Understanding the mechanism and activity of a novel biocide product

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Abstract

Healthcare associated infections account for hundreds of millions of infections worldwide every year[1]. Biocides are widely used as an infection control measure, however their effectiveness is being limited by the increasing prevalence of antimicrobial resistance[2].

It has been proposed that complex multi-component biocides could be used that act via multiple mechanisms of action. Bacteria would therefore need to develop resistance to multiple mechanisms simultaneously; potentially mitigating and slowing down the rate of resistance development. "SQ53" is a multi-component formulation made up from commercially-available biocides. The formulation demonstrates a very high level of activity, indicating a potential synergistic mechanism. The aim of this project is to investigate various biocide mixtures for potential synergism, aiming to optimise the biocides that are currently used and widely available.

Methodology

Miniumum inhibitory concentration (MIC) values were deduced via the broth-dilution method. SQ53 (JVS Products Ltd) was added to Tryptone Soya Broth (TSB) to achieve a range of final concentrations from 0.002% (v/v) to 10% (v/v). The broth was inoculated with bacterial stocks before overnight incubation at 37˚C.

Growth was visualised by turbidity in order to deduce the MIC. The minimum biocidal concentration (MBC) values were then deduced by subculturing out each condition onto Tryptone Soya Agar (TSA) plates that do not contain SQ53 and incubating overnight at 37˚C.

Transmission electron microscopy (TEM) samples were prepared by incubating *Klebsiella pneumoniae* BM04 and *Staphylococcus aureus* NSM7 in TSB containing 0.04% SQ53 overnight at 37˚C. The cell suspensions were fixed and embedded in resin[3] before being imaged on a Hitachi HT7700.

MIC & MBC Results

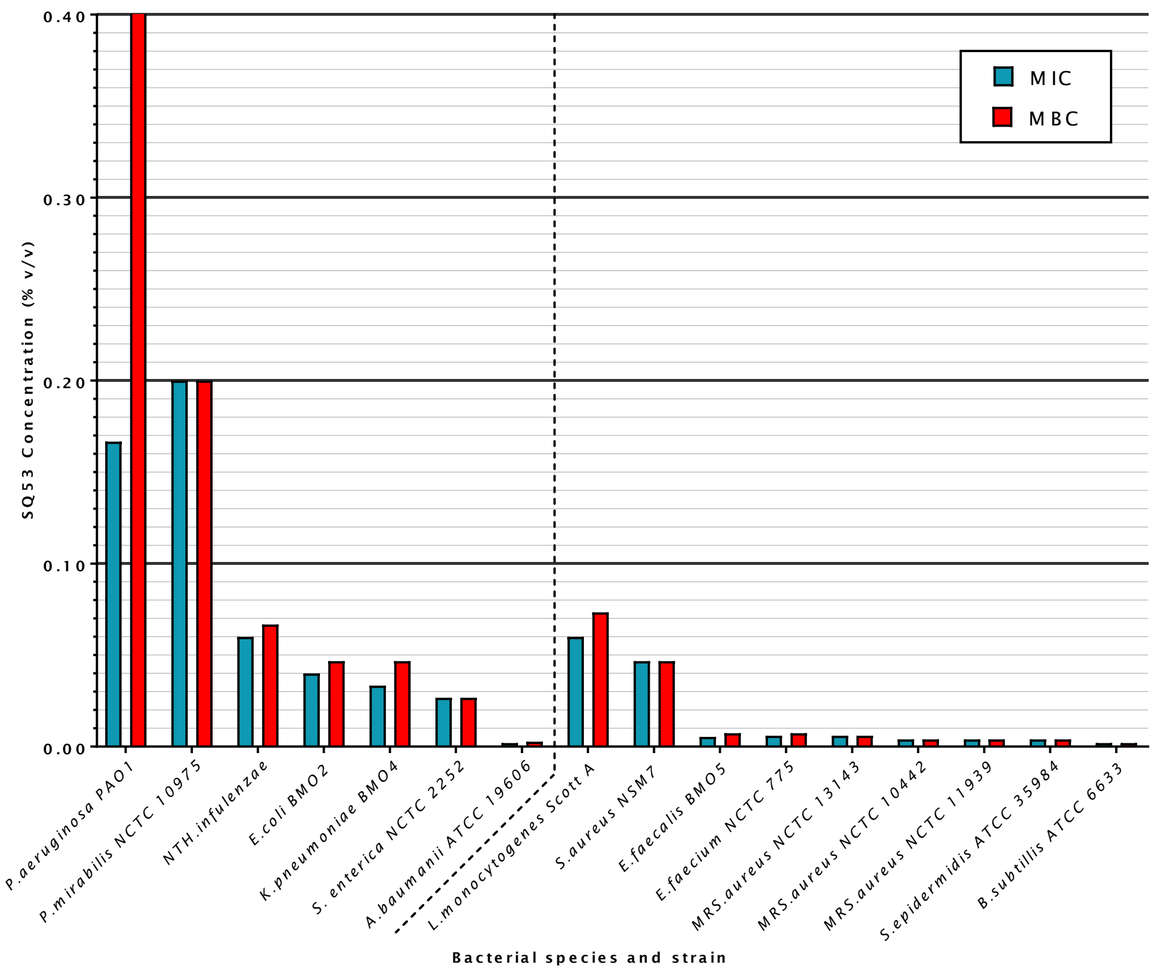

Figure 1. SQ53 MIC and MBC values for a range of clinically-relevant bacterial species, with Gram-negative species on the left of the dotted line and Gram-positive species on the right. The minimum concentration of SQ53 required to inhibit visible growth and kill bacteria as determined by the broth-dilution and subculture methods respectively.

