Adebiotech - Innovations to fight respiratory diseases
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Rapid identification of respiratory pathogens at patient's bedside
Feedback of FilmArray Pneumonia Panel Plus use

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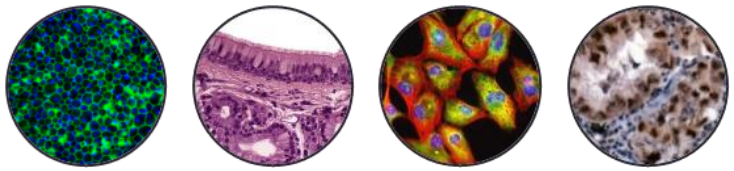

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What is a syndromic PCR?

- Symptom-driven PCR that targets probable pathogens
- Syndromic PCR = panel of potential targeted pathogens
  - meningitis/encephalitis, pneumonia, gastro-intestinal etc…
- Several manufacturers on the market (bioMérieux, Hologic…)

Syndromic PCR implementation at the Reims University Hospital

- 2018 : FilmArray® panel Meningitis/Encephalitis (FA-ME)
- 2021 : Novodiag (Hologic now) panel Gastro-intestinal (GE+)
- 2020: FilmArray® panel Pneumonia Plus (FA-PP)

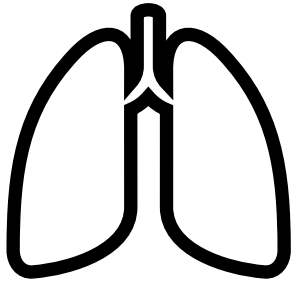
Syndromic PCR positioning in France

- **National Infection Prevention Strategy 2022-2025 and AMR**
  - **Action 22** : To develop new interventions to promote the proper use of antibiotics
  - **Sous action 22.1** : To rationalize and integrate the use in healthcare establishments of new rapid point-of-care microbiological diagnostics (multiplex PCR, rapid resistance detection systems, etc…) available on the market
- **Role of the French Microbiology and Infectious Diseases Societies (SFM/SPILF)**
  - Asked by DGOS to set up a task force

Syndromic PCR positioning at Reims University Hospital

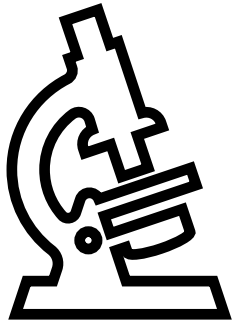
- **Questions for a rational use at Reims University Hospital**
  - Cost?
  - Service to the physicians (time to result, consideration, gaps…)?
  - Se/Spe compared to specific PCR (positioning)?
  - Emergency diagnosis or facilitating flux of operational procedures in the lab?

