



Screening for β -lactam resistance in *H. influenzae*

Erika Matuschek

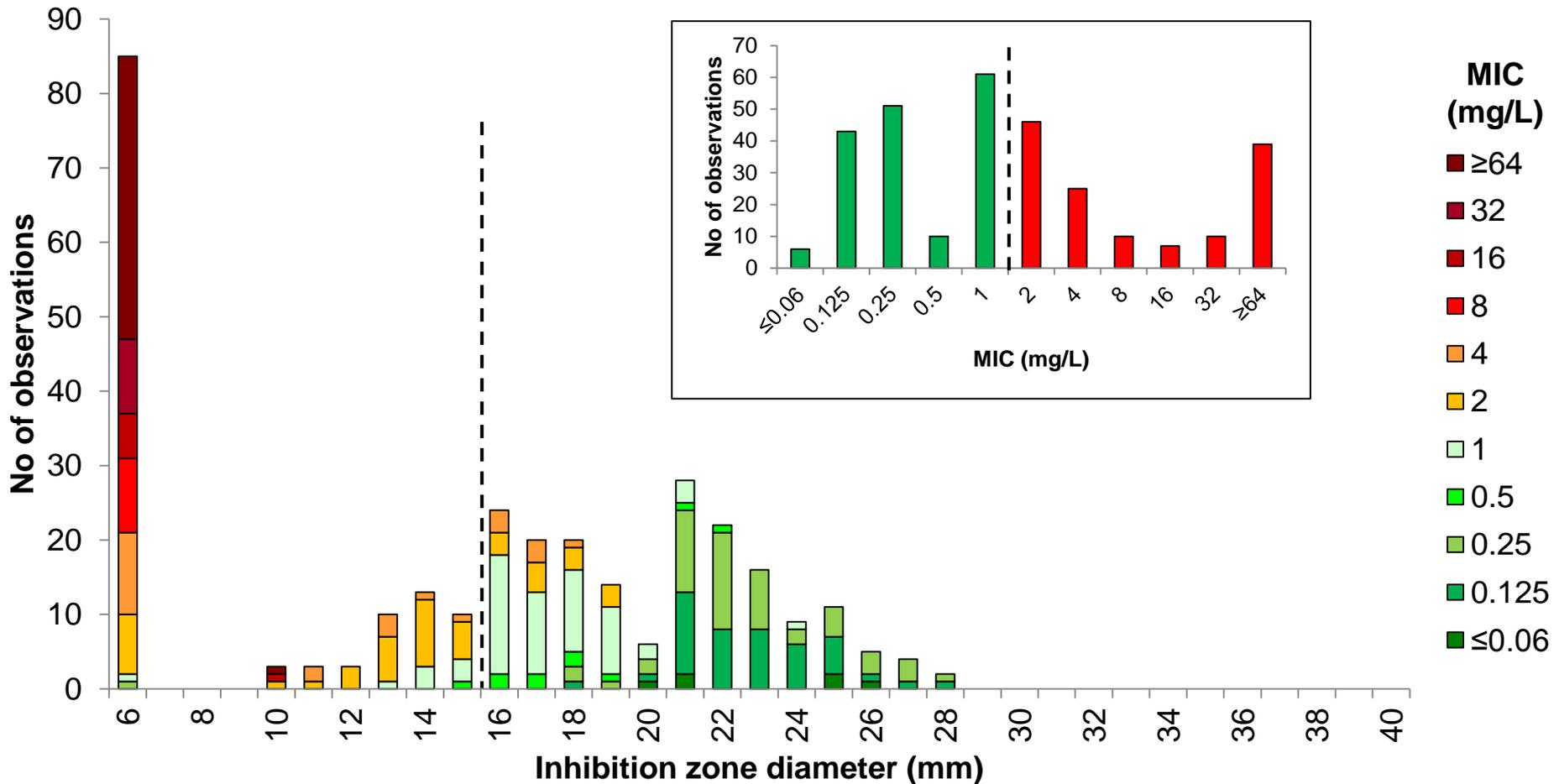
EUCAST Development Laboratory
(EDL)

β -lactam resistance in *H. influenzae*

- β -lactam resistance mechanisms:
 - β -lactamase
 - Mutations in penicillin-binding protein 3 (PBP3)
- Clinical breakpoints for aminopenicillins (ampicillin, amoxicillin and amoxicillin-clavulanic acid) intersect the PBP3 positive population.
 - Varying results with repeated MIC testing.
 - Poor separation between susceptible and low-level resistant isolates with disk diffusion.

