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Monitoring antibiotic resistant pathogens in hospital sinks and wastewater biofilms by culture-independent techniques *versus* conventional culturing methods

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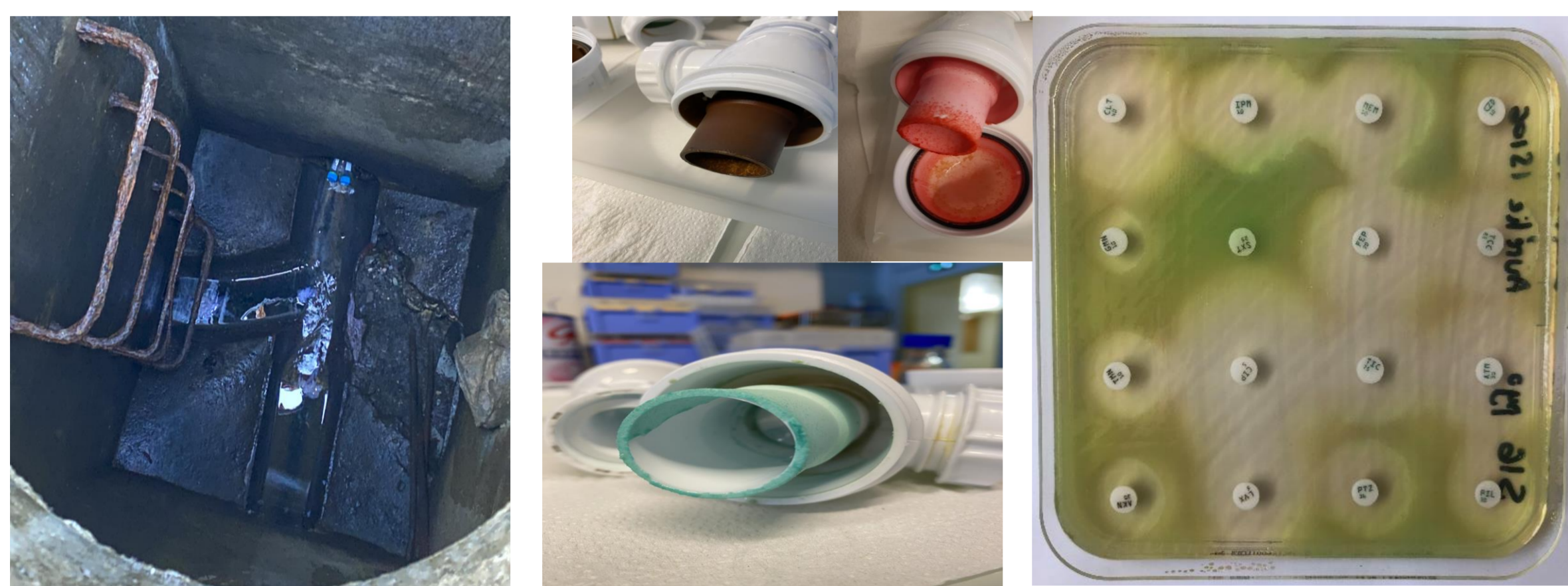
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1- Context

- Biofilms in the sink drains of hospital wards potentially contain nosocomial pathogens and antibiotic-resistant bacteria (ARB). They are also potential sources for the dissemination of ARBs to patients.
- Hospital wastewater (WW) and wastewater biofilms (WWBs) are hotspots for antibiotic resistant genes (ARGs) and ARB.