Flow charts for rational use of syndromic PCR in Reims



Let’s talk about fighting respiratory diseases…

…at the clin lab



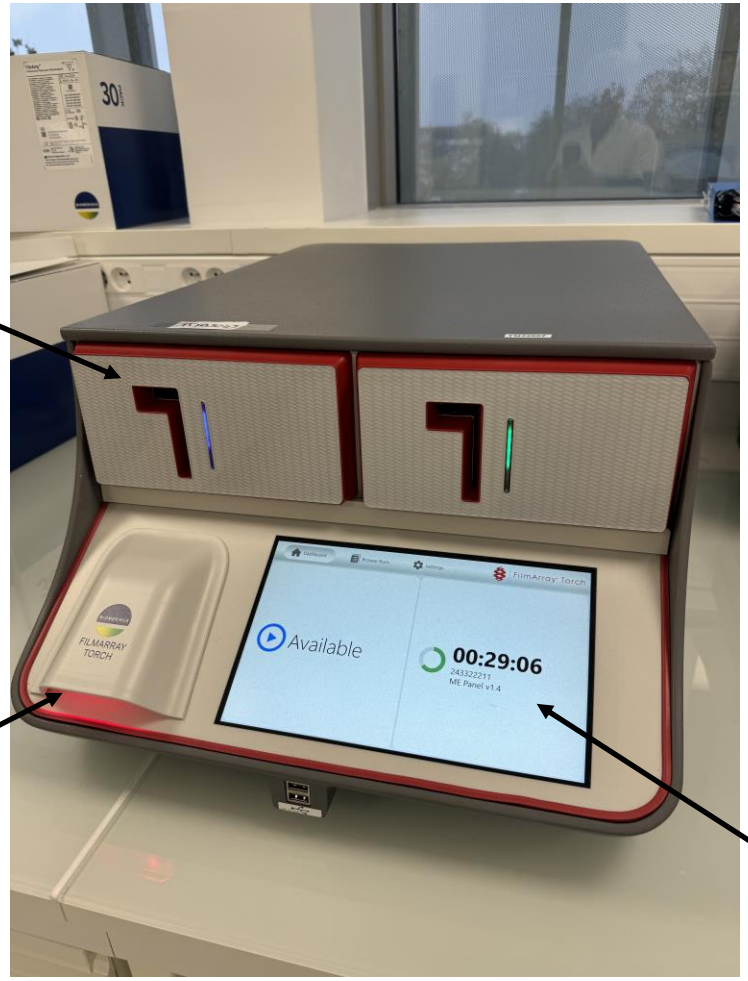
Lower respiratory tract infections (2021)

1. The world's most deadly communicable disease (other than COVID-19)
2. Ranked as 5th leading cause of death
3. 2.5 millions of death in 2021

