Adaptation of the capacity to form biofilm in *Staphylococcus aureus* isolates during the course of human chronic bone and joint infections

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Introduction

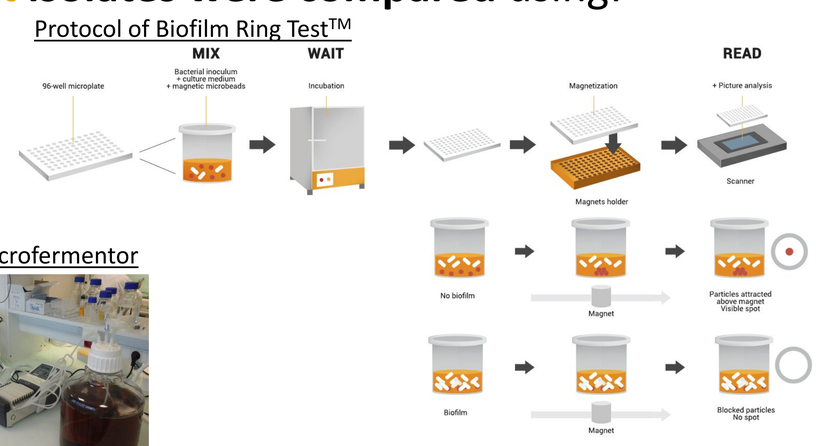
- Prosthetic joint infection (PJI) is associated with high rates of **chronicity** and **relapse** (10-20% of cases).
- One of the major bacterial mechanisms is **biofilm** formation, within which bacteria are protected from antimicrobials and host immune response.
- The present study aimed to determine and compare the biofilm formation ability of **3** pairs of isolates collected in 3 different patients during the **initial** and **recurrent** BJI episodes.

Materials and Methods

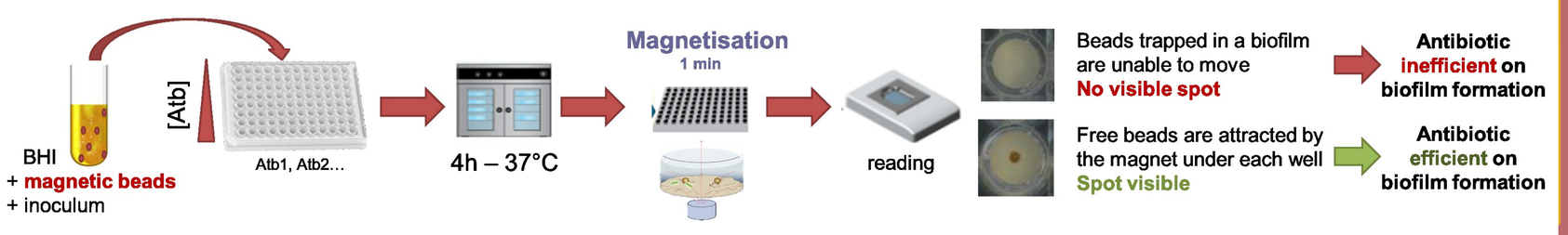
➢ **Three couples** (SM, GM and MD) of methicillin-susceptible *S. aureus* (MSSA) strains collected from patients with persisting or relapse of BJI were tested. The **biofilm formation capacity of the initial and recurrent isolates were compared** using:

- **Biofilm Ring Test™ assay** (EARLY KINETICS ADHESION)
  - using Brain Hearth Infusion media (BHI) – incubation time: 0, 2, 4, 6 and 24h
- **Crystal Violet assay** (MATURE BIOFILM CAPACITY)
  - using BHI + 1% glucose (BHIg) – incubation time : 24h
  - using a pool of human serum + 1% glucose (SERg)
    incubation time: 7, 14, 21 and 28 days
- **Microfermentors assay** on glass spatula (DYNAMIC BIOFILM CAPACITY)
  - using BHIg - evaluated by plate count – incubation time: 24h
- **Antibiofilmogram® assay**
  (BIOFILM MINIMAL INHIBITORY CONCENTRATION
  - using BHI – incubation time : 4h

Protocol of Biofilm Ring Test™

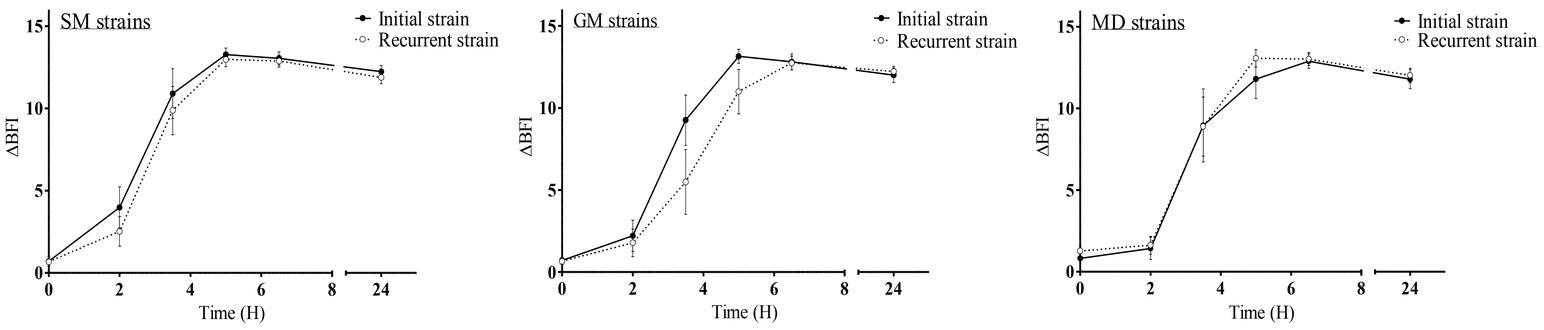


Picture of microfermentor



Results

Early biofilm formed of clinical *S. aureus* using the BioFilm Ring Test® technologie



