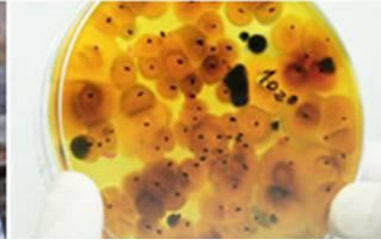


Training School
"Galleria mellonella
in biofilm research"



Bratislava, Slovakia,
11st September 2023



(Mixed) Biofilms and Infection.

Luisa Jordao

National Institute of Health Dr Ricardo Jorge

Environmental Health Department

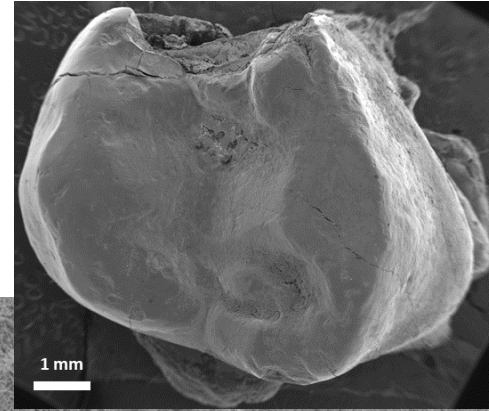
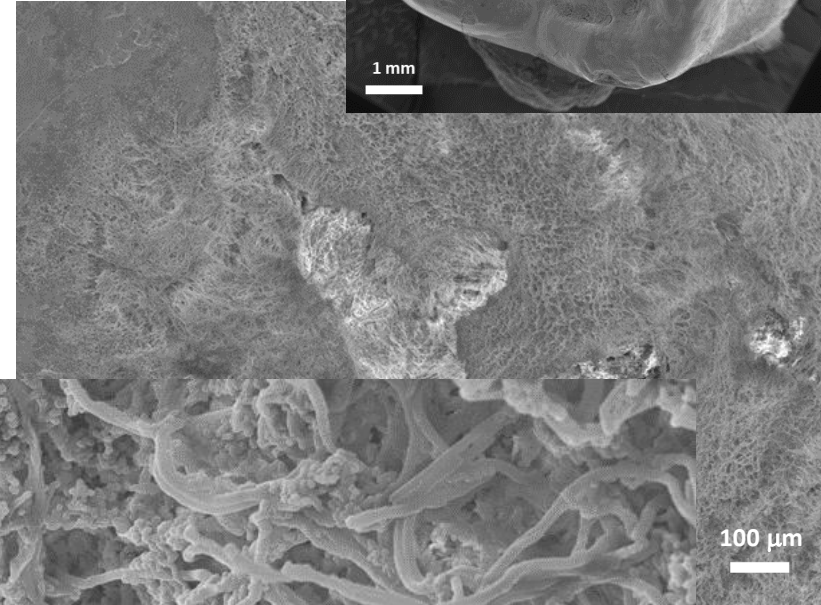
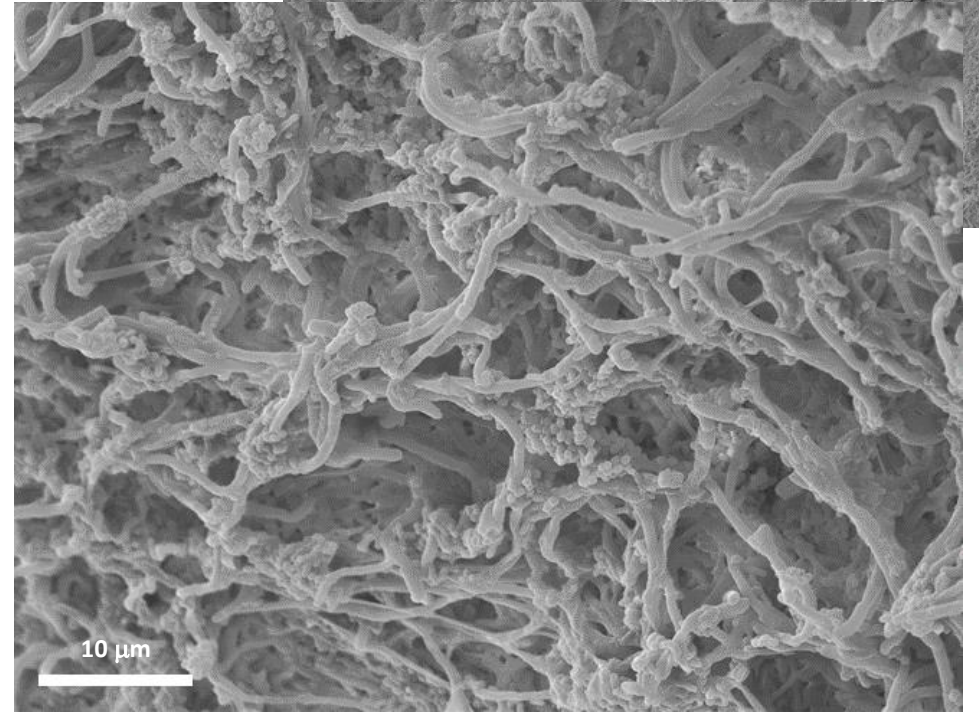
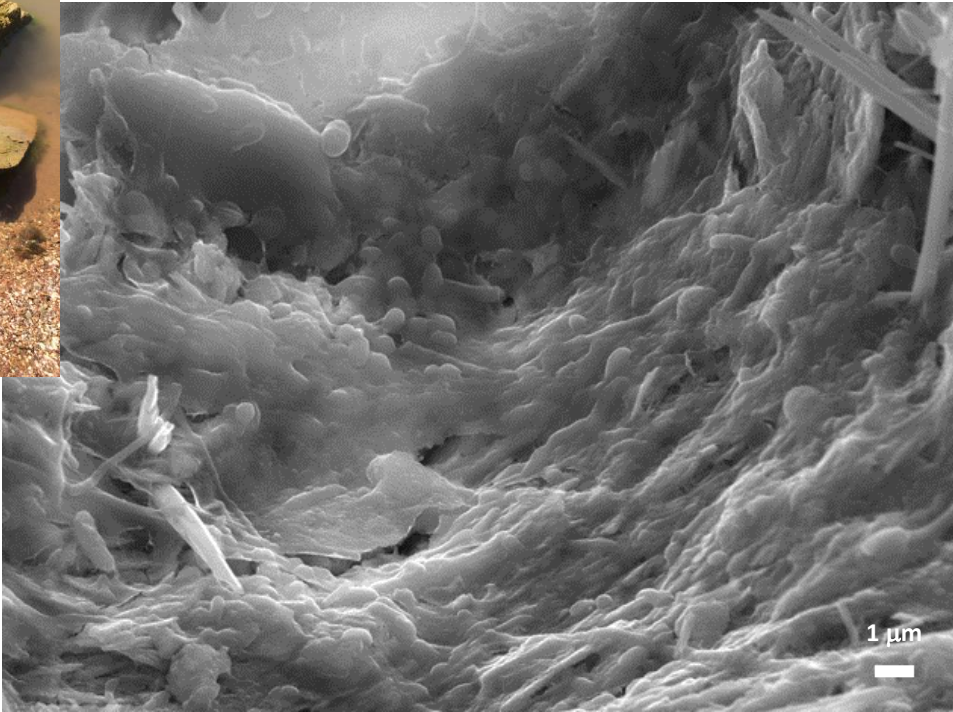
Lisboa, Portugal



Biofilms:

thin layers of microorganisms adhering to the surface of a structure, which may be organic or inorganic, together with the polymers that they secrete.

They are dynamic structures which experience different stages of organization with the ageing.



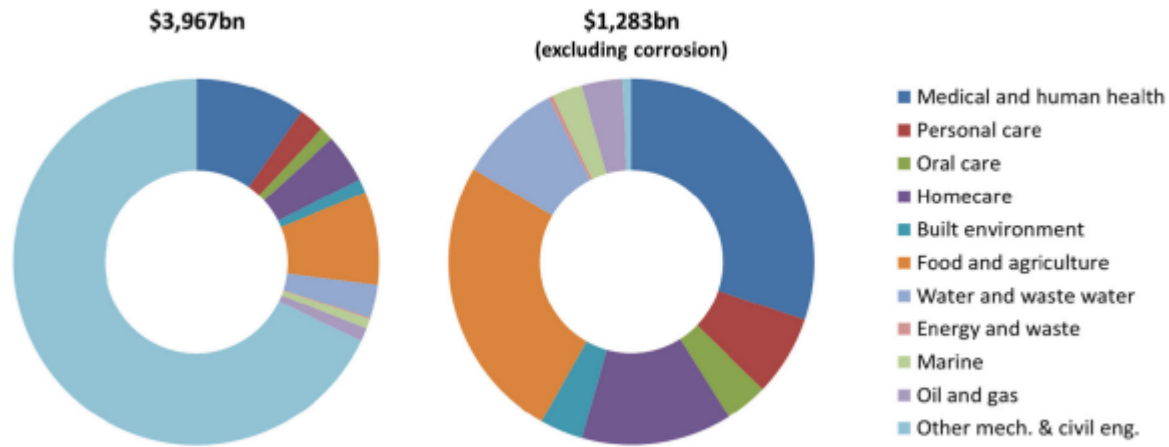
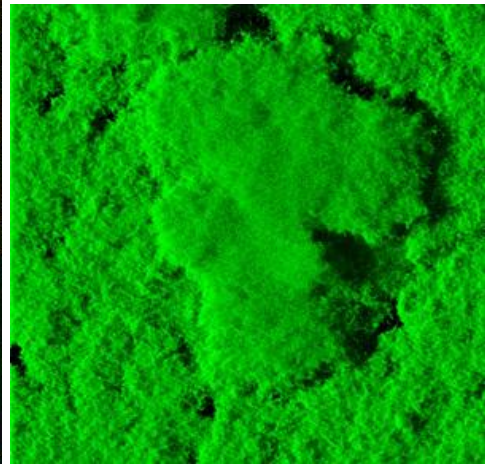
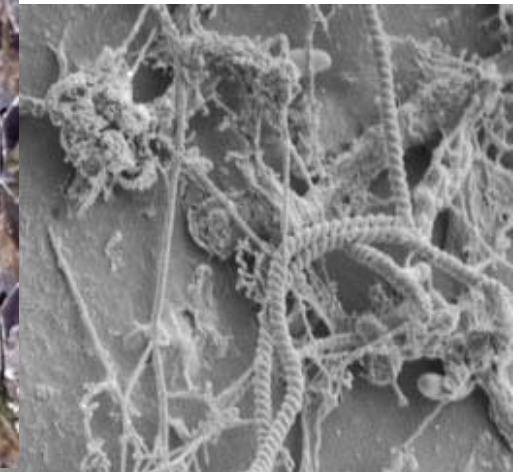
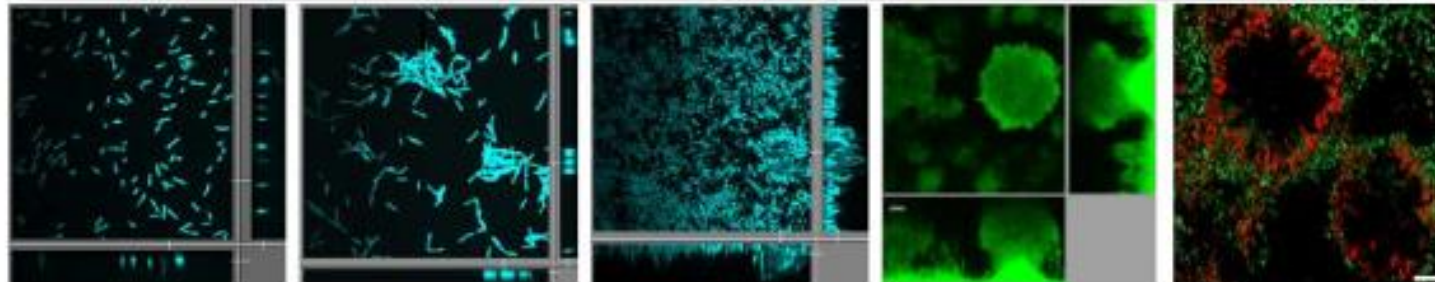
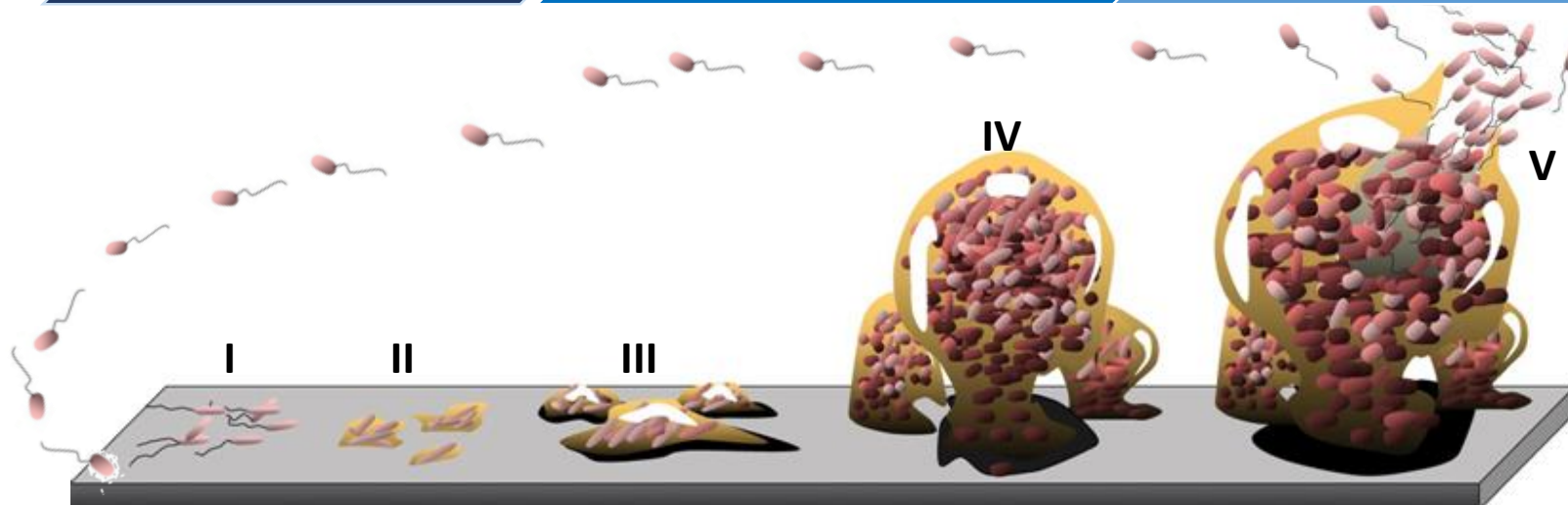
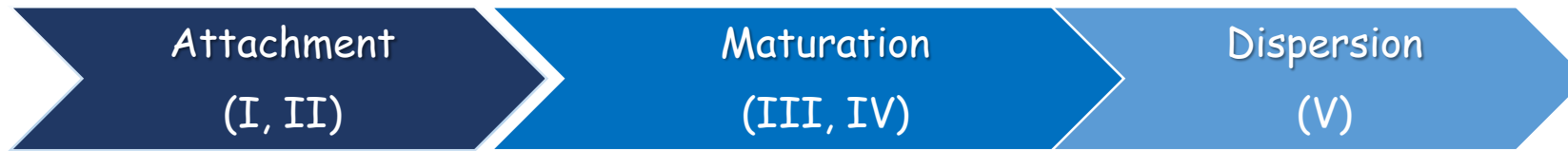