Ampicillin 2 μ g vs. MIC

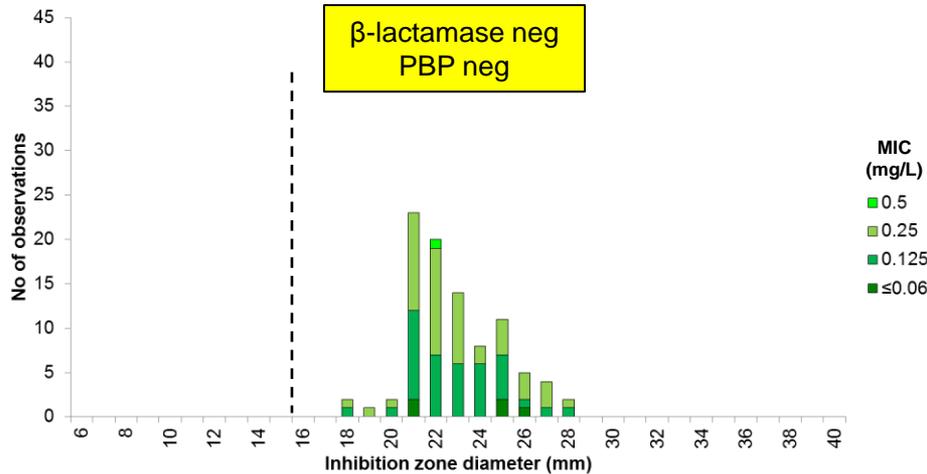
H. influenzae, 166 isolates (305 correlates)



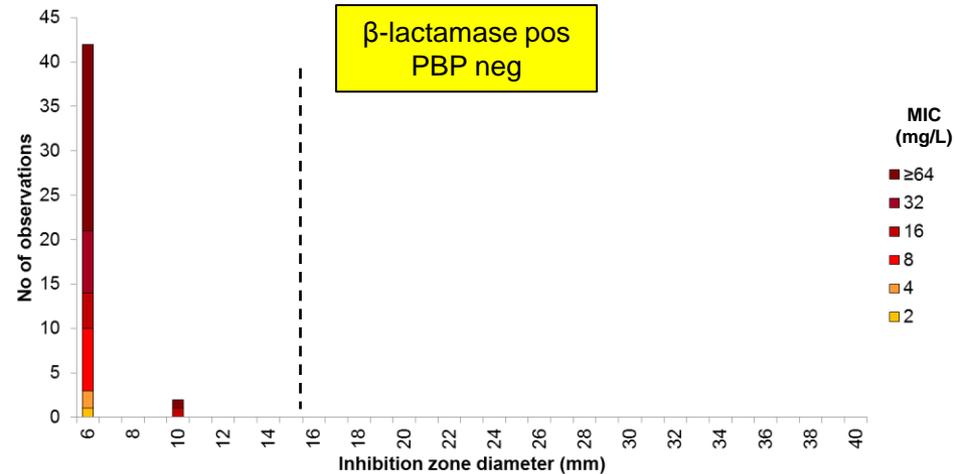
Breakpoints		ECOFF
MIC	$S \leq 1, R > 1$ mg/L	1 mg/L
Zone diameter	$S \geq 16, R < 16$ mm	

