

# Visualization-Combined Microbiome Detection (VCMD) method enable deep analyses of Central Venous Catheter Infections

Alexander Fuchs<sup>1</sup>, Anastasia Bragina<sup>1</sup>, Maria Sensen<sup>1</sup>, Philipp Stiegler<sup>2</sup>, Robert Krause<sup>2</sup> & Gabriele Berg<sup>1</sup>

<sup>1</sup>Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria

<sup>2</sup>Medical University of Graz, Department of Internal Medicine, Section of Infectious Diseases and Tropical Medicine, Graz, Austria

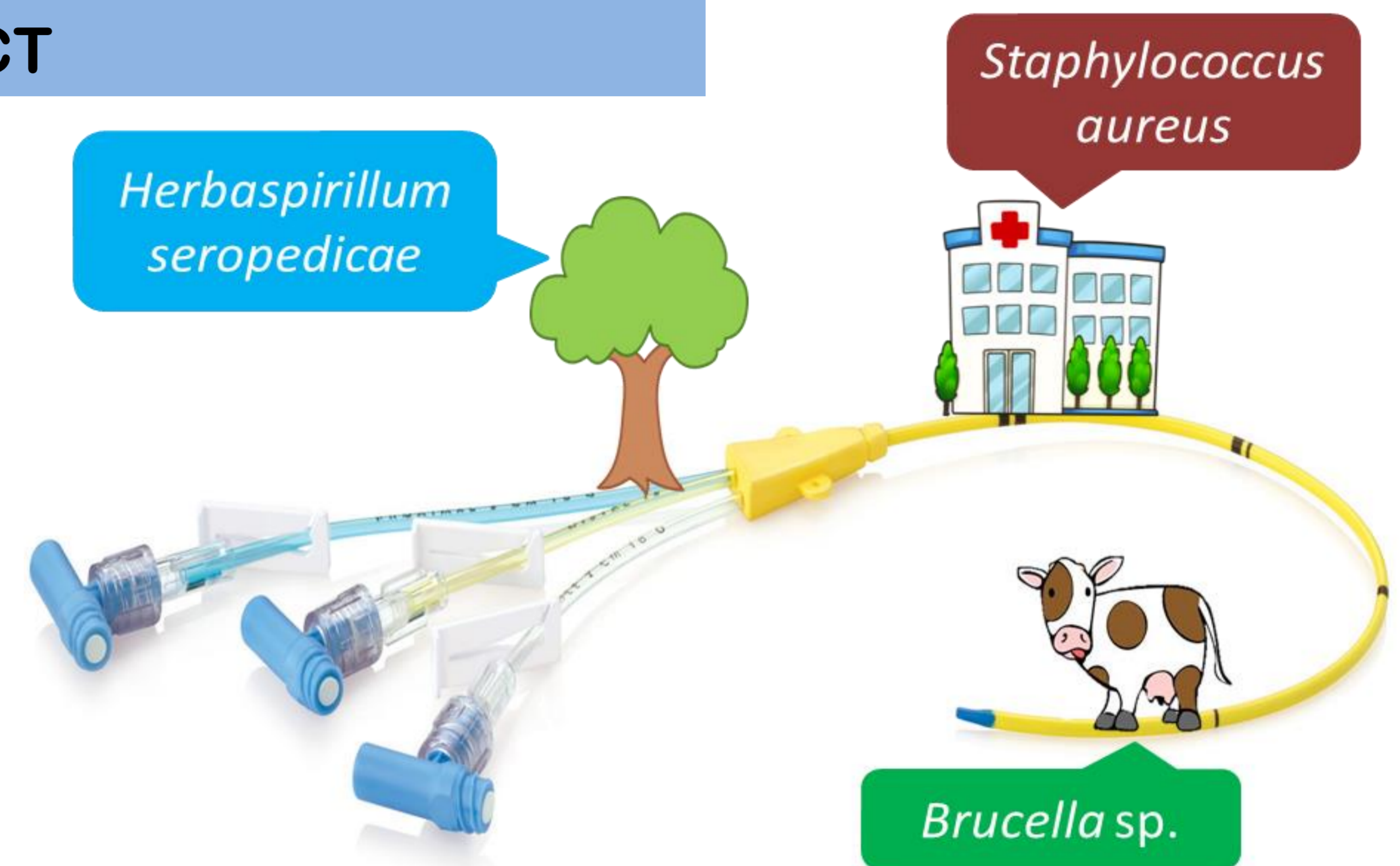
## ABSTRACT

Biofilms, bacteria-protective exopolysaccharide layers, are ubiquitous in environment and can also be found in different medical indwelling devices. Biofilm formation on central venous catheters (CVCs) in many cases leads to CVC-related blood stream infections (CRBSI), which results in higher mortality rate and increased treatment costs.