Fig. 1 Economic significance of biofilms by sector. Corrosion has been removed from the right chart to expand the viewing of the other sectors.

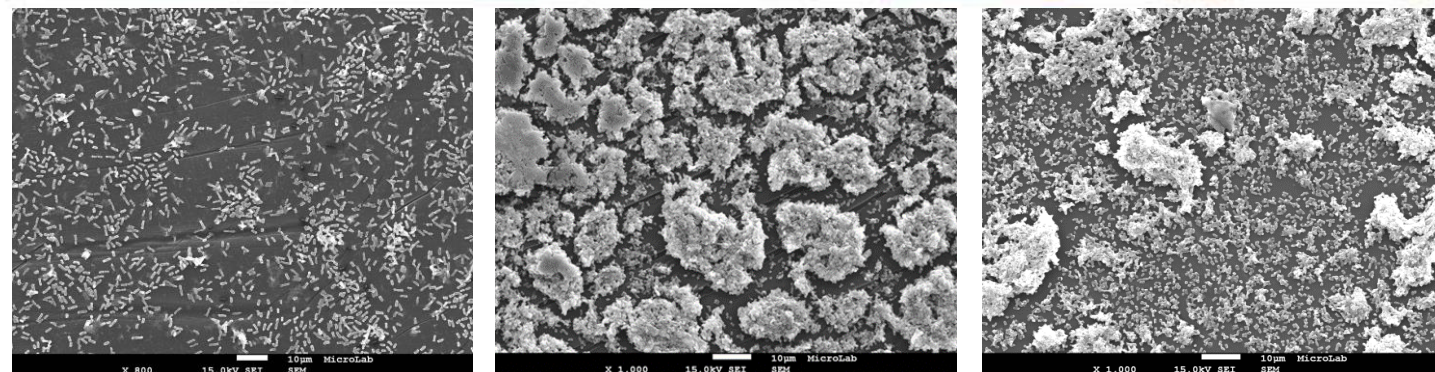
npj Biofilms and Microbiomes (2022) 8:42 ; <https://doi.org/10.1038/s41522-022-00306-y>






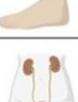
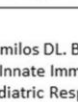


D. Davies



Adapted from: Bordi and Bentzmann, *Annals of Intensive Care*, 2011



Infection type	Common bacterial species	Reference example
 Endocarditis	Staphylococci, Streptococci, Enterococci > Gram negatives	Habib G et al, 2015
 Osteomyelitis	Staphylococci, mostly <i>S. aureus</i> > Gram negatives and anaerobes	Lew et al, 2004 Darouiche et al, 2004, Fantoni et al, 2019
 Periodontitis	<i>Streptococcus</i> , <i>Veillonella</i> , <i>Granulicatella</i> , <i>Neisseria</i> , <i>Haemophilus</i> , <i>Corynebacterium</i> , <i>Rothia</i> , <i>Actinomyces</i> , <i>Prevotella</i> , <i>Capnocytophaga</i> , <i>Porphyromonas</i> , and <i>Fusobacterium</i>	Larsen et al, 2017 Do et al, 2013
 Cystic fibrosis	<i>P. aeruginosa</i> , <i>S. aureus</i> > <i>Burkholderia</i> spp., <i>Stenotrophomonas maltophilia</i> , and <i>Achromobacter</i>	Elborn et al, 2016
 Chronic tonsillitis	<i>S. aureus</i> , alpha-hemolytic <i>Streptococcus</i>	Hamilos et al, 2019
 Ulcers, Skin wound	Polymicrobial > <i>P. aeruginosa</i> > <i>S. aureus</i>	Percival et al, 2012, Wolcott et al, 2016
 Urinary tract Infection	Enterobacterales	Vestby et al, 2020

Hamilos DL. Biofilm Formations in Pediatric Respiratory Tract Infection : Part 1: Biofilm Structure, Role of Innate Immunity in Protection Against and Response to Biofilm, Methods of Biofilm Detection, Pediatric Respiratory Tract Diseases Associated with Mucosal Biofilm Formation. *Curr Infect Dis Rep.* 2019 Feb 28;21(2):6. doi: 10.1007/s11908-019-0658-9. PMID: 30820766.

Microorganisms 2022, 10(7), 1259; <https://doi.org/10.3390/microorganisms10071259>

Device-associated infections

Healthcare-associated infections (HAIs) are infections that patients acquire during the course of receiving healthcare treatment for other conditions. These infections related to medical care can be devastating and even deadly.

SURVEILLANCE REPORT

Healthcare-associated infections acquired in intensive care units

Annual Epidemiological Report for 2019

Key facts

- In 2019, 8 874 (7.4%) of patients staying in an intensive care unit (ICU) for more than two days presented with at least one ICU-acquired healthcare-associated infection (HAI) under surveillance (pneumonia, bloodstream infection, or urinary tract infection).
- Of all patients staying in an ICU for more than two days, 4% presented with pneumonia, 3% with bloodstream infection (BSI), and 2% with urinary tract infection (UTI).
- Some 96% of pneumonia episodes were associated with intubation, 44% of BSI episodes were catheter-related, and 94% of UTI episodes were associated with the presence of a urinary catheter.
- The most frequently isolated microorganism was *Klebsiella* spp. in ICU-acquired pneumonia episodes, coagulase-negative staphylococci in ICU-acquired bloodstream infections, and *Escherichia coli* in ICU-acquired urinary tract infections.
- Antimicrobial use was empirical in 59% of days of therapy (DOTs), directed in 23% of DOTs, and prophylactic in 14% of DOTs.
- Some 11% of *Staphylococcus aureus* isolates were oxacillin-resistant (MRSA), and 14% of *Enterococcus* spp. isolates were glycopeptide-resistant. Resistance to third-generation cephalosporins was reported in 15% of *E. coli* isolates, 38% of *Klebsiella* spp. isolates, and 37% of *Enterobacter* spp. isolates. Carbapenem resistance was reported in 17% of *Klebsiella* spp. isolates, 26% of *P. aeruginosa* isolates, and 82% of *Acinetobacter baumannii* isolates.

Drinking water is considered one of the most overlooked and underestimated sources of healthcare associated infections (HAIs).

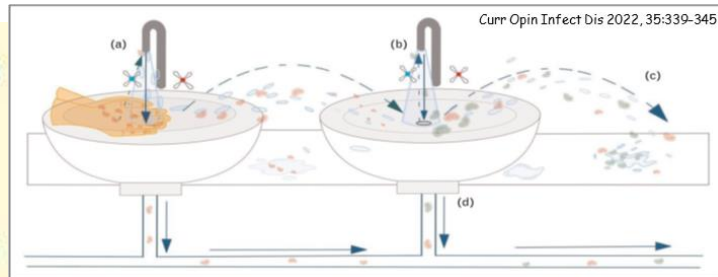
Legionella pneumophila

Pseudomonas aeruginosa

Mycobacterium avium

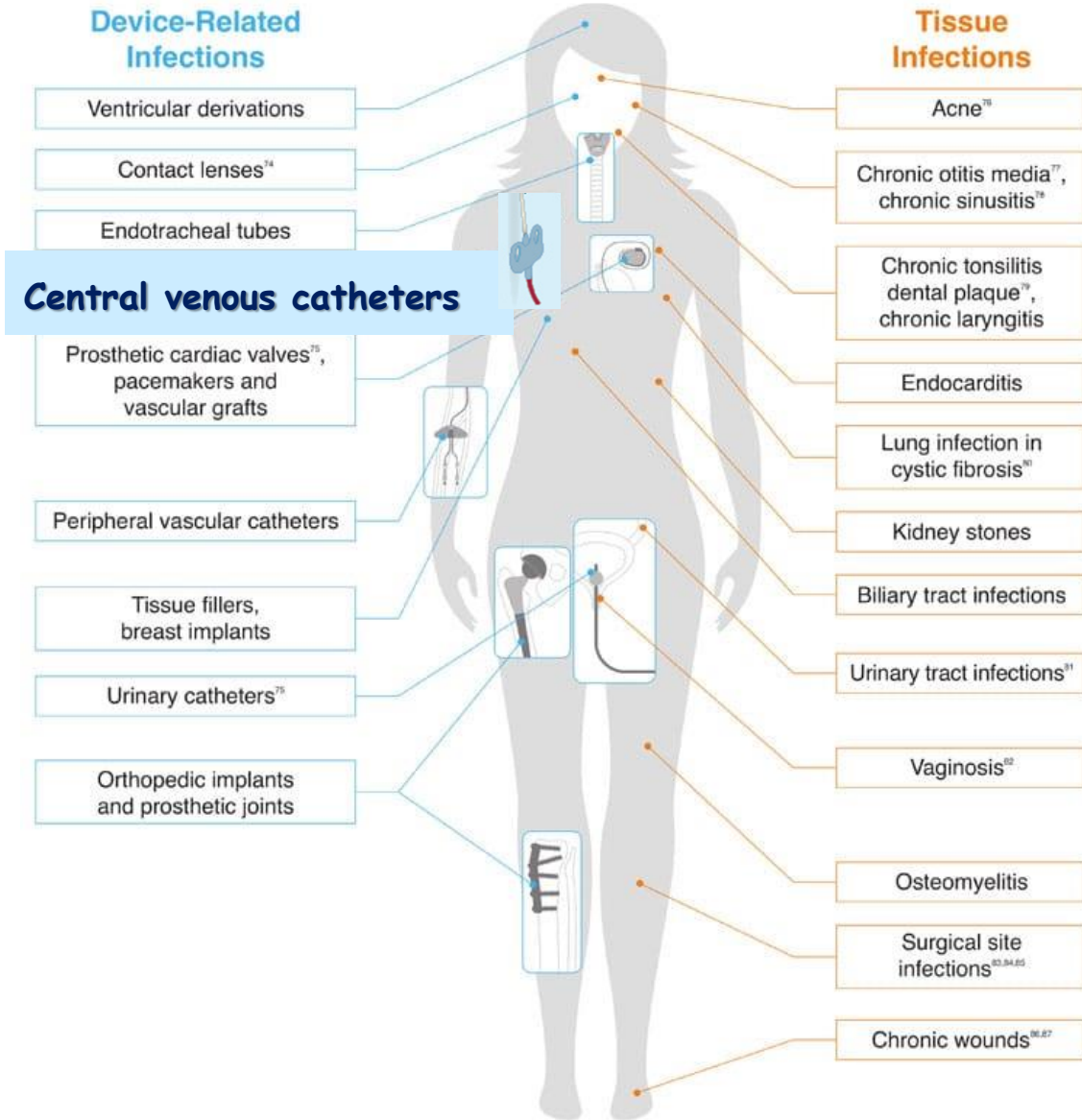
Species, such as *S. aureus*, *K. pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens* and *E. coli* have been found in premise plumbing and linked to waterborne HAI outbreaks.

