

P2775 EUCAST antimicrobial susceptibility testing: *Haemophilus influenzae* and beta-lactam agents

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Background: Due to increasing resistance related to mutations in penicillin binding proteins (PBP3) in *Haemophilus influenzae*, antimicrobial susceptibility testing (AST) versus beta-lactam agents is subject to several difficulties. The aim of this study was to review and improve EUCAST recommendations for disk diffusion testing of *H. influenzae* versus beta-lactam agents.

Materials/methods: AST was performed for all beta-lactam agents with EUCAST breakpoints, including the benzylpenicillin 1 unit screening disk, on a collection of *H. influenzae* (n=138) biased towards beta-lactam resistance. The isolates were from the worldwide SENTRY collection (JMI Laboratories, USA). All isolates were examined for beta-lactamase production with a nitrocefin disk and investigated for PBP3 mutations with PCR. Broth microdilution (BMD) was performed according to ISO 20776-1 on custom Sensititre panels (Thermo Fisher Scientific) using EUCAST broth for fastidious organisms, MH-F broth. Disk diffusion was performed according to EUCAST (MH-F media) using agar from BD (BBL) and Thermo Fisher Scientific (Oxoid). MIC-zone diameter correlations were evaluated vs. EUCAST Breakpoint Tables v 8.1, 2018.

Results: The following beta-lactam resistance mechanisms were identified among the tested isolates: No beta-lactam resistance (n=51), beta-lactamase only (n=23), PBP3 mutations only (n=55) and both beta-lactamase and PBP3 mutations (n=9). The benzylpenicillin screening disk correctly identified 86/87 isolates with beta-lactam resistance as screen positive. Screen-negative isolates were susceptible for all beta-lactam agents with EUCAST breakpoints, with the exception of cefuroxime which had two minor errors. The MIC-zone diameter correlations for each agent were excellent for isolates without beta-lactam resistance and those with beta-lactamase only. For isolates with PBP3 mutations, there was significant overlap between susceptibility categories with disk diffusion, in particular for ampicillin (Figure 1), amoxicillin-clavulanic acid, cefepime, cefpodoxime, cefuroxime and imipenem.

Conclusions: EUCAST recommends screening for beta-lactam resistance in *H. influenzae* with the benzylpenicillin disk. Screen-negative isolates have no beta-lactam resistance and can be reported susceptible for all beta-lactam agents with clinical breakpoints. For screen-positive isolates and agents with poor separation between susceptibility categories, Areas of Technical Uncertainty (ATU) have been suggested and approved for the EUCAST Breakpoint Tables v 9.0 (2019). This results in more reliable AST reports for *H. influenzae* versus beta-lactam agents.

Figure 1. Inhibition zone diameter distributions for *H. influenzae* and ampicillin 2 µg vs. ampicillin MIC presented per β-lactam resistance mechanism.
 EUCAST breakpoints (v 8.1, 2018) are shown as a dotted line.

