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► To cite this version:

Nathan Nicolau-Guillaumet, Laurent Dortet, Aymeric Jacquemin, Bruno Mourvillier, Anaëlle Muggeo, et al.. Lack of detection of *Klebsiella aerogenes* sub-species in lung infection by the BioFire® FilmArray® Pneumonia Panel plus. *Clinical Microbiology and Infection*, 2024, 10.1016/j.cmi.2024.02.008 . hal-04491624

HAL Id: hal-04491624

<https://hal.univ-reims.fr/hal-04491624>

Submitted on 13 Mar 2024

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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.**

4
5 Nathan NICOLAU-GUILLAUMET¹, Laurent DORTET^{2,3,4}, Aymeric JACQUEMIN², Bruno MOURVILLIER⁵,
6 Anaëlle MUGGEO¹ and Thomas GUILLARD^{1,*}

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

10 ²Team "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 ³ Bacteriology-Hygiene Unit, Assistance Publique-Hôpitaux de Paris, AP-HP Paris-Saclay, Bicêtre Hospital, Le
13 Kremlin-Bicêtre, France.

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

16 ⁵ 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

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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 10th,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.

106

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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- 131

132 **Figure 1.**

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

135

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.