Background and mechanisms

Reduced susceptibility to penicillin is caused by structural changes in penicillin-binding proteins (PBP), leading to decreased affinity for penicillin and other beta-lactam agents. This results in preserved activity of the PBPs in the presence of the antimicrobial agent, and the cell wall synthesis is not inhibited (1, 2). In pneumococci, five PBPs with high molecular weight (PBP 1A, 1B, 2A, 2B, 2X) and one with low molecular weight (PBP 3) are found. Changes in PBPs 1A, 2B and 2X have the strongest impact on penicillin susceptibility (3). Penicillin-degrading enzymes (beta-lactamases) have not been described in pneumococci.

Streptococcus pneumoniae can take up and incorporate DNA-fragments into its genome in a horizontal gene transfer process called transformation. Changes in PBPs can occur by incorporation of PBP-encoding gene-fragments from closely related species, such as streptococci belonging to the mitis group. This results in PBP-genes consisting of fragments with different origin, termed mosaic genes (4). Reduced penicillin susceptibility usually becomes detectable after multiple stepwise changes in PBP-genes and the clinical impact can range from the need to adjust beta-lactam dosing (I-category: susceptible, increased exposure) to resistance, where beta-lactam antibiotics cannot be used for treatment. Susceptibility to aminopenicillins and cephalosporins can also be affected in varying degrees.

In the 21st century, conjugate vaccines covering several pneumococcal serotypes have been included in vaccination programs for children and elderly in large parts of the world. This has affected the serotype distribution and epidemiology of resistant strains.

Method

Screening for susceptibility to all beta-lactams with activity against pneumococci is performed by disk diffusion using an oxacillin 1 µg disk.

From 2025, a benzylpenicillin 1 unit disk is used for SIR-categorization of benzylpenicillin in oxacillin screening positive isolates. MIC testing for benzylpenicillin has no added value. EUCAST recommends inclusion of the benzylpenicillin disk in the initial screening to avoid the time delay of two-step testing. The benzylpenicillin disk should only be read and interpreted if the oxacillin inhibition zone diameter is <20 mm.

Isolates with oxacillin inhibition zone diameter ≥20 mm (negative oxacillin screening test) can be reported susceptible (S or I) to all beta-lactams for which clinical breakpoints are available. For isolates with oxacillin inhibition zone diameter <20 mm (positive oxacillin screening test), see flowchart below.

EUCAST warning against the use of gradient tests for benzylpenicillin MIC in Streptococcus pneumoniae
In November 2019, a EUCAST warning was issued against the use of gradient tests for determining benzylpenicillin MIC in *Streptococcus pneumoniae*, since available gradient tests systematically underestimate benzylpenicillin MIC values in *S. pneumoniae* by one or more doubling dilutions. The warning is still valid, for which reason only broth microdilution should be used for MIC-testing of benzylpenicillin in pneumococci.

Interpretation when the oxacillin screening is positive (<20mm)

For interpretation of the oxacillin disk diffusion test, and suggested comments, see the flowchart below which can also be found at the bottom of the *S. pneumoniae* tab in the breakpoint table. When oxacillin screening is positive (inhibition zone <20 mm), reporting of reduced susceptibility to penicillin should not be delayed and phenoxymethylpenicillin should be reported as resistant (R) for all isolates.

Note that the SIR categorisation for benzylpenicillin and further testing algorithm for other beta-lactam agents is dependent on infection type:

Endocarditis and meningitis

All isolates with oxacillin inhibition zone <20 mm are reported resistant (R) to benzylpenicillin. Other relevant beta-lactam agents (cefotaxime, ceftriaxone and/or meropenem) can be reported susceptible for isolates with oxacillin inhibition zone diameter 9-19 mm. For isolates with an oxacillin inhibition zone diameter <9 mm, the MIC must be determined and interpreted according to breakpoints.

Other infections

Benzylpenicillin can be used for treatment of other infections when the isolate is classified in the I-category, as long as the dosage is appropriately adjusted (see table below and dosages tab in the breakpoint table). Benzylpenicillin should be reported susceptible, increased exposure (I) if the benzylpenicillin inhibition zone diameter is ≥14 mm, and resistant (R) if the benzylpenicillin inhibition zone diameter is <14 mm. A comment regarding appropriate dosing of benzylpenicillin should be considered when reporting isolates in the I-category (see recommendations in the flowchart of the national breakpoint table).