**The aim of our study** was to investigate the CVC colonization patterns of removed catheters from patients who developed infection by applying Visualization-Combined Microbiome Detection (VCMD) method. VCMD includes fluorescence *in situ* hybridization (FISH) in combination with confocal laser scanning microscope (CLSM) as well as sequencing based microbiome analyses.

### In summary:

1. By applying FISH we were able to visualize the inner biofilm structure of CVC.
2. We could detect a two-layer structure in relation to the inner surface of the catheter.
3. The bacterial communities tend to localize both in the soft layer close to the lumen of the catheter and in between the two layers.



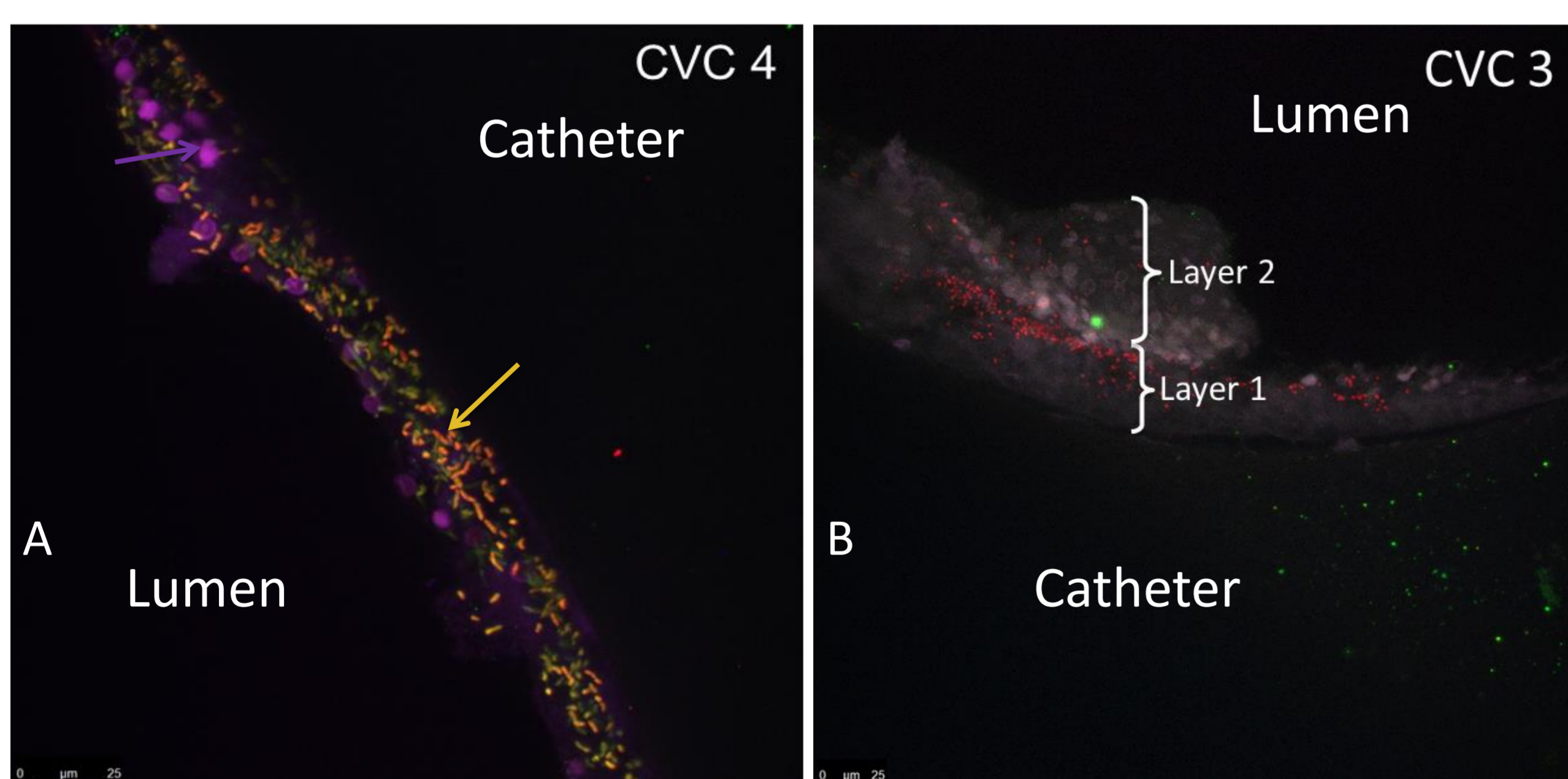
4. The microbiome analyses of the community shows three distinct origins of bacteria:
  - a/ environmental species e.g. *Herbaspirillum seropaedicae*, *Brucella sp.*
  - b/ human/hospital-related species, e.g. *Staphylococcus aureus*
  - c/ commensal human flora-related species, e.g. *Staphylococcus epidermidis*, *Enterococcus faecium*

