

1 **Letter to the Editor.**

2 **Lack of detection of *Klebsiella aerogenes* sub-species in lung infection by the BioFire®**  
3 **FilmArray® Pneumonia Panel plus.**

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5 Nathan NICOLAU-GUILLAUMET<sup>1</sup>, Laurent DORTET<sup>2,3,4</sup>, Aymeric JACQUEMIN<sup>2</sup>, Bruno MOURVILLIER<sup>5</sup>,  
6 Anaëlle MUGGEO<sup>1</sup> and Thomas GUILLARD<sup>1,\*</sup>

7  
8 <sup>1</sup>Université de Reims Champagne-Ardenne, INSERM, CHU de Reims, Laboratoire de bactériologie-Virologie-  
9 Hygiène hospitalière-Parasitologie-Mycologie, P3Cell, U 1250, Reims, France

10 <sup>2</sup>Team "Resist", INSERM Unit 1184, Faculty of Medicine, Université Paris-Saclay, Service de Bactériologie-  
11 Hygiène, Hôpital Bicêtre, 78 rue du Général Leclerc, 94275, Le Kremlin-Bicêtre, France.

12 <sup>3</sup> Bacteriology-Hygiene Unit, Assistance Publique-Hôpitaux de Paris, AP-HP Paris-Saclay, Bicêtre Hospital, Le  
13 Kremlin-Bicêtre, France.

14 <sup>4</sup> Associated French National Reference Center for Antibiotic Resistance, Carbapenemase-Producing  
15 Enterobacterales, Bicêtre Hospital, Le Kremlin-Bicêtre, France.

16 <sup>5</sup> Medical Intensive Care Unit, University hospital of Reims, Université de Reims Champagne-Ardenne, EA-4684  
17 CardioVir, France.

18  
19 **\*To whom correspondence should be addressed: Thomas Guillard**

20 Inserm UMR-S 1250 P3Cell  
21 45, rue Cognacq-Jay 51092 Reims cedex, France  
22 Fax: +33326784134.  
23 Phone: +33326787702.  
24 Fax: +33326784134.  
25 E-mail: [tguillard@chu-reims.fr](mailto:tguillard@chu-reims.fr)

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29 Number of figures: 1 + S1

30 Supplementary Tables : S1 and S2

31 Word count of the manuscript: 956/800

32 Number of references: 4/5

33 To the Editor,

34 *Klebsiella aerogenes* is involved in hospital-acquired pneumonia and ventilator-associated  
35 pneumonia. BioFire® FilmArray® Pneumonia Panel plus (FAPP) is an effective tool to rapidly detect the  
36 presence of *K. aerogenes* with excellent sensitivity and specificity [1,2] but some cases have reported  
37 unexplained lacks of detection. We were facing a FAPP on a Broncho Alveolar Lavage detecting  
38 Rhinovirus/Enterovirus but no bacteria (negative or under the limit of detection of  $10^{3.5}$  copy/mL) in a  
39 patient intubated and ventilated after 27 days of hospitalization in the intensive care unit (ICU). Based  
40 on this result and our previous experience of the FAPP in our laboratory [3], the physicians decided  
41 not to treat according to the FAPP result. Eighteen hours later, the positive  $10^5$  CFU/ml *K. aerogenes*  
42 monomicrobial culture allowed to start a treatment with cefepime but delayed for almost one-day. To  
43 gain insight, a short-read Whole Genome Sequencing (WGS) was performed on the 187 *K. aerogenes*  
44 strains with decreased susceptibility to carbapenems recovered from both screening and clinical  
45 specimens sent to the French National Reference Center (F-NRC) for antimicrobial resistance. Among  
46 these 187 strains, FAPP cassettes were inoculated with  $10^5$  CFU/ml of 16 isolates representative of  
47 the global genetic diversity of *K. aerogenes*. If the FAPP did not detect the presence of *K. aerogenes*, a  
48  $10^8$  CFU/ml solution was tested.

49 Phylogenetic analysis of our collection of 187 isolates allowed us to identify four clades of *K.*  
50 *aerogenes*, named *K. aerogenes stricto sensu*, *K. aerogenes-like 1*, *K. aerogenes-like 2* and  
51 *K. aerogenes-like 3* (Figure 1). These four clades possessed more than 95% Average Nucleotide  
52 Identity (Table S1) confirming that they all belonged to the same species and could be considered as  
53 distinct subspecies. Overall, 81% (n=151) belonged to *K. aerogenes stricto sensu* species, 11% (n=21)  
54 to *K. aerogenes-like 1*, 5% (n=9) to *K. aerogenes-like 2* and 3% (n=6) to *K. aerogenes-like 3*. We  
55 selected 16 isolates representing the diversity of the *K. aerogenes* epidemiology (Figure 1); FAPP  
56 detected and correctly quantified all *K. aerogenes stricto sensu* (8/8) and *K. aerogenes-like 3* (3/3) at  
57 low inoculum ( $10^5$  CFU/ml). Conversely, FAPP did not detect low inoculums of all *K. aerogenes-like 1*

58 (3/3) and *K. aerogenes-like 2* (3/3) (Figure 1). Interestingly, the high inoculum ( $10^8$  CFU/ml) of these  
59 two subspecies were unexpectedly detected but under-quantified, at  $10^5$  CFU/ml instead of  $10^8$   
60 CFU/ml.