(https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

FilmArray® BIOFIRE at Reims University Hospital

FA Torch module (duplex)
Up to 5 duplex modules



Barcode reader for cartridge identification

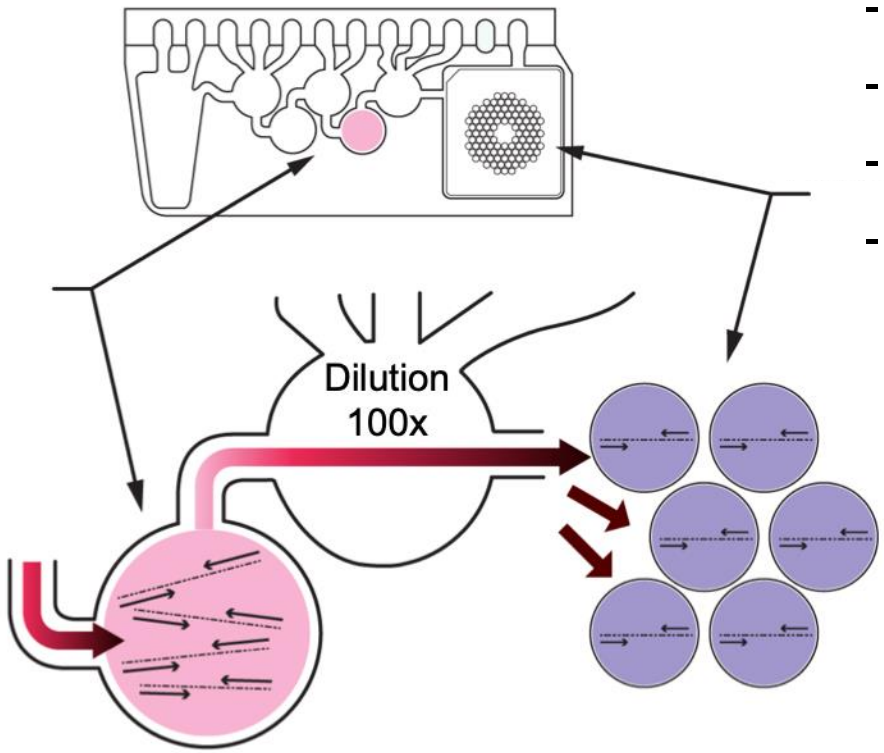
Screen to follow analyses

Principle of the FilmArray® BIOFIRE

Nested PCR

PCR 1 : multiplex

- Volume = 140 µl
- ~10 pairs of primers
- 27 cycles
- No detection

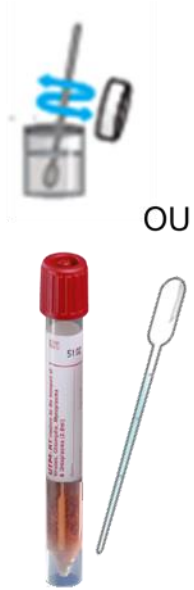


PCR 2 : monoplex

- Volume = 1 µl
- 1 well = 1 pair of primers for 1 targeted pathogen
- 30 cycles
- Detection by LC Green Plus

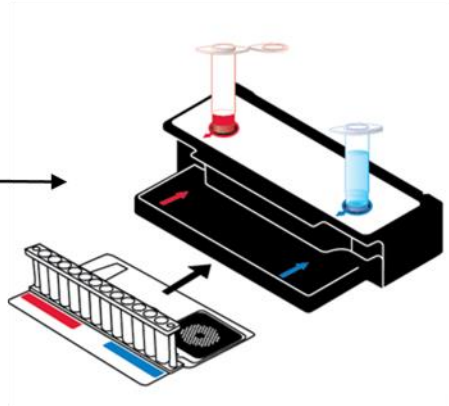
Workflow for a sample analysis

Sample

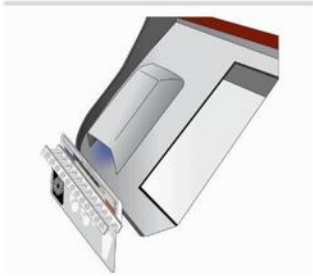


OU

Cartridge preparation



Identification



Analysis

FilmArray Pneumonia Plus (FA-PP)

BioFire® FilmArray®
Pneumonia Panel *plus*

2h

BACTERIA

(Semi-quantitative results)

Acinetobacter calcoaceticus-baumannii complex
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Moraxella catarrhalis
Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

ATYPICAL BACTERIA

(Qualitative results)

Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

VIRUS

Adenovirus
Coronavirus
Human metapneumovirus
Human rhinovirus/enterovirus
Influenza A virus
Influenza B virus
MERS CoV
Parainfluenza virus
Respiratory syncytial virus

AMR GENES

Methicillin resistance

mecA/C and MREJ

Carbapenemases

IMP
KPC
NDM
OXA-48 like
VIM

ESBL

CTX-M

Features of FA-PP - Meta-analysis

- **Se/Spe for 8968 tests within 30 studies**
  - Se = 94%
  - Spe = 98%
- **Taking into account pathogens included in the panel**
  - Se = 85%
  - Spe = 96%
  - PPV = 56%
  - NPV = 99%

Moy et al. Anaest Crit Care Pain Med. 2023(6):101300.

FA-PP positioning at the Reims University Hospital

- In the event of suspected Ventilator-Associated Pneumonia (VAP)
- In the event of suspected Community-Acquired Pneumonia (CAP) + Legionella (when urinary Lp1 antigen not possible to be done)
- In the event of suspected CAP due to *M. pneumoniae* (in case of emergency and weekends)

First lesson from the field…

…COVID-19

Use of FA-PP during COVID-19 (1)

Context

- Long stay in ICU
- Frequent Invasive Mechanical Intubations
- Frequent bacterial co-infections
- Overuse of FA-PP

What have we done?

- Study in Nancy and Reims (ICU)
- 90 patients included (PCR COVID-19 + and IMV) March - May 2020

