# Lack of detection of Klebsiella aerogenes sub-species in lung infection by the BioFire® FilmArray® Pneumonia Panel plus

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**Letter to the Editor.**

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

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To the Editor,

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

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

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

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

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

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

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

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

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

## Author contribution

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

## Transparency declaration

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## Statement on research ethics

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

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**Figure 1.**

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

**Figure S1.**

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