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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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1- Context

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



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

2- Aim

monitoring antibiotic resistant pathogens, especially emerging highly resistant bacteria (EHRB), in hospital sinks and WW biofilms using culture-dependent *versus* culture-independent approaches

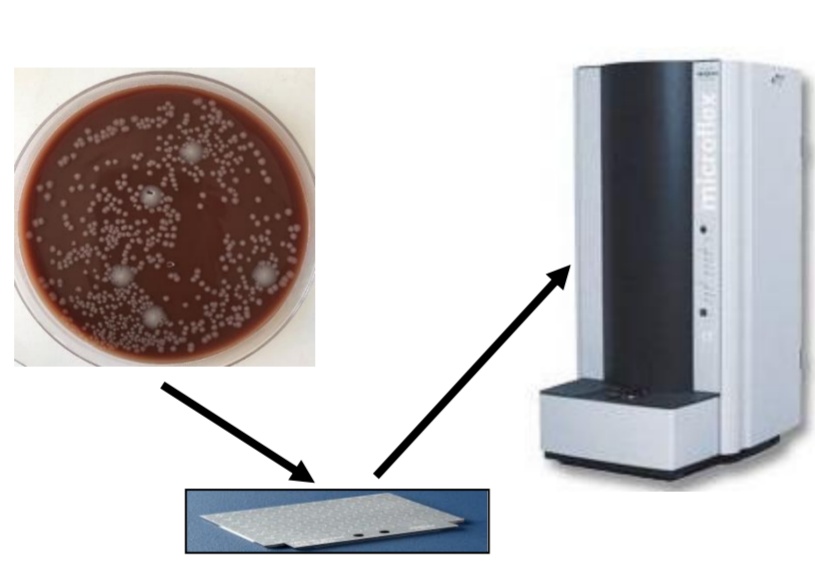
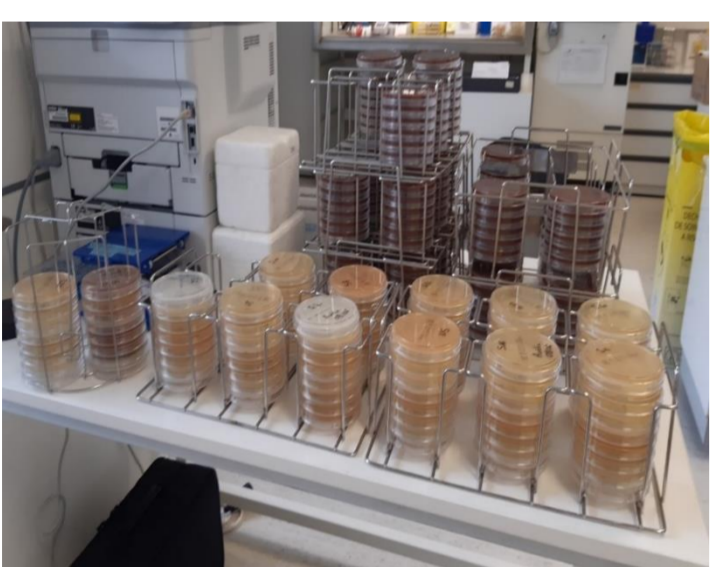
3- Methods

- Culture-dependent approach

Total aerobic flora numbering and identification
 → EHRB research

MALDI-TOF MS
 → identification of the bacterial species

Antibiotics susceptibility testing

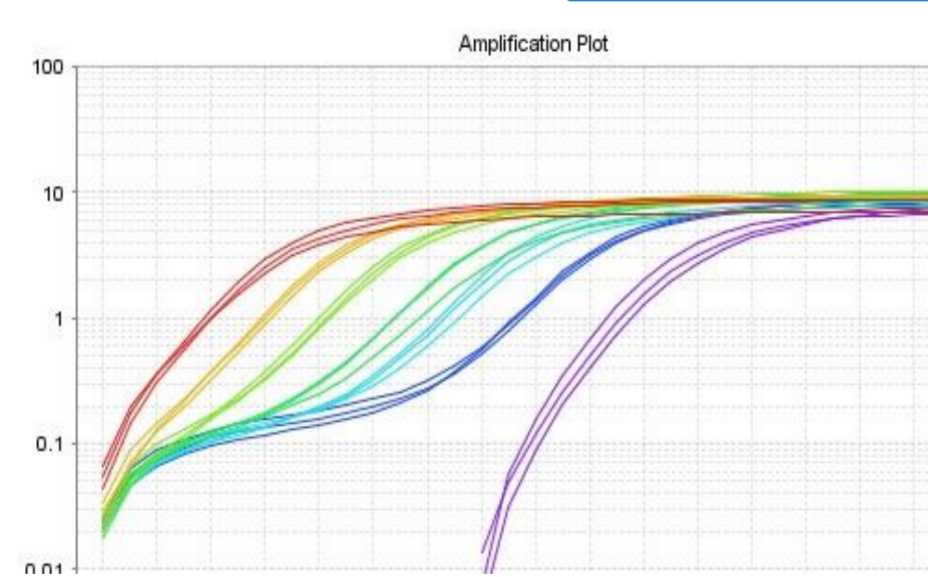


- Confirmation of Carbapenemase-Producing *Enterobacteriaceae* (CPE): Coris® Resist-5 O.K.N.V.I™ test = immunochromatography for the identification of OXA-48, KPC, NDM, VIM and IMP carbapenemases + MAST® CARBA test
- Confirmation of Glycopeptide Resistant Enterococci (GRE): PCR amplification of *VanA/VanB* genes