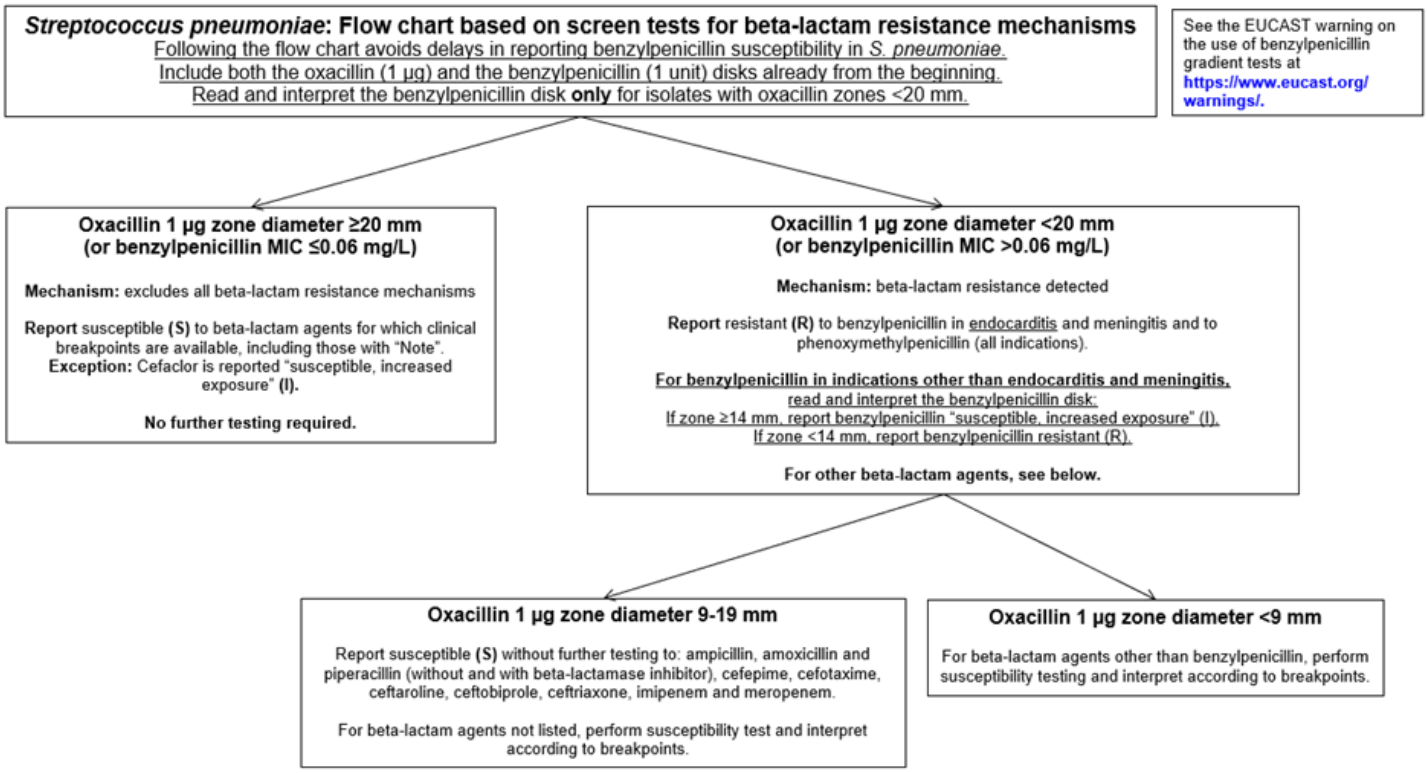
Other beta-lactam agents may be effective despite the presence of a resistance mechanism. Ampicillin, amoxicillin, piperacillin-tazobactam, cefepime, ceftaroline, ceftobiprole, cefotaxime, ceftriaxone, imipenem and meropenem can be reported susceptible (S) when the oxacillin zone diameter is ≥9 mm. However, SIR categorisation of cefuroxime must be based on MIC-value when the oxacillin zone diameter is <20 mm.

