

Whither triclosan?

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Triclosan has activity against many, but not all, types of Gram-positive and Gram-negative bacteria. It is bacteriostatic at low concentrations, but higher concentrations are bactericidal. *Pseudomonas aeruginosa* is highly resistant, whereas methicillin-resistant *Staphylococcus aureus* strains are inhibited over a range of ~0.1–2 mg/L. Triclosan shows significant activity against some mycobacteria, but is not sporicidal. Its growth-inhibitory properties result from an inhibition of enoyl reductase, FabI. Membrane-destabilizing effects are likely to be responsible for bacterial inactivation by higher concentrations. Resistance can arise from mutations in, and/or overproduction of, FabI, impermeability or efflux. Whilst triclosan resistance in laboratory experiments may be associated with changes in antibiotic susceptibility, comprehensive environmental surveys have not demonstrated any association between triclosan usage and antibiotic resistance. Triclosan has several important uses, and the future aim must be to retain these applications whilst eliminating the more frivolous and unnecessary ones.

Keywords: mechanisms of action, mechanisms of resistance, antibiotic resistance

Introduction

The phenylether or chlorinated bisphenol, triclosan, is an antimicrobial agent that has been employed for a variety of purposes for more than 20 years. It is used clinically and in oral hygiene products, and is incorporated into many types of cosmetic formulations.¹ Triclosan has a broad range of activity that encompasses many, but not all, types of Gram-positive and Gram-negative non-sporulating bacteria, some fungi,^{2,3} *Plasmodium falciparum* and *Toxoplasma gondii*.⁴ Its spectrum^{3,5} includes high activity (low MICs often of the order of 0.01–0.1 mg/L) against staphylococci, some streptococci, some mycobacteria, *Escherichia coli* and *Proteus* spp. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains may or may not show an elevated triclosan MIC.⁶ Enterococci are much less susceptible than staphylococci,⁵ and *Pseudomonas aeruginosa* is highly resistant.^{3,5} Bacterial spores are unaffected. Triclosan is bacteriostatic at low concentrations but higher levels are bactericidal.^{7,8}

In recent years, there has been renewed interest in the antibacterial properties of triclosan. This stems from the finding that triclosan had a more specific mechanism of action than hitherto realized,⁹ and that there might be a link between triclosan usage and antibiotic resistance.¹⁰ These aspects have since been examined extensively, and it is the purpose of this short paper to consider four aspects that pertain to this issue: (i) the mechanism(s) of action of triclosan; (ii) the mechanism(s) of bacterial resistance to triclosan; (iii) the possible association between triclosan usage in the clinical and domiciliary environments and antibiotic resistance; and (iv) its current and future usage.

Mechanism(s) of action of triclosan

Earlier studies, reviewed by Russell,¹¹ suggested that the bacterial cytoplasmic (inner) membrane was the major target for triclosan action. However, it was later demonstrated in *E. coli*, *S. aureus* and other triclosan-susceptible organisms that the growth-inhibitory activities of the phenylether resulted from blocking lipid synthesis by specifically inhibiting an NADH-dependent enoyl-acyl carrier protein (ACP) reductase, or FabI.^{9,12,13} The enoyl reductase, InhA, in *Mycobacterium smegmatis* was also found to be a target for triclosan action.¹⁴

The question then arises as to whether inhibition of a single enzyme by triclosan is responsible for its inhibitory and lethal actions.^{11,15,16} Triclosan is normally employed in practice at concentrations much greater than the MICs cited above against highly susceptible bacteria. At such concentrations triclosan is rapidly bactericidal,^{7,8} and this lethal activity extends to triclosan-resistant strains of *E. coli*.¹⁷ Triclosan-induced K⁺ leakage, indicative of membrane damage, occurs at bactericidal levels.^{7,8} Membrane-destabilizing effects have also been demonstrated by Villalain *et al.*¹⁸ Triclosan demonstrates a Z-pattern type of adsorption, which is indicative of the breakdown of a structure, presumably the membrane, and the generation of new adsorbing sites.¹⁹ As with other biocidal agents, triclosan possesses more than one type of action, and it is possible to delineate its growth-inhibitory and lethal effects.

