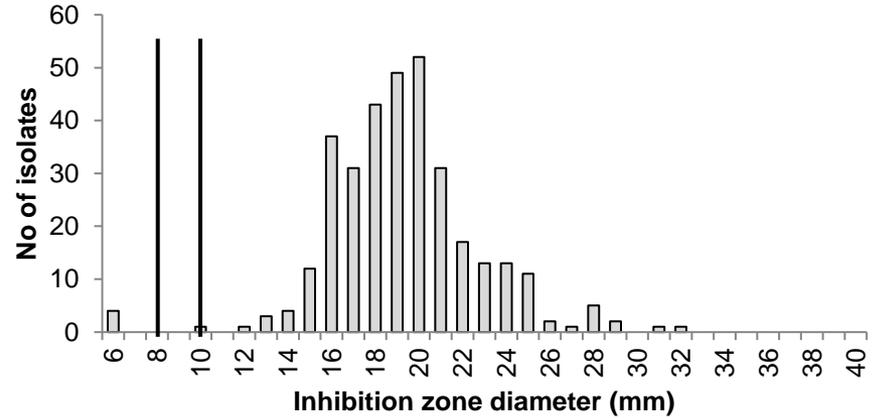
MIC  
Epidemiological cut-off (ECOFF): ID  
Wildtype (WT) organisms: -

Confidence interval: -  
104 observations

**Ampicillin**  
*E. casseliflavus*, 73 isolates

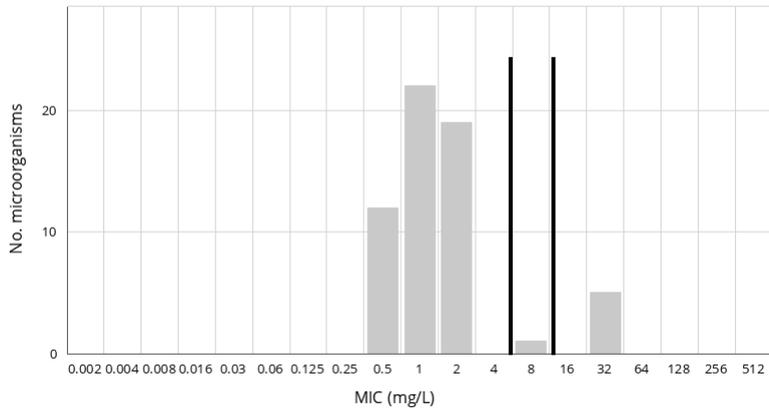


**Ampicillin 2 µg**  
*E. casseliflavus*, 334 isolates



Ampicillin / Enterococcus casseliflavus  
International MIC distribution - Reference database 2023-02-03  
Based on single distribution

