

Triclosan Promotes *Staphylococcus aureus* Nasal Colonization

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ABSTRACT The biocide triclosan is used in many personal care products, including toothpastes, soaps, clothing, and medical equipment. Consequently, it is present as a contaminant in the environment and has been detected in some human fluids, including serum, urine, and milk. *Staphylococcus aureus* is an opportunistic pathogen that colonizes the noses and throats of approximately 30% of the population. Colonization with *S. aureus* is known to be a risk factor for several types of infection. Here we demonstrate that triclosan is commonly found in the nasal secretions of healthy adults and the presence of triclosan trends positively with nasal colonization by *S. aureus*. We demonstrate that triclosan can promote the binding of *S. aureus* to host proteins such as collagen, fibronectin, and keratin, as well as inanimate surfaces such as plastic and glass. Lastly, triclosan-exposed rats are more susceptible to nasal colonization with *S. aureus*. These data reveal a novel factor that influences the ability of *S. aureus* to bind surfaces and alters *S. aureus* nasal colonization.

IMPORTANCE Triclosan has been used as a biocide for over 40 years, but the broader effects that it has on the human microbiome have not been investigated. We demonstrate that triclosan is present in nasal secretions of a large portion of a test population and its presence correlates with *Staphylococcus aureus* nasal colonization. Triclosan also promotes the binding of *S. aureus* to human proteins and increases the susceptibility of rats to nasal colonization by *S. aureus*. These findings are significant because *S. aureus* colonization is a known risk factor for the development of several types of infections. Our data demonstrate the unintended consequences of unregulated triclosan use and contribute to the growing body of research demonstrating inadvertent effects of triclosan on the environment and human health.

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Staphylococcus aureus is a human commensal and pathogen that colonizes the nares and throats of approximately 30% of the human population and was responsible for the deaths of approximately 19,000 people in the United States in 2005 (1). *S. aureus* infections range from relatively mild abscesses to more severe diseases such as endocarditis and bacteremia (1). *S. aureus* nasal colonization is a significant risk factor for several infections, including bacteremia, postoperative infections, and diabetic foot ulcer infections, and contributes to the spread of this pathogen in hospital environments (2). *S. aureus* nasal colonization is influenced by host and bacterial factors, with carriage rates differing among ethnic groups and families (3). The binding of host proteins such as keratin, fibrinogen, and collagen promotes *S. aureus* adherence and human colonization (4). Despite this knowledge, the reason why *S. aureus* successfully colonizes some individuals while others remain noncolonized remains an enigma.

The biocide triclosan is used in a vast number of personal care products, including soaps, toothpastes, kitchen surfaces, clothes, and medical equipment (5). Despite recent reviews of triclosan's potential impact on public health, it is still used widely and faces minimal regulation in the United States; as a result, triclosan is accumulating in the environment, as well as in human bodies (6).

Triclosan is readily absorbed by the gastrointestinal tract and oral mucosa, leading to the detection of sub-MICs of triclosan in human serum, urine, and milk (6–8). In a recent study, the concentration of triclosan in stream sediments was found to increase with urbanization and correlated with a significant change in the streambed microbial ecology (9). Triclosan targets the enoyl-acyl carrier protein reductase of the bacterial type II fatty acid synthesis (FASII) pathway and inhibits fatty acid biosynthesis (10). At higher concentrations, triclosan is thought to have broad antimicrobial effects through the disruption of cell membranes and less specific interactions with other proteins (11). In mammals, triclosan has been shown to disrupt the endocrine system by antagonizing estrogen and androgen receptors, as well as to elevate the resting cytosolic $[Ca^{2+}]$ in primary skeletal myotubules (12). Further analysis of the effect of triclosan on mammalian muscle function found that the presence of triclosan impairs the excitation-contraction coupling of cardiac and skeletal muscles (13). Strikingly, mice exposed to triclosan exhibited an up to 25% decrease in cardiac output and an 18% mean decrease in grip strength, demonstrating a striking effect of triclosan on muscle function (13).

