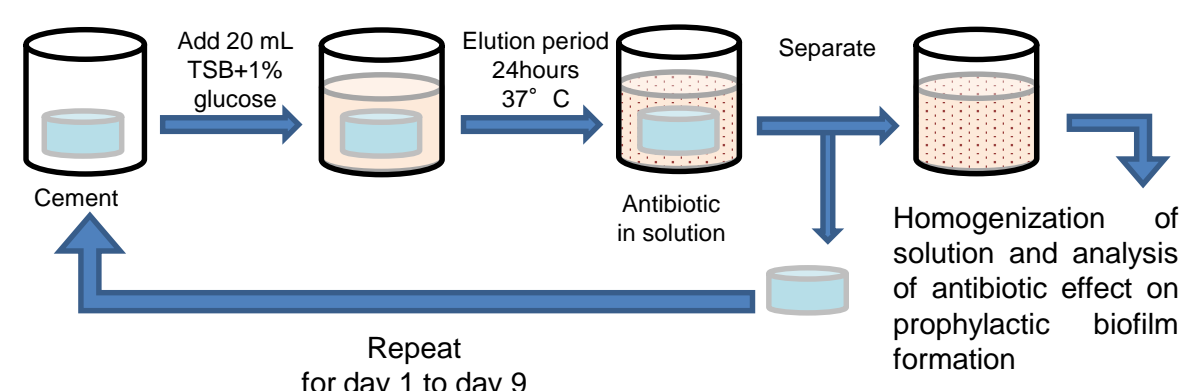


Background

A two-stage procedure can be proposed to manage prosthetic joint infections (PJI): after the infected prosthesis is removed, a bone cement spacer is inserted to avoid the filling by fibrous tissue. Following an adapted and effective antibiotherapy, the spacer is retrieved and a new prosthesis is implanted. Antibiotic-loaded bone cements (ALBCs) are frequently used in 2-stage procedures. They provide a local release of antibiotics to prevent the neoformation of biofilm. Here we propose to study *in vitro* the prophylactic effect of plain cement (no antibiotic), G cement (gentamicin), G+V cement (gentamicin+vancomycin) and G+C cement (gentamicin+clindamycin) in order to assess the interest of combining antibiotics in ALBCs to prevent biofilm formation.

MATERIAL & METHODS

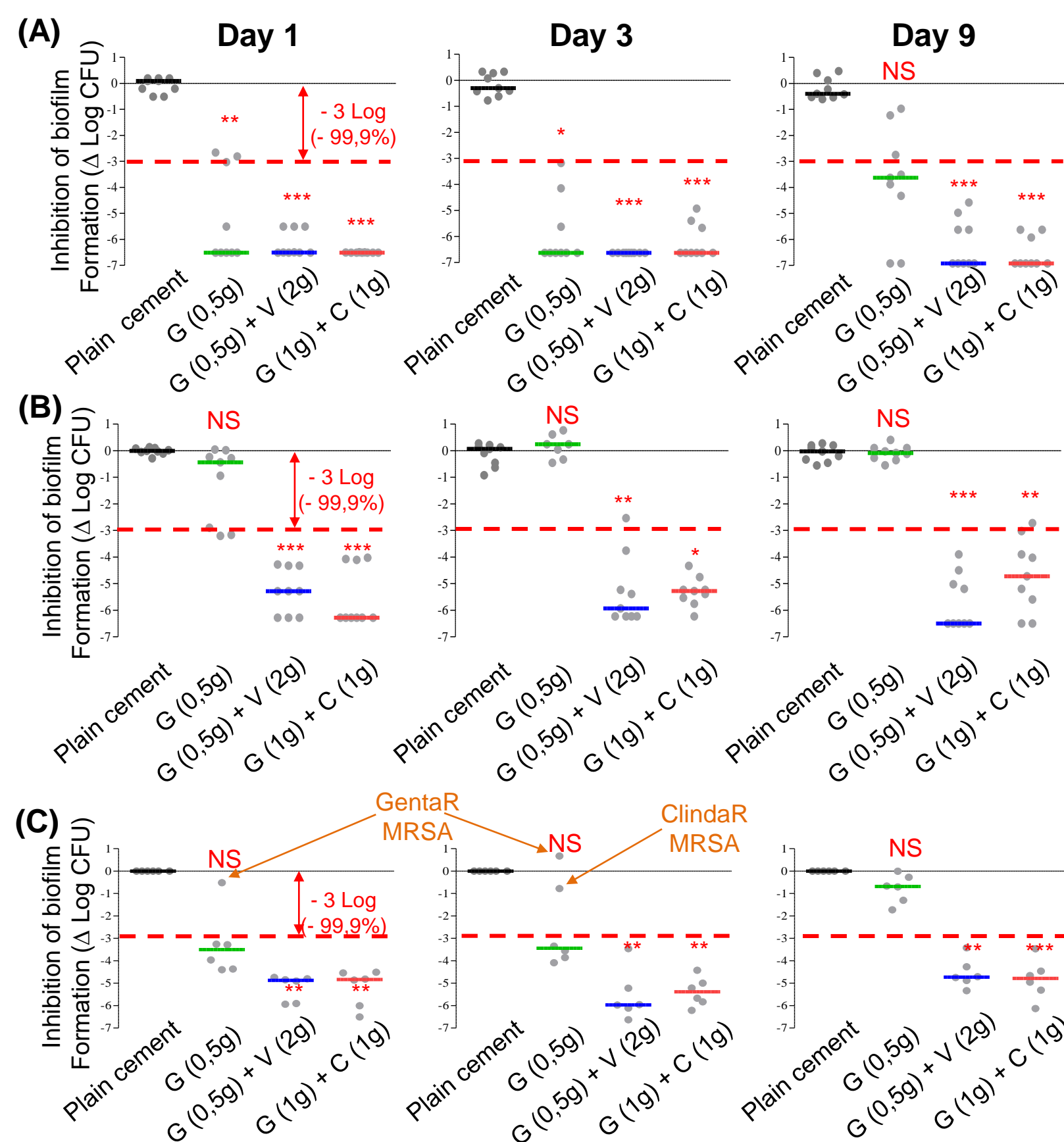
Standardized cylinders made from commercially available plain cement (no antibiotic), G cement (gentamicin), G+V cement (gentamicin and vancomycin) and G+C cement (gentamicin and clindamycin) were incubated in Tryptic Soy Broth with 1% glucose for 1 to 9 days at 37°C with medium changed each day. Biofilms of 6 clinical strains of *Staphylococcus aureus* and 5 clinical strains of *Staphylococcus epidermidis* with specific antibiotic susceptibilities were grown for 24h in a 96-well plate in elution solutions resulting from *in vitro* release from ALBCs at 1, 3 and 9 days. Biofilms were then softly rinsed using our homemade steam-based method (Tasse et al., 2018) and the number of viable cells in the biofilm was evaluated by plate counting.



