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Haemophilus influenzae with Non-Beta-Lactamase-Mediated Beta-Lactam Resistance: Easy To Find but Hard To Categorize

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INTRODUCTION

Beta-lactam resistance in *Haemophilus influenzae* is mediated through:

1. **Beta-lactamase production** (*bla*$_{TEM}$ or *bla*$_{ROB}$)
2. **Substitutions in penicillin-binding protein (PBP3)** (encoded by the *ftsI* gene):
   a) low-level PBP3-mediated resistance (low-rPBP3)-> mainly aminopenicillins affected
   b) high-level PBP3-mediated resistance (high-rPBP3)-> these strains express higher resistance to extended-spectrum cephalosporins

Susceptibility testing of *H. influenzae* has been characterized as «a tricky business»:

- the rPBP3 population overlaps the wild-type population in terms of MICs : *bla*-negative rPBP3 strains have ampicillin MICs of 0.58 to 16 mg/L, whereas the epidemiological cutoff (ECOFF) value is <=1 mg/liter
- the clinical relevance of current breakpoints is debated
- CLSI and EUCAST both use ECOFF to define susceptibility and divide the rPBP3 population by categorizing strains with ampicillin MICs of >1 mg/L (EUCAST) or >2 mg/L (CLSI) as resistant irrespective of dosage
- In addition, susceptibility testing is associated with uncertainty due to technical and biological variation (another testing method, differet test media etc.)

MATERIALS AND METHODS

Bacterial isolates: 154 *bla*-negative *H. influenzae* isolates-> 50 (32%) sPBP3 isolates with no resistance mutations and 104 (68%) low-rPBP3 isolates.

Gradient MIC: Ampicillin, amoxicillin and cefuroxime MICs were determined by Etest.

Disk Diffusion: Susceptibility categorization: disk diffusion was perfomed according to EUCAST recommendations using standard disks (AMP2 and CXM30). Screening for rPBP3 genotype: nine other discs were evaluated for their ability to identify isolates with the rPBP3 genotype.

Broth Micodilution (BMD): used as a gold standard.

RESULTS

Etest generally overestimated MICs at lower ranges and underestimated MIC at higher ranges, and agreement rates were low for all three agents.

By Etest, both essential and categorical agreement were generally poor (<70%), with high very major errors (VME) (CLSI, 13.0%; EUCAST, 34.3%) and falsely susceptible rates (FSR) (CLSI, 87.0%; EUCAST, 88.3%) for ampicillin.

Ampicillin (2 µg) with adjusted (+/-2 mm) zone breakpoints was superior to Etest for categorization of susceptibility to ampicillin (agreement, 74.0%; VME, 11.0%; FSR, 28.3%).

Conversely, Etest was superior to 30 µg cefuroxime for categorization of susceptibility to cefuroxime (agreement, 57.1% versus 60.4%; VME, 2.6% versus 9.7%; FSR, 7.1% versus 26.8%).

Benzylpenicillin (1 unit) (EUCAST screening disk) and cefuroxime (5 µg) identified rPBP3 isolates with highest accuracies (95.5% and 92.2%, respectively).

DISCUSSION

- In recent years, altered PBP3 has surpassed beta-lactamase as the most frequent beta-lactam resistance mechanism in *H. influenzae.*

- Commonly used acceptance criteria are 90% essential agreement for MIC determination and 1.5% VME and 3% ME for susceptibility categorization. These acceptance criteria were not fulfilled neither for Etest nor for disk diffusion!

- A change in ampicillin MIC breakpoints to avoid division of the rPBP3 population would improve categorical agreement with BMD MIC for both disc diffusion and Etest.

- Further investigations are needed to decide wheather a change in breakpoints is advisable- > a possibility woud be to define an intermediate category encompassing rPBP3 *bla*-negative isolates with ampicillin MICs up to 8 mg/L (this is supported by PK/PD calculations) but clinical data are needed.

- The authors suggest that *H. influenzae* isolates that are rPBP3 positive by screening be categorized as cefuroxime resistant and always be reported as ampicillin resistant in cases of meningitis.

- In addition, to minimize the clinical consequences of falsly susceptible results, the authors suggest adding a comment recommending high-dose aminopenicillin therapy or the use of other agents in severe infections caused by screening positive isolates categorized as suscepible to aminopenicillins by disk or gradient diffusion.

Weitere Fragen und Überlegungen

- Warum hat EUCAST trotz diesen besorgniserregenden Daten noch nichts an den Guidelines für *Haemophilus* verändert?

- In dieser Studie wurden nur Ampicillin, Amoxicillin und Cefuroxim untersucht. Wie ist die Empfindlichkeit auf andere Beta-Laktame (z.B. Piperacillin/Tazobactam) bei rPBP3-Stämmen?

- In dieser Studie hat PG1 Disk eine gute Sensitivität und Spezifität für Nachweis von rPBP3 genotype bei *bla*-negativen Stämmen gezeigt. Was machen wir mit *bla*-positiven Stämmen? Sollte man dafür noch eine zweite Disk für Screening benutzen (wie z.B. CXM5)?

- Ist die gleiche Problematik auch bei anderen *Haemophilus* Spezies vorhanden?

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