Comment on: Triclosan resistance in methicillin-resistant Staphylococcus aureus expressed as small colony variants: a novel mode of evasion of susceptibility to antiseptics

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Sir,

In their recent article, Bayston et al.1 provide an interesting report on the occurrence of small colony variants (SCVs) of Staphylococcus aureus during exposure to silicone impregnated with triclosan. In parallel with our prior report2 Bayston et al. describe the isolation of SCVs with low-level resistance to triclosan, reduced coagulase production, reduced haemolytic activity and did not detect auxotrophy. They recognize, as others have done, the possible clinical importance of SCVs and the potential role of triclosan in their generation. However, we would like to urge some caution in their interpretation of their data and consequently on the significance of the issues raised.

A significant difference between triclosan and other antimicrobials known to select for SCVs (principally aminoglycosides) is that it is used topically. It is thought that SCVs are unlikely to initiate infections, rather, once a wild-type infection has been established, antibiotic treatment selects for mutation to give the SCV phenotype/form. Hence, selection of SCVs by systemic antibiotics occurs once the bacteria have broached the skin and are at the site of infection, whereas triclosan-induced SCVs will presumably be on the skin surface. Thus, an important question is whether these SCVs would then be capable of ever initiating an infection in healthy individuals.

Extending this logic means the triclosan-induced SCVs formed at the skin surface may be transmissible by skin–skin contact, as wild-type S. aureus. This could provide a mechanism for them coming into contact with wounds or finding other routes through the epidermis. However, it would also leave them vulnerable to the current mechanisms of preventing transfer of infection, such as alcohol hand-washes and barrier systems such as gloves.

The association of SCVs with triclosan-impregnated polymers is possibly more worrying than selection of SCVs by topical preparations. For example, the use of triclosan-impregnated sutures3,4 will provide a selective pressure for SCV status in close proximity to a site of entry into the body, and the release of triclosan is less controlled, so the opportunity that triclosan concentrations are closer to those that select for SCVs is greater. Furthermore, during this method of triclosan delivery, the antimicrobial is not combined with surfactants or other ingredients found in topical formulations, hence triclosan will be acting without the synergistic effects of these chemicals.5 It has also been reported that surface-bound SCVs were highly resistant to the bactericidal action of oxacillin or vancomycin;6 consequently, it would be interesting to know the susceptibility of these SCVs while adhered to the silicone discs. However, in contrast to the authors’ comment, we believe that the use of materials impregnated with triclosan is not as widely established as some authors report. Triclosan is not used as a typical product protectant in plastic articles. Indeed, its use in plastic is limited to special articles that represent a niche market. Thus, the frequency and impact of these events is likely to be very low.

We found previously that the selection of SCVs by triclosan was concentration-dependent.7 It would be very interesting to know what the triclosan concentration in the discs was and at what rate it was released. This is also key to any speculation on their clinical impact.

Owing to the abnormal characteristics of S. aureus SCVs, they are easily confused with coagulase-negative staphylococci7 and potentially with various other slow growing bacterial genera. Additionally, they require extended incubation times and their slow growth rate leaves them liable to be ‘overgrown’ by faster growing organisms. For these reasons, we feel that it is necessary to unambiguously confirm that putative SCVs are indeed S. aureus and not contaminants. We have found nucleic acid-based methods useful for this.2

Bayston et al. state that triclosan acts by inhibiting FabI, an enzyme that executes the final step in the elongation cycle of bacterial fatty acid biosynthesis. It should be noted that although FabI is undoubtedly a target for low levels of triclosan, the antimicrobial also has other targets, such as the cytoplasmic membrane.8 Thus, we feel it would have been appropriate to have investigated the possibility that the triclosan resistance found in their SCVs was as a result of this phenotype and not through coincidental alterations to FabI or other targets.

Finally, we were concerned to see that the title of the article states that SCVs are ‘a novel mode of evasion of susceptibility to antiseptics’; however, their results report only on susceptibility to triclosan. As the paper presents no evidence that SCVs demonstrate resistance to other antiseptics, the title of the article is misleading. It clearly implies that SCV status, obtained as a result of triclosan exposure, confers resistance to multiple antiseptics when there is no evidence to support this hypothesis.

The role of S. aureus SCVs in morbidity and mortality is coming under increasing scrutiny. Whilst we welcome all research that aids our understanding of these curious infections, there is a danger that too much speculation on the clinical impact of SCVs based only on in vitro findings could restrict the use of an antimicrobial that has to date performed well during infection management situations. We would also recommend that studies unambiguously confirm that putative SCVs are indeed S. aureus and investigate alternative mechanisms of resistance in addition to making the association with the SCV phenotype.
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Transparency declarations

D. O. is an employee of Ciba Spezialitätenchemie Grenzach GmbH, Grenzach-Wyhlen, Germany. P. F. S. and M. J. D. have nothing to declare.

References


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Triclosan resistance in methicillin-resistant Staphylococcus aureus expressed as small colony variants: a novel mode of evasion of susceptibility to antiseptics—authors’ response

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Sir,

We welcome the opportunity to reply to the comments by Seaman et al.1 on our recent article.2 First, they say that we recognize, ‘as others have done, . . . the potential role of triclosan in [small colony variant (SCV)] generation’. In fact, apart from the parallel publication by Seaman et al.,3 we know of no other which points out the association between triclosan exposure and SCV generation.

Secondly, Seaman et al. state that SCVs are unlikely to initiate infections. Several studies have found persisting serious infections from which only SCVs have been isolated.4,5 Of course, these could be the result of antimicrobial chemotherapy as Seaman et al. recognize, but SCVs have also been found in chronic infection without antibiotic exposure and they can be induced experimentally by the intracellular milieu alone. In addition, there is some evidence that Staphylococcus aureus SCVs are at least as virulent as their wild-types6,7 and have successfully initiated infections in animal models.8-9 This assertion by Seaman et al. is therefore unfounded. Similarly, they state that as the SCVs would be on the skin surface, it is questionable whether they would be able to initiate an infection in healthy individuals. Even healthy people undergo surgery, and most surgical site infection is caused by staphylococci from the skin. Their comments regarding skin to skin transmission being prevented by alcohol hand washes and gloves fail to recognize that, although these are important, they are not perfect.

We agree with their comment that triclosan-impregnated polymers such as those used in sutures might constitute a risk of SCV generation; indeed, this was the implication of our paper. A range of items impregnated with triclosan are available, and several authors have pointed this out in recent papers.3,10,11 Seaman et al. ask what the triclosan concentration in the discs was. This was 0.3% (w/w), but the most important concentration in respect of attached bacteria is that in the Nernst mission being prevented by alcohol hand washes and gloves fail to recognize, but SCVs have also been found in chronic infection without antibiotic exposure and they can be induced experimentally by the intracellular milieu alone. In addition, there is some evidence that Staphylococcus aureus SCVs are at least as virulent as their wild-types6,7 and have successfully initiated infections in animal models.8-9 This assertion by Seaman et al. is therefore unfounded. Similarly, they state that as the SCVs would be on the skin surface, it is questionable whether they would be able to initiate an infection in healthy individuals. Even healthy people undergo surgery, and most surgical site infection is caused by staphylococci from the skin. Their comments regarding skin to skin transmission being prevented by alcohol hand washes and gloves fail to recognize that, although these are important, they are not perfect.

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Although triclosan is important in infection control, we consider that exposure to the antiseptic in the way described in our paper is clinically relevant, and reporting the generation of SCVs in this way is not ‘a danger of too much speculation’ as Seaman et al. suggest.

Transparency declarations

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