Clinical strains used in this study

Methicillin-susceptible <i>S.aureus</i> (MSSA)	Fig. (A)
Methicillin-resistant <i>S.aureus</i> (MRSA)	Fig. (C)
Gentamicin-resistant MRSA	
Vancomycin-resistant MRSA	
Clindamycin-resistant MRSA	
Clindamycin-resistant MRSA	
Methicillin-susceptible <i>S.epidermidis</i> (MSSE)	Fig. (F)
Methicillin-resistant <i>S.epidermidis</i> (MRSE)	
Gentamicin-resistant MRSE	
Vancomycin-resistant MRSE	
Clindamycin-resistant MRSE	

S.aureus

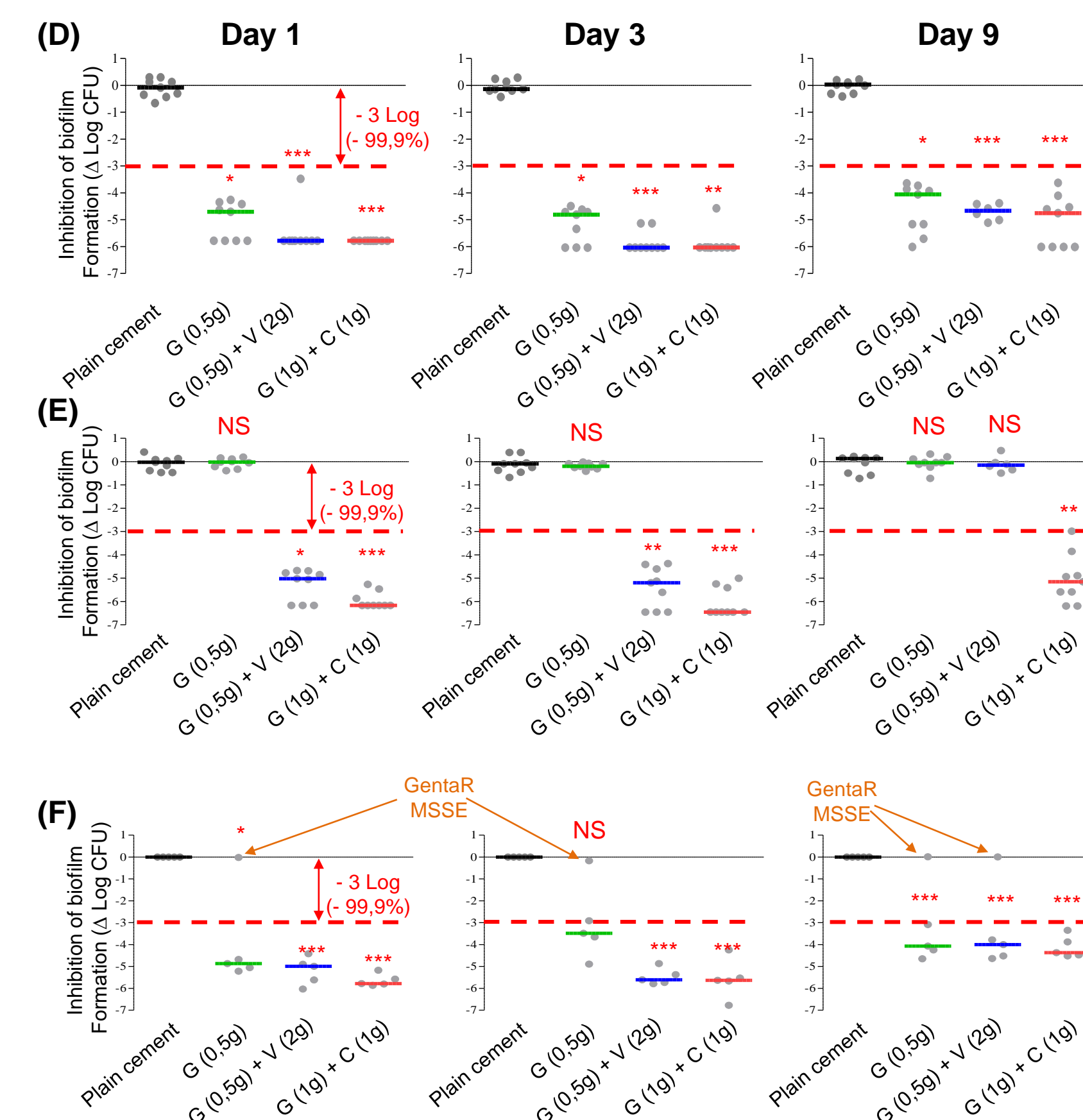


- G+V and G+C significantly decrease biofilm formation at D1, D3 and D9 in comparison with plain cement.
- G significantly decreases biofilm formation at Day 1, Day 3 but not at Day 9
- Combinations of antibiotics seem more effective than gentamicin alone against MSSA biofilm formation at Day 9

- G+V and G+C significantly decrease biofilm formation at D1, D3 and D9 in comparison with plain cement.
- G is not effective against GentaR MRSA biofilm formation
- Combinations of antibiotics seem more effective than gentamicin alone against GentaR MRSA biofilm formation

- Significant anti-biofilm effect for G+V and G+C at Day 1, Day 3 and Day 9
- G didn't have any anti-biofilm effect against all *S. aureus* strains
- G+V and G+C keep their anti-biofilm effect against GentaR MRSA and against ClindaR MRSA that turns gentamicin tolerant at Day 9

S.epidermidis



- G, G+V and G+C significantly decrease biofilm formation at D1, D3 and D9 in comparison with plain cement
- No gain of anti-biofilm effect for bi-antibiotic ALBCs compared to Gentamicin ALBC