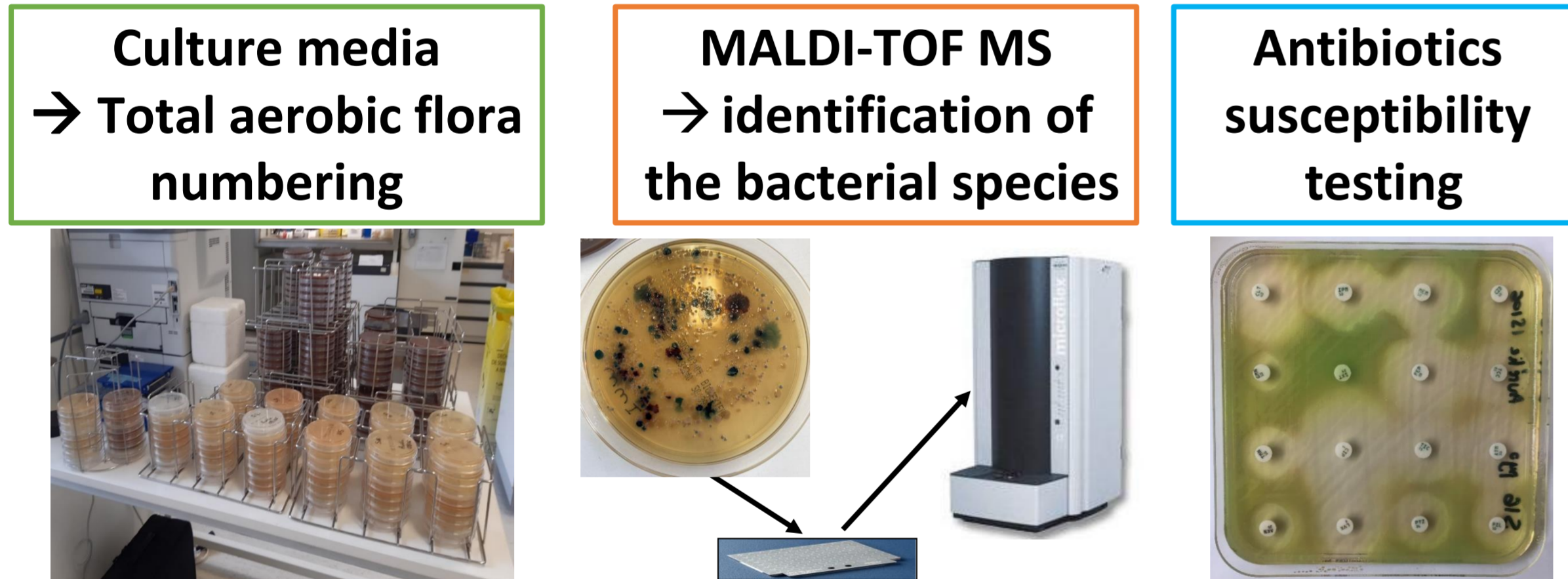
- However, in routine, sink biofilms, apart from the neonatal ICU (NICU), and WW are not analyzed at the Grenoble University Hospital:
 - no complete overview of ARB and opportunistic pathogens,
 - no overview of ARGs and associated mobile genetic elements (MGEs)

2- Aim of the study

Monitoring antibiotic resistant pathogens, mostly Emerging Highly Resistant Bacteria (EHRB) in hospital sinks, WW and WWB using culture-dependent *versus* culture-independent approaches

