



Detection of penicillin susceptibility in *S. aureus*

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Mentimeter question 1

Do you perform testing for penicillin susceptibility on *S. aureus* in your lab?

- No
- Yes, with EUCAST disk diffusion
- Yes, both disk diffusion and clover leaf test
- Yes, including test for blaZ
- Not relevant (don't work in a lab)

Mentimeter question 2

Do you know the current percentage of *S. aureus* isolates in your country/region which is penicillin susceptible?

- No
- <10%
- 10-25%
- 25-40%
- >40%

Agenda

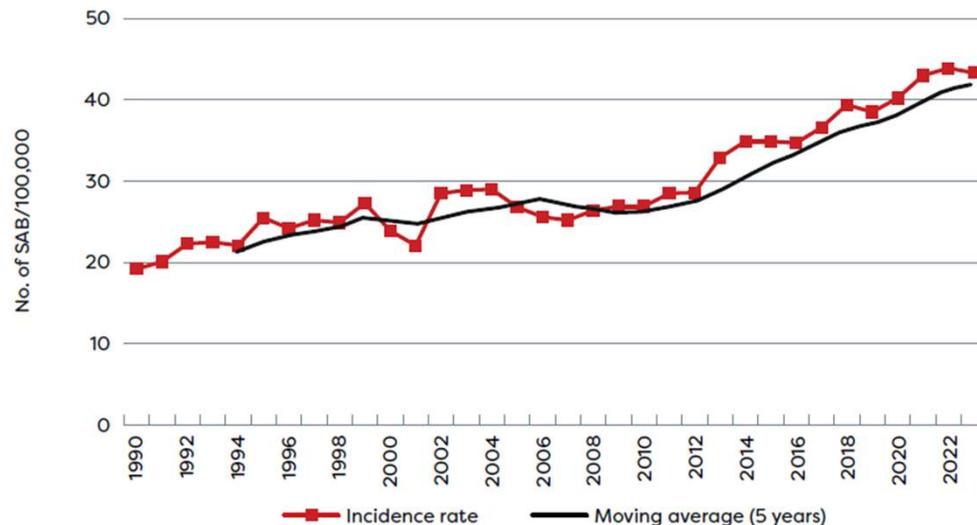
- Clinical context: a hot topic
- Molecular background: not so complicated
- Epidemiology: increasing susceptibility
- **Testing: disk diffusion, clover leaf, blaZ testing**

Staphylococcus aureus: a major human pathogen

S. aureus bacteraemia is both a common and serious infection



Figure 2. Incidence rate of SAB in Denmark per 100,000 inhabitants 1990-2023



2023: 30-day all-cause case fatality 24%

Staphylococcus aureus bacteraemia Cases in Denmark 2023 <https://antibiotika.ssi.dk/-/media/arkiv/subsites/antibiotikaresistens/stafylokokrapporter/staphylococcus-aureus-bacteraemia---cases-in-dk-2023.pdf>

Treatment of Penicillin-susceptible *S. aureus* (PSSA)



Invasive PSSA infections are treated with Benzylpenicillin.

Bacteraemia treatment: “The use of penicillin for the subset of MSSA that is penicillin-susceptible is currently not recommended but is the subject of an ongoing RCT”

Minter et al. Clin Infect Dis. 2023 Nov 30;77(11):e57–68.

Late breaker: results from the SNAP trial

Enrolment closed in August 2024 on advice of the data and safety monitoring committee

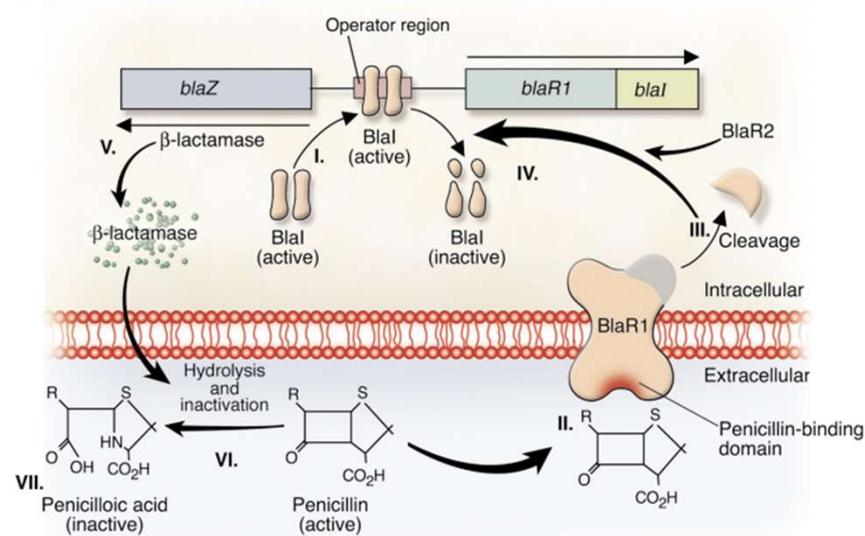
	281 adults with PSSA bacteraemia	
	benzylpenicillin i.v.	(flu)cloxacillin i.v.
Primary outcome: 90-day all-cause mortality	13.8%	21.5%
Secondary outcome: Acute kidney injury	10.9%	21.6%

pre-specified non-inferiority criterion not met, but
96.1% posterior probability of benzylpenicillin being non-inferior

“(...) indicates that benzylpenicillin should be preferred over (flu)cloxacillin for treatment of PSSA bacteraemia.”