Potential Transmission Routes from Water to Patients



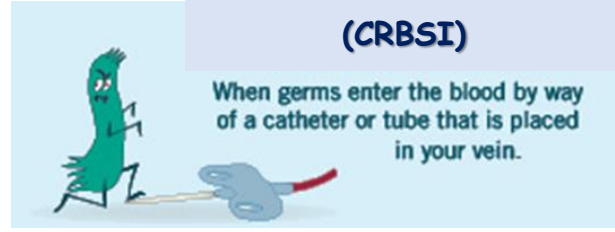
Each year on 5 May, the "SAVE LIVES: Clean Your Hands"

Chronic infections: Medical biofilms

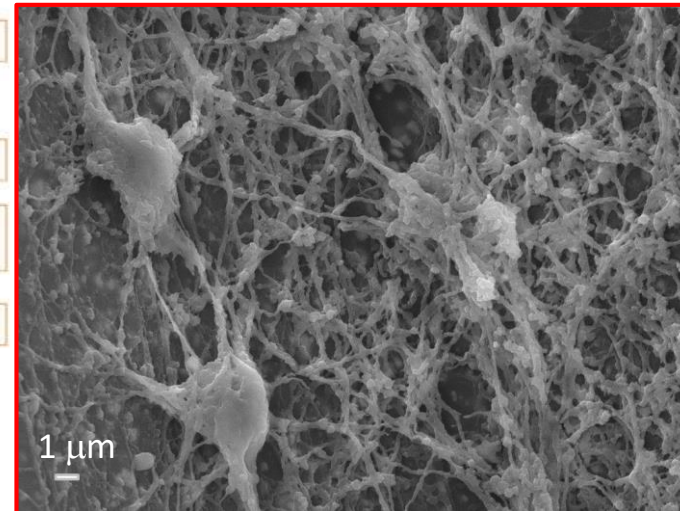
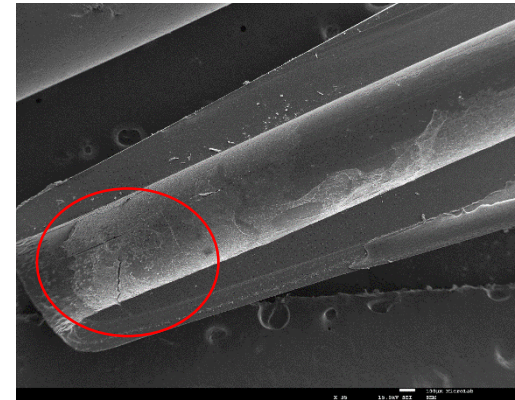


<https://biofilm.healthcare/where-do-biofilms-exist/>

Catheter related bloodstream infections (CRBSI)



<https://professionals.site.apic.org/infection-prevention-basics/learn-about-hais/>



Microorganisms	Portugal (n= 380)	Slovakia (n= 21)	Romania (n= 187)	Germany (n= 2974)	France (n= 2467)	U.K. (n= 103)	Total (n=7 929)
Coagulase-negative staphylococci	10.8	9.5	0	30.3	19.5	19.4	23.6
<i>Enterococcus</i> spp	7.9	9.5	9.1	20.2	12.4	13.6	14.9
<i>S. aureus</i>	9.7	9.5	18.2	13.9	12.0	16.5	12.0
<i>Klebsiella</i> spp	21.1	23.8	34.8	7.5	12.8	15.5	12.4
<i>E. coli</i>	6.3	4.8	4.3	8.5	11.6	13.6	9.2
<i>P. aeruginosa</i>	19.2	19.0	13.4	4.0	12.8	2.9	9.5
<i>Enterobacter</i> spp	10.0	4.8	1.1	4.6	14.1	3.9	8.3
<i>Candida</i> spp	6.8	4.8	0.0	6.4	0	10.7	4.5
<i>Serratia</i> spp	5.5	0.0	0.0	0.0	3.1	2.9	3.4
<i>Acinetobacter</i> spp	2.6	14.3	19.3	7.1	0.6	1.0	2.3

Adapted from Table 3: Number of isolates and percentages of the ten most frequently isolated microorganisms in ICU-acquired bloodstream infection (BSI) episodes by country, EU/ EEA, 2017, Healthcare-associated infections acquired in intensive care units - Annual Epidemiological Report 2019

Microbiologically confirmed CRBSI defined as a BSI occurring 48h before or after CVC removal, and a positive culture of the same microorganism from either a) CVC, or b) blood, or c) pus from the insertion site or differential delay positivity of blood samples.

<https://ecdc.europa.eu/en/publications-data/healthcare-associated-infections-acquired-intensive-care-units-annual>

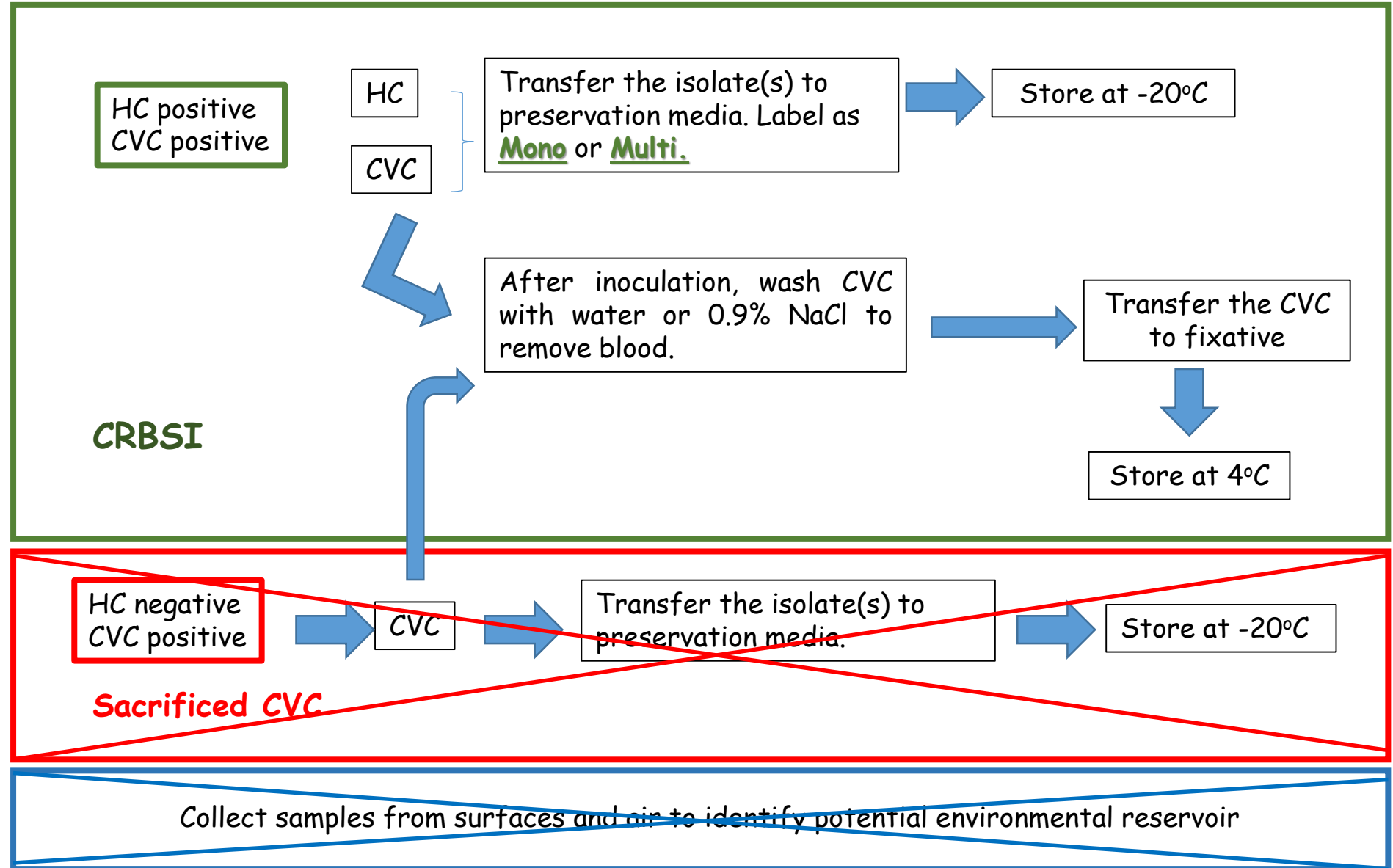
Study outline

A
149

B
262

C
350 beds

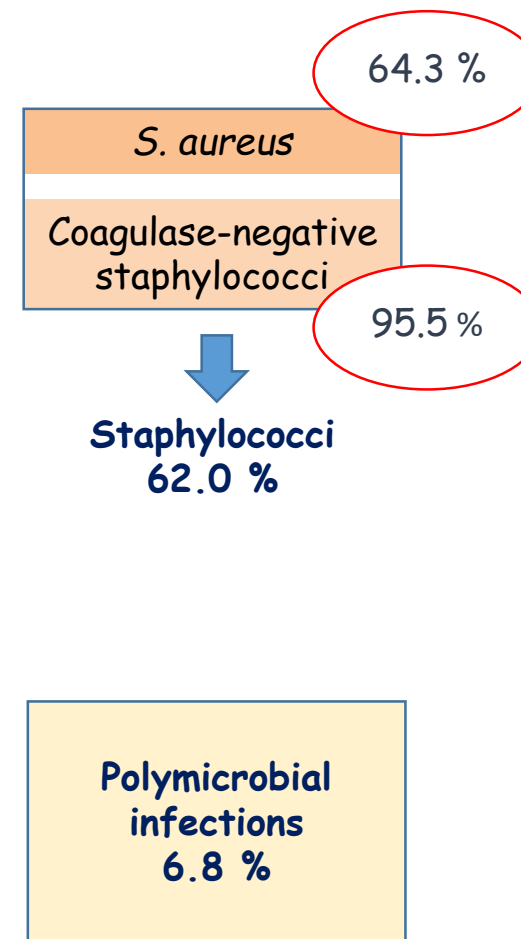
1 year
or
100 CRBSI cases



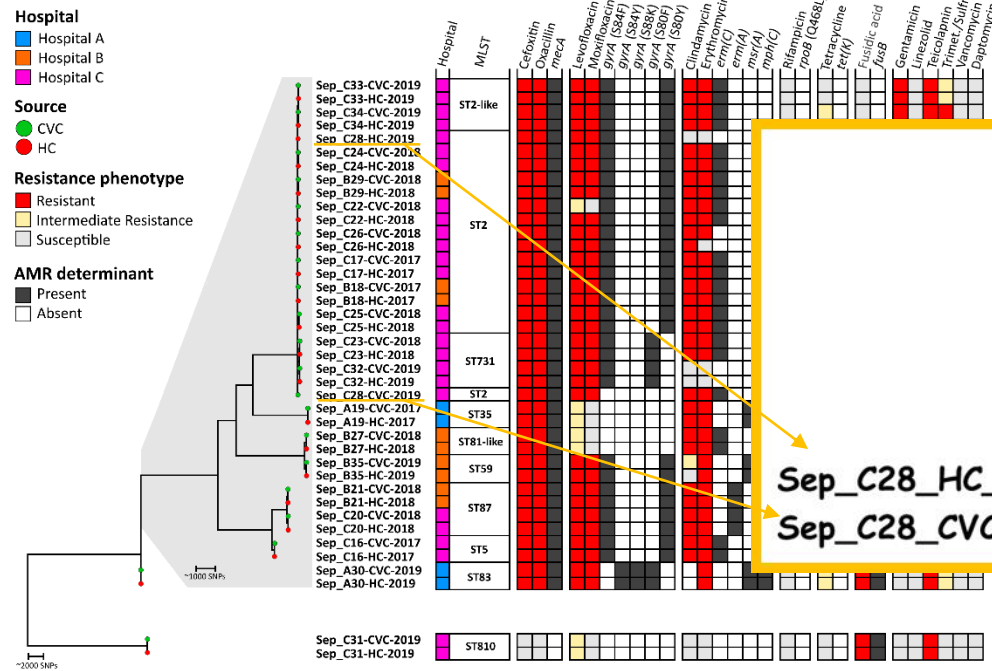
HC: hemoculture, CVC: central venous catheter