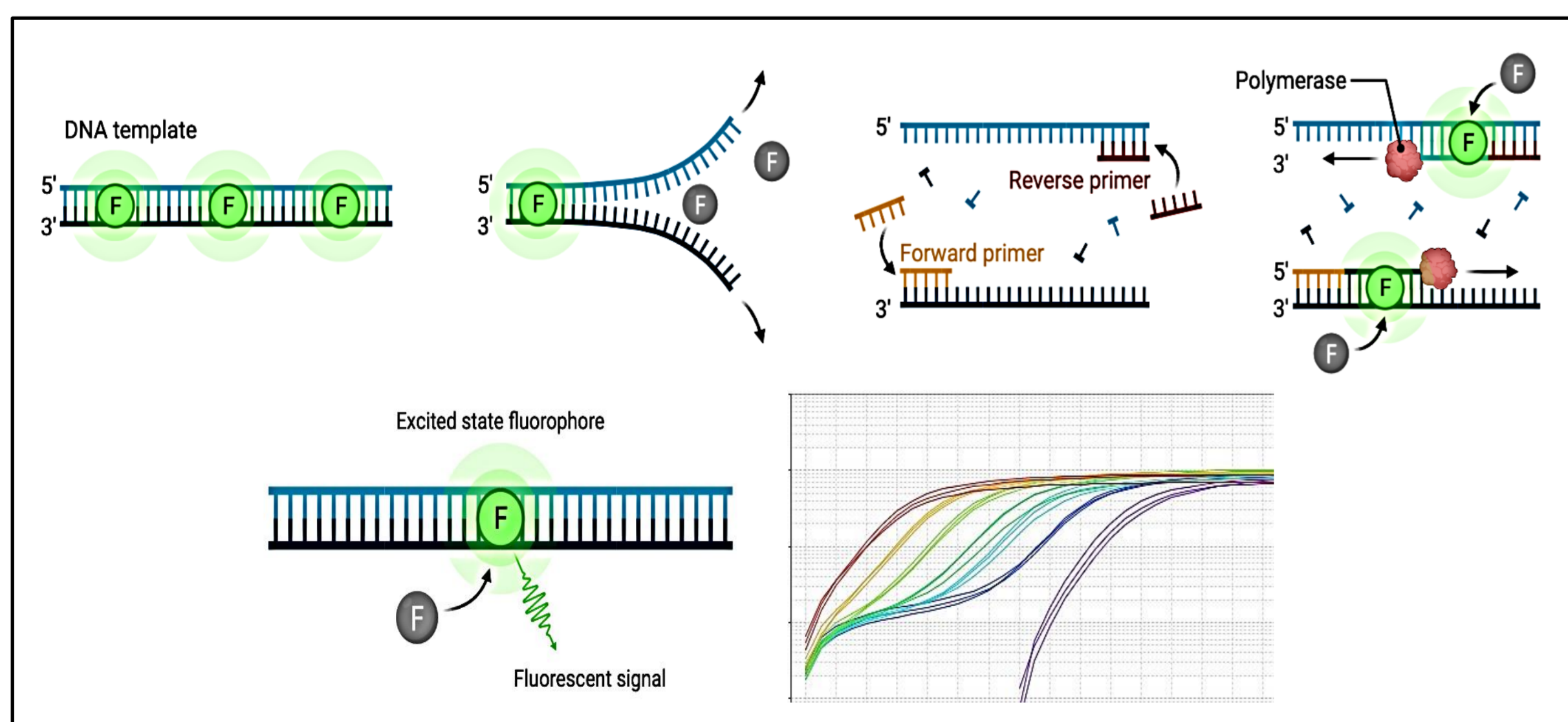
3- Methods

- Culture-dependent approach



- Confirmation of the presence of carbapenemases: "Coris Resist-5 O.K.N.V.I" test = immunochromatography for the identification of OXA-48, KPC, NDM, VIM and IMP carbapenemases
- Confirmation of vancomycin resistance: PCR amplification of the resistance corresponding genes

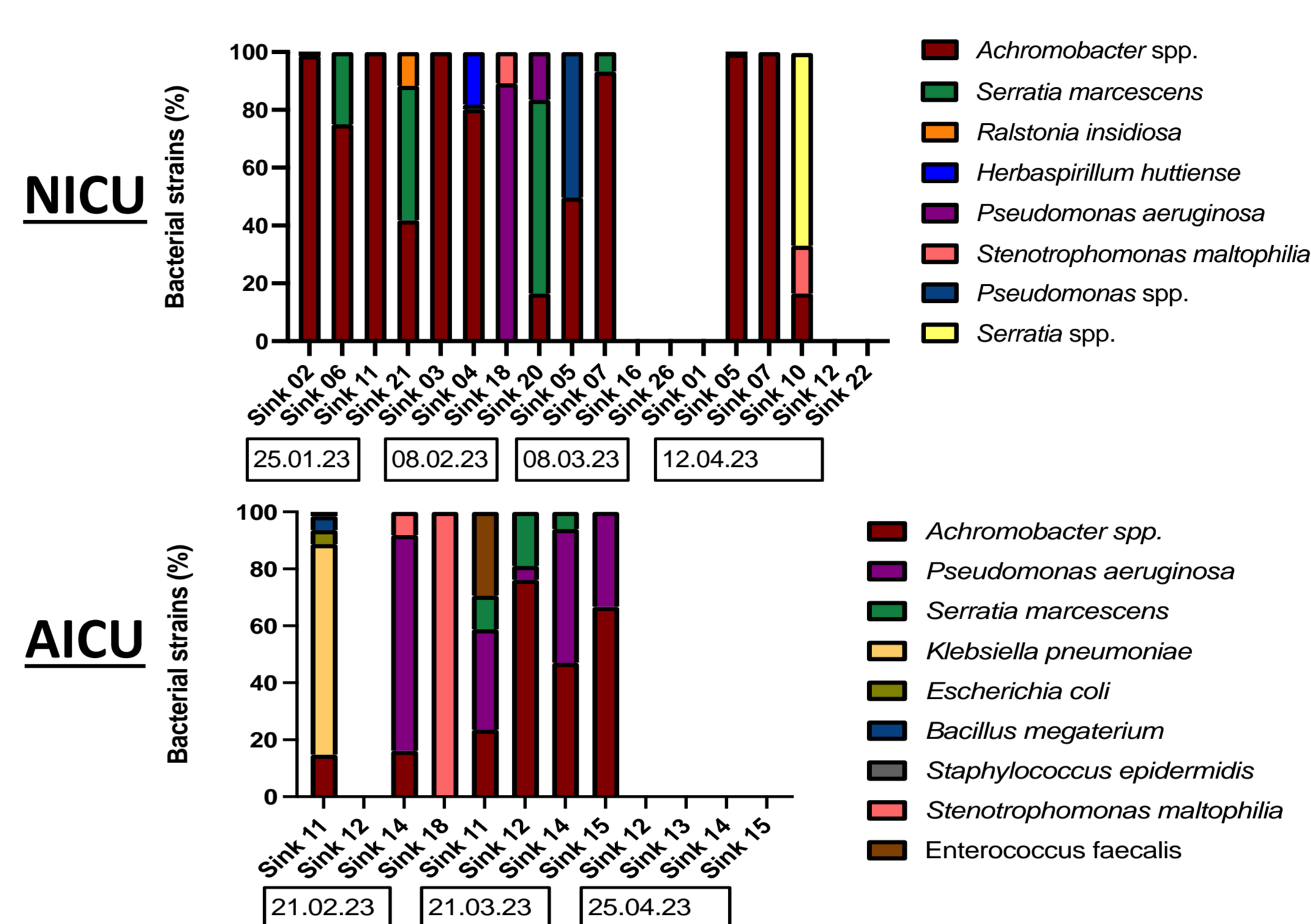
- Culture-independent approach: evaluation of the biofilm resistome by qPCR targeting 2 genes: *bla*_{OXA-like} and *INT11*



