

Reduced Triclosan Susceptibility in Methicillin-Resistant *Staphylococcus epidermidis*

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Triclosan MIC determination showed that recent *Staphylococcus aureus* clinical isolates ($n = 100$) were highly susceptible to triclosan, with a 50% minimal inhibitory concentration (MIC₅₀) of 0.12 µg/ml and a MIC₉₀ of 0.25 µg/ml. *Staphylococcus epidermidis* isolates ($n = 96$) were less susceptible, with a MIC₅₀ of 0.12 µg/ml and a MIC₉₀ of 8 µg/ml. Decreased susceptibility to triclosan was more prevalent among methicillin-resistant *S. epidermidis* than among methicillin-sensitive *S. epidermidis* isolates.

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is a widely used antimicrobial agent that is found in a variety of consumer goods and in antiseptic products that are designed for use in the clinical setting (4). Triclosan has been recommended for the control of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospital settings after being used successfully to control in-hospital MRSA outbreaks (2), and it is an additive biocidal agent used in some biomedical devices (9).

Recently, triclosan was determined to have a target-based mechanism, inhibiting the action of one of the enzymes of bacterial fatty acid biosynthesis, enoyl-acyl carrier protein reductase, the product of the *fabI* gene (11, 12). This finding has led to recent concern about the ability of microbes to become resistant to triclosan through both target-based (8, 14) and efflux-based (5, 6) mechanisms.

Typically, many bacterial species, including the staphylococci, are quite susceptible to triclosan. For *S. aureus*, a MIC at which 90% of the strains are inhibited (MIC₉₀) of 0.06 µg/ml was determined in a recent study of 232 clinical isolates (1). MRSA strains with reduced susceptibility to triclosan have appeared in clinical settings, with MICs of triclosan ranging from 1 to 4 µg/ml (1, 3). It has been suggested that hospital treatments may contribute to decreased susceptibility to triclosan. Cookson et al. isolated MRSA strains for which triclosan MICs were 2 to 4 µg/ml from patients who had been treated with daily triclosan baths (7). Others have also found clinical isolates of *S. aureus* with reduced triclosan susceptibility (3, 15), but the resistance levels and frequencies of such isolates remain low. The importance of the reduced triclosan susceptibility in the clinic is unclear.

Reduced susceptibility to triclosan has not been associated with reduced susceptibility to other antibiotic agents. In a previous laboratory study, up to 40-fold increases in the MICs of triclosan, from 0.025 to 1 µg/ml, but the isolates did not show altered susceptibility to vancomycin, beta-lactams, aminoglycosides, or tetracycline (15).

The use of triclosan to eradicate MRSA from patients by

reducing skin colonization, and the increasing use of triclosan as an antimicrobial additive in medical devices (9), motivates continued monitoring of triclosan susceptibility among hospital isolates. We report the triclosan MICs for 100 recent clinical isolates of *S. aureus* and 96 recent clinical isolates of *Staphylococcus epidermidis*, and we found that more than 20% of the *S. epidermidis* isolates showed decreased susceptibility to triclosan. To our knowledge, this is the first report of triclosan MICs for a population of clinical *S. epidermidis* isolates.

A panel of *S. aureus* and *S. epidermidis* strains was assembled from the extensive microbe collection of Focus Technologies (Herndon, Va.). The strains were identified to the species level by using standard identification algorithms. The *S. aureus* strains included 50 MRSA and 50 methicillin-sensitive *S. aureus* strains, and the *S. epidermidis* strains included 47 methicillin-resistant *S. epidermidis* (MRSE) and 49 methicillin-sensitive *S. epidermidis* (MSSE) strains. The methicillin resistance phenotype was identified by determining oxacillin MICs according to NCCLS guidelines (13). All strains were collected between January 2001 and August 2002 and represent unique, nonconsecutive isolates. To obtain a representative and diverse panel, we collected strains from 27 different states in the United States, from 13 different clinical specimen sources, and from male and female patients ranging from 1 to 94 years of age with an approximately equal distribution between inpatients and outpatients.

Antimicrobial susceptibility testing was conducted at Focus Technologies by using broth microdilution methodology in ac-

TABLE 1. In vitro susceptibilities of clinical staphylococcal isolates to triclosan

Organism (no. of isolates)	MIC (µg/ml) of triclosan		
	MIC ₅₀	MIC ₉₀	Range
<i>S. aureus</i> (100)	0.12	0.25	0.06–4.0
MSSA ^a (50)	0.12	0.12	0.06–2.0
MRSA (50)	0.12	0.25	0.06–4.0
<i>S. epidermidis</i> (96)	0.12	8	≤0.03–8.0
MSSE (49)	0.12	1	≤0.03–8.0
MRSE (47)	0.12	8	≤0.03–8.0

^a MSSA, methicillin-sensitive *S. aureus*.