- SQ53 is effective against Gram-negative bacteria at concentrations as low as 0.04% v/v.
- SQ53 is effective against Gram-positive bacteria at concentrations as low as 0.002% v/v.
- SQ53 is generally more effective against Gram-positive bacteria than Gram-negative bacteria.

TEM Results

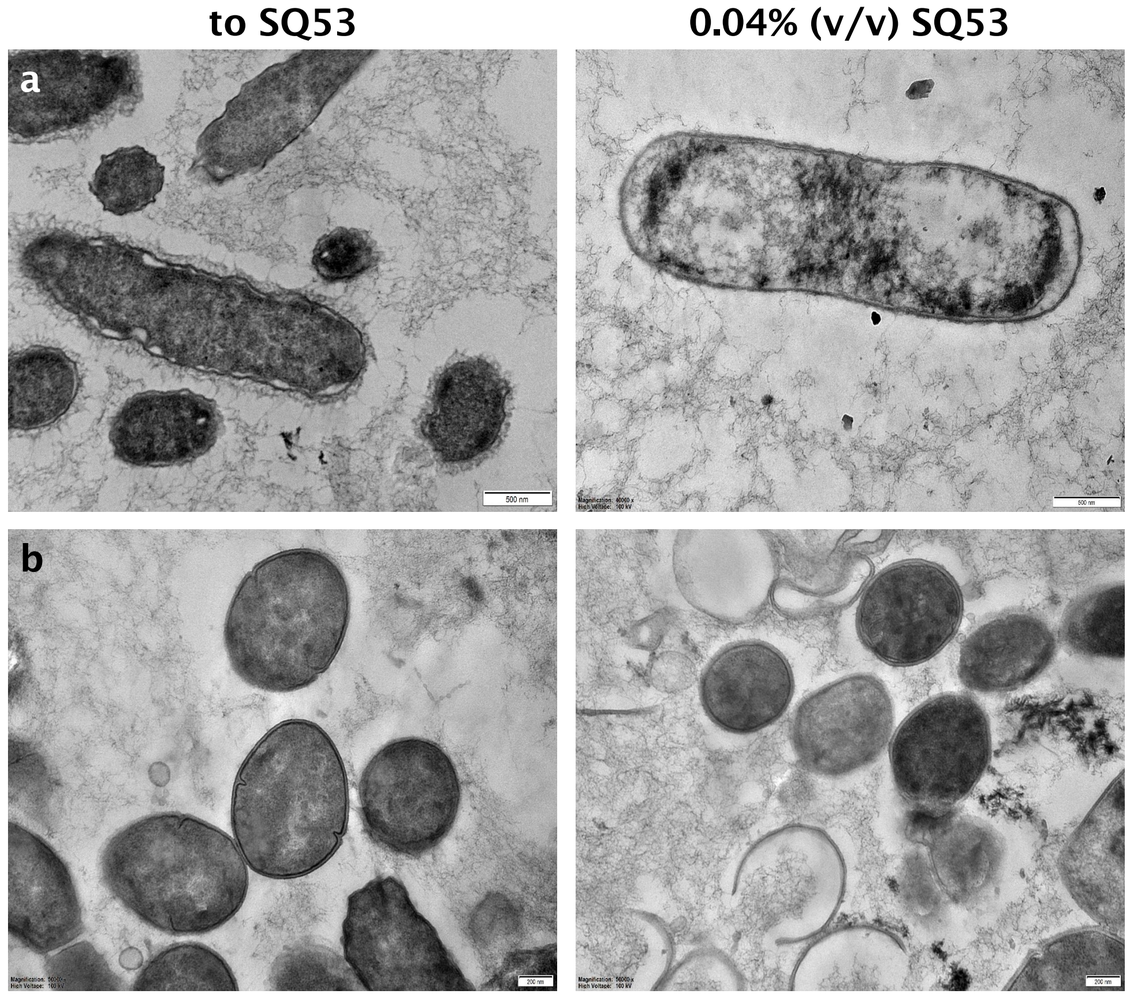

Figure 2. Images demonstrating the effect of SQ53 on *K.pneumoniae* BMO2 and *S.aureus* NSM7 captured via TEM. *K.pneumoniae* BMO2 (a) and *S.aureus* NSM7 (b) were cultured overnight at 37˚C in the presence or absence of 0.04% (v/v) SQ53. Cell suspensions were then fixed and embedded in resin before TEM images were captured at 40,000x (a) and 50,000x (b).

From the above images, our interpretation is:

- Bacteria exposed to SQ53 display severe membrane disruption.
- When exposed to SQ53, *K.pneumoniae* BMO4 exhibits a decrease in intracellular electron density, and the inner membrane appears to pull away from the outer membrane.
- SQ53 induces *S.aureus* NSM7 cell lysis.
- Dark electron-dense patches outside of the cell appear to be cellular debris.

Conclusions

- SQ53 is effective against both Gram-negative and Gram-positive bacterial species at low concentrations, demonstrating a high level of antimicrobial activity.
- Gram-positive bacterial species are generally more susceptible to SQ53 than Gram-negative species.
- The biocide formulation causes fatal disruption to bacterial membranes, and interacts with intracellular components.

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[1] Report on the Burden of Endemic Health Care-Associated Infection Worldwide. Geneva, World Health Organisation, 2011.
[2] B.Meyer, B.Cookson (2010) 'Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control?', Journal of Hospital Infection, 76(3), pp. 200-205.
[3] A.Page, J.Lagnado, T.Ford, G.Place (1994) 'Calcium alginate encapsulation of small specimens for transmission electron microscopy', Journal of Microscopy, 175(2), pp. 166-170.