3-years prospective study (March 2017- February 2020)

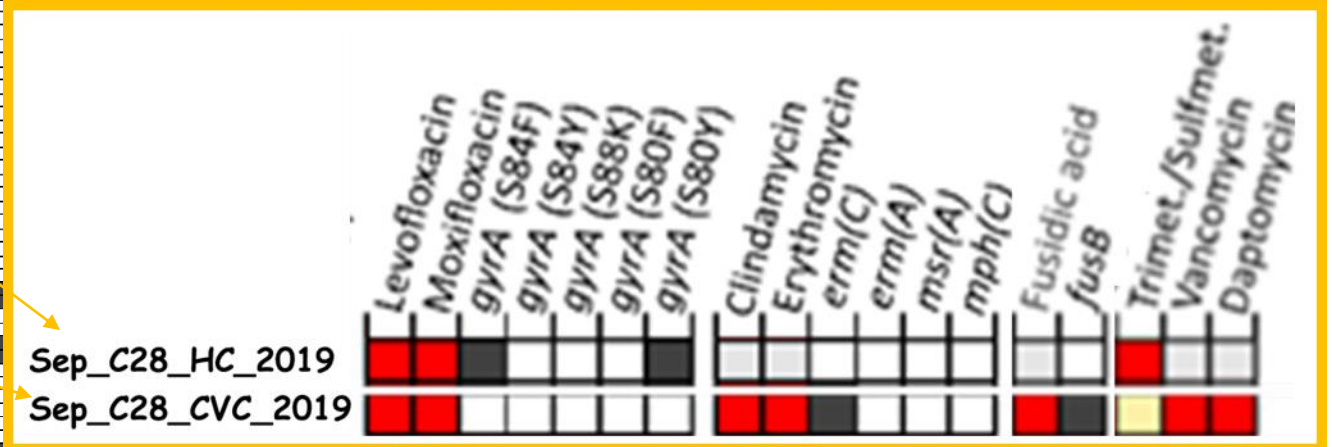
Etiological agent(s)	CRBSI [n (%)]			
	Hospital A	Hospital B	Hospital C	Overall
<i>S. aureus</i>	6 (40 %)	4 (28.5%)	4 (13.8%)	14 (24.1%)
<i>S. epidermidis</i>	3 (20%)	5 (35.7%)	13 (44.8%)	21 (36.2%)
<i>S. haemolyticus</i>		1 (7.1%)		1 (1.7%)
<i>K. pneumoniae</i>	3 (20%)	1 (7.1%)	6 (20.7%)	10 (17.2%)
<i>P. aeruginosa</i>			2 (6.9%)	2 (3.4%)
<i>Enterococcus faecalis</i>	1 (6.7%)			1 (1.7%)
<i>Serratia marcescens</i>	1 (6.7%)			1 (1.7%)
<i>Candida glabrata</i>			1 (3.4%)	1 (1.7%)
<i>Candida parapsilosis</i>		2 (14.3%)	1 (3.4%)	3 (5.1%)
<i>K. pneumoniae, S. epidermidis</i>			1 (3.4%)	1 (1.7%)
<i>S. marcescens, E. faecalis</i>		1 (6.7%)		1 (1.7%)
<i>S. marcescens, P. aeruginosa</i>	1 (6.7%)			1 (1.7%)
<i>E. cloacae, C. parapsilosis</i>			1 (3.4%)	1 (1.7%)
Total (n/ %)	15 (25.9%)	14 (24.1%)	29 (50.0%)	58 (100%)



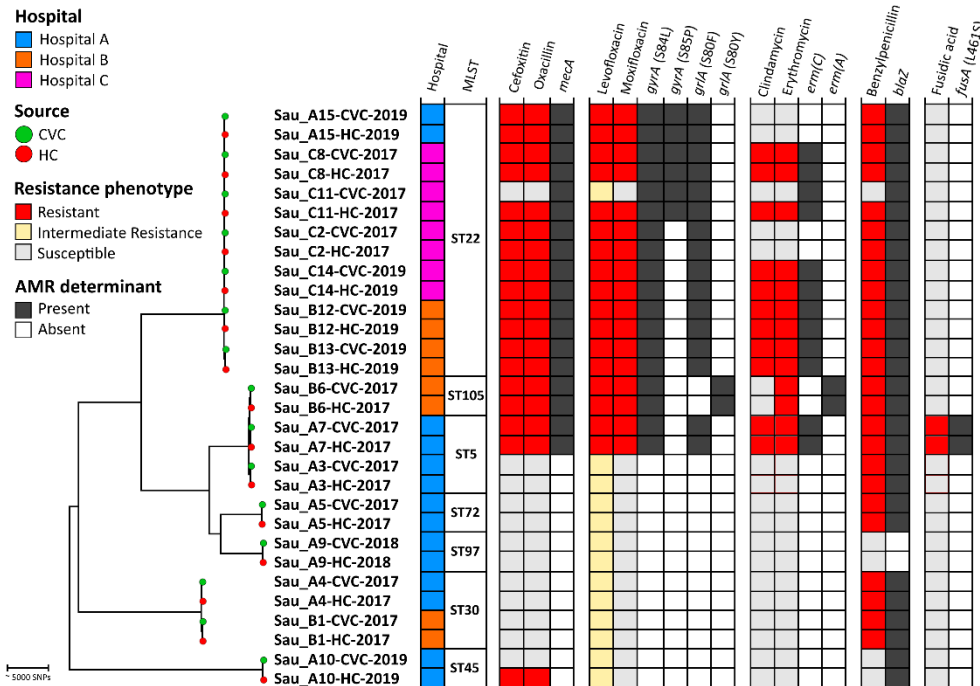
High rates of multidrug resistance (MDR)



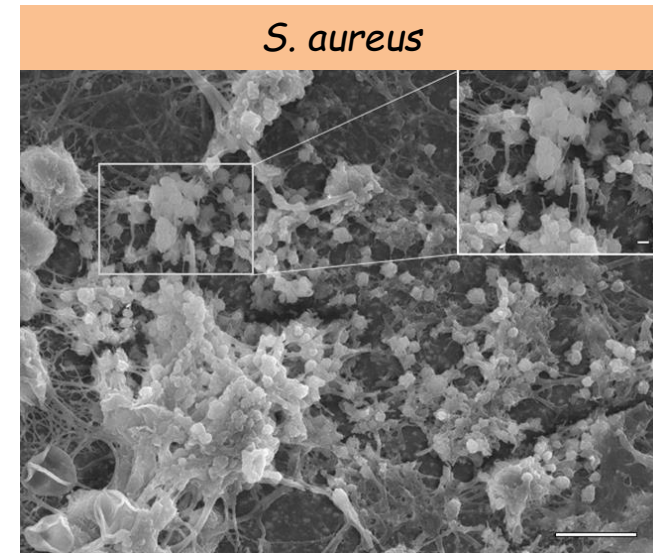
Coagulase-negative staphylococci *S. epidermidis*



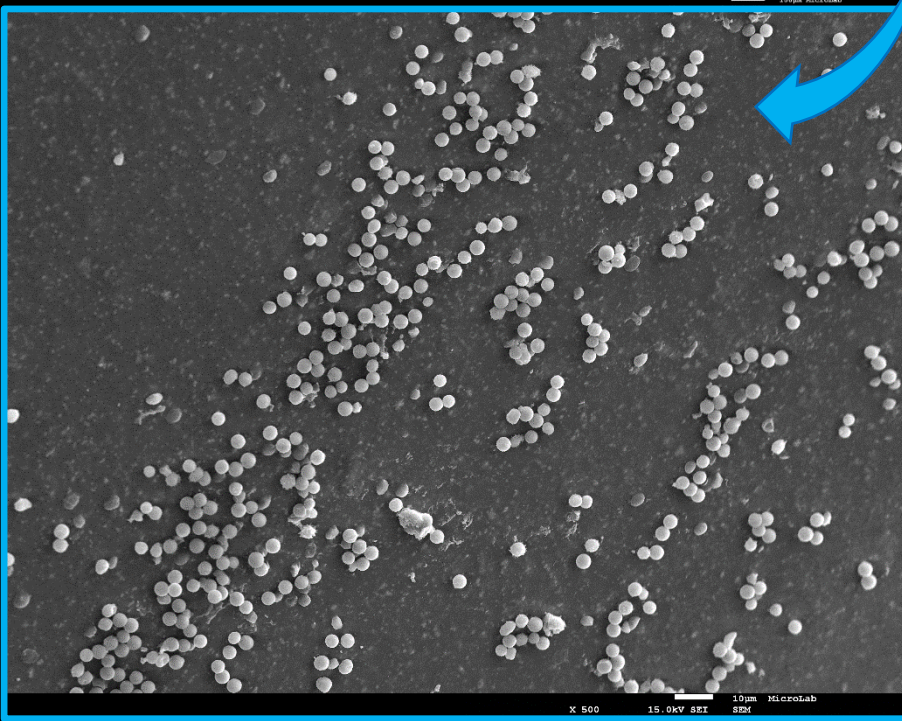
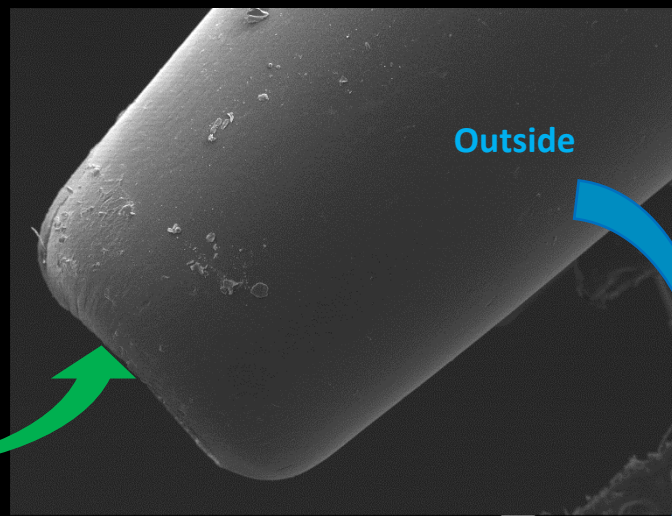
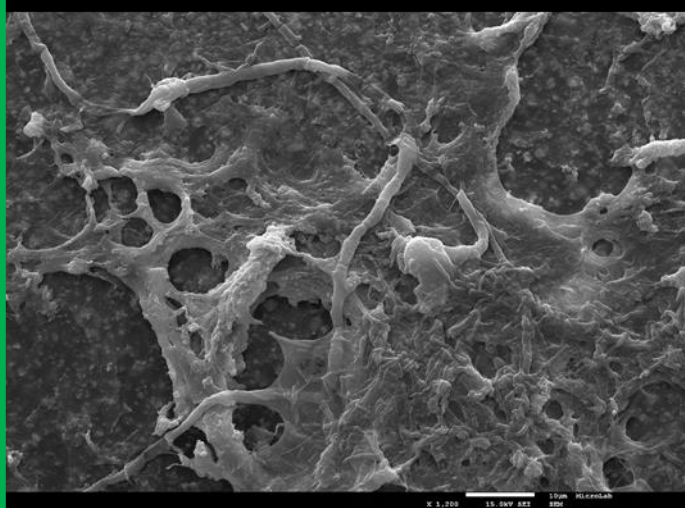
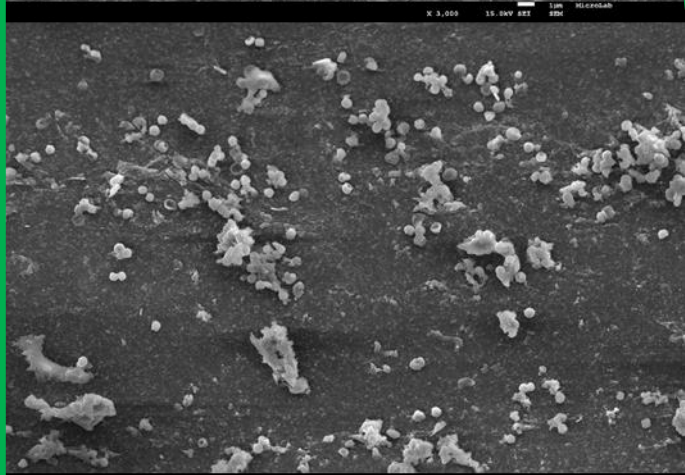
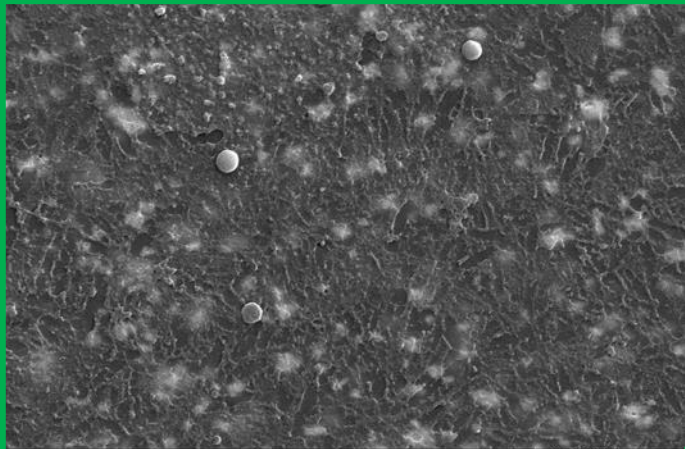
Biofilm positive: 50.0 %



