

ACTIVITY OF ANTIBACTERIAL CLAY AGAINST DRUG-RESISTANT BACTERIA

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Background: Oregon Mineral Technologies (OMT) Blue Clay has shown antibacterial properties in preliminary studies. Here, we assessed its *in vitro* activity against planktonic and biofilm bacteria.

Methods: Twelve bacterial strains representing species frequently associated with wound infections, including antibiotic resistant strains, were studied. For planktonic studies, bacteria were grown to 1.5×10^8 cfu/mL in 20% (5g/L) Luria broth (LB), diluted to 10^7 cfu/mL, and combined in a 1:1 ratio with OMT clay (200 mg/mL), OMT leachate, or water. Samples were incubated at 37°C with shaking, and quantitatively cultured at 0, 4, 8, 12, and 24h. For biofilm studies, biofilms grown on Teflon discs were incubated for 24h with either OMT Blue Clay (200 mg/mL), OMT leachate, or 10% LB, followed by quantitative culture. All testing was performed in triplicate. Statistical analyses were performed using the Wilcoxon rank sum test.

Results: Planktonic studies. Within 4h, the clay and leachate significantly reduced planktonic bacterial populations of all strains tested compared to controls ($p \leq 0.05$). By 24h, there was a ≥ 3 \log_{10} cfu/mL reduction for all strains tested, with the exception of leachate-treated *Staphylococcus aureus* IDRL-6169, for which there was a 2.5 \log_{10} cfu/mL reduction that was statistically significant compared to the control ($p=0.0369$). At 24h, *S. aureus* IDRL-6169 and *Escherichia coli* IDRL-10366 exhibited greater reductions when treated with clay *versus* leachate ($p = 0.0369$ and 0.0463 , respectively); for the remainder of the strains there was a less than 0.5 \log_{10} cfu/mL difference between the clay and leachate, a statistically non-significant difference. Biofilm studies. Clay exposure reduced population densities of all organisms tested compared with controls, of statistical significance. Leachate treatment also resulted in statistically significant population reductions compared with controls for all organisms except for *S. aureus* IDRL-6169. Clay *versus* leachate formulations supported statistically significant population reductions of greater magnitude for four of the twelve organisms tested: *S. aureus* IDRL-6169 ($p=0.0369$), *Staphylococcus epidermidis* RP62A ($p=0.0253$), *Enterobacter cloacae* IDRL-10306 ($p=0.0495$), and *E. cloacae* IDRL-10375 ($p=0.0495$).

Conclusion: OMT Blue Clay exhibited antimicrobial activity against all 12 bacterial strains tested in planktonic studies, and in addition, demonstrated activity against biofilms *in vitro*.