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

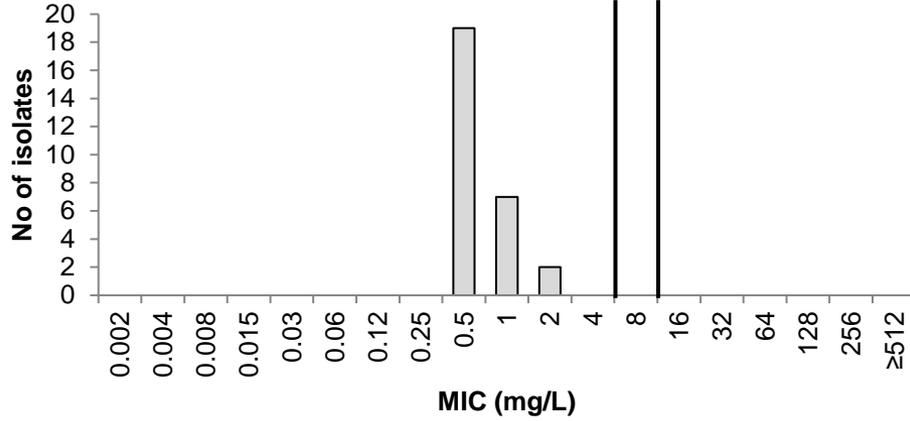


**Brytpunkter 4 / 8 mg/L**  
**Disk diffusion 10 / 8 mm**

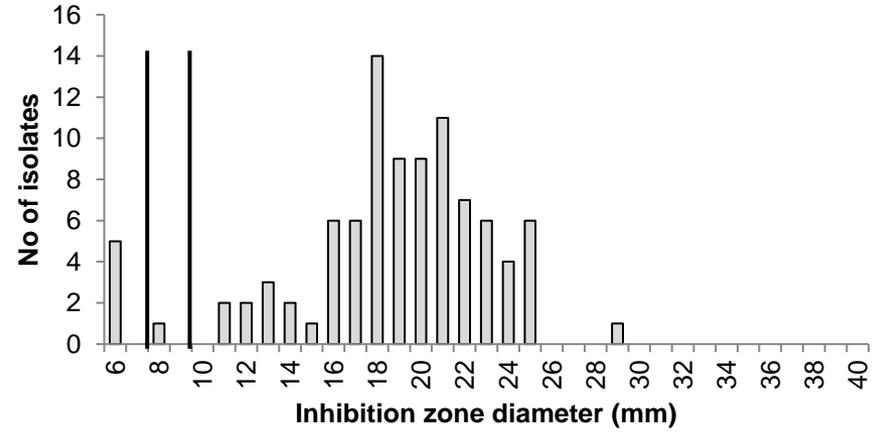
MIC  
Epidemiological cut-off (ECOFF): ID  
Wildtype (WT) organisms: -

