## ABSTRACT

Bacterial biofilms remain a major public health burden. Staphylococcus epidermidis biofilm is the predominant cause of biofilm-associated infections. Kisumu county has a high circulation of antibiotic resistance genes, which is attributable to S. epidermidis biofilm, necessitating effective S. epidermidis biofilm control. Given the high tendency of bacteria to develop resistance to antibiotics, S. epidermidis biofilm control using physico-chemical disinfection is a suitable approach. In Kisumu county, heat (60°C), 1.72 M sodium chloride (NaCl), 0.178 M sodium hypochlorite (NaOCl) and 1.77 M hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are the commonly used disinfectants. Studies on susceptibility of bacterial biofilms to disinfectants have focused on structurally or metabolically unique bacterial species; hence, offer limited insights on general biofilm disinfection. Despite S. epidermidis being a model and the most clinically relevant biofilm, its susceptibility patterns to the disinfectants remain undocumented. Mechanisms, including reduced diffusion through biofilm matrix, physiological heterogeneity within biofilm or persister cells are linked with high biofilm tolerance against antimicrobials. However, these mechanisms only provide partial explanations for biofilm's tolerance against fewer antibiotics, but not physico-chemical stresses, necessitating exploration of conclusive tolerance mechanisms. Although studies have implicated extracellular DNA (eDNA) and alternative sigma factor B ( $\sigma^{B}$ ) in planktonic cells' tolerance against stressors, their contribution in biofilm's (S. epidermidis included) tolerance against physico-chemical stress exposure remain unknown. Hence, the susceptibility patterns, eDNA release and  $\sigma^{B}$  activity of S. epidermidis biofilm in response to physico-chemical stress exposure were evaluated. One S. epidermidis isolate per skin swab of sixty-two Kisumu county residents was used to generate a pair of biofilm and planktonic cultures. A post-test study design was adopted. The pairs were exposed to 60°C, 1.72 M NaCl, 0.178 M NaOCl or 1.77 M H<sub>2</sub>O<sub>2</sub> for 30 and 60 min for susceptibility determination using standard plating. Further, the pairs were exposed to optimal physico-chemical stresses (50°C, 0.8 M NaCl, 5 mM NaOCl or 50  $\mu$ M H<sub>2</sub>O<sub>2</sub>) for 60 min for eDNA and  $\sigma^{B}$  activity quantification using qubit fluorometry and quantitative real-time PCR respectively. Statistical differences between groups were determined by t-tests using GraphPad Prism software. Significantly fewer S. epidermidis biofilms were killed upon exposure to 60°C, 1.72 M NaCl, 0.178 M NaOCl or 1.77 M H<sub>2</sub>O<sub>2</sub> than the planktonic cells (p < 0.0001). Unlike NaCl, biofilms exposed to 50°C, 5 mM NaOCl or 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> exhibited significantly higher eDNA yields and  $\sigma^{B}$  activity than planktonic cells (p < 0.05). These findings demonstrated that S. epidermidis biofilm was more tolerant to the disinfectants, and that eDNA and  $\sigma^{B}$  activities contributed to its tolerance against the disinfectants. Collectively, the findings could inform on development of efficient disinfection approaches against S. epidermidis biofilm by targeting eDNA and/or  $\sigma^{B}$ ; hence, reducing the burden and spread of antimicrobial tolerance.