Triclosan is embedded in many medical devices, such as su-

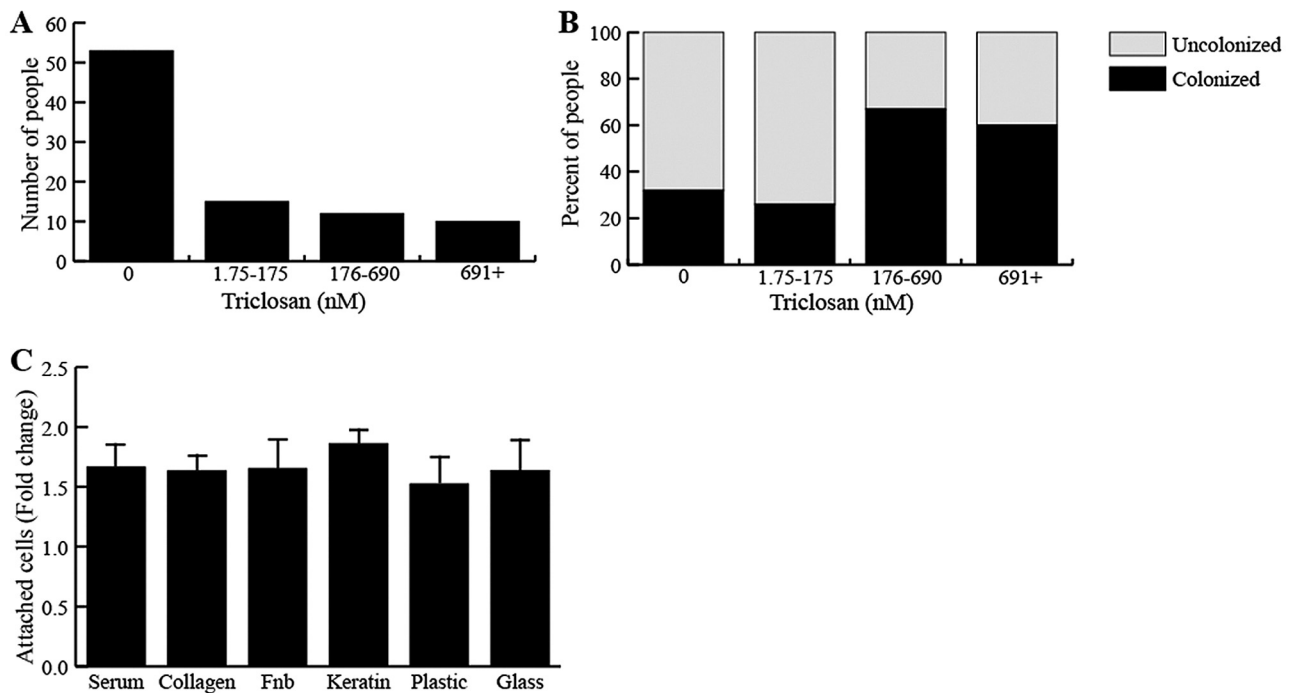


FIG 1 Triclosan influences interactions with the human nares. (A) Nasal secretions were collected from 90 healthy individuals, and an ELISA was performed to determine the concentration of triclosan in each secretion. (B) The same individuals were also swabbed for the presence of *S. aureus* colonization of the nares. The differences in *S. aureus* nasal colonization between ≤ 175 nM triclosan and >175 nM triclosan is statistically significant by ANOVA ($P < 0.01$). (C) Attachment assays were performed with human serum, collagen, fibronectin (Fnb), and keratin, which are found in nasal secretions, as well as with plastic and glass surfaces. *S. aureus* treated with 50 nM triclosan bound all of these host proteins and artificial surfaces to a greater extent than untreated cells did ($P < 0.05$ by *t* test).

tures and catheters, for the prevention of infections (14, 15). Triclosan baths are also a recommended method for decolonizing an individual who is colonized with *S. aureus* prior to surgery (16). Using triclosan in medical devices may lead to unexpected complications by affecting microbes exposed to sub-MICs of the biocide. It has been shown that long-term exposure of *S. aureus* to triclosan results in the induction of small-colony variants that are more resistant to antimicrobials (17). Furthermore, resistance to triclosan can be gained by *S. aureus* through mutations in the *fabI* gene, as well as other unknown Fab-independent ways (18). There is also debate about the essentiality of the type II fatty acid biosynthesis pathway that triclosan targets. In the presence of triclosan in human serum, *S. aureus* can modify and incorporate exogenous host fatty acids into cells, strengthening the argument against the essentiality of FASII in the host (19).

