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High Prevalence of Biocide Resistance Determinants in *Staphylococcus aureus* Isolates from Three African Countries

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We assessed the prevalence of six biocide resistance genes among 82 methicillin-resistant *Staphylococcus aureus* (MRSA) and 219 methicillin-susceptible *S. aureus* (MSSA) isolates from three African countries; the prevalence was very high for *sepA* (95.3%), *mepA* (89.4%), and *norA* (86.4%), intermediate for *lmrS* (60.8%) and *qacAB* (40.5%), and low for *smr* (3.7%). A significant association between biocide resistance genes and antibiotic resistance was observed, and a new cutoff MIC of ≥1 mg/liter for chlorhexidine nonsusceptibility was defined.

Biocides, as quaternary ammonium compounds (QACs) or biguanides, are widely used in infection control programs, including hand washing and skin decolonization prior to invasive procedures. However, its overuse led to the emergence of *Staphylococcus aureus* with decreased antiseptic susceptibility, which became a problem in hospitals in different regions of the world (1–3). Although biocide resistance might be multifactorial, multidrug resistance efflux pumps are the predominant mechanisms mediating cross-resistance to both antibiotics and biocides (4). Numerous genes encoding multidrug efflux pumps, such as *qacA*, *qacB*, *smr*, *norA*, *lmrS*, *mepA*, and *sepA*, have been described in clinical *S. aureus* isolates from humans, animals, and environmental samples (2, 5–10). Moreover, reduced susceptibility to chlorhexidine, one of the most frequently used biocides, is usually associated with both the *qacAB* and *smr* gene families (11, 12). Consequently, daily chlorhexidine baths in combination with mupirocin nasal ointment, a usual practice in preventive decolonization programs, and the usage of chlorhexidine hand soap as an infection control measure might be compromised (13). Although *qacAB* prevalence in methicillin-resistant *S. aureus* (MRSA) has been reported as highly varied, from <1% in a few Asiatic countries to 80% in Brazil (1, 2, 14–20), the geographic distribution of additional biocide resistance genes has been poorly studied and, to date, there are no data from the African continent, where the prevalence of MRSA is considerably high (up to 60%) (21).

In the present study, we aimed to fulfil this gap by determining the prevalence of six biocide resistance genes among representative *S. aureus* isolates from previous nasal carriage surveillance studies in Angola, São Tomé and Príncipe, and Cape Verde (21, 22). Antimicrobial resistance profiles, molecular characterizations, and information on the presence of virulence determinants were available for all isolates (21, 22). In order to obtain the highest degree of clonal variability, convenience samples of 82 MRSA and 219 methicillin-susceptible *S. aureus* (MSSA) isolates were selected, including one isolate from each pulsed-field gel electrophoresis (PFGE) subtype described in each country and during each screening period: (i) Angola, 57 MRSA and 62 MSSA isolates; (ii) Cape Verde, 6 MRSA and 99 MSSA isolates; and (iii) São Tomé and Príncipe, 19 MRSA and 58 MSSA isolates.

Internal fragments of six efflux pump genes (*qacAB*, *smr*, *norA*, *lmrS*, *mepA*, and *sepA*) were amplified by PCR in all isolates. The primer sequences, modifications of published protocols, and specific controls are listed in Table S1 in the supplemental material.

The chlorhexidine MIC and minimum bactericidal concentration (MBC) were determined for all isolates using a Clinical and Laboratory Standards Institute broth microdilution method (23). *S. aureus* strains ATCC 29213 and ATCC 12228 were used as quality controls.

Categorical variables were compared using Fisher's exact test, and prevalence increasing trends were verified by chi-square test using the GraphPad software version 6.0. In all cases, *P* values of <0.05 were considered statistically significant.

The global prevalence of six biocide resistance genes in the present *S. aureus* collection was very high for *sepA* (95.3%), *mepA* (89.4%), and *norA* (86.4%), intermediate for *lmrS* (60.8%) and *qacAB* (40.5%), and low for *smr* (3.7%). A similar distribution was observed in a comparison of the MRSA and MSSA populations (see Table S2 in the supplemental material), except for *sepA* and *qacAB*, which were more prevalent among MRSA isolates (*P* = 0.0137 and <0.0001, respectively). Although *qacAB* has commonly been reported among MRSA isolates (1, 3, 24, 25), no direct relation was described between methicillin resistance and *qacAB* prevalence (26).