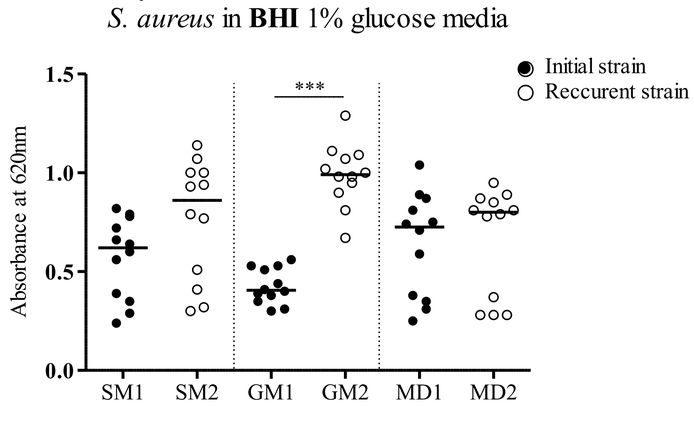
➡ **Early step** of biofilm formation **was similar** between initial and recurrent strains

	Cloxacillin	Teicoplanin	Vancomycin	Daptomycin	Fosfomycin	Ofloxacin	Rifampicin	Gentamicin	Fusidic Acid	Linezolid	Clindamycin
SM1	>16	16	>16	>8	>128	>64	< 0,0625	8	0,5	4	<0,125
SM2	>16	16	>16	8	>128	>64	< 0,0625	4	0,5	2	<0,125
GM1	4	4	>16	>8	>128	>64	< 0,0625	>8	>8	2	<0,125
GM2	8	>16	>16	>8	>128	>64	>8	>8	>8	>8	<0,125
MD1	>16	8	>16	>8	>128	>64	< 0,0625	>8	0,5	2	<0,125
MD2	>16	8	>16	8	>128	>64	< 0,0625	>8	0,25	2	<0,125

Interpretation according to CASFM-EUCAST breakpoint

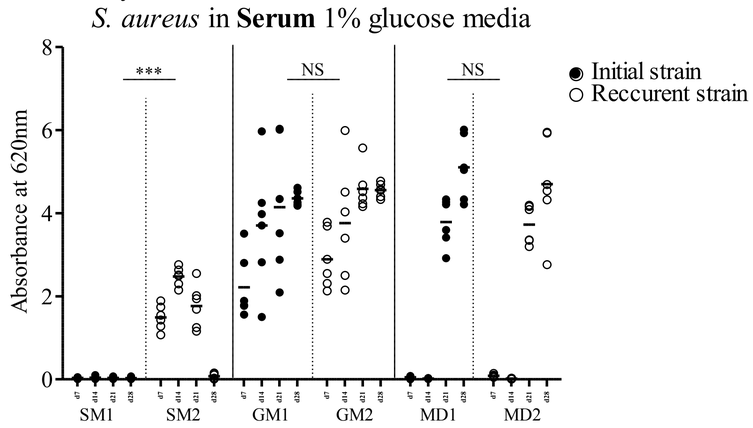
➡ **GM recurrent** isolate can form biofilm in presence of **higher concentration of rifampicin and linezolid** than initial isolate.

Crystal violet absorbance of clinical *S. aureus* in **BHI** 1% glucose media



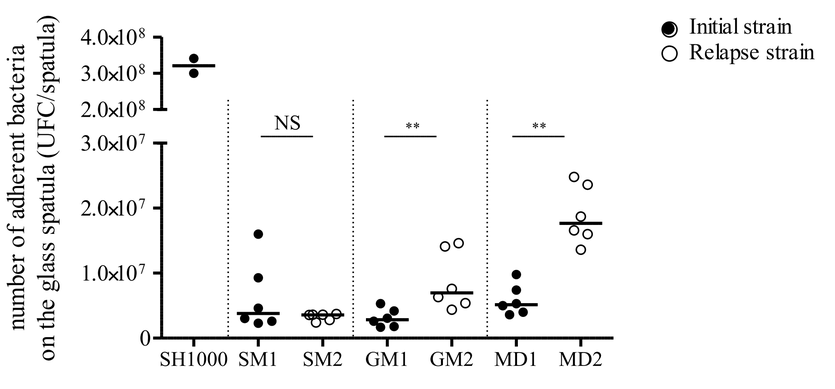
➡ **GM recurrent** isolate revealed a **higher** capacity to form **mature biofilm** in BHIg

Crystal violet absorbance of clinical *S. aureus* in **Serum** 1% glucose media



➡ **SM recurrent** isolate revealed a **higher** capacity to form **mature biofilm** in SERg

Biofilm formed on a glass spatula after 24h incubation in a **microfermentor** in BHIg1%



➡ **GM and MD recurrent** isolate revealed a **higher** capacity to form **biofilm** in dynamic model

Conclusion

- Our results suggest that ***S. aureus*** **PJI chronicization** is **associated** with an *in vivo* bacterial **adaptation/selection** regarding **biofilm formation**.
- Biofilm formation differed from one couple to another, depending of the experimental conditions, suggesting different adaptation processes.
- In any case, the enhanced capacity of biofilm formation affect the **recurrent** strains compared to **initial** stain **in each patient**.