For isolates with oxacillin zone diameter <9 mm, SIR-categorisation of amoxicillin and amoxicillin-clavulanic acid oral and i.v. is inferred from ampicillin (indications other than endocarditis and meningitis). Isolates classified in the I-category (susceptible, increased exposure) for ampicillin, amoxicillin or amoxicillin-clavulanic acid require high-dose treatment (see dosages tab in the breakpoint table).

Unexpected results

Reduced susceptibility (I/R) to 3rd generation cephalosporins (cefotaxime and/or ceftriaxone) is rare in pneumococci, at least in the European setting. Such isolates should be re-tested and, if the result is confirmed, sent to the national reference laboratory.

Flowchart (see also bottom of S. pneumoniae tab in the breakpoint table):



Referral to national reference laboratory

In all Nordic countries, all invasive isolates are sent to the respective reference laboratories.
Isolates with reduced susceptibility or resistance to 3 rd generation cephalosporins should be confirmed by a reference laboratory. In Iceland oxacillin screen positive isolates are sent to the reference laboratory.

References

1. Hakenbeck R, Tarpay M, Tomasz A. Multiple changes of penicillin-binding proteins in penicillin-resistant clinical isolates of Streptococcus pneumoniae. Antimicrob Agents Chemother. 1980 Mar;17(3):364-71.

2. Zighelboim S, Tomasz A. Penicillin-binding proteins of multiply antibiotic-resistant South African strains of Streptococcus pneumoniae. Antimicrob Agents Chemother. 1980 Mar;17(3):434-42.

3. Sanbongi Y, Ida T, Ishikawa M, Osaki Y, Kataoka H, Suzuki T, Kondo K, Ohsawa F, Yonezawa M. Complete sequence of six penicillin-binding protein genes from 40 *Streptococcus pneumoniae* clinical isolates collected in Japan. Antimicrob Agents Chemother 2004;48:2244-50.

4. Dowson CG, Hutchison A, Brannigan JA, George RC, Hansman D, Linares J, Tomasz A, Smith JM, Spratt BG. Horizontal transfer of penicillin-binding protein genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. Proc Natl Acad Sci USA 1989;86:8842-46.

Responsible for this document

NordicAST representatives, subgroup for gram positive bacteria, see http://www.nordicast.org/nordicasts-medlemmar

Changes

Date	Changes
2025-04-29	Text and flowchart updated according to breakpoints table v15.0 • Inclusion of benzylpenicillin disk from the beginning • Endocarditis added to meningitis in interpretation
2022-01-20	Text and flowchart updated according to breakpoint table v12.0: • amoxiclin with or without clavulanic acid p.o. and i.v. inferred from ampicillin (indications other than meningitis) • lower oxacillin screening breakpoint changed from 8 to 9 mm • for isolates with oxacillin inhibition zone 9-19 mm, MIC determination for cefotaxim, ceftriaxon and meropenem for meningitis treatment is no longer recommended • imipenem and meropenem added to flowchart (can be reported susceptible without further testing for isolates with oxacillin inhibition zone 9-19 mm) Minor linguistic changes
2021-03-29	Flowchart updated, Benzylpenicillin dosage for meningitis caused by *S. pneumonia* added as foodnote in table

2020-09-18	Translation to English, new title (old title: “Streptococcus pneumoniae. Påvisning av nedsatt penicillinkänslighet.”). Text revised, reflecting changes in the breakpoint table and new definition of I-category (susceptible, increased exposure). Algorithm table replaced by flow chart. Interpretation/dosing table adjusted. Added information on criteria which isolates are sent to reference laboratories in all Nordic countries. Update of persons responsible for the document.
2015-05-22	Justerat tabell "Bedömning baserat på bensylpenicillin MIC" samt uppdaterat tolkning för betalaktamantibiotika vid oxacillin 8-19mm
2015-03-20	Layoutjustering samt uppdatering av dokumentansvariga.
2014-03-06	Justerat tabell över tolkning av oxacillin zon för tydligare utseende.
2013-06-13	Tillfört tabell "Bedömning baserat på bensylpenicillin MIC"
2013-05-14	Nytt dokument baserat på Natås OB: AFAs anbefalte metode for påvisning av nedsatt penicillinfølsomhet hos Streptococcus pneumoniae. Versjon 1.2,2012 v