4- Study at the NICU and the AICU: culture-dependent approaches

- Analysis of the biofilms from the sinks of 30 patient rooms | Sampling from Jan. to May 2023
- Analysis of 8 hospital WW and 7 WWB

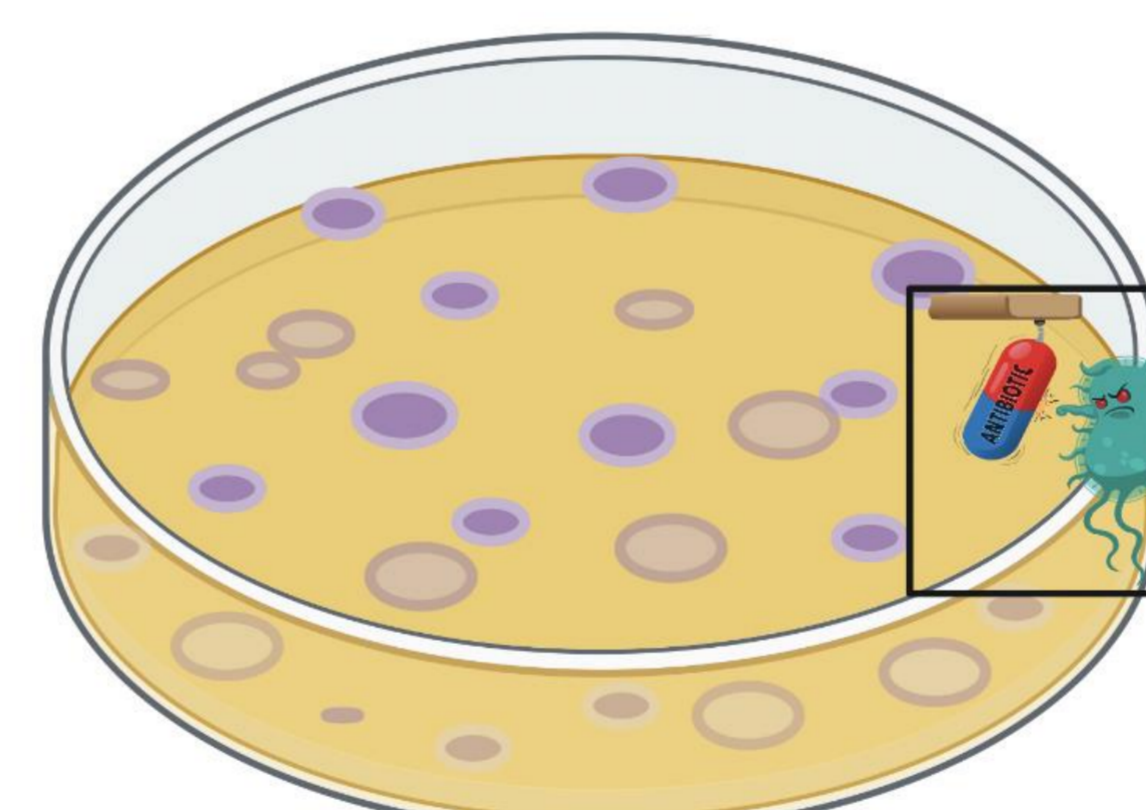
4.1- Culture-based analyses



- Except for a few germ-free sinks, the sinks globally revealed a low diversity in isolated bacterial clones: average of 3-6 [0 - 6] different bacterial species per sink in the two ICUs
- The most frequently isolated genus in both wards: *Achromobacter* spp. found in 72.2% (13/18) of the samples at the NICU and 50% (6/12) at the AICU

