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EMERGENCE OF AN HAEMOPHILUS PARAINFLUENZAE ISOLATE WITH DECREASED SUSCEPTIBILITY TO CEFOTAXIME PLUS FLUORQUINOLONES

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ABSTRACT

Background. *H. parainfluenzae* (Hpa) is commonly associated with respiratory and genitourinary tract infections. Decreased susceptibility (DS) or resistance to ampicillin is mediated by TEM ß-lactamase and/or mutations in PBP3 (*fstI* gen). To date, DS to cefotaxime associated with PBP3 mutations was described only in *H. influenzae* isolates from Japon and Spain. Recently, a TEM-15 extended-spectrum beta-lactamase was described in two Hpa isolates from South Africa. The first fluorquinolone-resistant Hpa was recently reported in Spain.

Aim. To describe the first Hpa isolate with DS to both cefotaxime and fluorquinolones.

Methods. Hpa M11065 was recovered from a 2-year-old cystic fibrosis patient. Disc diffusion and MIC were evaluated according to CLSI guidelines. ß-lactamase activity was evaluated by nitrocefin. PCR and DNA sequencing of *bla*TEM, *fstI* and quinolone-resistance determining region (QRDR) of *gyr*A and *par*C genes were performed. Ciprofloxacin MIC was evaluated with and without 12.5 mg/L of reserpine.

Results. Hpa M11065 was ß-lactamase positive and presented BLPACR phenotype (β-lactamase positive amoxicillin clavulanate resistant) with MIC (mg/L) of: ampicillin (256); amoxicillin-clavulanate (8), cefuroxime (8), cefotaxime (4), trimethoprim-sulfamethoxazole (0.06) and chloramphenicol (0.5). The isolate harboured *bla*TEM-1 and mutations in PBP3: Asn526Lys, Ser385Thr, Val511Ala, Ile519Val and Asp551Leu. M11065 showed no halo with nalidixic acid disc and a ciprofloxacin MIC of 0.5 mg/L. Substitutions in QRDR of both *gyr*A (Ser84Tyr) and *par*C (Ser84Phe) genes were detected. Addition of reserpine did not reduce the MIC of ciprofloxacin, discarding the contribution of efflux pumps.

Conclusions. To our knowledge this is the first description of Hpa showing DS to cefotaxime associated to PBP3 mutations and DS to fluorquinolones due to QRDR mutations.

AIM

To describe the first *Haemophilus parainfluenzae* isolate with decreased susceptibility to both cefotaxime and fluorquinolones.

Materials & Methods

Clinical isolate. *H. parainfluenzae* M11065 was recovered from sputum recovered from a 2-year-old cystic fibrosis patient. The isolate was submitted to National Reference Laboratory (INEI) for further characterization.

Susceptibility assays. Disc diffusion and MIC were evaluated according to CLSI guidelines.

Efflux pump inhibitory assay. Ciprofloxacin MIC was evaluated with and without 12.5 mg/L of reserpine.

ß-lactamase activity. Nitrocefin assay was used to evaluate ß-lactamase activity.

Molecular techniques. PCR and DNA sequencing of blaTEM, fstI and quinolone-resistance determining region (QRDR) of gyrA and parC genes were performed.

CONCLUSIONS

Results

BLPACR phenotype
(β-lactamase positive amoxicillin-clavulanate resistant)

Table 1. Antimicrobial susceptibility profile

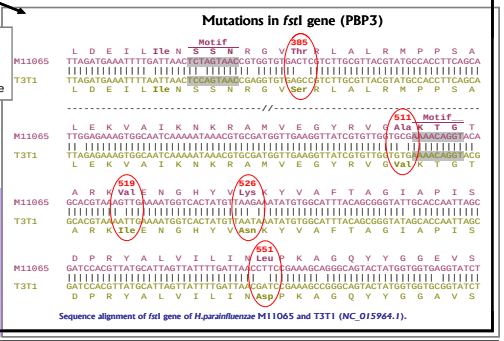
Antimicrobial	MIC in mg/L
Ampicillin	256 (R)
Amoxicillin-clavulanate	8 (R)
Cefuroxime	8 (I)
Cefotaxime	4 (NS)
TMS*	0.06 (S)
Chloramphenicol	0.5 (S)

R= Resistant; I= Intermediate; S= Susceptible;
NS= Non-susceptible; *TMS= trimethoprim-sulfamethoxazole

Nitrocefin assay → β-lactamase Positive → PCR → *bla*TEM β-lactamase → DNA sequencing → *bla*TEM-1 variant

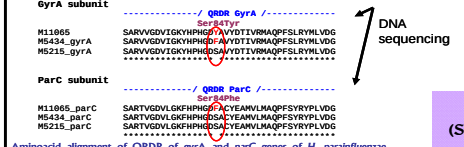
DNA sequencing → *fstI* gene (PBP3)

- Serine- 385→Threonine
- Valine- 511→Alanine
- Isoleucine- 519→Valine
- Asparagine- 526→Lysine
- Aspartic acid- 551→ Leucine



- *H. parainfluenzae* M11065 displayed BLPACR phenotype.
- High level of ampicillin resistance was due to bla_{TEM-1}.
- Decreased susceptibility to amoxicillin-clavulanate, cefuroxime and cefotaxime was associated with PBP3 mutations.
- As previously described in *H. influenzae*, the combination of Group II (Asp526Lys) and Group III (Ser385Thr) *fstI* mutations were related with decreased susceptibility to cefotaxime (MIC=4 mg/L).

Decreased susceptibility to Fluorquinolones



Aminoacid alignment of QRDR of *gyrA* and *parC* genes of *H. parainfluenzae* M11065 (DS-FQ), M5434 (DS-FQ) and M5215 (S-FQ).

- Ciprofloxacin MIC = 0.5 mg/L (Non susceptible)
- No halo was observed with nalidixic acid disc
- Inhibitory efflux pump assay: Addition of reserpine did not reduce the ciprofloxacin MIC, discarding the contribution of efflux pumps.

Substitutions in QRDR of both *gyr*A (Ser84Tyr) and *par*C (Ser84Phe) genes were associated with decreased susceptibility to fluorquinolones

1- *H. parainfluenzae* M11065 showed a BLPACR phenotype with decreased susceptibility to cefotaxime, due to the presence of at least two PBP3 mutations related to this phenotype (Ser385Thr, Asn526Lys).

2- *H. parainfluenzae* M11065 showed decreased susceptibility to ciprofloxacin associated with mutations in QRDRs of both *gyr*A (Ser84Tyr) and *par*C (Ser84Phe) genes.

3- To our knowledge, this is the first description of *H. parainfluenzae* with simultaneous decreased susceptibility to cefotaxime and ciprofloxacin.

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