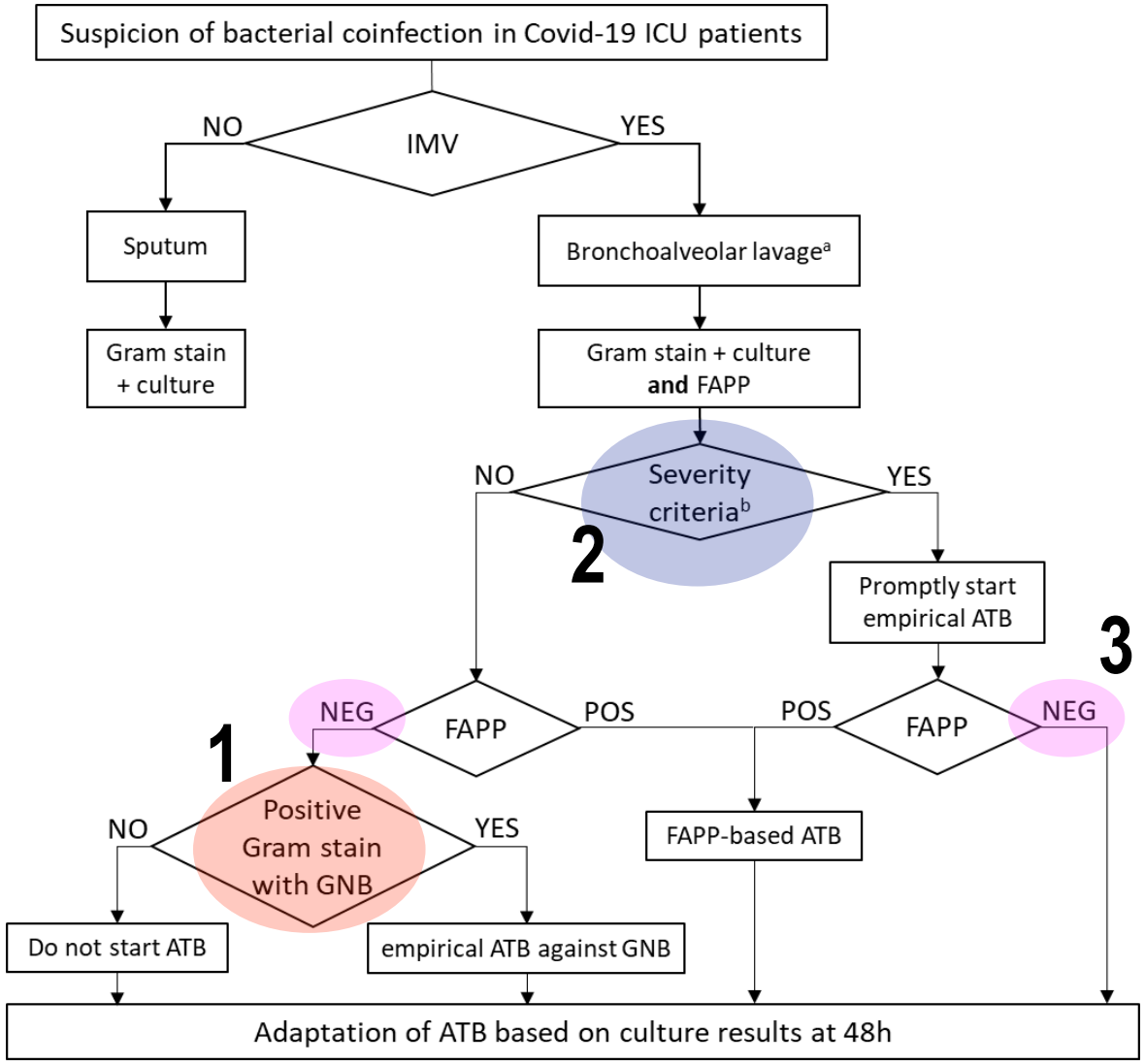
Use of FA-PP during COVID-19 (2)

Major Results

- 90 eligible patients (74 EndoTracheal Aspirates and 45 BronchoAlveolar Lavage)
- 5% CAP and 40% VAP (clinically confirmed)
- Bacteria detected in 45% by FA-PP and 39% by culture
- Adequacy of bacterial detection between FA-PP and culture
  - 96% for BAL and 80% for ETA (p = 0.017)

Novy at al. Diagn Microbiol Infect Dis. 2021 Nov;101(3):115507.