Except for *sepA*, which was prevalent in all countries (*P* = 0.1249), the distributions of other genes were significantly different (see Table S2 in the supplemental material); namely, a higher percentage of *qacAB* was detected in Angola (56.3%, *P* < 0.0001). The *smr* gene, sporadically reported in Europe and Asia (1, 2), was
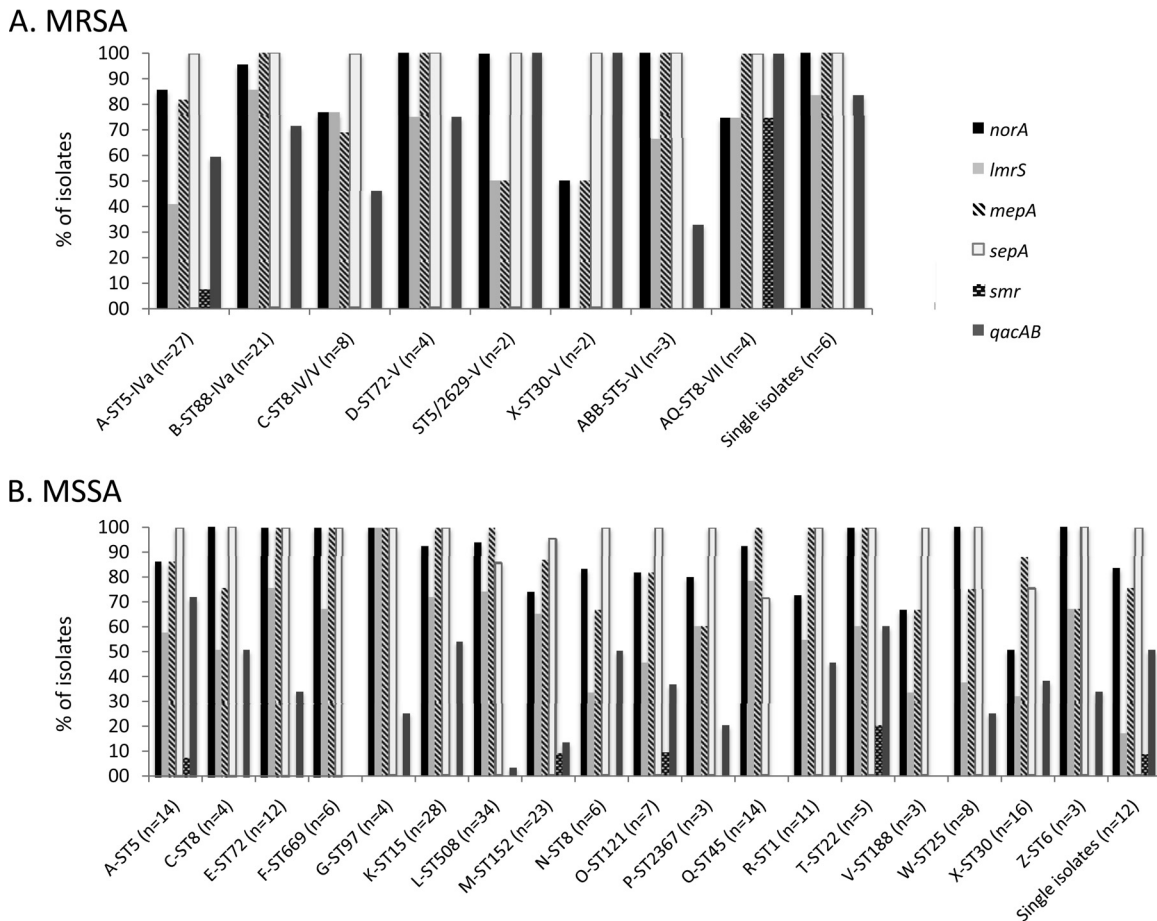
A. MRSA

B. MSSA

FIG 1 Distribution of the six biocide resistance genes by major MRSA (A) and MSSA (B) clonal lineages circulating in Angola, São Tomé and Príncipe, and Cape Verde.

detected only among MSSA isolates from Angola (5.9%) and Cape Verde (3.8%).

