Haemophilus influenzae and beta lactam resistance

Background and mechanisms

Reduced susceptibility to beta-lactams in *Haemophilus influenzae* may be caused by transferable beta-lactamases (most commonly TEM-1, more rarely ROB-1), or chromosomal mechanisms [1].

Beta-lactamase-mediated resistance affects penicillins only and is inhibited by beta-lactamase inhibitors (e.g. clavulanic acid or tazobactam). Chromosomally mediated resistance may also confer resistance to cephalosporins and carbapenems and is not inhibited by beta-lactamase inhibitors. The two mechanisms may occur separately or concomitantly.

The most common mechanism for chromosomally mediated beta-lactam resistance in *H. influenzae* is amino acid substitutions in the transpeptidase region of penicillin binding protein 3 (PBP3) due to mutations in the *ftsI* gene. This results in reduced affinity and reduced susceptibility to beta-lactams, in particular aminopenicillins and cephalosporins, for which PBP3 is the primary target.

Based on the presence or absence of distinct amino acid substitutions, isolates with PBP3-mediated resistance (rPBP3) may be classified as low-level or high-level resistant (low-rPBP3 or high-rPBP3). The distinction is clinically important because high-rPBP3 isolates, in contrast to low-rPBP3 isolates, often are resistant to 3rd and 4th generation cephalosporins. Low-rPBP3 isolates are frequent in Scandinavia and represent the predominant resistance genotype in Europe. Recent studies have shown expansion of international high-rPBP3 clones expressing resistance to cefotaxime and ceftriaxone in Nordic countries [2].

Screening

Screening for beta-lactam resistance is performed by disk diffusion. Benzylpenicillin 1 unit (PCG1) is used for primary screening. If PCG1 is categorized as screening positive, a beta-lactamase test is performed. Amoxicillin-clavulanic acid (2-1 µg) should be used to screen for PBP3-mediated resistance in beta-lactamase positive isolates [3-4].

Benzylpenicillin 1 unit (PCG1) will detect both beta-lactamase and PBP3-mediated resistance. In screening positive isolates:

- Perform a test for beta-lactamase.
- In beta-lactamase negative results, the beta-lactam resistance mechanism is from PBP3 changes alone.
- In beta-lactamase positive strains, the zone for amoxicillin-clavulanic acid (2-1 µg) is inspected to determine whether the isolate also contains PBP3 changes in addition to beta-lactamase mediated resistance.

Based on the screening test results, it may be deduced with a high degree of reliability whether an isolate lacks resistance mechanisms or if one or both mechanisms are present [3-4].

Because amoxicillin-clavulanic acid (2-1 µg) has lower sensitivity and specificity for detection of PBP3-mediated resistance compared to PCG1 [5], the disks should only be used for screening of beta-lactamase positive isolates.

Susceptibility categorization and interpretation

A flowchart for interpretation and reporting is provided below and in the NordicAST breakpoint table.

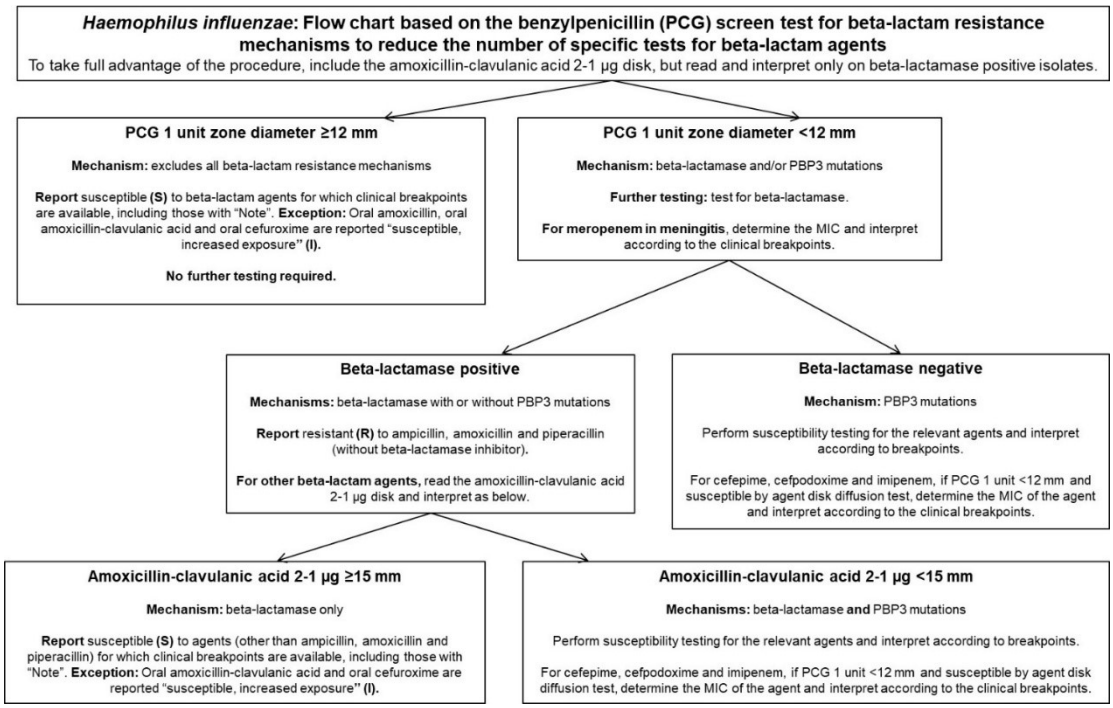
Separate MIC breakpoints are provided for meropenem in meningitis and should be performed in all isolates that are screening positive for beta-lactam resistance.

Amoxicillin, amoxicillin-clavulanic acid and cefuroxime in oral formulations should always be reported as "susceptible, increased exposure (I)" when deemed susceptible.