- G+V and G+C significantly decrease biofilm formation at D1 and D3 in comparison with plain cement
- G+C keep its anti-biofilm effect even at D9
- No anti-biofilm effect for G
- Combinations of antibiotics (especially G+C) are more effective than gentamicin alone

- Significant anti-biofilm effect for G, G+V and G+C at D1, D3 and D9
- Combinations of) seem more effective than gentamicin alone against GentaR MSSE
- G+C keep its anti-biofilm effect against GentaR MSSE (that turns vancomycin tolerant at Day 9)

Effect of ALBCs on (A) MSSA biofilm formation, (B) Gentamicin-resistant MRSA biofilm formation, (C) *S. aureus* biofilm formation (all strains merged), (D) MRSE biofilm formation, (E) Gentamicin-resistant MSSE biofilm formation and (F) *S. epidermidis* biofilm formation (all strains merged) Three independent experiments in triplicate for each condition: one strain (9 values) for (A)(B)(D) and (E) and six and five strains (one mean by strain) for (C) and (F) respectively, * means $p < 0.05$ compared to plain cement, Kruskal-Wallis test with a Dunn's post hoc test.

CONCLUSION

Our *in vitro* results suggest that combining gentamicin with vancomycin or clindamycin increase the prophylactic anti-biofilm effect compared to ALBCs loaded with gentamicin alone. These findings are especially relevant as G+C and G+V cement are still effective against gentamicin, vancomycin and clindamycin-resistant strains. This study was financially supported by Heraeus Medical but the company was not involved in the experimental process and the data analysis