S. aureus



Biofilm positive: 48.6 %



Inside

Outside

X 500 15.0kV SSI 10µm MicroLab SEI

Polymicrobial
infections
6.8 %

♀ 65 years old

E. cloacae complex and
C. parapsilosis

Dwell time: 4 days
Insertion at Subclavian
vein

♂ 72 years old

Serratia marcescens and
Enterococcus faecalis

Dwell time: 13 days
Insertion at Jugular vein

S. marcescens: 3rd generation
cephalosporins (R)

E. faecalis pan susceptible

♂ 61 years old

Klebsiella pneumoniae and
S. epidermidis

Dwell time: Not known
Insertion at Jugular vein

♀ 74 years old

Serratia marcescens and
Pseudomonas aeruginosa

Dwell time: 4 days
Insertion at Jugular vein

S. marcescens: Carbapenem
(R) and 3rd cephalosporins (I)

P. aeruginosa: Penicillin (R)

K. pneumoniae: Carbapenem
(R) and 3rd cephalosporins (R)

MRSE

Polymicrobial CRBSI case

♀ 65 years old

E. cloacae complex and

C. parapsilosis

Dwell time: 4 days

Insertion at Subclavian vein

Comparative genomics confirmed the isogenicity of CVC/HC isolate pair thus supporting the scenario of **CVC-mediated bloodstream bacterial/ fungal coinfection**.

C. parapsilosis isolates were confirmed *in silico* to belong to group I-*C. parapsilosis sensu stricto*¹.

E. cloacae complex isolates belong to ST599 and were identified as ***Enterobacter bugandensis*** (% of nucleotide identify to the type strain of 99.58% for *rpoB*, 98.67% for *gyrB*, 99.70% for *infB*, 99.57% for *atpD*, 99.51% for *hsp60* and 99.86% for 16SrRNA)².

¹Tavanti, A et al. *Candida orthopsilosis* and *Candida metapsilosis* Spp. Nov. to Replace *Candida parapsilosis* Groups II and III. *Journal of Clinical Microbiology* 2005, 43, 284-292.

² Singh, N.K. et al. Multi-Drug Resistant *Enterobacter bugandensis* Species Isolated from the International Space Station and Comparative Genomic Analyses with Human Pathogenic Strains. *BMC Microbiology* 2018, 18, 1-13

Antibiotic	Source	Central Venous Catheter		Hemoculture	
		MIC (mg/L)	Phenotype	MIC (mg/L)	Phenotype
Ticarcillin		≥ 128	R	≥ 128	R
Piperacillin/ Tazobactam		≥ 128	R	≥ 128	R
Ceftazidime		≥ 64	R	≥ 64	R
Ceftolozane/ Tazobactam		2	R	1	S
Cefepime		0.25	S	≤ 0.12	S
Aztreonam		16	R	16	R
Imipenem		≤ 0.25	S	≤ 0.25	S
Meropenem		≤ 0.25	S	≤ 0.25	S
Amikacin		2	S	≤ 1	S
Gentamicin		≤ 1	S	≤ 1	S
Tobramycin		≤ 1	S	≤ 1	S
Ciprofloxacin		≤ 0.06	S	≤ 0.06	S
Levofloxacin		≤ 0.12	S	≤ 0.12	S
Tigecyclin		≤ 0.5	S	≤ 0.5	S
Trimethoprim/Sulfamethoxazole		≤ 20	S	≤ 20	S
Colistin		> 8	R	> 8	R

Similar antimicrobial susceptibility profiles

Resistance to at least five different antimicrobials including different beta-lactam antibiotics and colistin.

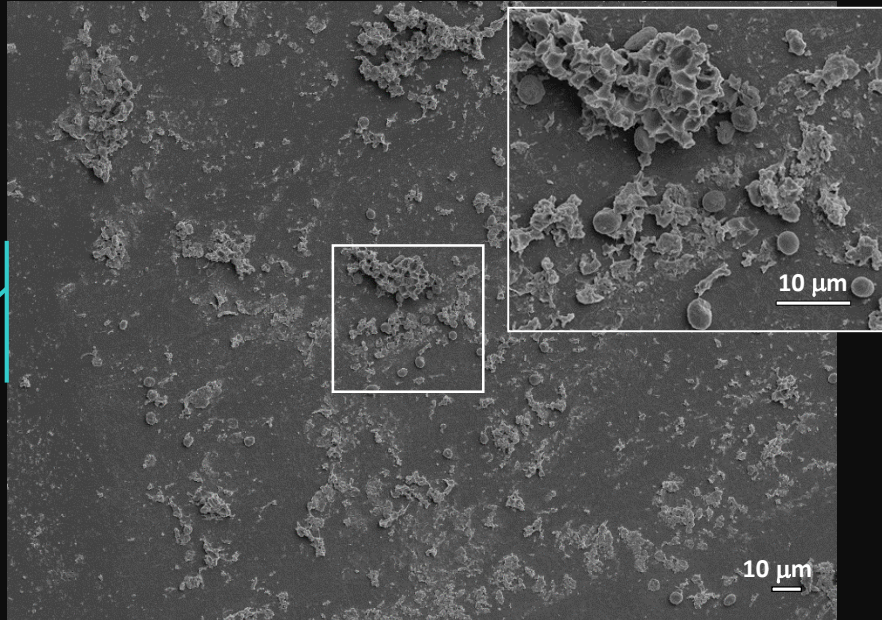
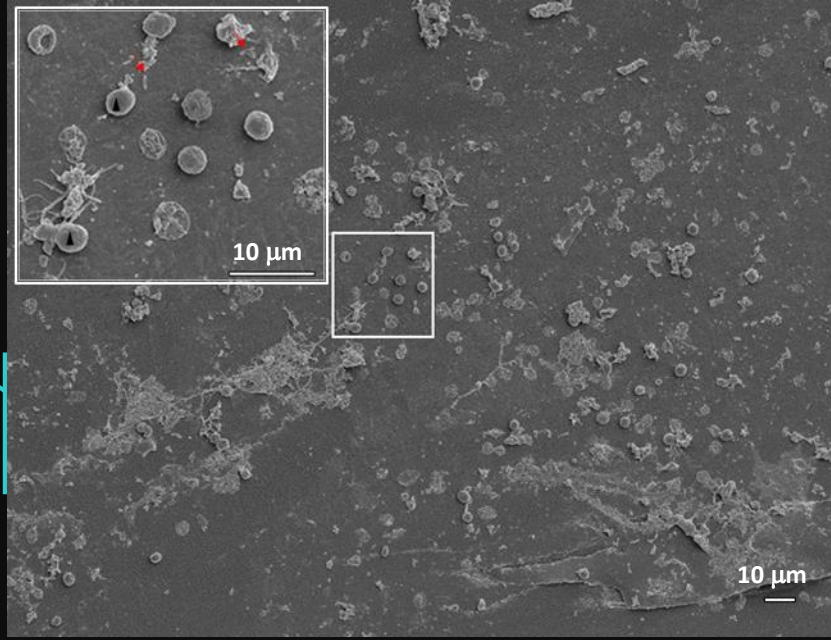
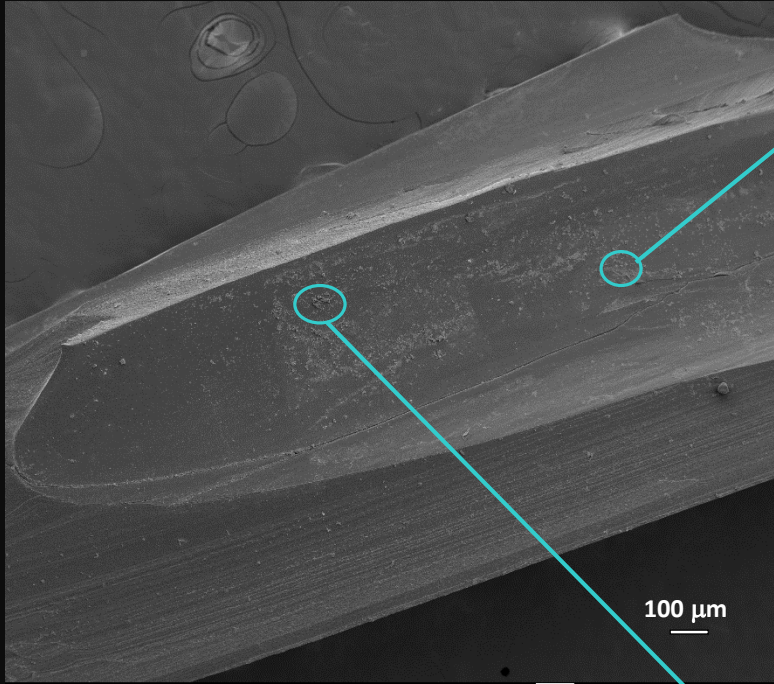
CD	Antibiotic	MIC (mg/L)	Phenotype	MIC (mg/L)	Phenotype	BLAST	%IDENTITY	HIT (AMR / Virulence gene)	HIT DESCRIPTION	HIT ACCESSION
	Ceftolozane / Tazobactam	2	R	1	S					
Eccom	Aztreonam	16	R	16	R					54792.1-1620
Eccom	Imipenem	≤ 0.25	S	≤ 0.25	S					0048:4338625-4340269
Eccom	Meropenem	≤ 0.25	S	≤ 0.25	S					54792.1
Eccom	Amikacin	2	S	≤ 1	S					4AN01000063.1
Eccom	Gentamicin	≤ 1	S	≤ 1	S					3785:4208384-4209544
Eccom	Tobramycin	≤ 1	S	≤ 1	S					
Eccom	Ciprofloxacin	≤ 0.06	S	≤ 0.06	S					
Eccom	Levofloxacin	≤ 0.12	S	≤ 0.12	S					
Eccom	Tigecyclin	≤ 0.5	S	≤ 0.5	S		77.34	Escherichia_coli_ampH	AmpH is a class C ampC-like beta-lactamase and penicillin-binding protein ide	AP012030.1:395554-396712
Eccom	Trimethoprim/Sulfamethoxazole	≤ 20	S	≤ 20	S		89.7	(Bla)blaACT-6	(Bla)blaACT-6	FJ237366:41-1186
Eccom	Colistin	> 8	R	> 8	R		89.7	ACT-6	ACT-6 is a beta-lactamase found in Klebsiella pneumoniae	FJ237366:41-1187
Eccomp_CVC_2017_38730	Beta-lactamase						100	blaACT-49	cephalosporin-hydrolyzing class C beta-lactamase ACT-49	NG_050712.1
								RESFINDER		
							100	blaACT-6_1	blaACT-6	FJ237366