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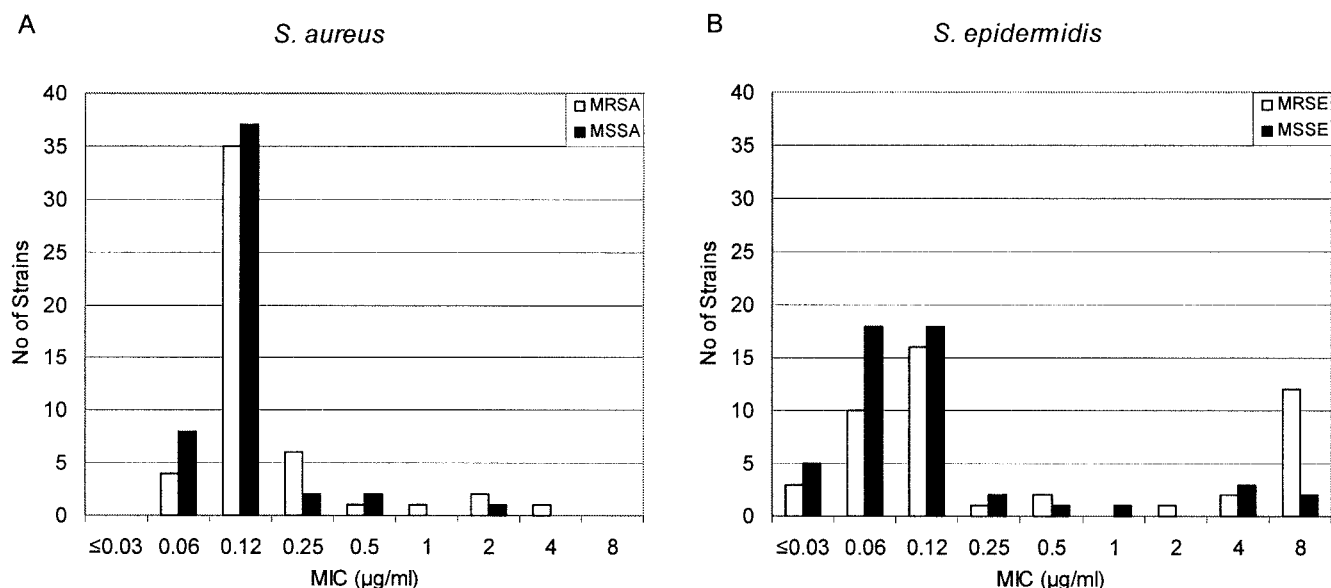


FIG. 1. Distribution of triclosan MICs among *S. aureus* (A) and *S. epidermidis* (B) strains. Open bars indicate methicillin-resistant strains, and solid bars indicate methicillin-sensitive strains.

cordance with NCCLS guidelines (13). Triclosan serial doubling dilutions covered the concentration range between 0.03 and 32 µg/ml.

The MICs for 100 *S. aureus* and 96 *S. epidermidis* recent clinical isolates were determined, and the results of these tests are summarized in Table 1. The majority of the *S. aureus* clinical isolates were highly susceptible to triclosan, with the MIC₅₀ being 0.12 µg/ml and the MIC₉₀ being 0.25 µg/ml. In contrast, the MIC₅₀ for the *S. epidermidis* isolates was 0.12 µg/ml and the MIC₉₀ was 8 µg/ml, indicating substantial heterogeneity in the *S. epidermidis* population.

The distribution of MIC results for the *S. aureus* and *S. epidermidis* strains is shown in Fig. 1. Of the *S. epidermidis* strains the, triclosan MICs were above 0.5 µg/ml for 22%. This finding is in contrast to the *S. aureus* results, in which the triclosan MICs were greater than 0.5 µg/ml for only 5%. For the *S. epidermidis* isolates, decreased susceptibility to triclosan was more prevalent among the MRSE strains than among the MSSE strains: the triclosan MICs were greater than 0.5 µg/ml for 15 of the 47 MRSE isolates (32%), were only 6 of the 49 MSSE isolates (12%). As with the MRSE strains, the majority of the *S. aureus* strains with reduced susceptibility to triclosan (4 of 5) possessed the MRSA phenotype.

Our results confirm a relatively low proportion of *S. aureus* clinical isolates with reduced susceptibility to triclosan. We found that for 5% of the *S. aureus* population ($n = 100$), triclosan MICs were greater than 0.5 µg/ml. These results are very similar to those from other studies of *S. aureus* clinical isolates, in which triclosan MICs were greater than 0.5 µg/ml for 4.3% ($n = 232$) and 7.5% ($n = 186$) (1, 3).

The *S. epidermidis* isolates include a higher percentage of isolates with reduced triclosan susceptibility than do the *S. aureus* isolates. The reasons for the larger population of *S. epidermidis* isolates with decreased triclosan susceptibility may be greater selective pressure and exposure to triclosan (be-

cause of frequent contact with triclosan-containing antimicrobial products) of *S. epidermidis* strains than of *S. aureus* strains. Alternatively, the mechanisms and frequencies of resistance may be different in *S. epidermidis* and *S. aureus*.

The strains selected here were chosen to be diverse and representative, a design which may have introduced unintended biases. Nonetheless, we believe that these results warrant additional follow-up studies aimed to further understand and explore the extent of *S. epidermidis* strains with decreased susceptibility and the molecular mechanisms by which these strains are reducing their susceptibility to triclosan.

The importance of staphylococci with reduced susceptibility to triclosan in the clinical setting is uncertain. While the amount of triclosan found in products is generally quite high (2.5 mg/ml is typical in soaps [10]), the potency of triclosan appears to be reduced in the formulated product. Levy showed that the potency of triclosan against *Escherichia coli* was reduced by 10- to 20-fold in a soap formulation (10). Thus, the decreased susceptibility of *S. epidermidis* to triclosan observed here may pose some increased risk to patients. We conclude that caution should be observed in the use of triclosan-containing agents as antiseptics expected to limit the growth of *S. epidermidis* strains and that additional studies to understand the association between reduced triclosan susceptibility and methicillin resistance are warranted.

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