Beta-lactam susceptibility in Haemophilus influenzae: the connection between genotype and phenotype

Vladimira Hunic‡, Josiane Reist†, Urs Schibli‡, Janina Linnik§, Adrian Egli†

†University Hospital of Basel; Clinical Microbiology
‡Bakt. Inst. Olten AG
§Division of Clinical Microbiology, University Hospital Basel

Background: Current EUCAST breakpoints for aminopenicillins in H. influenzae often lead to false susceptible results using disk diffusion and Etest. Furthermore, the screening algorithm of EUCAST does not separate between aminopenicillin susceptibility groups (BLNAS, BLNAR, BLPAR, and BLPACR). We evaluated different screening discs to improve categorization of aminopenicillin susceptibilities, and aimed to predict the impact of genetic polymorphisms on beta-lactam binding based on the PBP3 crystal structure.

Material/methods: 51 clinical isolates of H. influenzae were tested. We determined beta-lactamase production with BBL™ Cefinase™ Discs (Beckton Dickinson) and MICs for ampicillin, amoxicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam and cefuroxime with Etests (bioMérieux). Screening discs used were: penicillin 1U and 10U, ampicillin 2µg, amoxicillin 2µg, amoxicillin/clavulanic acid 3µg (2:1), cefuroxim 5µg and 30µg. Strains were genotypically characterized by TEM-1 PCR and key polymorphisms in the ftsI gene as previously described. This allowed a gold standard categorization into gBLNAS, gBLNAR, gBLPAR, and gBLPACR. The impact of polymorphisms on the beta-lactam binding site were predicted based on PBP3 crystal structures (PDB ID Code 3pbr).

Results: Based on the PBP3 amino-acid sequence, the investigated strains were classified into groups: wild type (n=16), I (n=2), II (a, b and d; n=20) or M (miscellaneous, n=13). No isolates from group II c or III were found. All strains in the M group show lower MICs to all antimicrobials compared to groups I and II (except beta-lactamase producing isolates). Interestingly, all strains belonging to group I, II and M showed a very low MIC for piperacillin/tazobactam (<0.016-0.125 mg/l). Highest sensitivity and specificity for phenotypic resistance classification was achieved with combination of penicillin 1U, cefuroxime 30µg and cefuroxime 5µg: gBLNAS 100% and 93.9%, gBLNAR 90.5% and...
100%, gBLPAR 90.9% and 100%, gBLPACR 100% and 98.0%, respectively. 81% of gBLNAR strains were categorized as amoxicillin susceptible and 9.5% as ampicillin susceptible. All gBLNAR (n=21) isolates were categorized as amoxicillin/clavulanic acid susceptible. Of 51 isolates tested, 12 (24%) were positive with Cefinase™ test and TEM-1 PCR. PBP3 alterations in the crystal structure indicated that mutations of the M group were localized in distance to the beta-lactam binding site, whereas the localization of the group I and II alterations were closer to the binding site.

**Conclusions:** The new three disc screening algorithm shows high sensitivity and specificity for the classification of *H. influenzae* strains into four aminopenicillin susceptibility groups. Not all PBP3 alterations corresponded to a resistant phenotype. Based on our findings, we assumed strains belonging to M group as phenotypically equal to wild type as interaction with the beta-lactamase binding site seemed less likely. A revision of EUCAST breakpoints is needed because genotypic BLNAR strains (belonging to group I and II) are categorized as susceptible for amoxicillin, ampicillin and amoxicillin/clavulanic acid.