Ampicillin vs. resistance mechanism

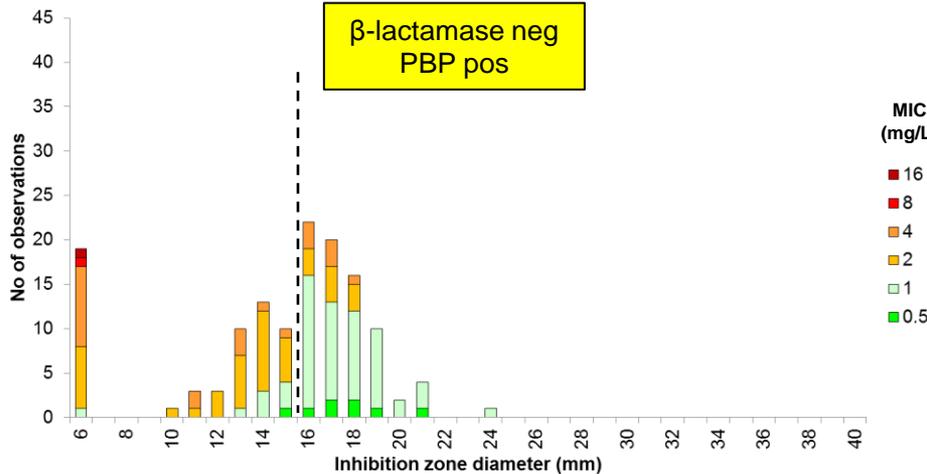
Ampicillin 2 µg vs. MIC
H. influenzae, 51 isolates (92 correlates)



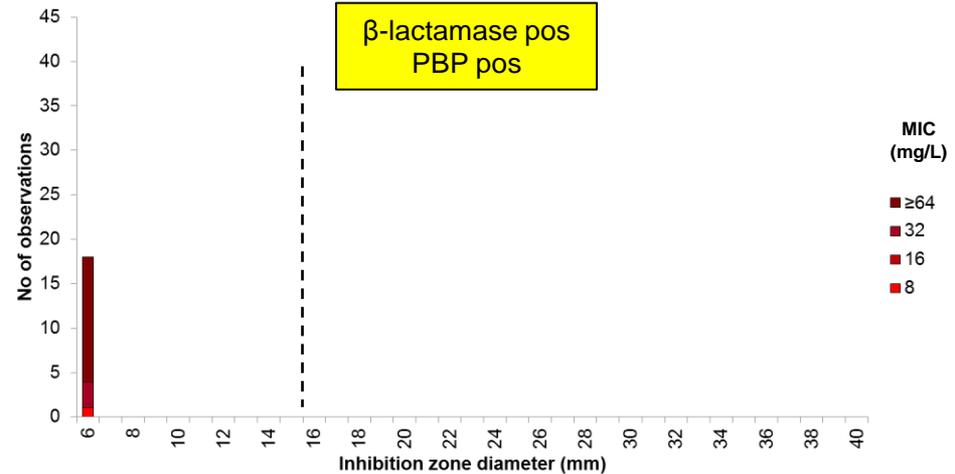
Ampicillin 2 µg vs. MIC
H. influenzae, 23 isolates (44 correlates)



Ampicillin 2 µg vs. MIC
H. influenzae, 68 isolates (134 correlates)