- Culture-independent approaches: evaluation of the biofilm resistome

High-throughput (HTP) qPCR targeting >90 ARGs and MGEs

qPCR targeting 2 genes: *bla*_{OXA} and *INT1*



Microbiota analysis by full length 16S rDNA sequencing

4- Pilot study at the Neonatal ICU and ongoing study at the Adult ICU: culture-dependent approaches

- Analysis of the biofilms from the sinks of 26 patient rooms, of hospital WW and WW biofilms

→ 3 [2 - 6] different bacterial species per sink: *Achromobacter* spp., *Serratia marcescens*, *Burkholderia complex cepacia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were the most frequent

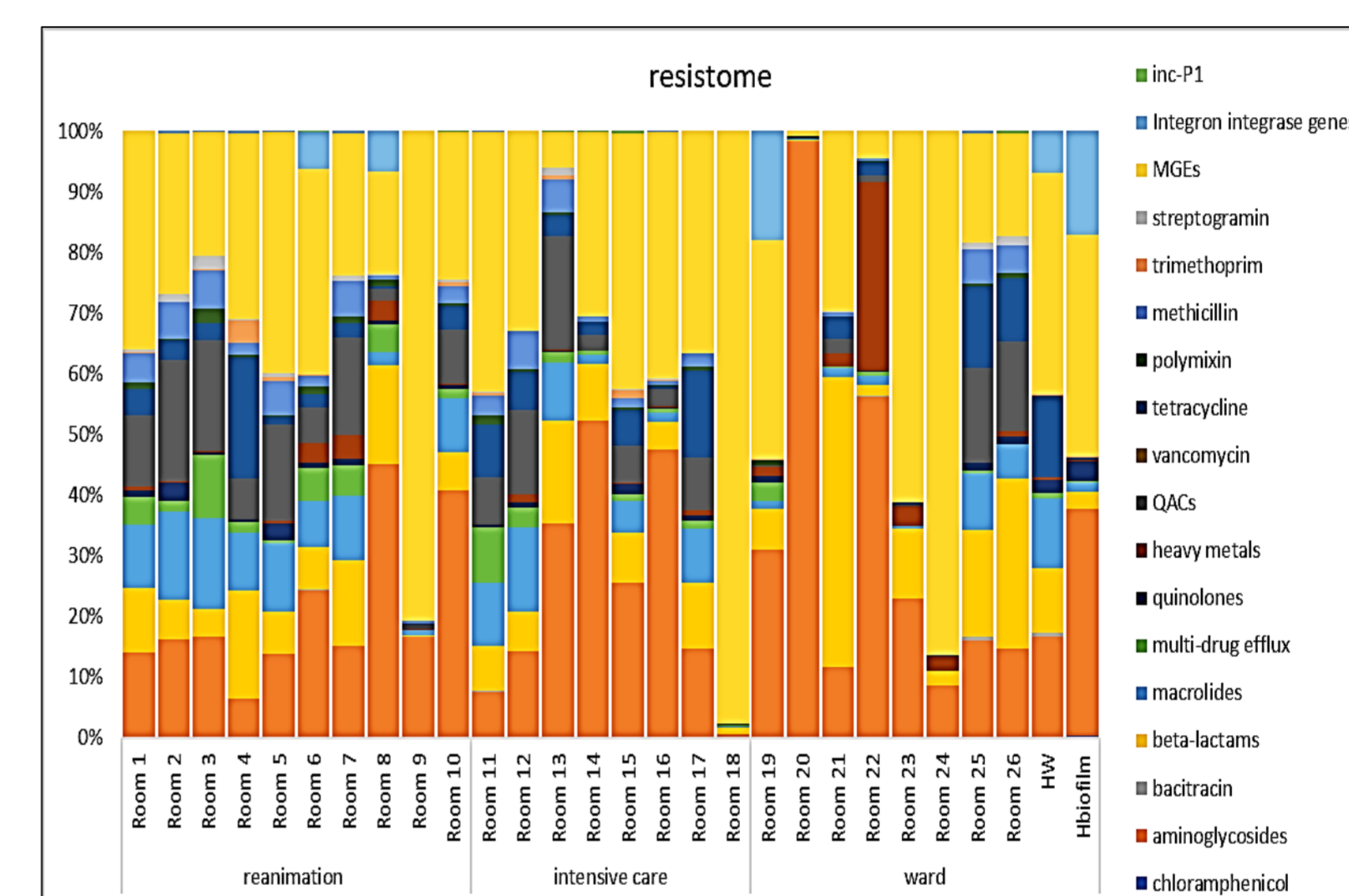
→ 9 bacterial species in the WW biofilm, 13 bacterial species + 1 yeast in the WW