Confidence interval: -  
59 observations

**Ampicillin**  
***E. durans*, 28 isolates**

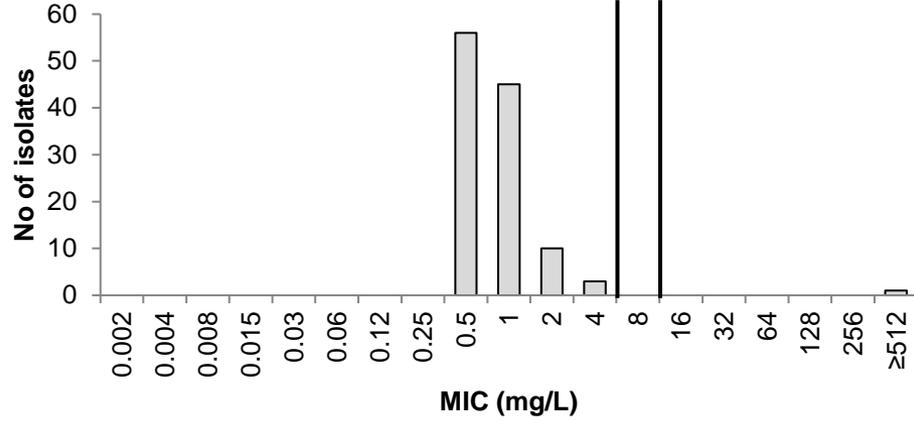


**Ampicillin 2  $\mu$ g**  
***E. durans*, 95 isolates**

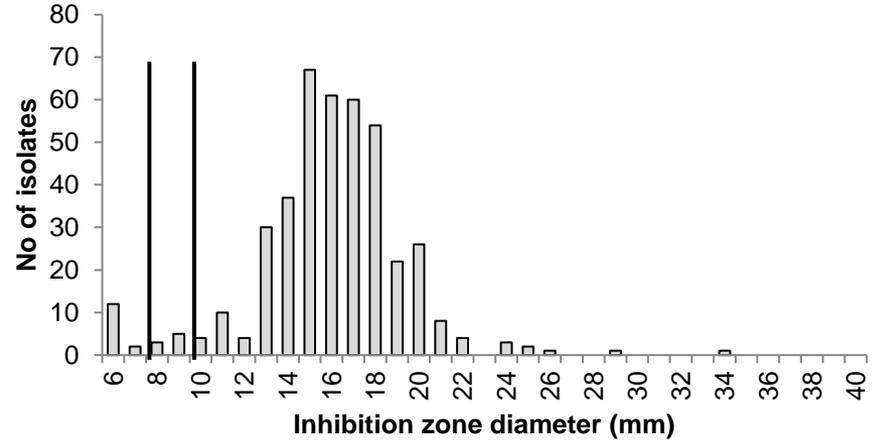


Brytpunkter 4 / 8 mg/L  
Disk diffusion 10 / 8 mm

**Ampicillin**  
***E. gallinarum*, 115 isolates**

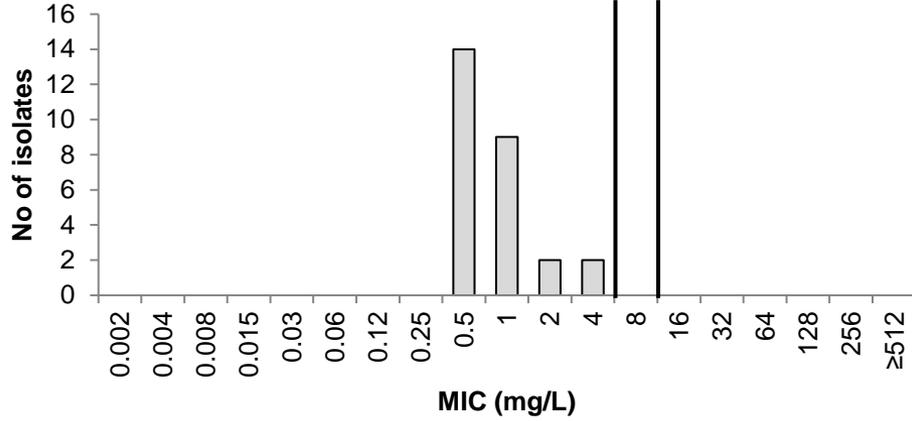