Here, we investigated if triclosan can be detected in human nasal secretions and if its presence increases the risk of *S. aureus* nasal colonization. We hypothesized that triclosan would be present in the nasal secretions of some individuals because it has been detected in serum, a major component of nasal secretions (20). We collected nasal secretions from 90 healthy adults and performed enzyme-linked immunosorbent assays (ELISAs) to detect the levels of triclosan present. We found that 37 (41%) of the 90 people in our sample population had detectable levels (≥ 1.75 nM) of triclosan in their nasal secretions (Fig. 1A). To investigate if the presence of triclosan in nasal secretions relates to colonization with *S. aureus*, we concurrently swabbed the nares of our sample population and detected *S. aureus* colonization by using a selective

and differential medium for *S. aureus* isolation, mannitol salt agar. A positive trend between the presence of triclosan in nasal secretions and colonization with *S. aureus* was observed (Fig. 1B). Individuals without triclosan or with levels of <175 nM had *S. aureus* carriage rates of 32 and 27%, respectively, which were in line with previous studies; however 64% of the individuals with >176 nM triclosan in their nasal secretions were colonized with *S. aureus* (Fig. 1B). Analysis of variance (ANOVA) revealed that significantly more individuals with >176 nM triclosan than individuals with <175 nM triclosan had *S. aureus* nasal colonization ($P < 0.01$).

Previous work has shown that attachment to host proteins is important for *S. aureus* nasal colonization (21). Therefore, we performed attachment assays to determine if the presence of triclosan induced the binding of *S. aureus* to host proteins (22). *S. aureus* grown in the presence of triclosan displayed significantly greater attachment to all of the host proteins tested than did non-triclosan-exposed cells (Fig. 1C). Along with host proteins, we also tested the ability of triclosan to induce the attachment of *S. aureus* to plastic (vinyl) and glass surfaces. We determined that triclosan-treated cells had significantly greater attachment to both plastic and glass surfaces than controls did (Fig. 1C).

To further investigate the role that triclosan may be playing in nasal colonization, we used a cotton rat model of *S. aureus* nasal colonization (23). Rats were gavaged with 1 ml of triclosan resuspended in corn oil at a concentration of 100 mg/kg/day or an oil control for 3 consecutive days. On day 5, rats were nasally inoculated with either a small inoculum (10^5 CFU) or a large inoculum

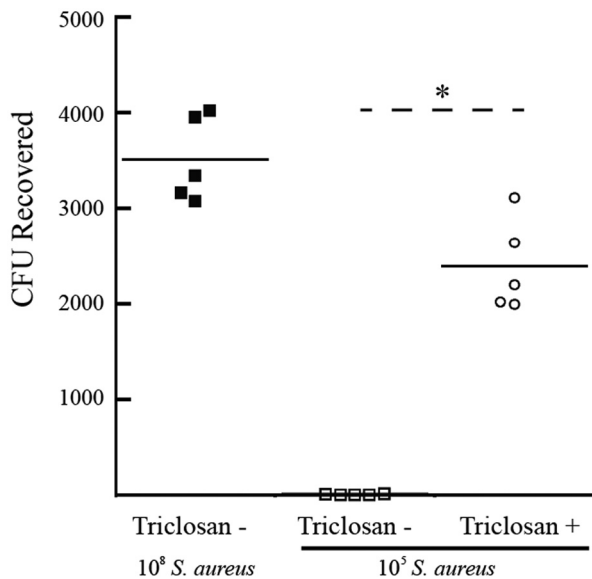


FIG 2 Triclosan-gavaged rats are more susceptible to *S. aureus* nasal colonization. Cotton rats were gavaged with triclosan or an oil control for 3 days. On day 5, they were challenged with either a small inoculum (10^5 CFU) or a large inoculum (10^8 CFU) of *S. aureus*. On day 12, their noses were removed and *S. aureus* bacteria were enumerated. Rats gavaged with triclosan were unable to clear the small inoculum, whereas the oil-gavaged rats were able to clear the bacteria from their noses. *, $P < 0.01$ by *t* test.