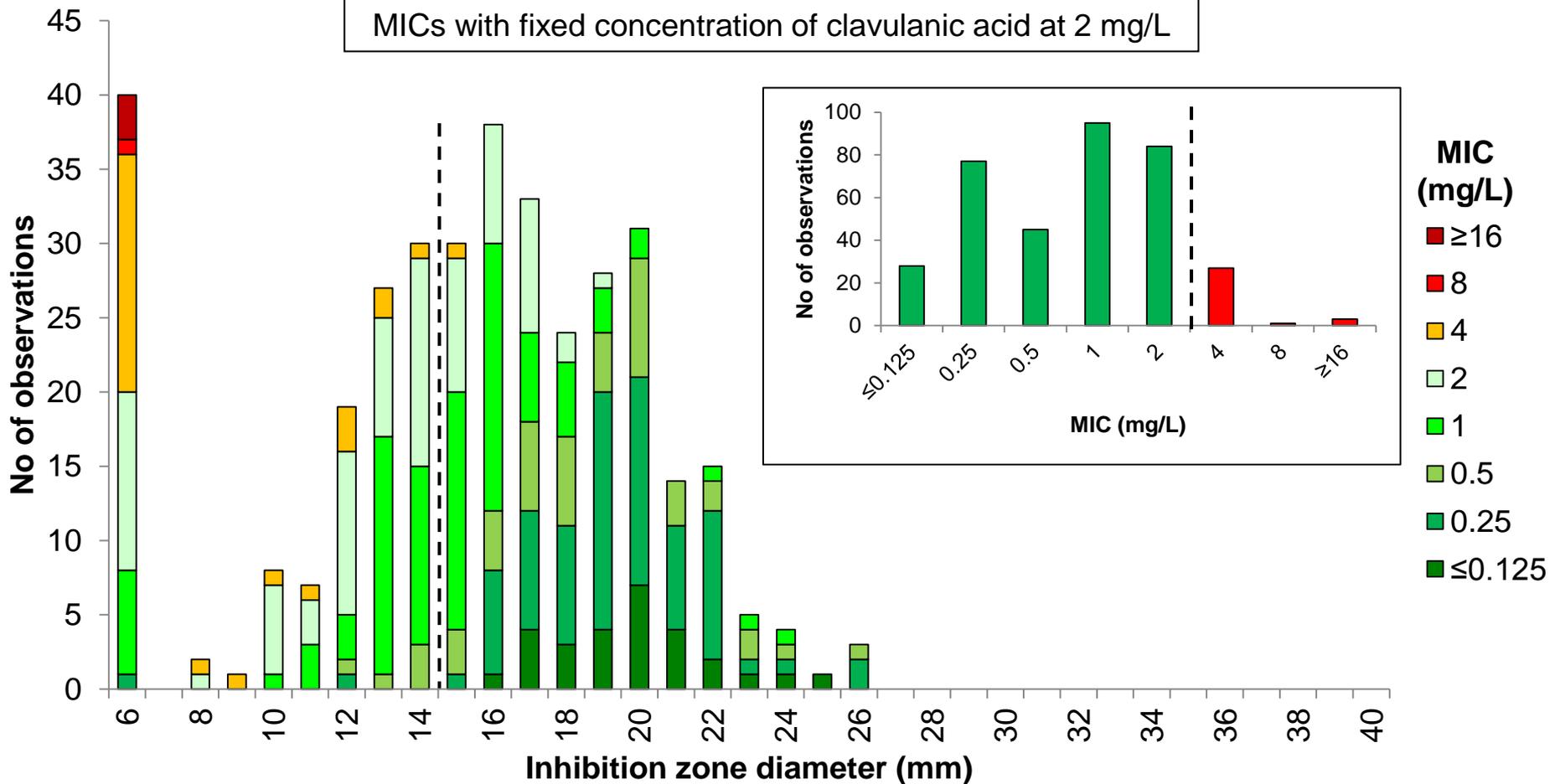


Ampicillin 2 µg vs. MIC
H. influenzae, 9 isolates (18 correlates)



Breakpoints	ECOFF
MIC	S≤1, R>1 mg/L
Zone diameter	S≥16, R<16 mm

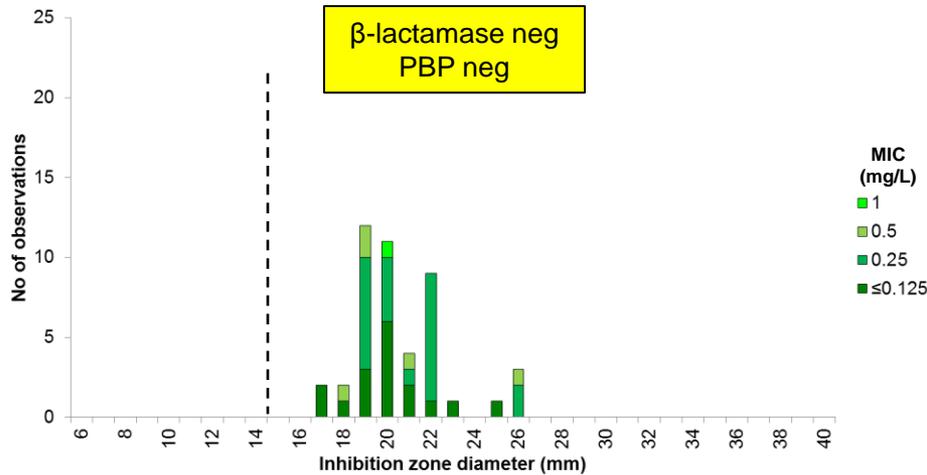
Amoxicillin-clavulanic acid 2-1 µg vs. MIC *H. influenzae*, 167 isolates (360 correlates)