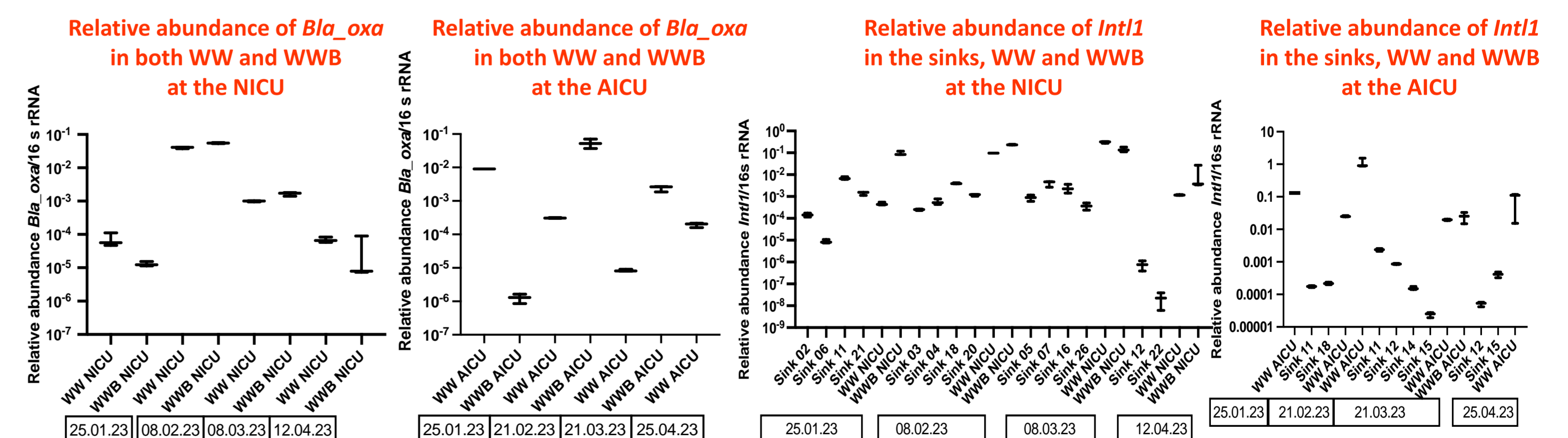
4.2- Isolation of EHRB by conventional culture-dependent approach

- No EHRB isolated in sink drain biofilms
- 16 strains isolated from WW and WWB biofilms from both ICUs



- NICU: 1 strain
 - 1 OXA-48 + strain of *Citrobacter freundii* complex (*C. freundii*)
- AICU: 15 strains, among which
 - 2 VIM + strains of *Citrobacter amalonaticus* complex (*C. farmeri*)
 - 5 OXA-48 + strains of *Citrobacter freundii* complex (*C. freundii*)
 - 3 OXA-48 + strains of *Citrobacter freundii* complex (*C. braakii*)
 - 1 VIM + strain of *Citrobacter freundii* complex (*C. braakii*)

4.3 Culture-independent approaches: evaluation of the biofilm resistome (*bla*_{OXA-like} and *Int11* genes)



- Bla*_{OXA-like} genes detected in 100% (15/15 samples) of WW and WWB samples from both ICUs
- Int11* detected in 77.7% (14/18 samples) of the analyzed sink biofilms and in 100% (8/8 samples) of WW and WWB samples at the NICU
- Int11* detected in 66.7% (8/12 samples) of the analyzed sink biofilms and in 85.7% (6/7 samples) in WW and WWB samples at the AICU

7- Conclusions

- Hospital sink, WW and WWB constitute a potential source of nosocomial pathogens such as *Serratia* spp and *Citrobacter* spp
- Hospital sink, WW and WWB constitute a potential source of EHRB and ARGs as well as MGEs (*Int11*)