(10^8 CFU) of *S. aureus*. On day 12, their noses were removed and *S. aureus* bacteria were enumerated. We observed that triclosan-exposed rats were more susceptible to colonization with *S. aureus* than were rats not exposed to triclosan (Fig. 2). The triclosan-exposed rats were significantly more susceptible to colonization with the small inoculum (10^5), whereas the control rats needed the larger inoculum (10^8) to become colonized.

Together, our data demonstrate that triclosan can be present in the nasal secretions of healthy human adults and that its presence in nasal secretions trends positively with *S. aureus* nasal colonization. Triclosan also promotes the binding of *S. aureus* to human proteins and artificial surfaces. Furthermore, in an *in vivo* model of nasal colonization, triclosan-exposed rats are more susceptible to colonization with *S. aureus*. In light of the significant use of triclosan in consumer products and its widespread environmental contamination, our data combined with previous studies showing impacts of triclosan on the endocrine system and muscle function suggest that a reevaluation of triclosan in consumer products is urgently needed (12, 13, 19, 24).

Methods. (i) Nasal colonization model and human nasal secretion collection. The cotton rat nasal colonization model described by Kokai-Kun was used in this study (23). Briefly, rats were gavaged with 1 ml of triclosan resuspended in corn oil at a concentration of 100 mg/kg/day or an oil control for 3 consecutive days. On day 5, rats were nasally inoculated with either a small inoculum (10^5 CFU) or a large inoculum (10^8 CFU) of *S. aureus* strain SH1000. On day 12, their noses were removed and *S. aureus* bacteria were enumerated. All animal experiments were conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the University of Michigan University Committee on Use and Care of Animals

(approval number 10394). Statistical significance was determined with a *t* test.

Nasal secretions were collected from a convenience sample of 90 adult volunteers in Ann Arbor, MI. Written informed consent was provided by study participants (University of Michigan Institutional Review Board approval number IRB00001996). The anterior nares were swabbed, and the swabs were streaked onto mannitol salt agar to determine colonization by *S. aureus*. Secretions were collected by thoroughly swabbing the anterior and posterior nasal passageways with a sterile cotton swab (Remel BactiSwab). The tip of the swab was cut from the shaft, placed in a ridged Eppendorf tube, and centrifuged to obtain secretions. The volumes of secretions obtained varied from ~50 to ~200 μ l. Collected secretions were vortexed and sonicated to break up clumps and then passed through a 0.22- μ m syringe filter. Secretions were stored at -20°C until they were analyzed by ELISA (Abraxis, Warminster, PA). Statistical significance was determined by ANOVA (Macanova version 5.05).

(ii) Attachment assay. The binding of *S. aureus* to surfaces coated with human serum, collagen, fibronectin, and keratin was tested as previously described (22). All reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Briefly, untreated 96-well plates (Nunc 265301) were incubated with 100 μ l of 1% human serum, 0.2% type IV collagen from human placenta, 0.01% fibronectin from human plasma, and 0.2% keratin from human epidermis for 24 h while shaking at 4°C . The plate was then washed three times with 1% bovine serum albumin. A 100- μ l volume of *S. aureus* strain SH1000 cells grown for 24 h in the presence or absence of a sub-MIC (50 nM) of triclosan or control dimethyl sulfoxide was incubated in plates for 1 h statically at 37°C . At the end of the treatment period, wells were washed three times with phosphate-buffered saline (PBS) to remove nonadherent cells. Attached cells were fixed with 2.5% glutaraldehyde in PBS for 1 h statically at 37°C . Attached cells were then stained with 0.1% crystal violet for 30 min at room temperature (RT), washed three times with water, and then quantified by resuspension in acidified ethanol and measurement of absorbance at 570 nm. Statistical significance was determined with a *t* test.

To assay for *S. aureus* binding to surfaces, glass or plastic coverslips were placed in 12-well plates (Costar 3513). Three milliliters of triclosan-treated or control *S. aureus* was incubated in the plates for 1 h statically at 37°C . At the end of the treatment, wells were washed three times with PBS to remove nonadherent cells. Attached cells were fixed with 2.5% glutaraldehyde in PBS for 1 h statically at 37°C . Attached cells were then stained with 0.1% crystal violet for 30 min at RT, washed three times with water, transferred to a clean container, and quantified by resuspension in acidified ethanol and measurement of absorbance at 570 nm. Statistical significance was determined with a *t* test.

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