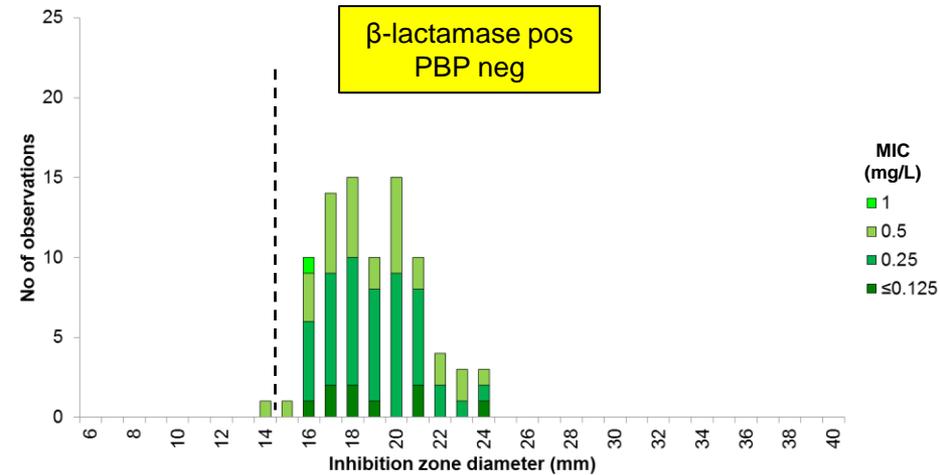
Breakpoints		ECOFF
MIC	S ≤ 2, R > 2 mg/L	2 mg/L
Zone diameter	S ≥ 15, R < 15 mm	

Amoxicillin-clav vs. resistance mechanism

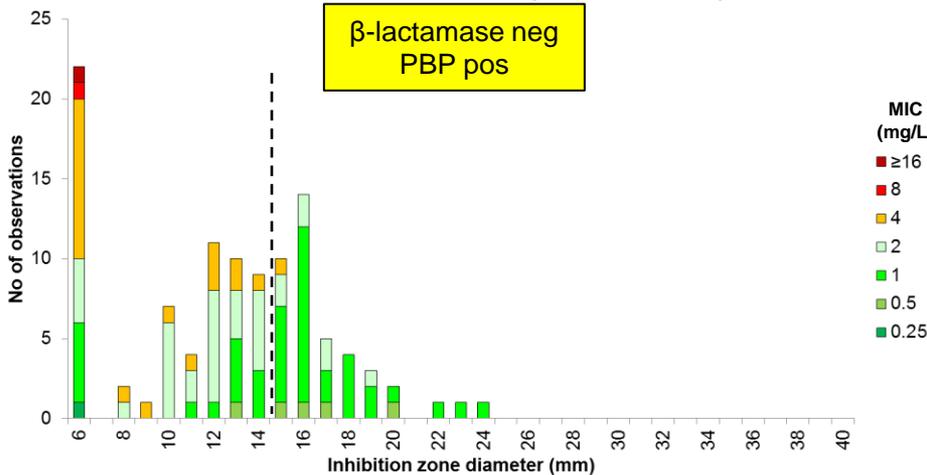
Amoxicillin-clavulanic acid 2-1 µg vs. MIC
H. influenzae, 45 isolates



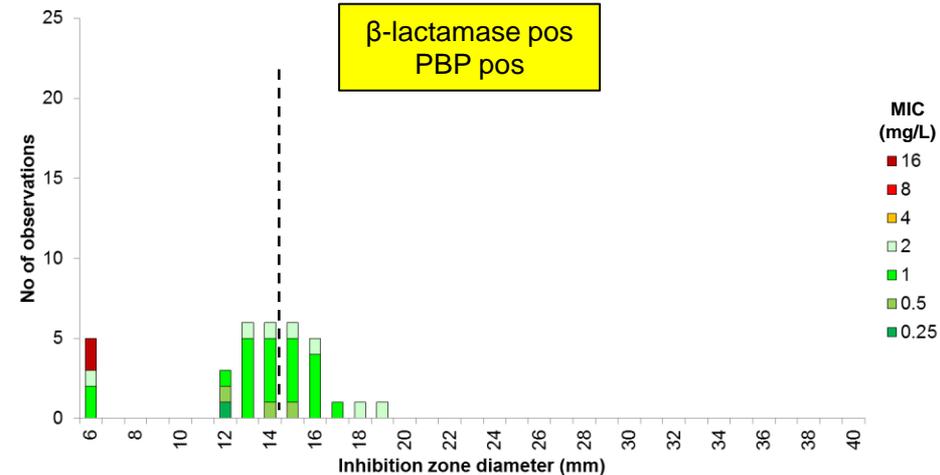
Amoxicillin-clavulanic acid 2-1 µg vs. MIC
H. influenzae, 23 isolates (86 correlates)



