Medium supplementation with magnesium prevented the induction of dormancy in biofilms of clinical and commensal isolates of *Staphylococcus epidermidis*

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Staphylococcus epidermidis is a commensal bacterium that colonizes the skin and mucous membranes, being the most prevalent staphylococcal species found in humans. However, *S. epidermidis* has the ability of colonize indwelling medical devices establishing biofilms, which makes this bacteria a common cause of bacteraemia particularly in immunocompromised individuals and neonates [1][2]. The presence of high amounts of dormant bacteria (viable but non-culturable cells, VBNC) is a hallmark of biofilms, making them more tolerant to antimicrobials and elusive to the host immune response [3]. Glucose (G) is commonly used *in vitro* to induce a biofilm mode of growth in *S. epidermidis* cultures [4] due to the medium acidification derived from carbon metabolism [5]. Previous studies in our laboratory demonstrated that the accumulation of VBNC bacteria inside *S. epidermidis* biofilms could be prevented by supplementation of the medium with magnesium (Mg) (unpublished data). In the present work, a flow cytometric live/dead staining [6], in combination with the spread-plate method, were used to evaluate the effect of magnesium in preventing dormancy on glucose excess-grown biofilms in 53 strains of international clinical, Portuguese clinical, and Portuguese commensal isolates of *S. epidermidis*. Culture supplementation with Mg^{2+} prevented the accumulation of death bacteria inside the biofilms in all the groups of strains tested, being the proportion of live bacteria ($SYBR^{+}PI^{-}$) significantly increased by Portuguese clinical isolates. The quantification of colony forming units (CFUs) revealed that Mg hampered the induction of dormancy in all the groups of strains studied (International clinical G: $3{,}6\pm0{,}8\times10^{7}$ vs. G+Mg: $2{,}6\pm0{,}5\times10^{8}$, Portuguese clinical $6{,}8\pm2{,}0\times10^{7}$ vs. $4{,}3\pm1{,}4\times10^{8}$, Portuguese commensal $7{,}2\pm1{,}5\times10^{7}$ vs. $2{,}6\pm0{,}4\times10^{8}$ CFUs/mL). These results confirmed that dormancy modulation is widespread in different *S. epidermidis* isolates obtained from clinical and commensal sources, suggesting that this is indeed an important mechanism of *S. epidermidis* biofilm physiology.

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