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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 The material is original, unpublished, and has not been simultaneously submitted to another medical journal.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

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

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23 *Sir,*

24 The potential role of *Haemophilus parainfluenzae* as a sexually transmitted genitourinary pathogen
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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29 emerged from the diffusion of plasmid-mediated β -lactamases (TEM-1, ROB-1). Later, isolates
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33 patterns [2]. Resistance to carbapenems is considered exceptional [2-4], and so far, not reported in
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75 *parainfluenzae* strain T3T1 (GenBank accession no. FQ312002) (i.e., Gly11Ala; Val34Met;
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83 We described the first 3-GC-resistant *H. parainfluenzae* strain from Italy. More importantly,
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131 **LEGEND TO THE FIGURES**

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133 **Supplemental Figure 1.** NCBI protein BLAST results of PBP3 (i.e., query *H. parainfluenzae*
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147 not available

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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*.

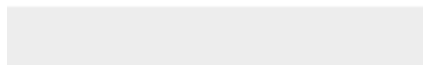


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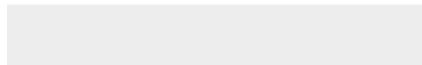


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