The molecular background of penicillin resistance in methicillin-susceptible *S. aureus*

- *blaZ* gene encodes serine-beta-lactamase
- Inducible: Regulated by *blaR1* and repressor *blaI*
- Located on transposon Tn552 or Tn552-like elements on a large plasmid or integrated into chromosome
- Four types: A, B, C, D with different kinetic profiles (related to amino acid residues 128 and 216)



Foster TJ. FEMS Microbiology Reviews, fux007, 41, 2017, 430–449

Lowy FD. J Clin Invest. 2003 May;111(9):1265-73.

Zygmunt DJ et al. Antimicrob Agents Chemother. 1992 Feb;36(2):440-5.

Voladri RKR et al. J Bacteriol. 1996 Dec;178(24):7248-53.

Epidemiology: global

SENTRY program 1997 - 2016

191 460 *S. aureus* isolates

from 427 centers (Americas, Europe, Asia-Pacific region)

Table 2. Temporal Trend in Percent Susceptibility to Selected Older Antimicrobials Among *S. aureus* Isolates, Stratified by Methicillin Resistance (SENTRY Program, 1997–2016)

Antimicrobial Agent	% Susceptible by Time Interval					Overall
	1997–2000	2001–2004	2005–2008	2009–2012	2013–2016	
MSSA						
Penicillin	14	19	20	23	26	21

Diekema D et al. Open Forum Infect Dis 2019 Mar 15;6(Suppl 1):S47-S53.

Epidemiology: penicillin susceptibility in the Nordic countries



Bacteremia isolates: increasing penicillin susceptibility from 23% in 2014 to **32% in 2023**

DANMAP 2023



Lund blood isolates:

2008/2009 (n=100): 57% penicillin susceptible

2014/2015 (n=140): 29% penicillin susceptible

2018-2020 (n=1998): **31% penicillin susceptible**

Aldman et al. Infect Dis (Lond) 2017 Jun;49(6):454-460.

Aldman et al. Int J Antimicrob Agents 2022 Apr;59(4):106567.



32% beta-lactamase negative, 68% positive

blood culture isolates in 2023 (n=1,695)

NORM / NORM-VET 2023



2008-2024: 10-20% Penicillin susceptible (**2024: 12,5%**)

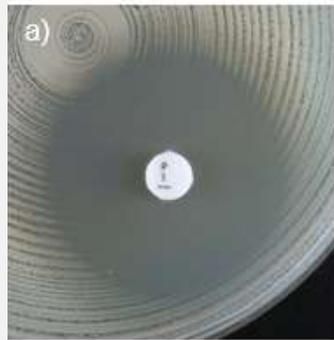
Personal communication Kristján Helgason

Phenotypic AST

EUCAST Disk diffusion

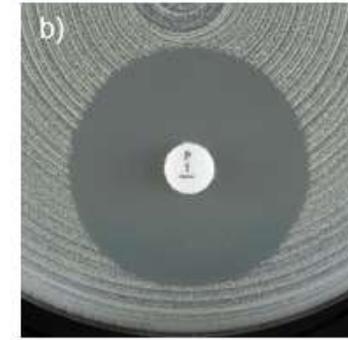
Penicillin S

Inhibition zone ≥ 26 mm
AND "beach"



Penicillin R

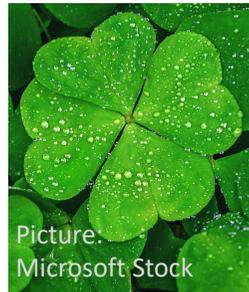
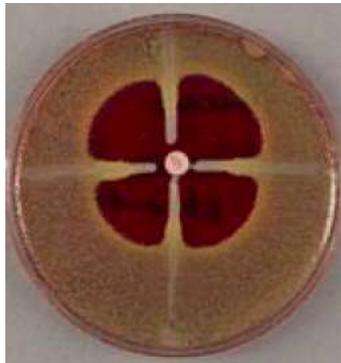
Inhibition zone < 26 mm
OR "cliff"



NordicAST addition: "inspect zone edge **or perform clover leaf test**"

Clover leaf test

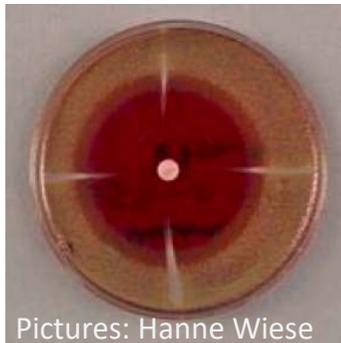
Seeding with Penicillin-susceptible *S. aureus* (PSSA)
cross: test strain



Test
positive



Penicillin R



Test
negative



Penicillin S

Ørstavik, Acta Path Microbiol Scand, 1971. 79:855-856.