61 The discrepancies observed regarding the detection and the quantification of *K. aerogenes stricto*  
62 *sensu* / *K. aerogenes-like 3* compared to *K. aerogenes-like 1* / *-like 2* solutions may suggest that the  
63 PCR primers were specific to genes present only in *K. aerogenes stricto sensu* and *K. aerogenes-like 3*  
64 species or significant polymorphism that alters the binding efficiency of the primers.

65 Of note, the WGS comparison of the *K. aerogenes* subspecies identified 96 genes present in *K.*  
66 *aerogenes stricto sensu* and *K. aerogenes-like 3* strains but absent in *K. aerogenes-like 1* and *-like 2*.  
67 Since the FAPP runs are not fully accessible for users, our run related to the clinical case described  
68 here was analysed by bioMérieux/BioFire® who evidenced late cycle thresholds ( $C_t$ ). Despite no  
69 further explanation being provided, it was in agreement with a poor/bad annealing of the primers to  
70 the DNA, leading to a poor amplification. In addition, the detection but false under-quantification of  
71 the high inoculums of *K. aerogenes-like 1* and *-like 2* despite of quantifications usually precise for *K.*  
72 *aerogenes* according to the literature [1], reinforced our hypothesis of primers annealing to variable  
73 DNA sequences between the *K. aerogenes* subspecies.

74 In our collection, *K. aerogenes-like 1* and *K. aerogenes -like 2* subspecies represented 16% (n=30) of  
75 the whole *K. aerogenes* addressed to the F-NRC. It suggested that up to 16% of *K. aerogenes*  
76 respiratory infections could be not diagnosed by FAPP according to the clonal populations circulating  
77 in the different hospitals or areas of interest. Even if the 187 fully sequenced strains were multidrug  
78 resistant strains, which do not represent the entire epidemiology of *K. aerogenes*, our collection  
79 homogenously covered 9.4% (67/708) of all the *K. aerogenes* STs reported in the pubMLST  
80 (<https://pubmlst.org/organisms/klebsiella-aerogenes>) the October 10<sup>th</sup>,2023, (Figure S1) including  
81 the most prevalent clonal complexes. In addition, extended spectrum beta-lactamase or  
82 carbapenemase-producing *K. aerogenes* isolates are increasingly described. It turns out that our

83 results are therefore not so far from the *K. aerogenes* epidemiology of medical interest. We set out  
84 to determine whether this result was comparable to the diagnoses we had made in our laboratory at  
85 the Reims University Hospital since we used the FAPP. Over 3 years from January 2020 to December  
86 2022, we analysed 1786 BAL from 646 patients in ICU, and 824 FAPP were performed. Among the  
87 BAL (Table S2), 43 were positive for *K. aerogenes*: 46.5% (n=20) were detected by FAPP and isolated  
88 within culture, 28% (n=12) were only detected by FAPP, 7% (n=3) were found on culture but without  
89 FAPP detection and 18,5% (n=8) were found on culture with no FAPP performed. Eventually, of the  
90 35 FAPP with a diagnosis of *K. aerogenes* respiratory infections, 9% (n=3) were negative and  
91 corresponded to *K. aerogenes-like 1* and *-like 2* sub-species, which is not far from the 16% of F-NRC  
92 strains, considering our limited patient sample.

93 Shortening treatment delay should be considered essential to reduce respiratory infections mortality.  
94 However, the under-detection by the FAPP of respiratory infections caused by *K. aerogenes*  
95 subspecies could lead to a delay in the implementation of the antibiotherapy and to an increased  
96 mortality, all the more because of the *K. aerogenes* association with poor clinical outcomes [4].

97 Collectively, these results are likely to encourage BioFire® to adjust their primers or PCR assay for  
98 better detection of all *K. aerogenes* subspecies. However, as we proposed previously for non-targeted  
99 pathogens [3], the culture at 48h should be taken into account when considering the antimicrobial  
100 therapy.

101 **Author contribution**

102 Conceptualization, N.N-G., L.D. and T.G.; methodology, N.N-G., L.D. and T.G.; validation, N.N-G., L.D.  
103 A.M., B.M. and T.G; writing original draft preparation, N.N-G., L.D. and T.G.; writing review and  
104 editing, N.N-G., L.D. A.M., B.M. and T.G; All authors have read and agreed to the published version of  
105 the manuscript.

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107 **Transparency declaration**

108 No author has any conflict of interest in relation to this publication.

109

110 **Funding**

111 No external funding was received

112

113 **Statement on research ethics**

114 This study describes bacterial strains and therefore does not require patients' informed consent.

115

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132 **Figure 1.**

133 **Detection of *Klebsiella aerogenes* strains by BioFire® FilmArray® Pneumonia Panel plus according to**  
134 **their phylogeny.**

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136 **Figure S1.**

137 **Phylogenetic relationship of *K. aerogenes* sequence types.** Representation of the phylogenetic  
138 relationship of *K. aerogenes* sequence types (STs) (n=708) reported in the pubMLST  
139 (<https://pubmlst.org/organisms/klebsiella-aerogenes>) the October 10th,2023. The main clonal  
140 complex (CC1 to CC8) of *K. aerogenes stricto sensu* are surrounded by a blue dotted line.