## VCMD METHOD

### VISUALIZATION OF CVC COLONIZATION PATTERN

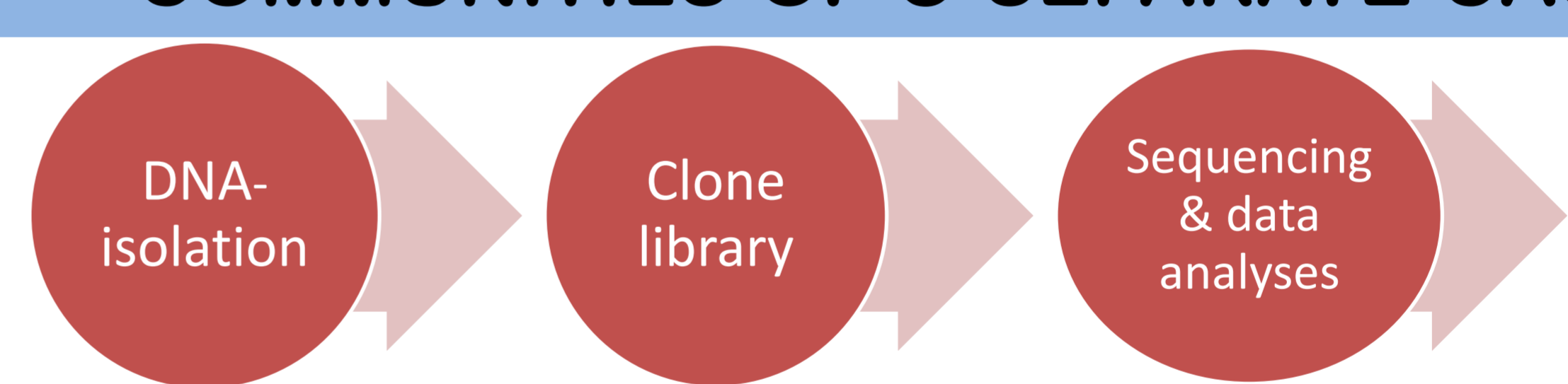


- Successful visualization of the intact biofilm on CVC using FISH-CLSM approach (Fig. 2A)
- Biofilm architecture: two polysaccharide layers with embedded bacterial and blood cells
- Two-layer biofilm structure: Bacteria located on distinct regions (Fig. 2 B)

