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Emergence of *Haemophilus parainfluenzae* resistant to third-generation cephalosporins in Italy: potential role of PBP3 and PBP5 substitutions in high-level resistance

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To Jean-Marc Rolain,
Editor in Chief
International Journal of Antimicrobial Agents

September 2, 2020

Enclosed please find the revision (R3) of the manuscript entitled “*Emergence of Haemophilus parainfluenzae resistant to third-generation cephalosporins in Italy: potential role of PBP3 and PBP5 substitutions in high-level resistance*”, which I am re-submitting as a *Letter* to *International Journal of Antimicrobial Agents*.

The text has been revised, as required by the Section Editor. I am providing the files with the highlighted text, a clean copy and the point-by-point reply to Reviewers’ comments.

The revised manuscript has been seen and approved by all the authors. The material is original, unpublished, and has not been simultaneously submitted to another medical journal.

We hope that the information provided with this manuscript will be interesting to the audience of *International Journal of Antimicrobial Agents*, and that it will meet with the Editor’s approval. We look forward to hearing from you at your earlier convenience.

Yours sincerely,

Luigi Principe

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POINT BY POINT REPLY TO REVIEWERS' COMMENTS

Section Editor:

SPAdes, Canu, and ParSNP have been published in peer-reviewed journal and their authors should get credit for using the product of their research. As previously requested, please insert regular citations to published articles for all these bioinformatics tools, even if the number of citations then exceeds the usual limit for letters.

Reply: References have been added (#6, #7, #8), as required.

HIGHLIGHTS

- The spread of resistance traits in *H. parainfluenzae* is a matter of concern
- Here we describe the first case of resistance to 3-GCs in *Haemophilus* spp. in Italy
- Several AA substitutions in PBP3 and PBP5 were detected in 3-GCs-resistant isolate
- AA substitutions in PBP5 were not so far reported in 3-GCs-resistant *Haemophilus* spp.
- Empirical therapy without microbiological investigations could be harmful to patients

**Emergence of *Haemophilus parainfluenzae* resistant to third-generation cephalosporins in
Italy: potential role of PBP3 and PBP5 substitutions in high-level resistance**

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25 is well recognized, even though indistinctive clinical symptoms make difficult the differentiation of
26 urethritis due to *Haemophilus* spp. from other pathogens [1]. Of note, *H. parainfluenzae* represents
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28 antimicrobial treatment due to emergence of resistance traits. In particular, resistance to β -lactams
29 emerged from the diffusion of plasmid-mediated β -lactamases (TEM-1, ROB-1). Later, isolates
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32 with variable phenotypic resistance profiles depending on the specific amino acid (AA) substitution
33 patterns [2]. Resistance to carbapenems is considered exceptional [2-4], and so far, not reported in
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35 With regard to Italy, though surveillance data are scarce and not updated, resistance to 3-GCs has
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39 In 2018, a 45-years-old Italian male with purulent urethral discharge was examined for sexually
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75 *parainfluenzae* strain T3T1 (GenBank accession no. FQ312002) (i.e., Gly11Ala; Val34Met;
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131 **LEGEND TO THE FIGURES**

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Table 1: Antibiotic susceptibility profile of *Haemophilus parainfluenzae* (LC/1315.18)

Antibiotic	MIC values, mg/L (S, I, R)	
	Etest	Broth microdilution
Ampicillin		4 (R)
Amoxicillin/clavulanate	24 (R)	>2/2 (R)
Ceftriaxone	2 (R)	2 (R)
Cefotaxime	> 32 (R)	>2 (R)
Ciprofloxacin		0.016 (S)
Doxycycline		2 (I)
Levofloxacin		0.06 (S)
Meropenem	2 (S)	2 (S)
Penicillin		>1 (IE)
Tetracycline		1 (S)
Trimethoprim/sulfamethoxazole		≤0.12/2.37 (S)
Ertapenem		0.125 (S)
Imipenem	0.75 (S)	

Abbreviations: MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible; IE, insufficient evidence. Since no specific criteria are currently available by EUCAST, results were interpreted according to those recommended for *H. influenzae*.

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Supplementary data

Supplemental Figure 1.pdf

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Supplementary data
Supplemental Figure 2.pdf

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Supplementary data
Supplemental Figure 3.pptx

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