**Ampicillin 2 µg**  
***E. gallinarum*, 417 isolates**

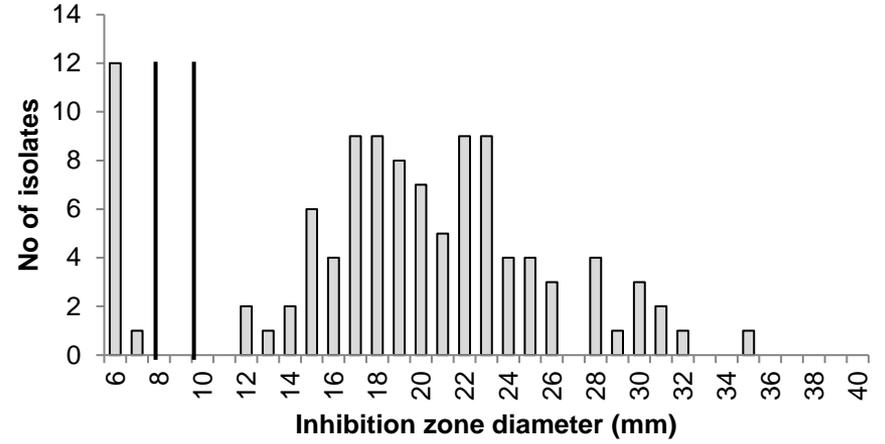


Brytpunkter 4 / 8 mg/L  
Disk diffusion 10 / 8 mm

**Ampicillin**  
***E. hirae*, 27 isolates**

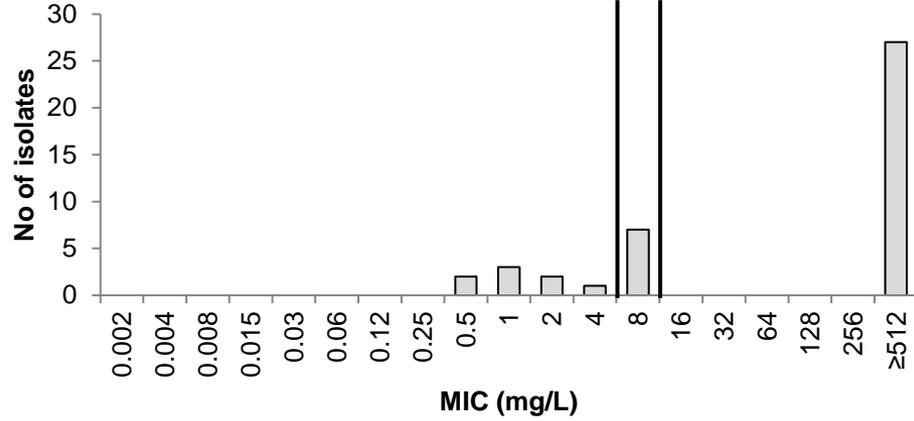


**Ampicillin 2  $\mu$ g**  
***E. hirae*, 107 isolates**

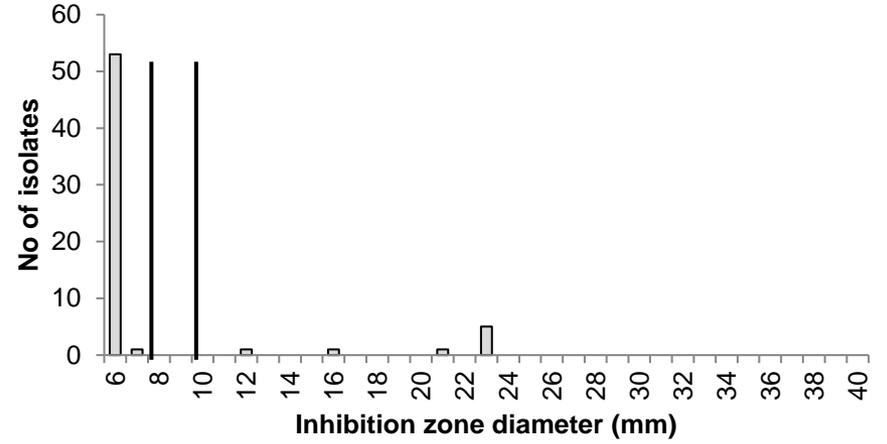


Brytpunkt 4 / 8 mg/L  
Disk diffusion 10 / 8 mm

**Ampicillin**  
***E. raffinosus*, 42 isolates**



**Ampicillin 