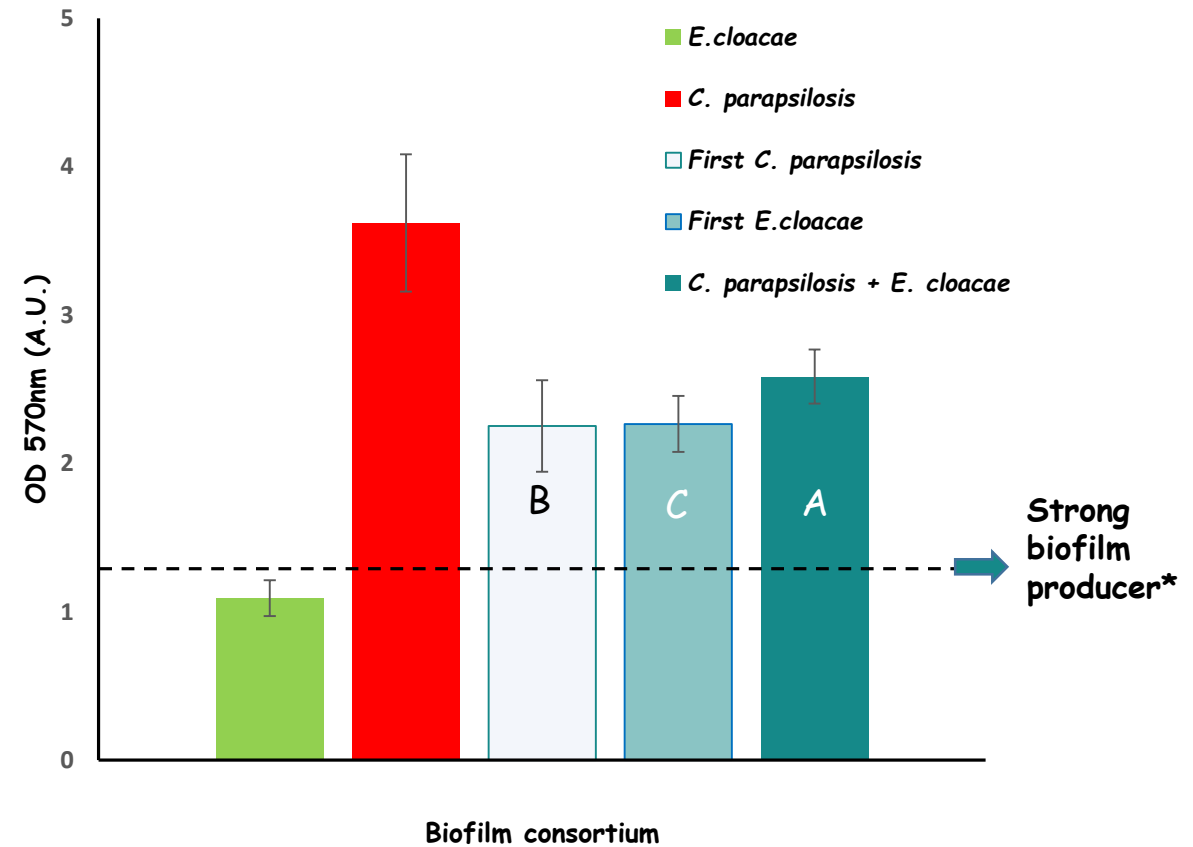
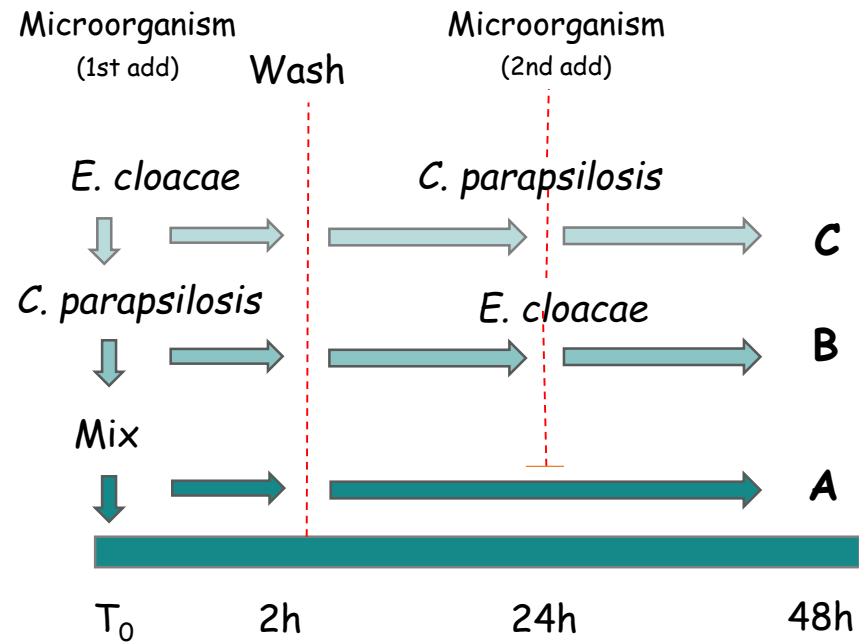
Ceftolozane / Tazobactam: CVC resistant and HC susceptible

SNP- mediated rpoS mediation was not confirmed

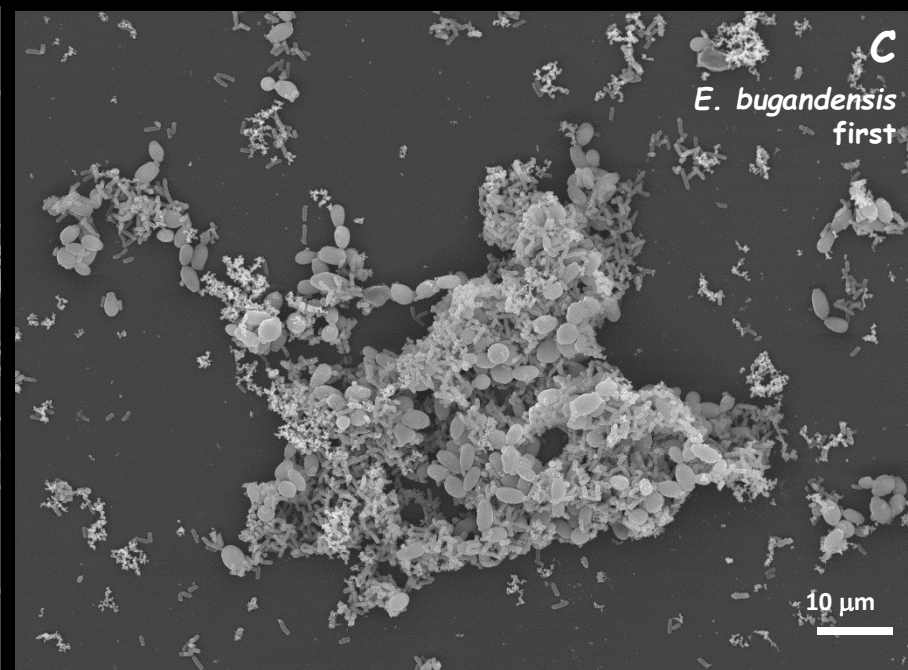
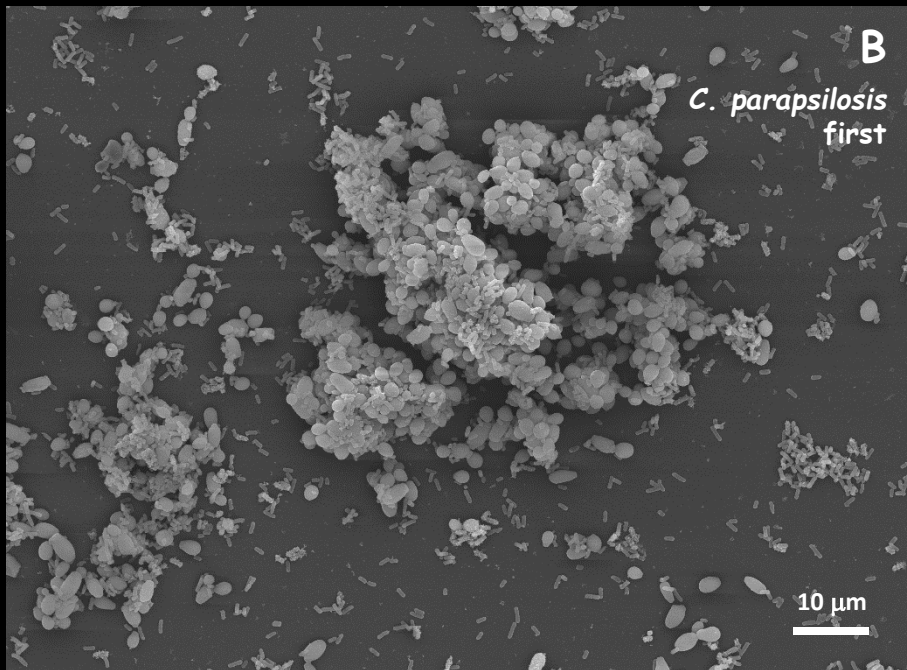
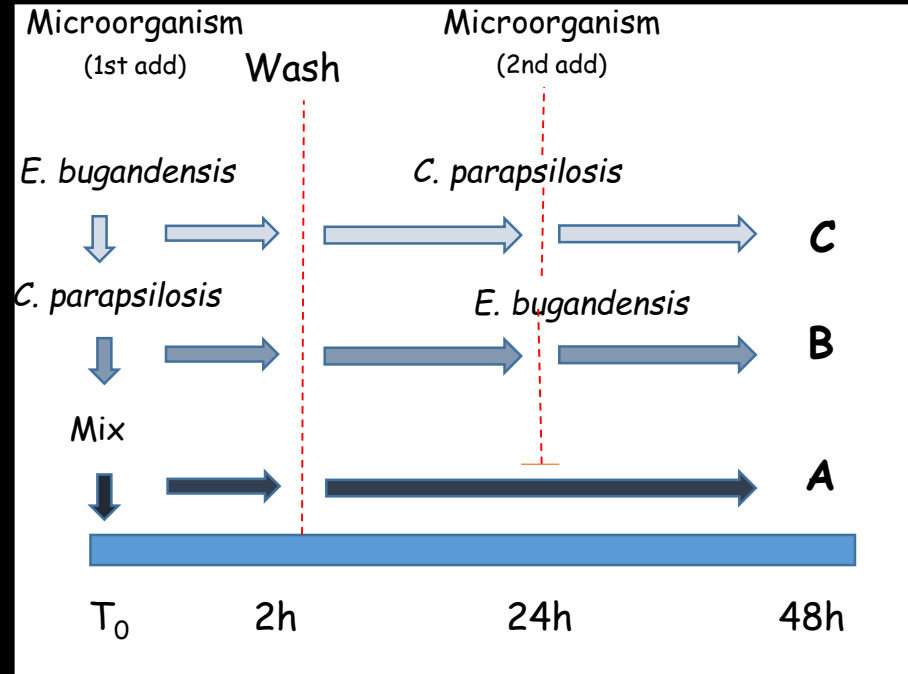
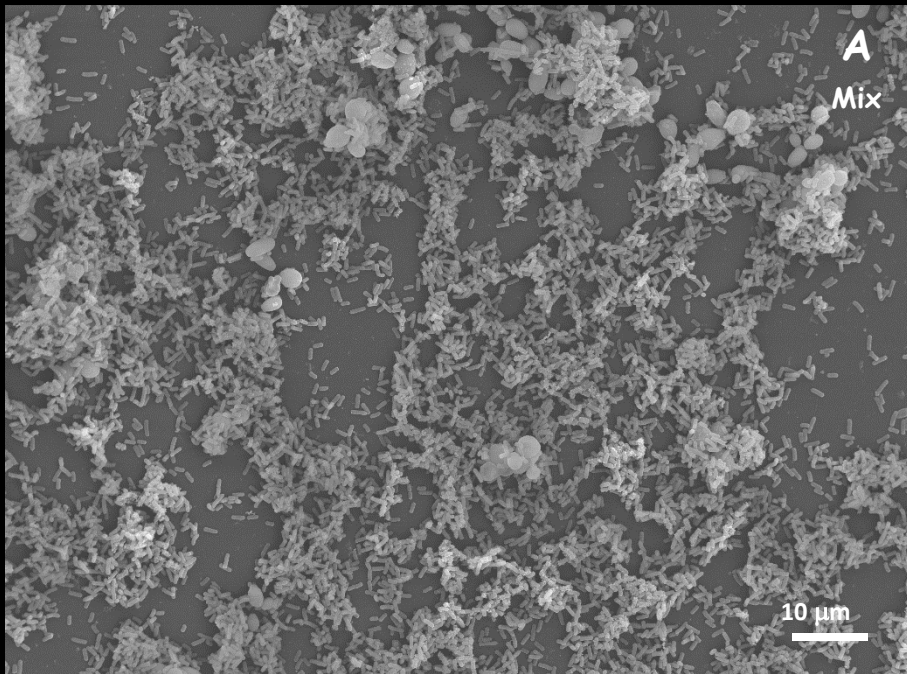
Antimicrobial agent	Source	Central Venous Catheter		Haemoculture	
		MIC (mg/L)	Phenotype	MIC (mg/L)	Phenotype
Fluconazole		> 256	R	>256	R
Amphotericin B		0.032	S	0.125	S
Anidulafungin		0.50	S	0.50	S