→ No EHRB isolated during this campaign

5- Culture-independent approaches

HTP qPCR targeting ARGs and associated MGEs

Example of the resistome in sink biofilms: analysis of 95 ARGs and MGEs



Full-length 16S rRNA sequencing (Nanopore)
 → Microbiota analysis: species level resolution

11 analyzed samples:

> 100 different identified species / sample
 → Added value of this technology newly developed in the laboratory:

- identification of various *Achromobacter* species: *A. insolitus*, *A. marplatensis*, *A. denitrificans*, *A. animicus*, *A. ruhlandi*, *A. piechaudii*, *A. aloeverae*
- identification of various *Serratia* species in addition to *Serratia marcescens*: *S. nematodiphila*, *S. ficaria*, *S. symbiotica*, *S. entomophila*, *S. fonticola*, *S. ureilytica*

High diversity of ARGs including carbapenemases encoding genes, extended spectrum β-lactamase (ESBL) genes and MGEs

6- Follow-up study at the Neonatal and the Adult ICUs

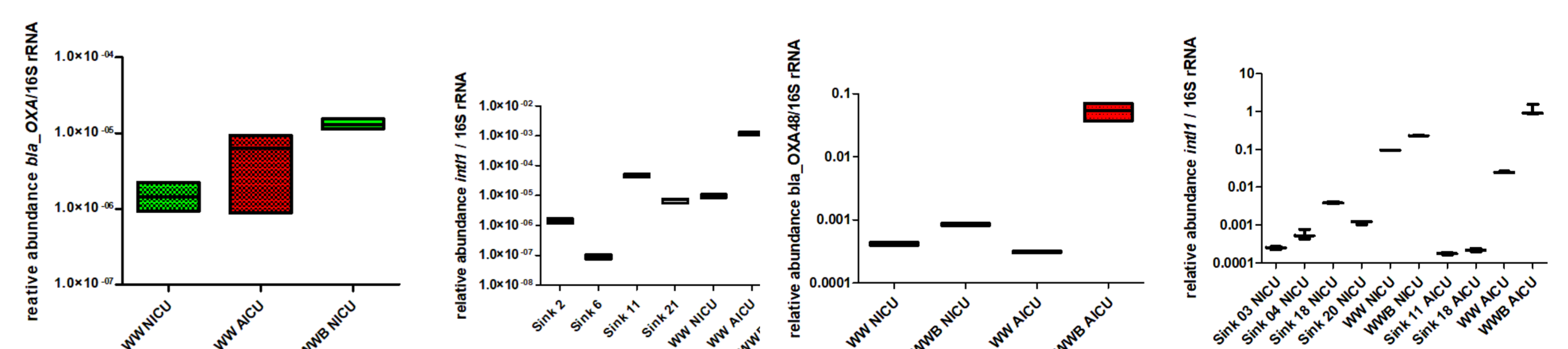
- Culture-dependent approaches

Sampling date	Sample	Ward	Results
25.01.23	WW biofilms	Neonatal ICU	OXA48+ <i>Citrobacter freundii</i>
25.01.23	WW	Adult ICU	VIM+ <i>Citrobacter farmeri</i> OXA48+ <i>Citrobacter freundii</i> OXA48+ <i>Citrobacter freundii</i>
21.02.23	WWB	Adult ICU	VIM+ <i>Citrobacter farmeri</i> Oxa-48+ <i>Citrobacter freundii</i>
21.02.23	WW	Adult ICU	VIM+ <i>Enterobacter bugandensis</i> OXA-48+ <i>Citrobacter freundii</i>

→ 3 [0 - 6] different bacterial species per sink
 → 13 [10 - 16] different bacterial species per WW and WW biofilms

→ 8 strains of CPE
 → No GRE

- Culture-independent approaches: evaluation of the biofilm resistome



- Int1* detected in 83,3% of the analyzed samples (10/12 samples)
- Int1* most abundant in WW biofilms from both the adult and the neonatal ICUs

7- Conclusions

- Hospital sink and WW biofilms constitute a potential source of nosocomial pathogens such as *Serratia* spp and *Citrobacter* spp
- Hospital sink and WW biofilms act as a reservoir for ARGs and MGEs, which are impossible to assess by routine culturing approaches
- Hospital WW and WW biofilms contain carbapenemase-producing *Enterobacteriaceae* such as *Citrobacter* spp: novel cause of concern ?