When screening results indicate PBP3-mediated resistance, susceptibility testing with interpretation according to clinical breakpoints should be performed for relevant agents individually.

The stepwise character of PBP3 resistance leads to close or overlapping susceptible and resistant populations when testing beta-lactam susceptibility in these isolates. Hence, an area of technical uncertainty (ATU) is needed in many situations see in the breakpoint table and at: https://www.eucast.org/ast_of_bacteria/calibration_and_validation.

Minimum Inhibitory Concentration (MIC) determination of *H. influenzae* by gradient tests sometimes produce misleading results in beta-lactamase negative populations [5-9]. Studies indicate that Etest® may underestimate ampicillin MIC by 1-2 dilutions for beta-lactamase negative isolates with ampicillin MIC close to the breakpoint [5-8, 10]. In a Norwegian study of such isolates, EUCAST disk diffusion was superior to Etest for susceptibility categorization to ampicillin, with higher categorical agreement rate (74.0% vs. 64.3%) and a significantly lower falsely susceptible rate (28.3% vs. 88.3%) [5].



References

[1] Tristram S, Jacobs MR, Appelbaum PC: Antimicrobial resistance in Haemophilus influenzae. Clin Microbiol Rev 2007, 20: 368-389.

[2] Skaare D, Anthonisen IL, Zecic N, Jenkins A, Caugant DA, Ranheim TE, Sundsfjord A, Hegstad K; Study Group on Exceptional Phenotypes in Haemophilus influenzae. Genomic profiling of cefotaxime-resistant *Haemophilus influenzae* from Norway and Sweden reveals extensive expansion of virulent multidrug-resistant international clones. Front Microbiol. 2025 Jul 29;16:1601390. doi: 10.3389/fmicb.2025.1601390. PMID: 40800114; PMCID: PMC12341455.

[3] Sondergaard A, Petersen MT, Fuursted K, Norskov-Lauritsen N: Detection of N526K-substituted penicillin-binding protein 3 conferring low-level mutational resistance to beta-lactam antibiotics in Haemophilus influenzae by disc diffusion testing on Mueller-Hinton agar according to EUCAST guidelines. J Antimicrob Chemother 2012, 67: 1401-1404.

[4] Garcia-Cobos S, Arroyo M, Perez-Vazquez M, Aracil B, Oteo J, Campos J: Evaluation of the EUCAST disc diffusion susceptibility testing method for Haemophilus influenzae based on the resistance mechanism to beta-lactam antibiotics. J Antimicrob Chemother 2013, 68: 159-163.

[5] Skaare D, Lia A, Hannisdal A, Tveten Y, Matuschek E, Kahlmeter G, Kristiansen B-E: Haemophilus influenzae with non-beta-lactamase-mediated beta-lactam resistance: easy to find but hard to categorize. Journal of Clinical Microbiology 2015, 53: 3589-95.

[6] Barry AL, Fuchs PC, Brown SD: Identification of beta-lactamase-negative, ampicillin-resistant strains of Haemophilus influenzae with four methods and eight media. Antimicrob Agents Chemother 2001, 45: 1585-1588.

[7] Billal DS, Hotomi M, Yamanaka N: Can the Etest correctly determine the MICs of beta-lactam and cephalosporin antibiotics for beta-lactamase-negative ampicillin-resistant Haemophilus influenzae? Antimicrob Agents Chemother 2007, 51: 3463-3464.

[8] Garcia-Cobos S, Campos J, Roman F, Carrera C, Perez-Vazquez M, Aracil B et al.: Low beta-lactamase-negative ampicillin-resistant Haemophilus influenzae strains are best detected by testing amoxicillin susceptibility by the broth microdilution method. Antimicrob Agents Chemother 2008, 52: 2407-2414.

[9] Mushtaq S, Warner M, Cloke J, Afzal-Shah M, Livermore DM: Performance of the Oxoid M.I.C.Evaluator Strips compared with the Etest assay and BSAC agar dilution. Journal of Antimicrobial Chemotherapy 2010, 65: 1702-1711.

[10] Tristram SG: A comparison of Etest, M.I.C.Evaluator strips and CLSI broth microdilution for determining beta-lactam antimicrobial susceptibility in Haemophilus influenzae. Journal of Antimicrobial Chemotherapy 2008, 62: 1464-1466.

Responsible for this document

NordicAST representatives, subgroup for fastidious bacteria, see NordicAST homepage.

Changes

Version	Changes
2026-05-20	Updated flowchart, text on testing for strains suspected for meningitis and background on epidemiology of high rPBP3 in Nordic countries
2023-11-30	Updated according to current EUCAST/NordicAST screening and testing algorithm and flowchart included in document.
2019-03-21	Removed recommendation to refer isolates with resistance to extended spectrum cephalosporins. Removed comment on unreliable results for aminopenicillins. Updated flowchart and notes. Document responsible updated.
2017-12-20	Added recommendation regarding rPBP3 and cefuroxime (interpretation and flowchart)
2016-12-19	Emphasized that susceptibility to amoxicillin should be inferred from ampicillin.
2016-10-06	English version added. Document responsible from Finland and Iceland added.
2016-03-17	Reference added (Skaare et al, JCM 2015). Clarification regarding amoxicillin p.o. (infer from ampicillin).
2015-03-20	Removed version number from Changes table (date only)
2015-01-01	Added CXM30 as alternative screening disk for beta-lactamase positive isolates. Equation of gradient MIC and disk diffusion for SIR-categorization of screening positive isolates. New comment regarding screening positive isolates and S-categorization to aminopenicillins. Updates on epidemiology, terminology and references.
2013-01-01	Minor text changes
2012-01-01	New document