Amoxicillin-clavulanic acid 2-1 µg vs. MIC
H. influenzae, 66 isolates (107 correlates)



Amoxicillin-clavulanic acid 2-1 µg vs. MIC
H. influenzae, 9 isolates (34 correlates)

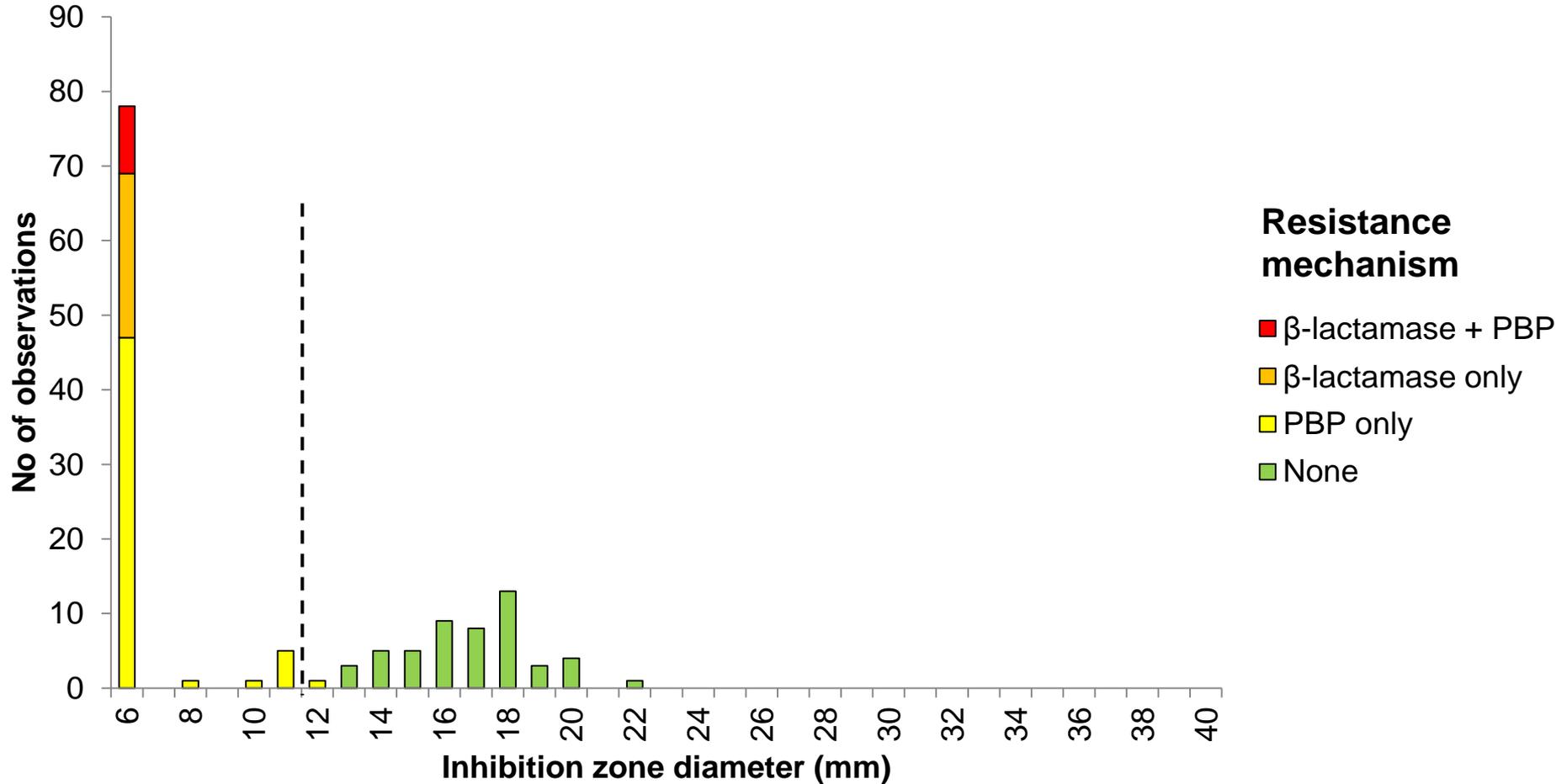


Breakpoints	ECOFF
MIC	2 mg/L
Zone diameter	S ≤ 2, R > 2 mg/L S ≥ 15, R < 15 mm

β -lactam resistance in *H. influenzae*

- The benzylpenicillin 1 unit disk detects all β -lactam resistance mechanisms, but cannot differentiate between β -lactamase and PBP3 mutations.
 - Cefaclor 30 μ g added to NordicAST screening algorithm
 - Difficult to read (fuzzy zone edges)
 - No clinical breakpoints (“dash”)
 - Cefuroxime 30 μ g can be used instead of cefaclor 30 μ g, but this disk is also difficult to read

Benzylpenicillin 1 unit vs. β -lactam resistance mechanism *H. influenzae*, 137 clinical isolates



Improved screening algorithm

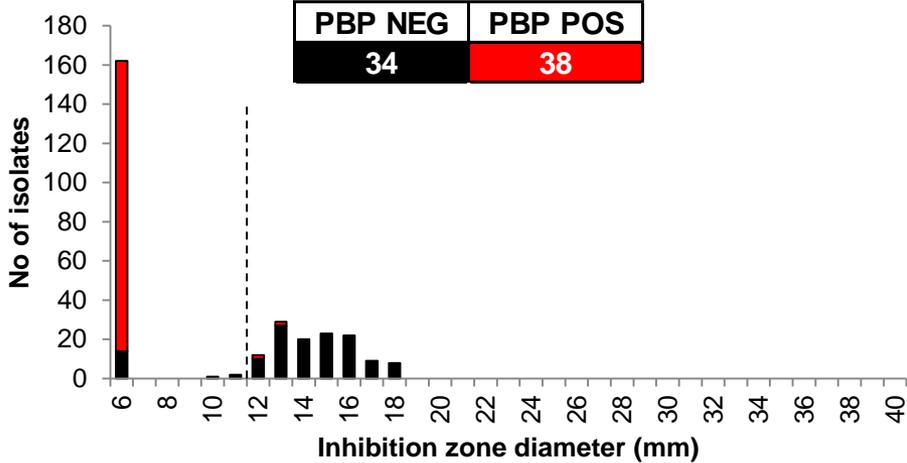
