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Letter to the Editor

## Bactericidal activity of octenidine against *Staphylococcus aureus* harbouring genes encoding multidrug resistance efflux pumps

*Sir,*

The bactericidal activity of the antiseptic octenidine dihydrochloride (OCT) against several antimicrobial-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), including isolates resistant to mupirocin [1], as well as the most relevant multidrug-resistant Gram-negative pathogens in healthcare facilities [2], has been shown previously. Resistance to biocides is usually associated with a reduction in the intracellular concentration of the drug, either by a decrease in cell wall permeability or through multidrug efflux pumps that are able to extrude different classes of antimicrobial agents from the cytoplasm, including biocides or antiseptic molecules. Multidrug efflux pumps have been frequently described among *S. aureus* leading to therapeutic and/or decolonisation failure and the spread of resistant pathogens. In *S. aureus*, various biocide resistance genes have been identified, located either on plasmids (e.g. *qacAB* and *smr*) or on the chromosome (including *norA*, *sepA*, *lmrS* and *mepA*) [3].

In this study, the in vitro bactericidal activity of OCT against MRSA and methicillin-susceptible *S. aureus* clinical isolates harbouring different genes encoding multidrug efflux pumps, all associated with decreased efficacy of disinfectants, was investigated for the first time.

Isolates were selected from the Staphylococcal Culture Collection of the Molecular Genetics Laboratory (Instituto de Tecnologia Química e Biológica, Oeiras, Portugal), belonging to different sequence types and tested for the presence of *norA*, *sepA*, *mepA*, *lmrS*, *qacAB* and *smr* genes by PCR [4] (Table 1).

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) to OCT (Schülke & Mayr GmbH, Norderstedt, Germany) were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. The efficacy of OCT was analysed according to European Standard guideline EN 13727:2012 + A1, a quantitative suspension test to evaluate chemical disinfectants and antiseptics [6]. Samples were incubated for different contact times (30 s or 5 min) with OCT diluted to final test concentrations ranging from 0.001% to 0.1% (w/w). Following the given contact time, 1 mL of sample was added to 9 mL of neutralising solution (0.1% tryptone, 0.85% NaCl, 3% Tween 80, 0.3% lecithin, 3% saponin and 0.1% histidine) to terminate the activity of OCT without interfering with bacterial growth and then serial dilutions were spread onto neutralising agar plates. Colonies were counted following incubation for 24 h and 48 h at 37 °C and the reduction factor was determined. Tests were also performed with 0.3% mucin (Sigma-Aldrich, St Louis, MO) and with 3 g/L bovine serum albumin (BSA) (Sigma-Aldrich) + 3 mL/L ovine erythrocytes (Oxoid, Madrid, Spain) to simulate different organic challenges commonly found on mucous membranes or wounds that may reduce the bactericidal effectiveness of biocides. A 5 $\log_{10}$ reduction was considered effective within 5 min according to EN 13727 [6]. *Staphylococcus aureus* ATCC 6538 was used as a standard strain for quality control in the biocide efficacy tests.

OCT at very low concentrations of 0.001% (=10 ppm) and 0.01% (=100 ppm) with no organic load was fully effective against all tested *S. aureus* isolates within 30 s of contact time only (Table 1). However, the presence of 3 g/L BSA + 3 mL/L erythrocytes or even the more challenging 0.3% mucin reduced the bactericidal activity of OCT for some, but not all, staphylococcal isolates in a time-dependent manner. All *S. aureus* isolates tested showed an OCT MIC of 0.5 μg/mL and an MBC of 1 μg/mL. A delay in the efficacy of different wound antiseptics, including polyhexanide biguanide, povidone-iodine and OCT, has been previously reported for bacterial strains without genes associated with biocidal resistance when using a high albumin concentration [7]. Noteworthy, in this study we showed that OCT is highly effective even in the presence of a high organic load against all *S. aureus* isolates at clinically used concentrations (0.05% and 0.1%) and at a short contact time, independently of the presence of different genes encoding multidrug efflux pumps. The efficacy of OCT against isolates harbouring genes encoding efflux pumps is of major importance since significant frequencies of the principal genes responsible for antiseptic resistance in *S. aureus* (plasmid-borne genes *qacAB* and *smr*) have been reported all over the world (up to 42% and 44% in Europe, 83% and 77% in Asia, 40.5% and 3.7% in Africa and 2% and 7% in Canada [3,4]). The current finding is of clinical relevance as several healthcare facilities have started to implement preventative strategies, such as whole-body patient decontamination on intensive care units or prior to surgery, based on antiseptics such as OCT in order to reduce infections within hospitals. In view of impending antimicrobial resistance associated with higher morbidity and mortality as well as increased therapeutic costs, it is also important to regularly monitor the efficacy of widely used antiseptics.

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**Table 1**
Characteristics of six *Staphylococcus aureus* isolates and $\log_{10}$ reduction factor (RF) for octenidine (OCT) determined under different organic load conditions.

| Isolate | Multidrug efflux pump genes | ST | MRSA/MSSA | Organic load | Contact time | $\log_{10}$ RF at OCT concentration of: | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 0.001% | 0.01% | 0.05% | 0.1% |
| ATCC 6538 | *sepA*, *mepA*, *norA* | 464 | MSSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes[a] | 30 s | 4.14 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | 3.61 | 2.79 | ≥5 | ≥5 |
| | | | | | 5 min | 3.46 | 4.62 | ≥5 | ≥5 |
| ANG565 | *sepA*, *mepA*, *norA*, *qacAB* | 15 | MSSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | 4.13 | 4.39 | ≥5 | ≥5 |
| | | | | | 5 min | 4.13 | 4.39 | ≥5 | ≥5 |
| ANG646A | *sepA*, *mepA*, *norA*, *lmrS* | 152 | MSSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | 3.93 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | 3.93 | ≥5 | ≥5 | ≥5 |
| CV479 | *sepA*, *mepA*, *norA*, *lmrS*, *qacAB*, *smr* | 1633 | MSSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes | 30 s | 4.77 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| HPV56 | *sepA*, *mepA*, *norA*, *lmrS*, *qacAB* | 247 | MRSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes | 30 s | 4.26 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | 4.83 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | 4.91 | ≥5 | ≥5 | ≥5 |
| ANG688 | *sepA*, *mepA*, *norA*, *lmrS*, *smr* | 8 | MRSA | No organic load | 30 s | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | ≥5 | ≥5 | ≥5 | ≥5 |
| | | | | BSA + erythrocytes | 30 s | 3.90 | ≥5 | ≥5 | ≥5 |
| | | | | | 5 min | 4.76 | ≥5 | ≥5 | ≥5 |
| | | | | Mucin 0.3% | 30 s | 3.98 | 3.90 | ≥5 | ≥5 |
| | | | | | 5 min | 3.98 | ≥5 | ≥5 | ≥5 |

ST, sequence type; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; BSA, bovine serum albumin.
OCT efficacy was determined according the EN 13727 for clean (no organic load) and dirty (BSA + erythrocytes and mucin 0.3%) conditions, with a 5 $\log_{10}$ reduction considered effective within 5 min of contact time.
[a] 3 g/L BSA + 3 mL/L ovine erythrocytes.

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## Competing interests

None declared.

## Ethical approval

Not required.

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