Time for biocide stewardship?

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To the Editor – we wish to comment on the article by Lee *et al* [1]. We welcome this important work emphasising the spread of pathogenic multidrug resistance *Staphylococcus epidermidis* globally but wish to highlight some critical findings derived from the deposited genomic data which have been partially reported in the supplementary information (Supplementary Table 1, sheet D) but not discussed in the article.

The study by Lee *et al* supports the concerns raised in numerous smaller scale and local studies that *S. epidermidis* is a dominant reservoir of multidrug resistance genes [2]. The authors' main focus is the prevalence of *rpoB* associated with resistance to rifampicin widely used in medical devices. We wish to draw the readers' attention to the high carriage of biocide resistance genes as well as heavy metal resistance in association with staphylococcal cassette chromosome *mec (*SCC*mec)* elements in hospital-adapted multidrug resistant *S. epidermidis*.

Biocide resistance. We focussed our analyses on i) *qacA/B* encoding an efflux pump for a variety of lipophilic cations and strongly associated with reduced microbial susceptibility to chlorhexidine [3], ii) the plasmid-borne *ileS2* gene and the core-genome V588F mutation in IleS responsible for high- and low-level mupirocin resistance, respectively [4]. Chlorhexidine is one of the most commonly used biocides in the community as well as hospital decontamination and infection control, with applications ranging from mouthwashes to impregnated catheters and skin/mucosal surface decontamination in intensive care units. Equally mupirocin is widely employed for decontamination of nasal surfaces in intensive care. Whilst these measures have shown remarkable effectiveness in control of bloodstream infections including those related to MRSA, MSSA and coagulase-negative Staphylococci [5], resistance to mupirocin and declining susceptibility to chlorhexidine has been reported worldwide [3, 6]. Resistance to biocides has raised concerns of selection of multidrug resistant bacteria associated with hospital-acquired infections with a most alarming report suggesting that exposure to chlorhexidine is associated with resistance to last resort antibiotic colistin [7].

The prevalence of *qacA/B* in *S. aureus* reported over the last decade is very variable although the drivers for high carriage are not clear. While some studies continue to report declining susceptibility to chlorhexidine and increasing prevalence

of *qacA/B* in MRSA from a range of clinical settings [8, 9], other studies, including our own work, report low presence of *qacA/B* in *S. aureus* from screening samples and bacteraemia patients [10, 11]. However, we have shown a high prevalence (80%) of *qacA/B* in *S. epidermidis* isolates from blood cultures randomly collected over 6 years in an intensive care unit in Scotland, with *qacA/B* carriage coinciding with reduced susceptibility to chlorhexidine [11]. Importantly the majority of *qacA/B* isolates in this study belonged to multidrug resistant clone ST2, the same lineage that dominated in the collection of isolates studied by Lee *et al*. We have also identified genetic determinants of resistance to mupirocin (*ileS2*, V588F mutation in IleS) and/or triclosan (*sh-fabI,* F204L mutation in FabI) in 65% of *qacA/B S. epidermidis* isolates [11]. Notably, in some cases mupirocin resistance genes were co-located with *qacA/B* on variants of known MRSA mobile elements raising the possibility of horizontal transfer of multidrug resistance genes between *S. epidermidis* and *S. aureus* [11].

We have analysed the genomic data published by Lee *et al* (ENA, Bioproject PRJEB12090, PRJNA470534 and PRJNA470752) for carriage of the *qacA/B* genes, the IleS-V588F mutation, the *ileS2*(*mupA*) gene, the FabI-F204L mutation and the *sh-fabI* gene. We did not analyse either *ileS* or *fabI* promoter mutations, thus underestimating resistance to mupirocin and triclosan. Analysis of the 226 clinical isolates in Lee *et al* revealed significant enrichment of biocide resistance in the three hospital-adapted multidrug resistant lineages of this global collection of pathogenic *S. epidermidis*, namely ST2, ST2-BPH0662 and ST23. The proportion of *qacA/B* positive isolates belonging to the ST2 and ST2-BPH0662 clones (85% and 78%, respectively) was significantly higher ($p = 0.004$, Fisher's exact test) than that detected in all other isolates cumulatively (68%). The ST23 and the ST2-BPH0662 lineage showed increased carriage of the core-genome IleS-V588F mupirocin resistance mutation compared to the other isolates (56% ST23, 48% ST2-BPH0662, 10% in other isolates; $p < 0.0001$ for both lineages) but not of the plasmid-borne *ileS2* (*mup*A) gene. There were no significant differences in the carriage rate of *sh_fabI* and FabI-F204L mutations pointing to a lesser role of triclosan, a biocide primarily used in household products.

SCC*mec* and heavy metal resistance. Our analysis revealed that all isolates belonging to the ST2 BPH0662 lineage described in Lee *et al* carry an SCC*mec* element orthologous to the SCC*mec* associated with the copper and mercury resistance mobile element (COMER) in community-associated MRSA USA300 [12]. In ST2 BPH0662 it presents as a composite element comprising an SCC*mec* type III and a COMER-like locus which, in addition to copper and mercury resistance, carries cadmium and arsenic resistance gene clusters that are not present in the MRSA SCC*mec* COMER element. Of note, decreased susceptibility to copper has been recently linked to increased resistance of COMER-positive *S. aureus* to macrophage killing and the success of the USA300 clone [13].

The global and sustained dominance of three hospital-adapted multidrug resistant lineages with high carriage of chlorhexidine and mupirocin resistance genes raises concerns of selection of multidrug resistance by these two biocides. Further, carriage of SCC*mec* in association with metal resistance genes points to selection of multidrug resistant *S. epidermidis* which may display enhanced fitness and resistance to innate immunity by analogy with *S. aureus*. Indeed, we have previously demonstrated that antibiotic use is an important predictor of prevalence density of dominant multidrug resistant clones [14] – we speculate a similar effect by intensive use of metals, chlorhexidine and mupirocin as previously suggested [15]. To support or refute this claim we recommend that future genomic analysis of hospital-acquired multidrug resistant *S. epidermidis* should include analysis of biocide resistance genes. Functional studies are required to evaluate the role of heavy metal resistance genes related to SCC*mec* elements in *S. epidermidis*.

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