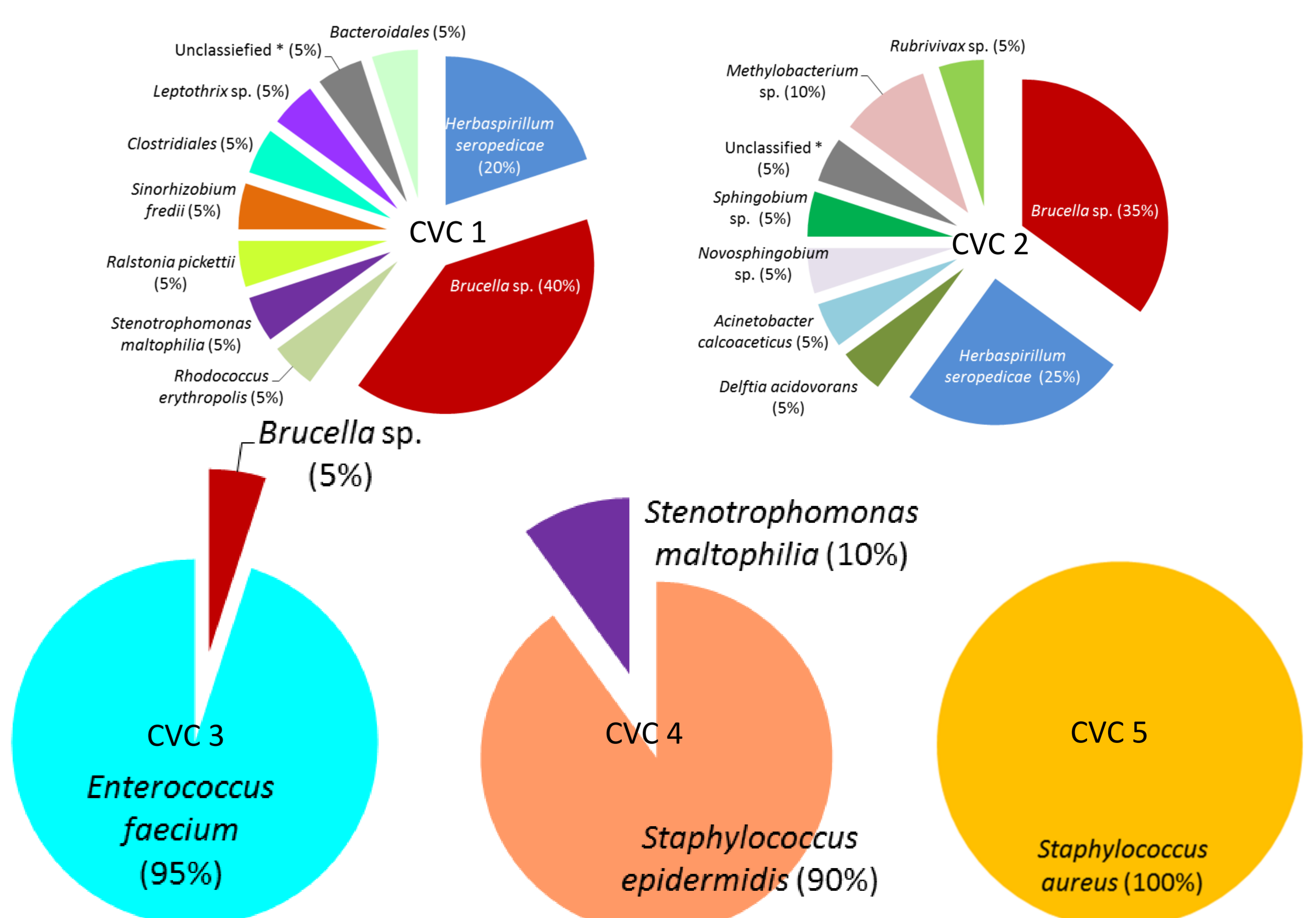


**Figure 1:** CVC-associated biofilms visualized by using FISH-CLSM. Orange: *Gamma*proteobacteria; violet: blood cells; red: all bacteria.

### ANALYSES OF DIVERSE MICROBIAL COMMUNITIES OF 5 SEPARATE CASES



- 16S rRNA gene (27f/1492r) clone libraries : 120 sequences
- Dominant bacterial taxa:
  - Alphaproteobacteria (*Brucella sp.*)
  - Betaproteobacteria (*Herbaspirillum seropaedicae*)
  - Firmicutes (*Staphylococcus sp.*, *Enterococcus sp.*)
- *Brucella sp.* was detected solely using cultivation-independent methods



**Figure 2:** Taxonomic composition of the CVC-associated biofilms. Analyzed CVCs were obtained from different patients of unknown health status. 20 sequences per sample were analyzed. Taxonomic assignment was done using nucleotide database of the NCBI server.

## CONCLUSION

Cultivation independent VCMD method has a potential to reveal the source/mode of infection by identifying the detailed structure and composition of the bacterial community. With the help of this application we may gain deeper understanding of CVC-related infections comparing to routine clinical procedures.

## REFERENCES

1. Catheter image: <http://www.delexpharma.com/www/?q=content/central-venous-catheters-0>
2. Grube M, Cardinale M, Vieira de Castro Jr J, Müller H, Berg G. (2009). Species-specific structural and functional diversity of bacterial communities in lichen symbioses. ISME J 3: 1105–1115.