Polymicrobial periprosthetic joint infection: eradication with different antimicrobials in vitro

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INTRODUCTION:

The treatment of polymicrobial periprosthetic joint infection (PJI) can be a significant challenge. Antibiotics contained within the cement spacer do not ensure 100% eradication of the infection, as their concentration decreases substantially over time. Alternative antimicrobial molecules, such as silver ions or xylitol, can be implemented for the treatment of chronic PJIs since they act against biofilm through different mechanisms, facilitating its eradication. This study aimed to compare, in vitro, the antimicrobial activity of cement loaded with antibiotics alone versus cement loaded with antibiotics and alternative antimicrobial molecules for the eradication of polymicrobial infections.

METHODS: Standard ATCC 25923 strains of methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa (PA), and Candida albicans (CA) were selected to form polymicrobial biofilms. The following antimicrobials were incorporated into bone cement: vancomycin, imipenem, and silver nitrate (SN). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for each microorganism. The concentration of each antimicrobial released from cement with and without xylitol was measured at 24 hours. Monomicrobial and mixed 48-hour biofilms of MRSA, PA, and CA were formed on sponges immersed in Mueller-Hinton broth (MHB). The antimicrobial activity of different combinations of antimicrobials—vancomycin, imipenem, xylitol, and SN—incorporated into cement was studied on these biofilms. Samples were collected over two weeks to count colony-forming units (CFUs). The assays were performed in triplicate with controls, and the delta CFU/ml for each antimicrobial combination was calculated using Stata v15.0 software. CFU values were categorized based on the logarithmic reduction achieved after sample plating. Kruskal-Wallis and Mann-Whitney tests were used to compare CFU counts obtained from the different antimicrobial combinations in the cement.

RESULTS: In the monomicrobial biofilm model, the combinations of imipenem + SN and imipenem + SN + xylitol eradicated PA by day 3. Furthermore, the imipenem + xylitol combination was significantly superior to imipenem alone by day 3. The vancomycin + SN + xylitol combination was superior for MRSA eradication compared to other combinations. For CA, SN alone and SN + xylitol achieved complete eradication by day 3. In polymicrobial biofilms, the combinations of imipenem + vancomycin + xylitol, imipenem + vancomycin + SN and imipenem + vancomycin + SN + xylitol were more effective in eradicating PA than imipenem + vancomycin alone. For MRSA, all combinations achieved complete and sustained eradication over time. However, biofilm eradication for CA was incomplete with the imipenem + vancomycin + SN and imipenem + vancomycin + SN + xylitol combinations. The imipenem + vancomycin + SN + xylitol combination was significantly superior to the same combination without xylitol.

DISCUSSION AND CONCLUSION:

SN and xylitol significantly increased antibiotic concentration acting synergistically. However, these substances are adjuvants and are not meant to replace current antibiotics and antifungals . SN demonstrated a broad-spectrum action and could be incorporated in the future for use in cases of chronic polymicrobial infections, although in vivo studies are needed to demonstrate its effectiveness and safety.

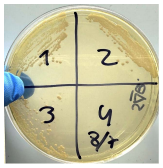

Table 3: CFU/ml count of monomicrobial biofilms

Substrate	Incubation time in days				
	0	3	6	9	14
Control	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]
Imipenem	10^7 [IQR: 10^7; 10^7]	10000 [IQR: 10000; 10000]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Imipenem + Xylitol	10^7 [IQR: 10^7; 10^7]	1040 [IQR: 1040; 1040]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Imipenem + SN	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Imipenem + SN + Xylitol	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin	10^7 [IQR: 10^7; 10^7]	1040 [IQR: 1040; 1040]	0 [IQR: 0; 260]	0 [IQR: 0; 260]	0 [IQR: 0; 0]
Vancomycin + Xylitol	10^7 [IQR: 10^7; 10^7]	1040 [IQR: 1040; 1040]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + SN	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + SN + Xylitol	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]

Table 4: CFU/ml count of *Pseudomonas aeruginosa* from polymicrobial biofilms during the 14-day period

Substrate	Incubation time in days				
	0	3	6	9	14
Control	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]
Vancomycin + Imipenem	10^7 [IQR: 10^7; 10^7]	2.2x10^[IQR: 2x10^; 2x10^]	7000 [IQR: 7000; 7000]	2.7x10^5 [IQR: 2x10^5; 2x10^5]	2x10^5 [IQR: 2x10^5; 2x10^5]
Imipenem + Xylitol	10^7 [IQR: 10^7; 10^7]	2x10^5 [IQR: 2x10^5; 2x10^5]	15000, 15000]	2x10^5, 2x10^5]	2x10^5, 2x10^5]
Imipenem + SN	10^7 [IQR: 10^7; 10^7]	10000 [IQR: 8000; 10000]	9000 [IQR: 8000; 10000]	10000 [IQR: 8000; 10000]	10000 [IQR: 8000; 10000]
Vancomycin + Xylitol	10^7 [IQR: 10^7; 10^7]	1700 [IQR: 1400; 2000]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + Imipenem + SN	10^7 [IQR: 10^7; 10^7]	1400 [IQR: 1000; 2000]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]

The values represent the median of the 3 trials with their respective interquartile range (IQR).

Table 5: CFU/ml count of *Staphylococcus aureus* from the polymicrobial biofilms over the 14-day period

Substrate	Incubation time in days				
	0	3	6	9	14
Control	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]
Vancomycin + Imipenem	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Imipenem + Xylitol	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + SN	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + Xylitol	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]
Vancomycin + Imipenem + SN	10^7 [IQR: 10^7; 10^7]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]	0 [IQR: 0; 0]

The values represent the median of the 3 trials with their respective interquartile range (IQR).

Table 6: CFU/ml count of *Candida albicans* from the polymicrobial biofilms over the 14-day period

Substrate	Incubation time in days				
	0	3	6	9	14
Control	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]
Vancomycin + Imipenem	10^7 [IQR: 10^7; 10^7]	2.2 10^ [IQR: 2x10^; 2x10^]	2x10^5 [IQR: 2x10^5; 2x10^5]	2.7x10^5 [IQR: 2x10^5; 2x10^5]	2x10^5 [IQR: 2x10^5; 2x10^5]
Imipenem + Xylitol	10^7 [IQR: 10^7; 10^7]	10^7 [IQR: 10^7; 10^7]	2x10^5 [IQR: 2x10^5; 2x10^5]	2.3x10^ [IQR: 2x10^; 2x10^]	2x10^ [IQR: 2x10^; 2x10^]
Imipenem + SN	10^7 [IQR: 10^7; 10^7]	5000 [IQR: 5000; 5000]	25000 [IQR: 20000; 30000]	25000 [IQR: 20000; 30000]	200000 [IQR: 150000; 250000]
Vancomycin + Xylitol	10^7 [IQR: 10^7; 10^7]	5000 [IQR: 5000; 5000]	25000 [IQR: 20000; 30000]	25000 [IQR: 20000; 30000]	200000 [IQR: 150000; 250000]
Vancomycin + Imipenem + SN	10^7 [IQR: 10^7; 10^7]	5000 [IQR: 5000; 5000]	25000 [IQR: 20000; 30000]	25000 [IQR: 20000; 30000]	200000 [IQR: 150000; 250000]

The values represent the median of the 3 trials with their respective interquartile range (IQR).