- Evaluation of benzylpenicillin 1 unit + clavulanic acid (5 or 10 μg) disks to distinguish between β -lactam resistance mechanisms in *H. influenzae*.
- Two isolate collections with known status of β -lactamase (nitrocefin-based test) and PBP3 mutations (sequenced by Thegerström and Resman, Lund, Sweden).

Study layout

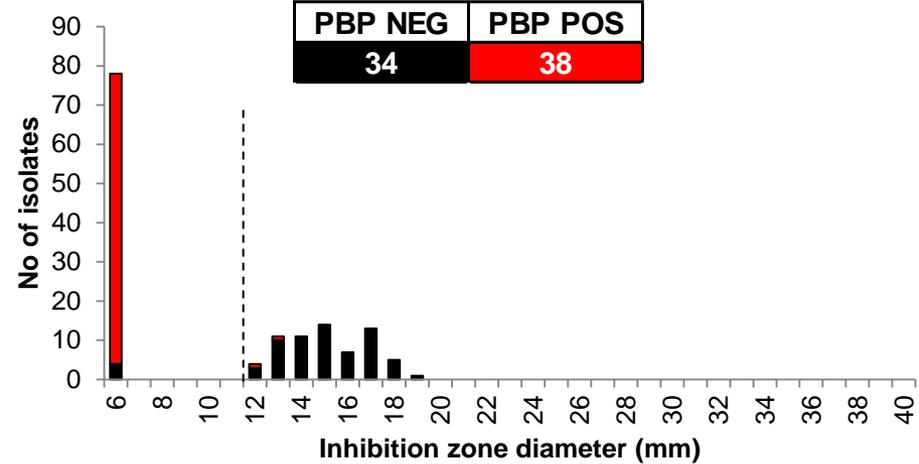
- Isolate collections:
 1. **LUND COLLECTION.** Isolates selected to represent different resistance mechanisms (n=99) (Thegerström and Resman, Lund, Sweden)
 - 72 beta-lactamase positive isolates of which 38 with PBP3 mutations
 2. **MULTI-LAB.** Isolates from the EUCAST multi-lab study (n=137)
 - 12 European laboratories, local isolates
 - 40 beta-lactamase positive isolates of which 10 with PBP3 mutations
- Disk diffusion at EDL
 - PCG 1 unit and PCG-clav (5 and 10 µg clavulanic acid) disks from two manufacturers
 - MH-F plates (Oxoid and BBL MH agar tested in parallel)