Flow chart for use of FA-PP



Highlights

1. Importance of the direct examination
2. Severity criteria = ATB before or after FA-PP
3. Decision when FA-PP negative

Severity criteria : septic shock or severe ARDS

Novy at al. Diagn Microbiol Infect Dis. 2021 Nov;101(3):115507.

Bullet points for rational use of FA-PP

1. **Training for mastering FAPP by the intensivist is required for successful utilization in the daily routine practice.**
   - Only 52% of treatment decisions based on FA-PP
2. **FAPP should be performed on BAL to avoid over-diagnosis of bacterial coinfection**
   - FA-PP and conventional cultivation better matched with BAL than endotracheal aspirates (ETA)
3. **Conventional culture should be systematically performed in parallel**
   - Not all AMR genes are detected by FA-PP (e.g. for ESBL only *bla*$_{\text{CTX-M}}$)
   - Overdetection of MRSA (6 detected and 3 confirmed)
4. **Therapeutic decision must be re-evaluated with the result of 2-days conventional culture**
   - BAL culture = 5 days. No bacteria with culture that were not targeted by FA-PP at H48.

Novy at al. Diagn Microbiol Infect Dis. 2021 Nov;101(3):115507.

Second lesson from the field…

…an unfortunate case report

False negative FA-PP

Case report

- 79 y/o patient in ICU with Invasive Mechanical Ventilation (IMV)
- FA-PP positive with Rhinovirus/Enterovirus => no antibiotic
- D+1: Positive BAL culture (10^5 CFU/ml *Klebsiella aerogenes*)
- D+2: Antibiotic susceptibility testing showed a 3GC-Resistant *K. aerogenes* (HyperAmpC)

Despite use of rapid test, delay in treatment….

Why have we missed *K. aerogenes* while in the FA-PP panel?

Nicolau-Guillaumet et al. Clin Microbiol Infect. 2024 Feb 16:S1198-743X(24)00083-1.

Lack of *K. aerogenes* detection: a known issue?

YES

- 3 papers in Pubmed
- FA-PP neg / culture pos = 3, 1 and 8 cases

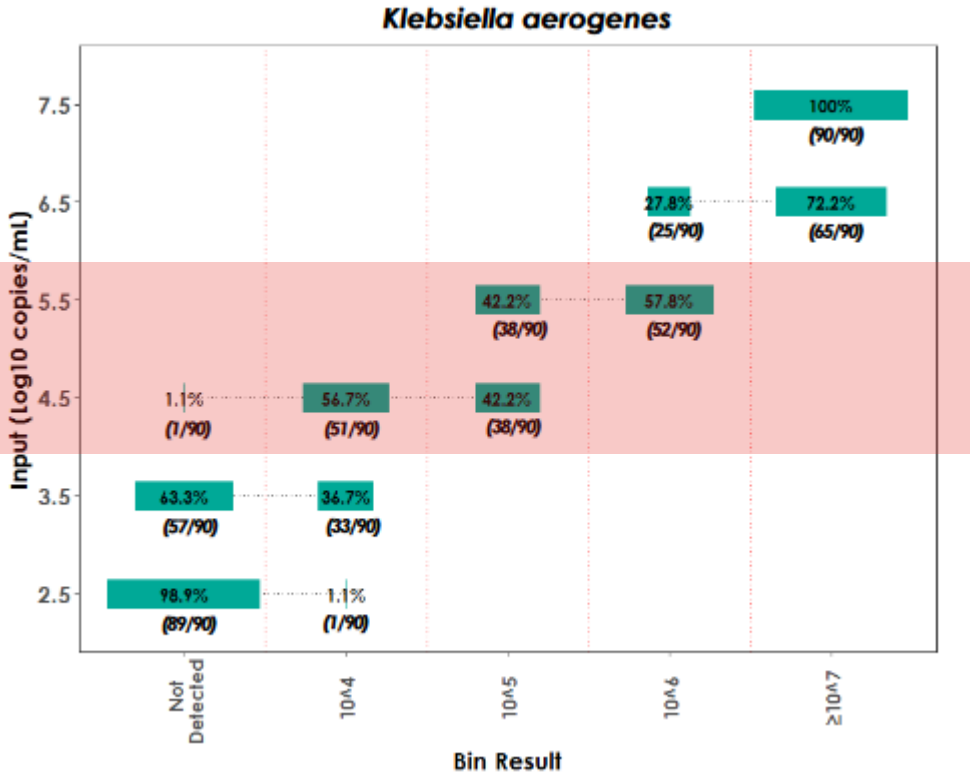
Table 1 Summary of total, BioFire Pneumonia *plus* (PN*plus*) Panel, and standard of care (SOC) detections