2 µg**  
***E. raffinosus*, 107 isolates**



Brytpunkter 4 / 8 mg/L  
Disk diffusion 10 / 8 mm

# JMI (USA) – Sentry data (non-truncated data circled)

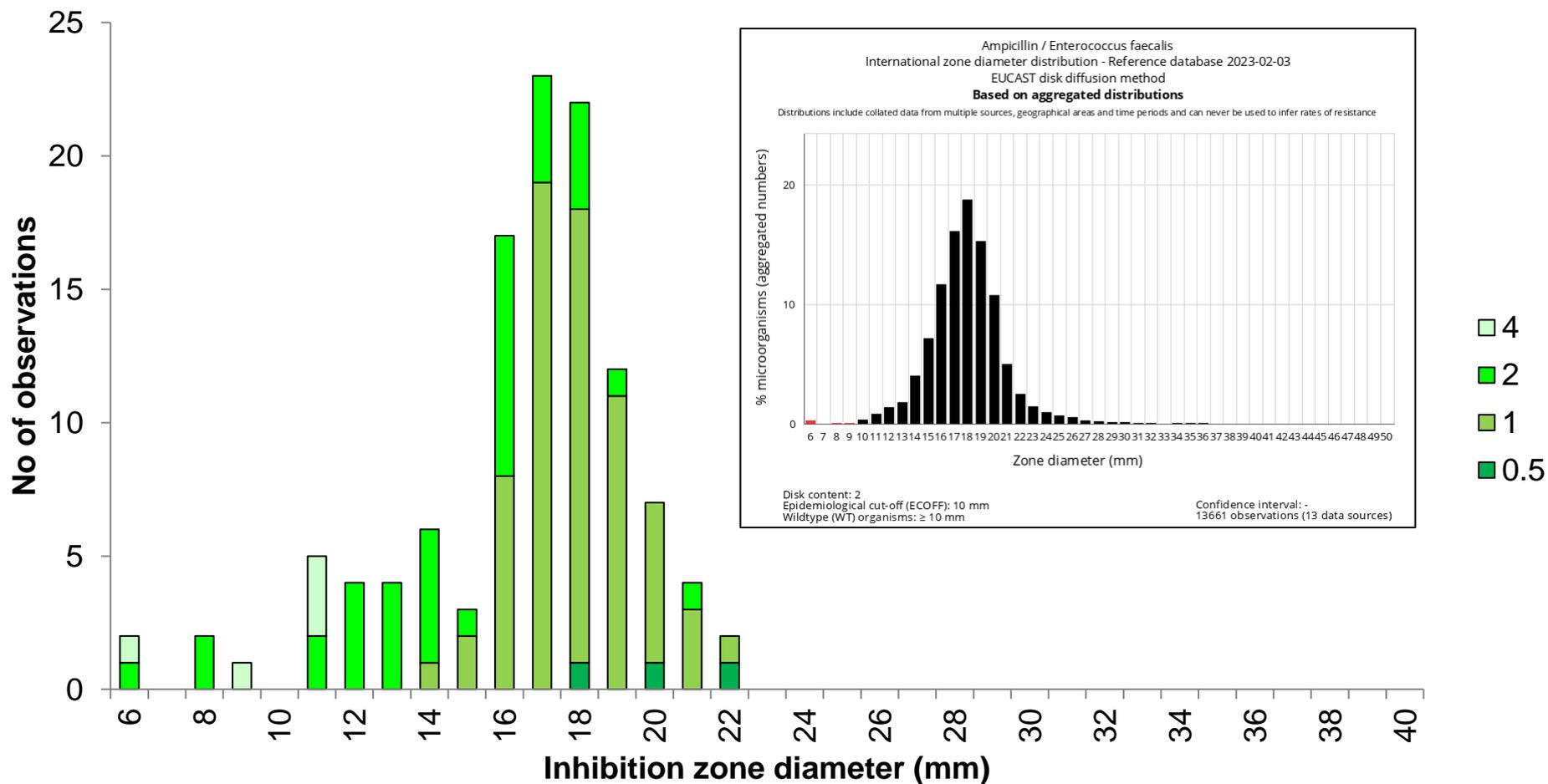
MIC distribution (and EUCAST breakpoints; S=green, R=red) of antimicrobial agents tested against Enterococcus spp. isolates in the SENTRY program (excluding Enterococcus faecalis and Enterococcus faecium) collected during