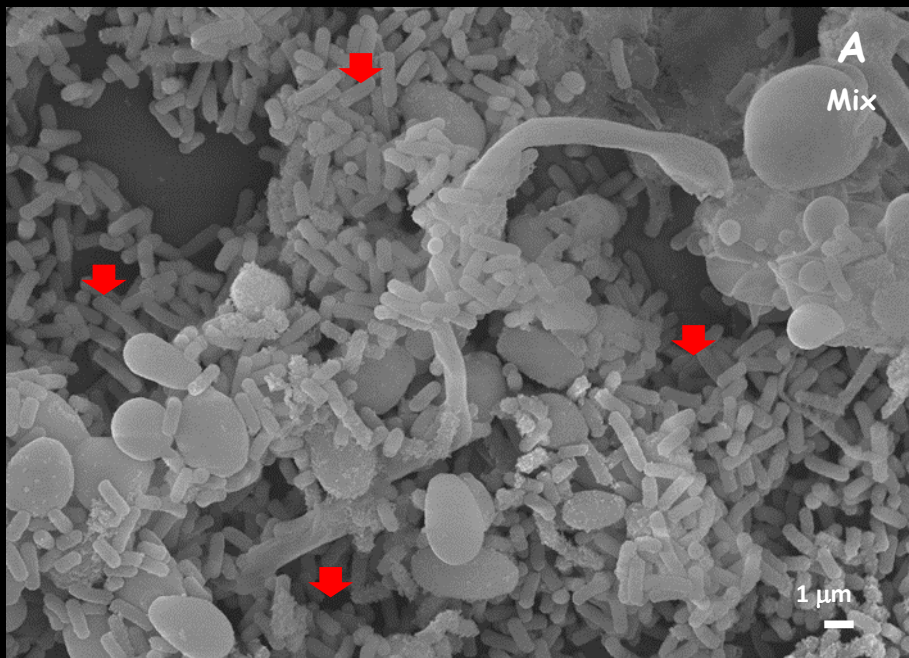
ERG11 gene harbored Y132F and R398I mutations due to the following nucleotide substitutions A395T and G1193T, respectively.





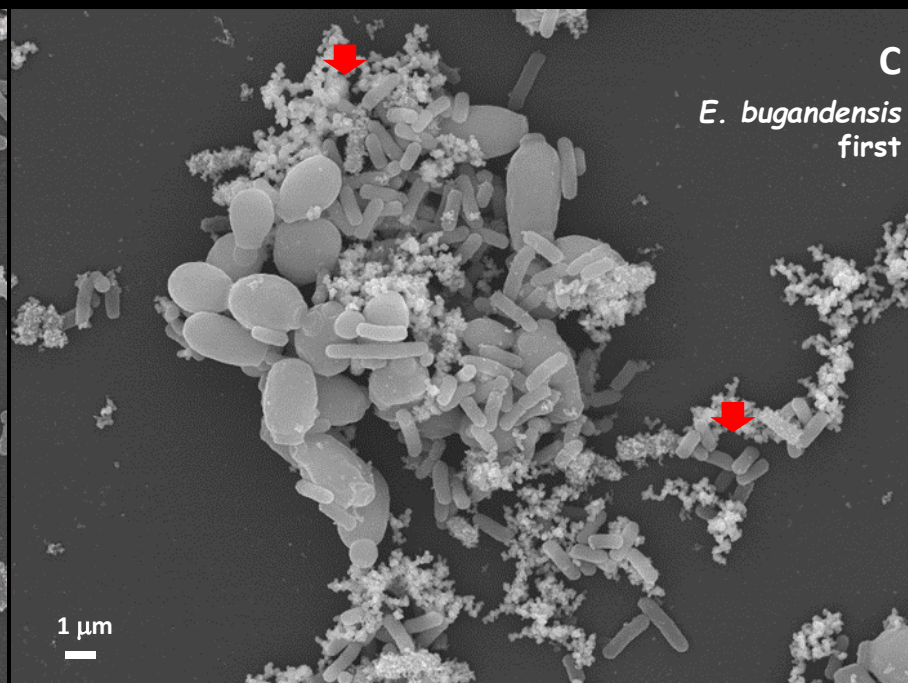
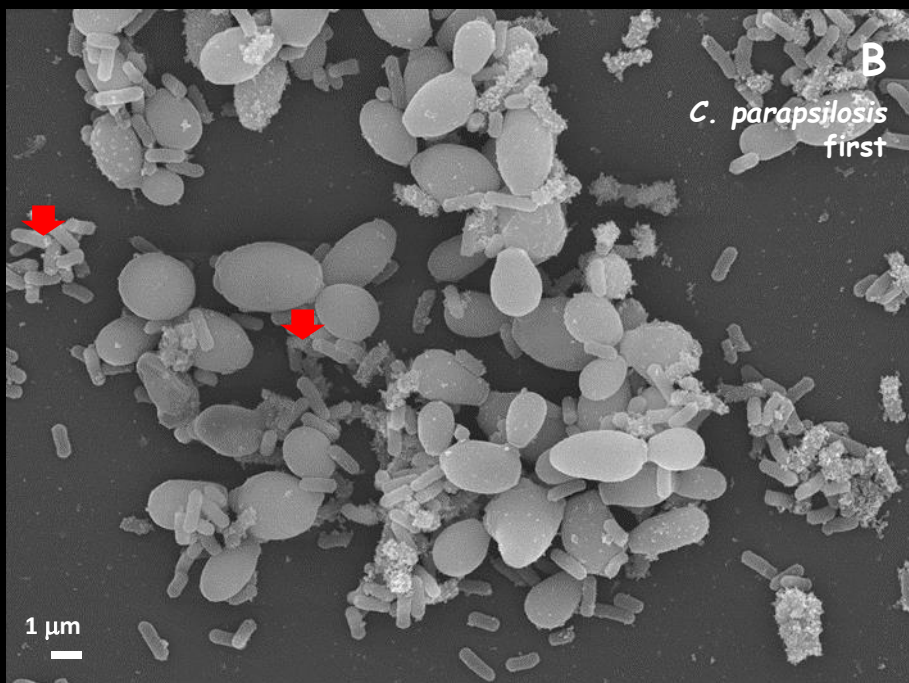
* Stepanovic S et al., *Apmis* 2007;115(8):891e9



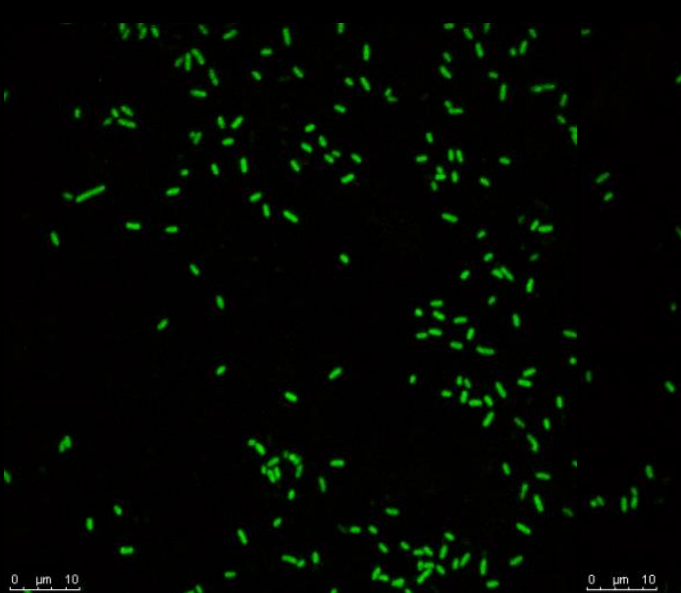


Biofilms started by a mixture of *C. parapsilosis* and *E. bugandensis* (A) are denser than those started by one of the microorganisms (B and C).