	Number SOC (+) PN*plus* (+)	Number SOC (−) PN*plus* (+)	Number SOC (+) PN*plus* (−)	Number total (+)
Acinetobacter calcoaceticus-baumannii complex	78	45	2	125
Adenovirus	3	41	1	45
Chlamydia pneumoniae	4	3	0	7
Coronaviruses[b]	5	46	1	52
Enterobacter cloacae complex	102	99	10	211
Escherichia coli	150	113	19	282
Haemophilus influenzae	194	291	19	504
Human metapneumovirus	5	5	1	11
Rhinovirus/enterovirus	57	308	3	368
Influenza A	24	20	1	45
Influenza B	2	0	0	2
Klebsiella aerogenes	29	33	8	70
Klebsiella oxytoca	26	45	12	83
Klebsiella pneumoniae group	179	76	15	270
Legionella pneumophila	43	6	6	55

Gastli N et al. Clin Microbiol Infect 2021;27:1308–14.
Lee SH et al. J Microbiol Immunol Infect 2019;52:920–8.
Ginocchio CC et al. Eur J Clin Microbiol Infect Dis 2021;40:1609–22

NO

- FA-PP evaluation by bioMérieux



Murphy CN, et al. J Clin Microbiol 2020;58.

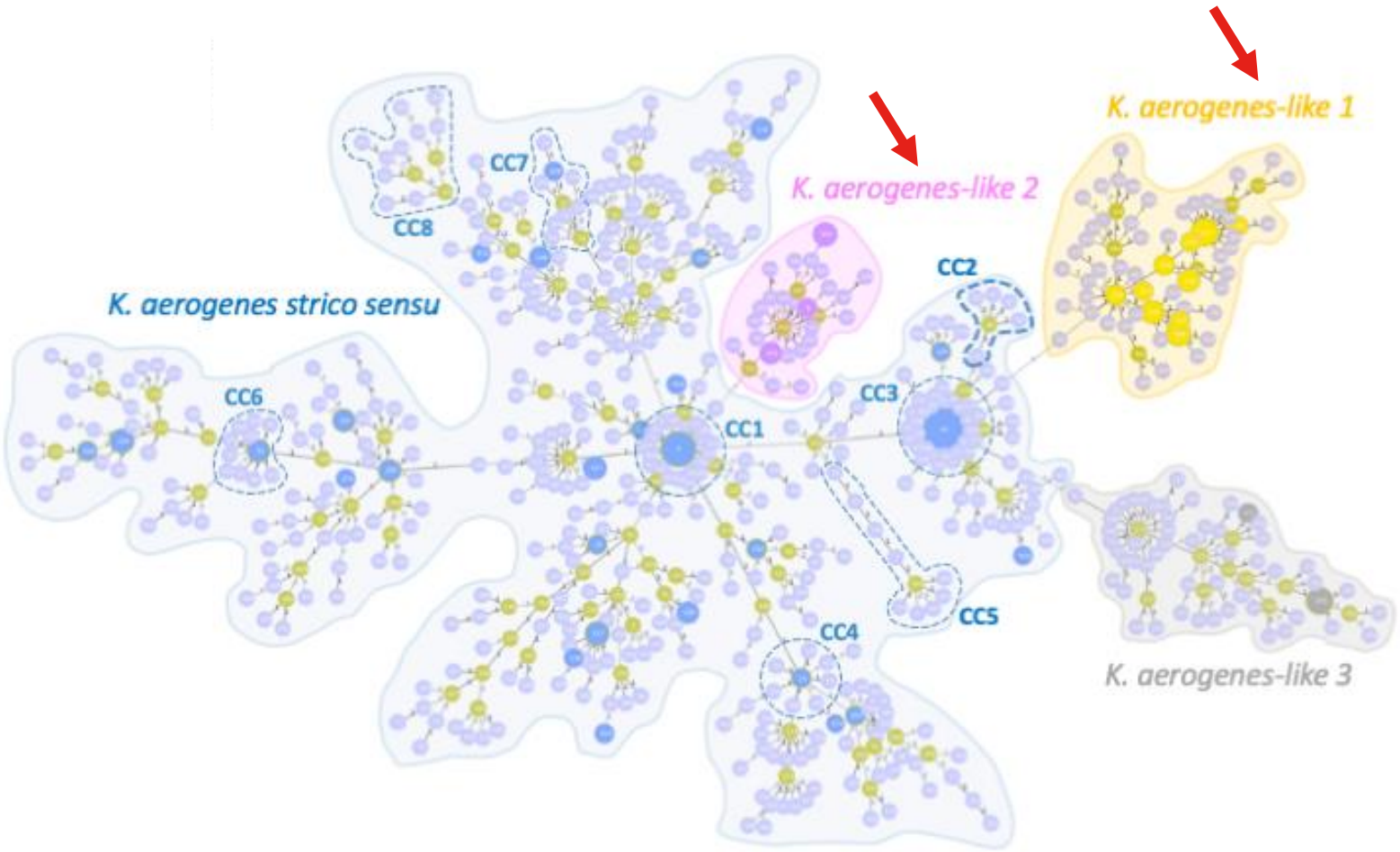
Lack of *K. aerogenes* detection: a frequent issue?