## Antimicrobial

Agent	0,02	0	0,1	0,1	0,3	0,5	1	2	4	8	16	32
Ampicillin						240	90	20	7	10		
Ceftaroline					134	114	59	56	29	7		
Doxycycline		3	22	31	2	0	1	8	26	9	2	
Levofloxacin						106	123	115	20			
Linezolid					10	76	202	112	3			
Minocycline							246	50	48	23		
Moxifloxacin		19	22	46	146	63	12	10	5			
Teicoplanin								397	1	1	2	
Tetracycline							232	0	2	7		
Tigecycline	21	172	129	73	8	1						
Vancomycin						216	17	26	107	33	0	

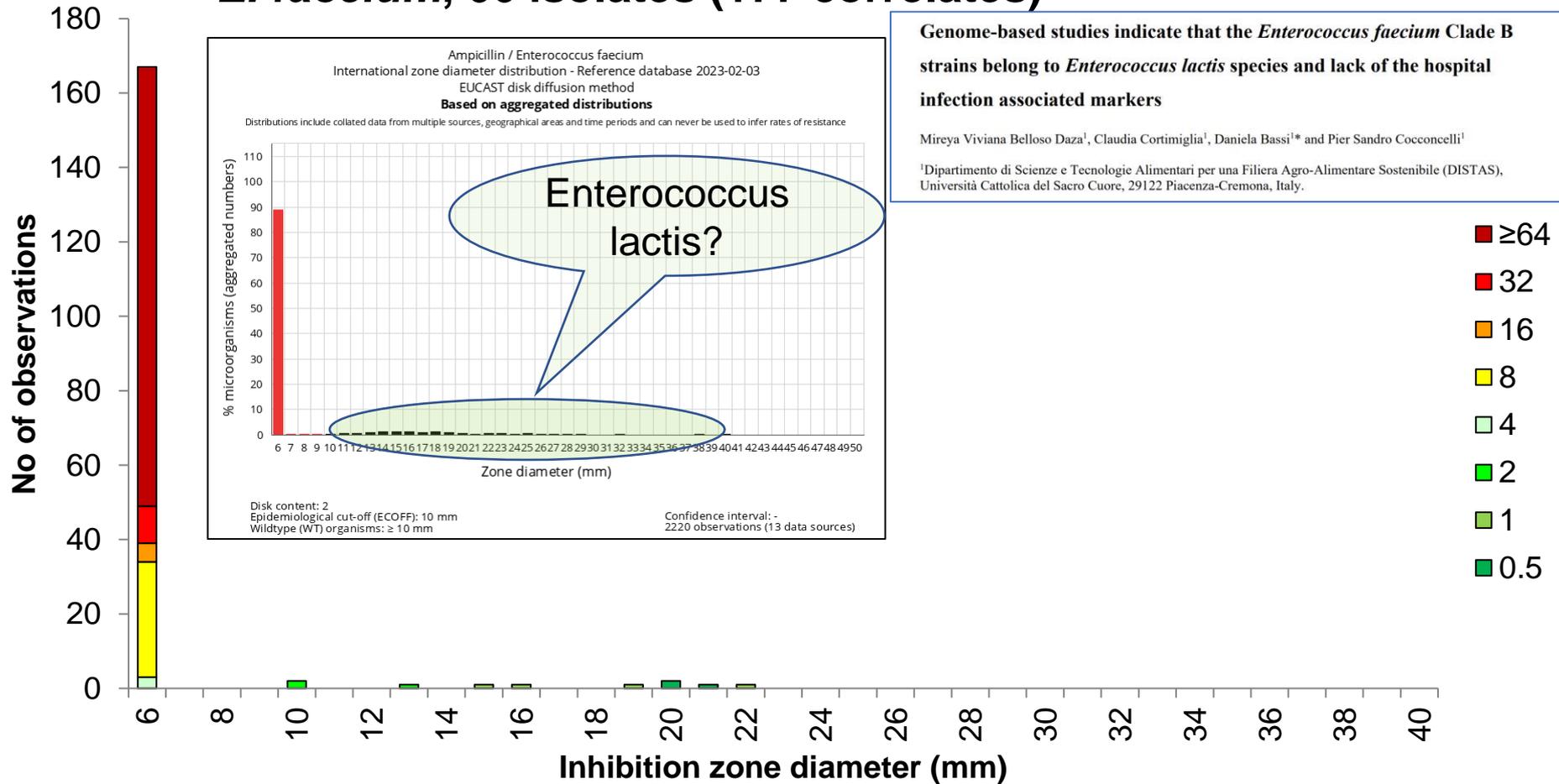
# Ampicillin 2 µg vs. MIC

## *E. faecalis*, 50 isolates (114 correlates)



# Ampicillin 2 µg vs. MIC

## *E. faecium*, 66 isolates (177 correlates)



Genome-based studies indicate that the *Enterococcus faecium* Clade B strains belong to *Enterococcus lactis* species and lack of the hospital infection associated markers

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- ≥64
- 32
- 16
- 8
- 4
- 2
- 1
- 0.5

Article

# Differentiating between *Enterococcus faecium* and *Enterococcus lactis* by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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**Abstract:** Unlike *Enterococcus faecium* strains, some *Enterococcus lactis* strains are considered potential probiotic strains as they lack particular virulence and antibiotic resistance genes. However, these closely related species are difficult to distinguish via conventional taxonomic methods. Here, for the first time, we used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with BioTyper and in-house databases to distinguish between *E. faecium* and *E. lactis*. A total of 58 reference and isolated strains (89.2%) were correctly identified at the species level using MALDI-TOF MS with in-house databases. However, seven strains (10.8%) were not accurately differentiated as a single colony was identified as a different species with a similar score value. Specific mass peaks were identified by analyzing reference strains, and mass peaks at  $10,122 \pm 2 m/z$ ,  $3650 \pm 1 m/z$ , and  $7306 \pm 1 m/z$  were unique to *E. faecium* and *E. lactis* reference strains, respectively. Mass peaks verified reproducibility in 60 isolates and showed 100% specificity, whereas 16S rRNA sequencing identified two different candidates for some isolates (*E. faecium* and *E. lactis*). Our specific mass peak method helped to differentiate two species, with high accuracy and high throughput, and provided a viable alternative to 16S rRNA sequencing.

**Keywords:** *Enterococcus faecium*; *Enterococcus lactis*; MALDI-TOF MS; identification; differentiation; specific mass peak; in-house database



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**Citation:** Kim, E.; Yang, S.-M.; Kim, H.-J.; Kim, H.-Y. Differentiating between *Enterococcus faecium* and *Enterococcus lactis* by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. *Foods* **2022**, *11*, 1046. <https://doi.org/10.3390/foods11071046>