Lund collection, 72 isolates, all β -lactamase positive

PCG-clav 1 unit-5 μ g

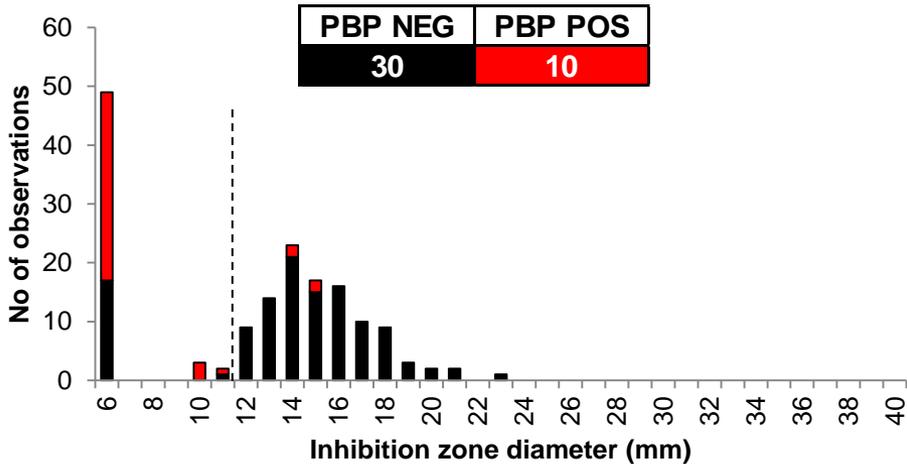


PCG-clav 1 unit-10 μ g

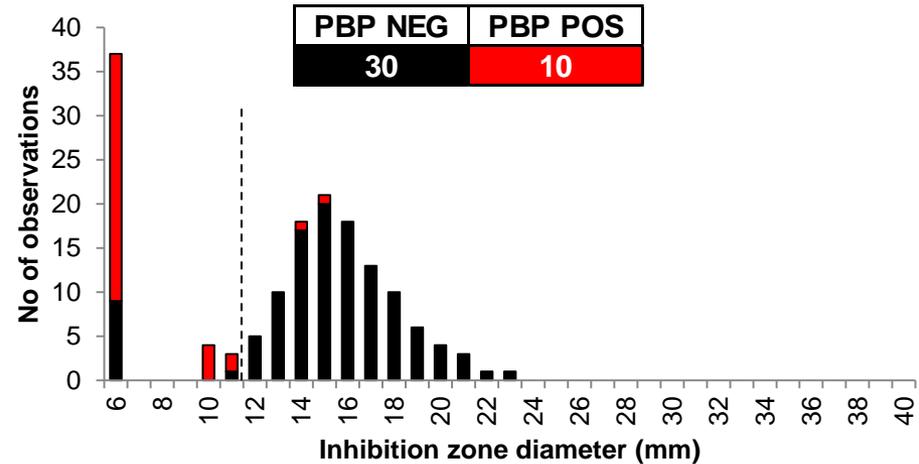


Multi-laboratory study, 40 isolates, all β -lactamase positive

PCG-clav 1 unit-5 μ g



PCG-clav 1 unit-10 μ g



Conclusions

- Combined screening with PCG 1 unit and PCG-clavulanic acid 1 unit-10 µg will improve the screening algorithm for β -lactam resistance in *H. influenzae*.
 - Exchange cefaclor for PCG-clav 1 unit-10 µg in NordicAST algorithm.
 - To be further evaluated in a multi-laboratory study and possibly included in EUCAST BP table v 9.0 (2019).
- The problem with overlap between susceptible and low-level resistant *H. influenzae* for aminopenicillins remains and will be further investigated.

**Disk diffusion test with
benzylpenicillin 1 unit disk and
benzylpenicillin 1 unit + 10 µg clavulanic acid**