Although the presence of some biocide resistance genes has been associated with major *S. aureus* clonal lineages (9, 13, 18, 19, 27, 28), this was not the case in our collection, in which all genes but one (*smr*) were present among the 14 MRSA clonal types (Fig. 1A). Concerning the major clones currently circulating in the three African countries (A-ST5-IVa, B-ST88-IVa, and C-ST8-IV/V) (21), *mepA*, *norA*, and *qacAB* were predominant not only in the B-ST88-IVa lineage (100%, 95%, and 77%, respectively) but in the majority of A-ST5-IVa isolates (81%, 85%, and 59%, respectively) as well. Moreover, *lmrS* was widespread between the B-ST88-IVa (86%) and C-ST8-IV/V (77%) lineages and also found in the A-ST5-IVa lineage (41%). Therefore, biocide resistance genes are highly prevalent among major MRSA clonal lineages in these African countries.

The MSSA population showed higher clonal lineage variability (30 clones) than that of the MRSA population (21), with a parallel variability in the distribution of biocide resistance genes (Fig. 1B). Although a direct correlation between the presence of a biocide resistance gene and a specific clone was not traced, *qacAB* was poorly represented in two of the most predominant MSSA lineages (Q-ST45 and M-ST152) and absent in V-ST188 (Fig. 1B).

In this study, biocide resistance genes were significantly associated with antibiotic resistance (see Table S3 in the supplemental material). *qacAB* was associated with resistance to β-lactams (cefoxitin), aminoglycosides, rifampin, trimethoprim-sulfamethoxazole, and chloramphenicol, which has also been reported in *S. aureus* clinical isolates in China (3). *qacAB* and *smr* resistance genes are located on mobile genetic elements, namely, plasmids that frequently carry other antibiotic resistance genes, which results in the coselection of antibiotic-resistant bacteria (4, 8, 29). Rifampin resistance, which was high in these African countries (21), was associated with the presence of four out of the six antiseptic resistance genes (see Table S3). Rifampin resistance is driven by mutations in the *rpoB* gene, and several reports have suggested that the fitness burden associated with rifampin resistance might influence the prevalence of specific resistance genotypes in clinical strains of *S. aureus* (30). Although these suggestions might justify the maintenance of antiseptic genes as an advantage for bacterial persistence in this environment, this association should not be excluded as a confounding parameter.

The MIC for chlorhexidine ranged between 0.125 and 8 mg/liter in the entire collection, although the overwhelming majority of the isolates (90%) showed an MIC of 0.5 to 1 mg/liter. Higher MICs of 4 and 8 mg/liter were observed in three Angolan isolates only. Similar chlorhexidine MICs (0.5 to 1 mg/liter) were found in

MRSA and MSSA populations (85.0% [70/82 isolates] of MRSA versus 91.8% [201/219 isolates] of MSSA; $P = 0.1285$), as previously reported for MRSA isolates from human and animal origins (10, 31). To date, there is no breakpoint consensus to define biocide-reduced susceptibility, and independent reports have proposed different epidemiological cutoff (ECOFF) values based on the normal distribution of MICs in different *S. aureus* populations (27, 32, 33). In our study, an MIC cutoff of ≥1 mg/liter was defined, since <1% of the isolates showed an MIC of ≥2 mg/liter. In Cape Verde, 40% of the *S. aureus* isolates showed a chlorhexidine MIC of ≥1 mg/liter, which is significantly higher than the values observed in São Tomé and Príncipe (34%) and Angola (23%) ($P = 0.0186$). Chlorhexidine nonsusceptibility still seems to be low in these African countries, despite the high percentage of biocide resistance genes detected in *S. aureus* isolates there. The presence of *qacAB* and *norA* genes was not associated with increased chlorhexidine nonsusceptibility in our collection ($P = 0.7069$ and 0.7196, respectively), although these associations have commonly been described in staphylococci (11).

One of the limitations of our study was the lack of availability of quantitative data concerning biocide usage. Although the prevalence of biocide-resistant isolates in the African population might be underestimated due to the use of convenience samples, these data show that the use of antiseptics might be selecting for antibiotic-resistant strains and assisting their survival in the health care environment, which is of major concern for future infection control programs.

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