Clover leaf test: some practicalities

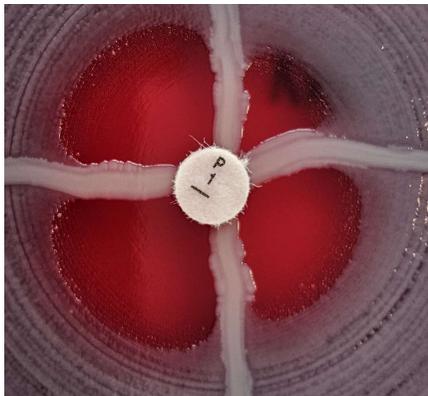
1. Seeding/flooding versus streaking the plates with PSSA

Ørstavik 1971:

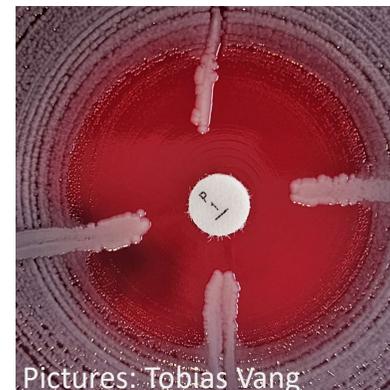
seeding by flooding with a 3 ml suspension, surplus removed by a pipette, dried 1h RT

Herlev/DK: 0,5 McFarland suspension, plate rotator

2. Inoculum of the test isolate matters!



high inoculum
(streak from colony)



low inoculum
(streak from 0,5
McFarland suspension)

Performance of phenotypic tests

345 *S. aureus* isolates

199 PSSA blood culture isolates from US (testet penicillin S by MIC)

85 PSSA blood culture isolates from DK (testet penicillin S by disk diffusion & clover leaf)

61 challenge MSSA (CDC)

Tests performed at SSI: *blaZ* PCR (golden standard), EUCAST disk diffusion, clover leaf test

EUCAST disk diffusion (zone diameter + edge): 98% sensitivity

Clover leaf test: 100 % sensitivity and 100% specificity

1/85 isolates from Danish Dept. of Clin. Microbiology had been reported false susceptible

Skov et al. J Antimicrob Chemother 2021; 76: 1487–1494

Performance of the EUCAST disk diffusion method

Multi-center study (SNAP: Australia, New Zealand, Canada, Singapore, Israel)

19 laboratories tested according to EUCAST

9 *S. aureus* isolates, sent in triplicates

selected based on phenotypic susceptibility determined by VITEK 2 (MIC \leq 0.125 mg/L)

6 *blaZ* positive, 3 *blaZ* negative

1 *blaZ* positive isolate with inhibition zone 28mm and sharp zone edge

	Susceptible <i>n</i> (%)	Resistant <i>n</i> (%)	Total <i>n</i>
EUCAST methodology (<i>n</i> = 19 laboratories)			
<i>blaZ</i> PCR detected	37 (11)	305 (89)	342
<i>blaZ</i> PCR not detected	170 (99.4)	1 (0.6)	171
Total	207	306	513

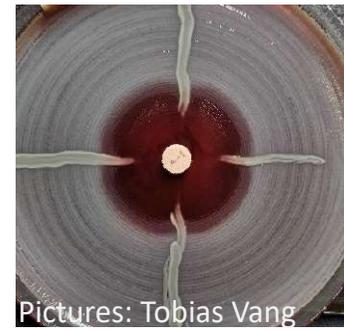
(VME)

Phenotypic testing vs. genotypic testing on blood culture vials

Study setup:

- *blaZ* PCR performed directly on 201 positive blood culture vials
- Routine phenotypic AST (EUCAST disk diffusion, S confirmed with clover leaf test)

	blaZ PCR	EUCAST disk diffusion	Clover leaf test
n = 101	neg	S	negative
n = 99	pos	R	NA
n = 1	pos	S	positive



Pictures: Tobias Vang

Take home messages

- Penicillin-susceptibility testing of *S. aureus* is clinically important, as benzylpenicillin is the increasingly preferred treatment
- The percentage of susceptible isolates in the Nordic countries is appr. 30% in recent data, but local differences occur
- The EUCAST disk diffusion method performs very well, but:
- For invasive isolates, consider to supplement with the clover leaf test or molecular *blaZ* testing