- January 2020 – December 2022
- 786 BAL / 646 patients / 824 FA-PP

	FA-PP (+) Culture (+)	FA-PP (+) Culture (-)	FA-PP (-) Culture (+)	No FA-PP Culture (+)	Total
K. aerogenes	20	12	3	8	43
FA-PP quantification	10^4 to $>10^7$	10^4 to 10^6	-	-	
Culture quantification	$<10^3$ to 10^8	-	5.10^3 to 10^5	$<10^3$ to 10^6	

Nicolau-Guillaumet et al. Clin Microbiol Infect. 2024 Feb 16:S1198-743X(24)00083-1.

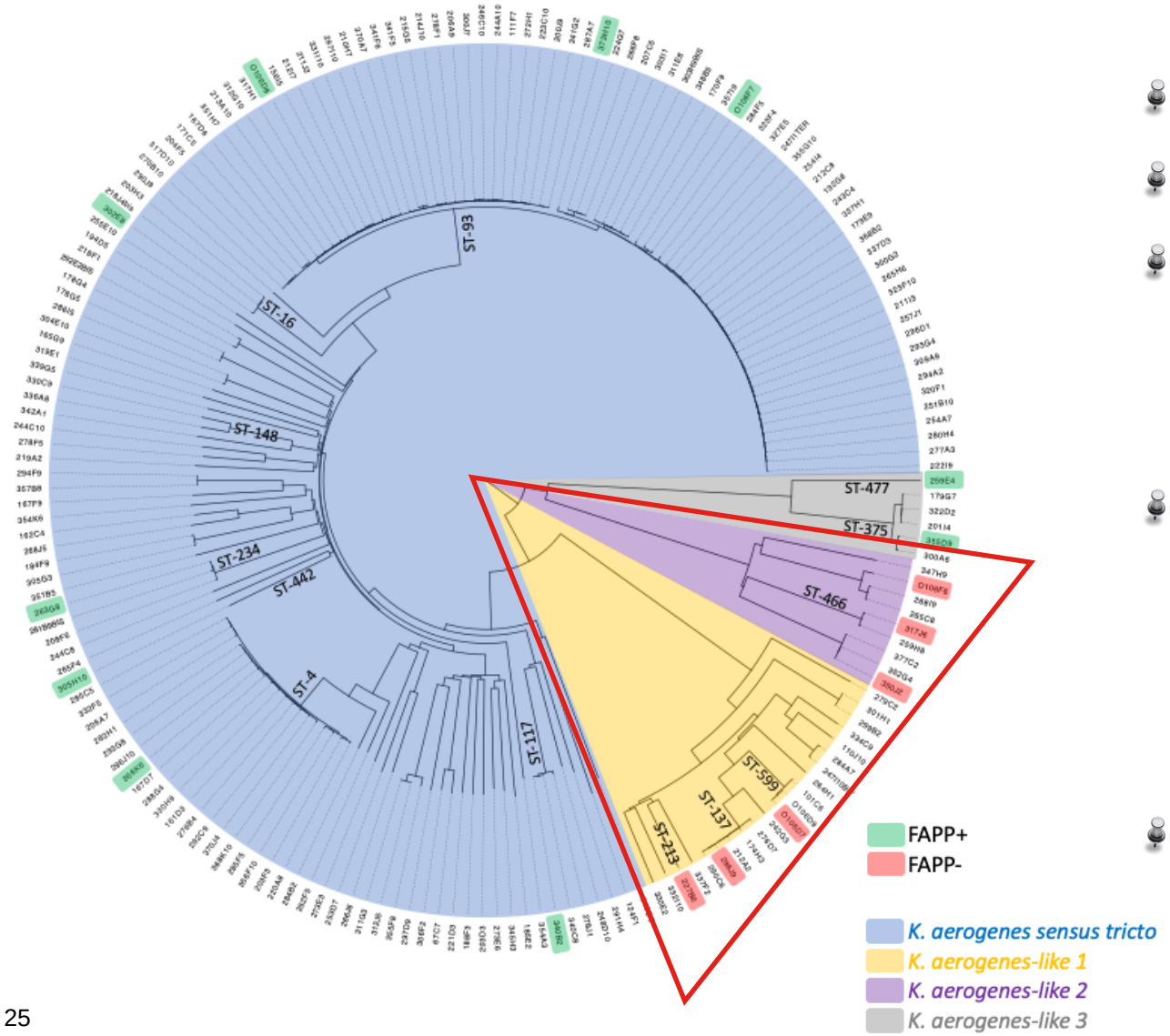
Genomes of *K. aerogenes* and FA-PP (1)



- 187 isolates
- Whole Genome Sequencing
- 3 sub-species of *K. aerogenes*
- 16% of *K. aerogenes like 1* and *2*

Nicolau-Guillaumet et al. Clin Microbiol Infect. 2024 Feb 16:S1198-743X(24)00083-1.

Genomes of *K. aerogenes* and FA-PP (2)



- Saline solution with 10^5 CFU/ml
- Inoculum checked by culture
- If FA-PP negative => solution with 10^8 CFU/ml

- *K. aerogenes* at 10^5 UFC/ml = FA-PP négative
  => *K. aerogenes* like 1 et like 2
  => NB: 16% of all the *K. aerogenes*

- If higher inoculum (10^8 CFU/ml) => FA-PP positive (but false quantification: 10^5 CFU/ml)

Nicolau-Guillaumet et al. Clin Microbiol Infect. 2024 Feb 16:S1198-743X(24)00083-1.

Take home message

1. **You should know** the panel and what you miss
   - *Moganella* spp., *Hafnia* spp., *Serratia* spp.

2. **You should know** the limits of quantification limits compared with conventional culture

3. **You should know** what to do with a positive FA-PP (ATB) and a negative FA-PP (other Δg)

4. **You should know** that a negative FA-PP with 48h negative culture = stop ATB

Nicolau-Guillaumet et al. Clin Microbiol Infect. 2024 Feb 16:S1198-743X(24)00083-1.