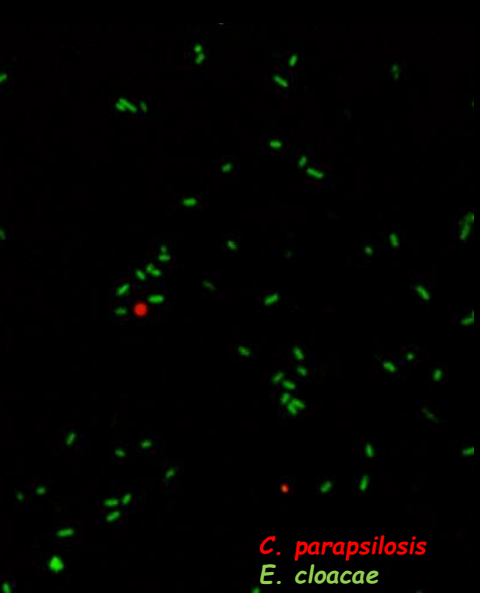
E. bugandensis starts biofilm assembly



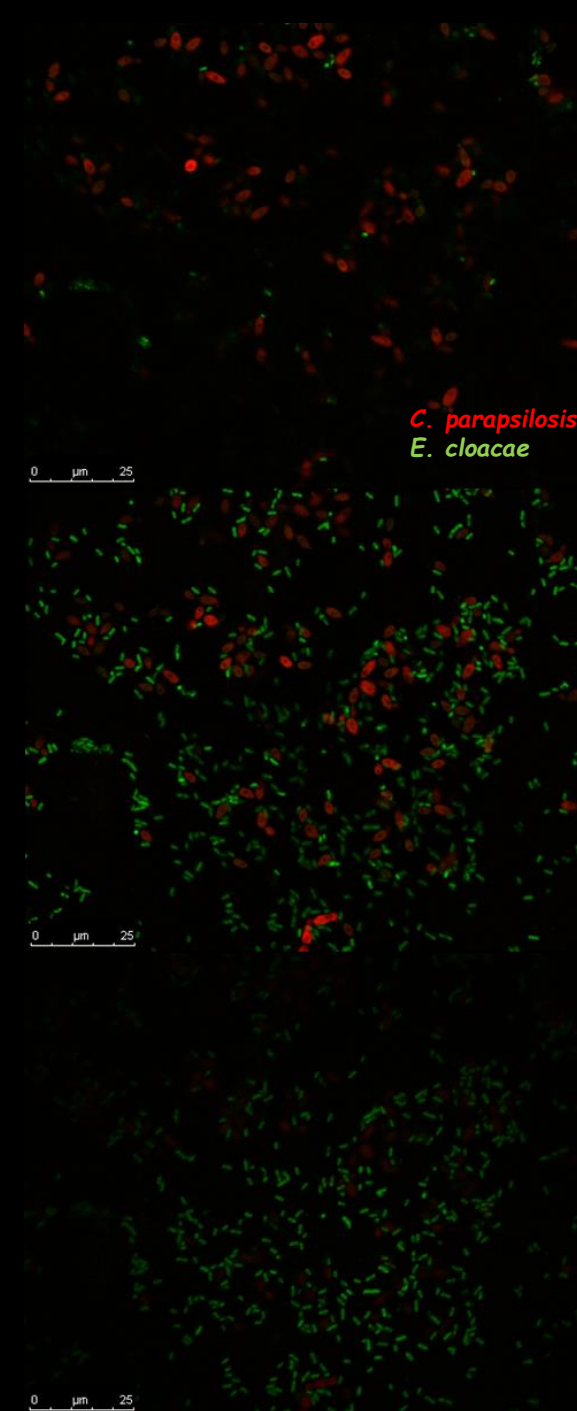
Environmental isolates
Glass



Bottom layer



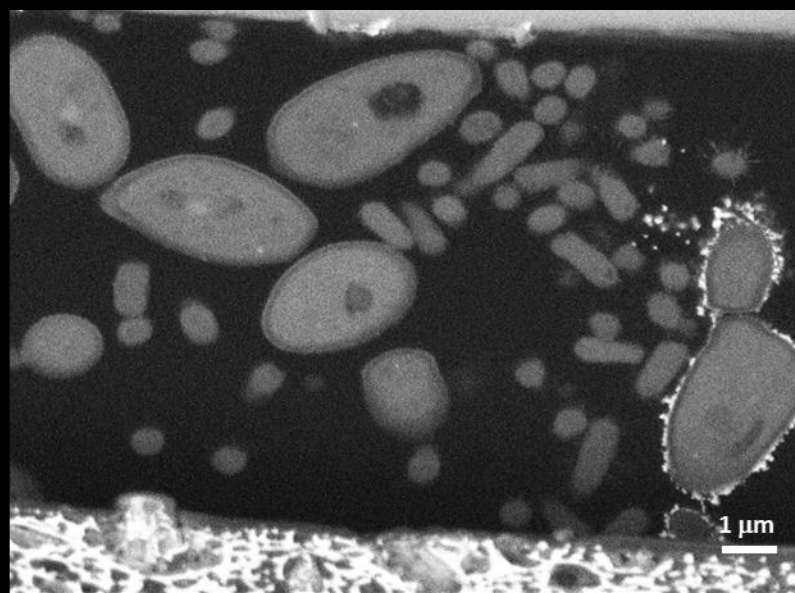
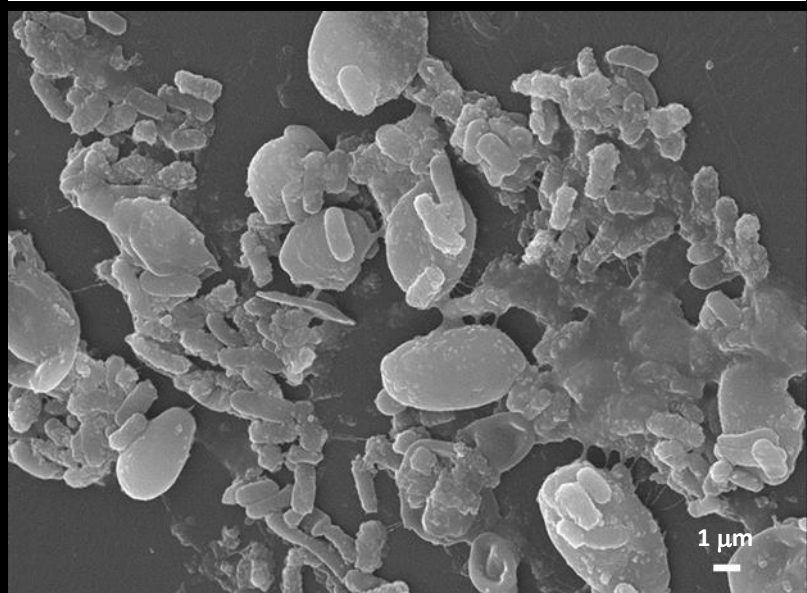
Top layer

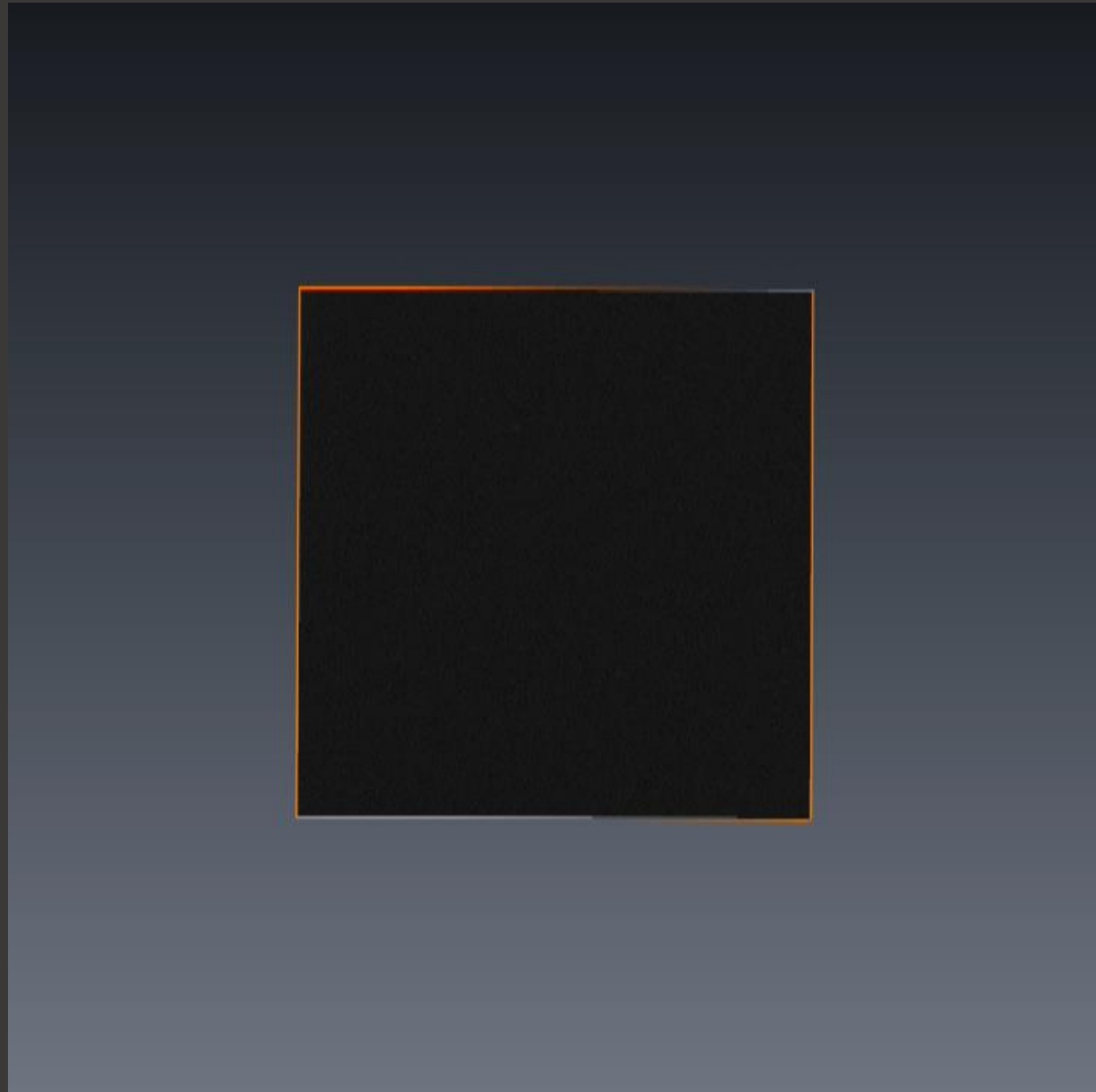


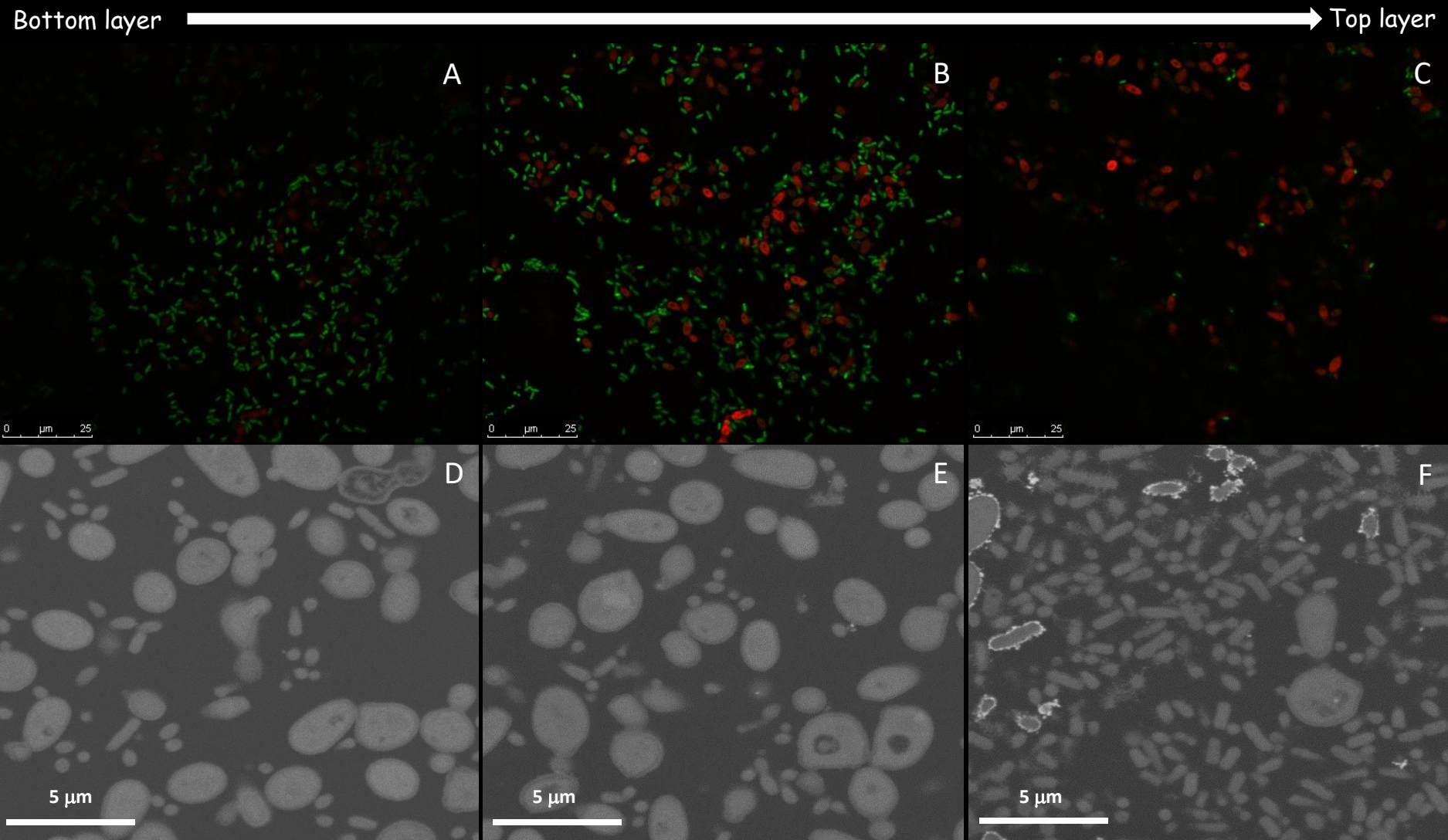
Glass
Top
layer

Bottom
layer

Polyurethane (CVC)







Different biofilm phenotypes on different materials.

FINAL REMARKS:

- Staphylococci are main CRBSI etiological agents with concerning rates of AMR.
- WGS could be a key tool to confirm the linkage between CVC and BSI. WGS also provides important insight on the genetic diversity and AMR signature of CRBSI etiological agents.
- The ability of these microorganisms to colonize CVCs might contribute to their success, but more prospective *in vitro* and *in vivo* studies are required to clarify the role of biofilms on CRBSI and other foreign-body related infections.
- Surface and microorganism's are key determinants for the biofilm phenotype.



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