Bacterial resistance to triclosan

In the laboratory, triclosan-resistant bacteria can be produced fairly readily by serial passage in increasing triclosan concentrations¹⁷ or

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by isolation of resistant colonies within growth inhibition zones around a triclosan disc.^{7,8} Resistance may be due to overproduction of enoyl reductase, an insensitive form of enzyme or to changes in cellular impermeability in *E. coli*.¹⁷

In *P. aeruginosa*, which is intrinsically resistant to triclosan, resistance could be due to a non-susceptible enoyl reductase (both triclosan-susceptible and -non-susceptible enzymes have been found²⁰), an outer membrane permeability barrier or efflux. The permeabilizer, ethylenediamine tetraacetic acid (EDTA), does not increase susceptibility to triclosan,²¹ and efflux has been stated to be the major reason for triclosan non-susceptibility.^{22–24}

MRSA strains may or may not show elevated triclosan MICs.^{6–8,25} Fan *et al.*²⁶ found that all *S. aureus* strains with elevated triclosan MICs overexpressed FabI production by three- to five-fold, and that strains with the highest MICs (1–2 mg/L) also had mutations in FabI.

Possible association between triclosan and antibiotic resistance

The overexpression of *marA*, *soxS* or *acrAB* in laboratory or clinical strains of *E. coli* reduces their susceptibility to triclosan, and also to ampicillin, tetracyclines and fluoroquinolones.¹⁰ The increases in resistance in terms of MIC are, however, low. Exposure of a triclosan-susceptible mutant of *P. aeruginosa* to triclosan switches on an efflux pump that renders the cells highly resistant to ciprofloxacin.²² However, there is no evidence that triclosan usage is responsible for clinical ciprofloxacin resistance in either human or veterinary medicine.²³

Susceptibility of MRSA strains to triclosan has changed little over a 10 year period,⁶ and there does not seem to be any association between triclosan response in MRSA and other strains of *S. aureus* and antibiotic susceptibility or resistance.^{7,8}

Both triclosan and the important antitubercular drug, isoniazid, inhibit enoyl reductase in *M. smegmatis* and *Mycobacterium tuberculosis*.^{14,27,28} In the former, mutations in the *inhA* gene produce increases in resistance to both triclosan and isoniazid. Triclosan binds in a similar manner to the enoyl reductase (InhA) of mycobacteria and that of *E. coli*. The InhA from *M. tuberculosis* is 36% identical and 65% similar to EnvM, and 87% identical and 97% similar to the InhA from *M. smegmatis*.¹⁴ Nevertheless, there are differences in the effects of triclosan and isoniazid^{27,28} such that inhibitors targeted at the enoyl substrate binding site could produce effective drugs against isoniazid-resistant strains of *M. tuberculosis*.²⁷

It has been emphasized that laboratory studies have a useful role to play in evaluating mechanisms of action of and resistance to biocides, including triclosan, but that these should, wherever possible, be related to the clinical and other uses of these agents.^{15,16} Do biocides therefore select for antibiotic resistance?²⁹ Certainly, there are some similarities in the manner in which bacteria resist the action of both types of antibacterial agents.³⁰ Several authors have purported to show a relationship between the use of triclosan (or other biocides) and antibiotic resistance.^{31–33} Others have cast doubt on this proposal,^{16,29,34,35} whilst emphasizing that, like all biocides, triclosan should be used only when appropriate.

Some recent surveys on the use of triclosan and other biocides add weight to these doubts. In the first,³⁶ conducted over a 10 year period, it was found that there was no relationship between triclosan usage and antibiotic resistance in MRSA and *P. aeruginosa*. In the second,³⁷ it was shown that there were no significant differences in overall titres of bacteria, potential pathogens or frequencies of anti-

biotic resistance in a single-time analysis of homes that did or did not use surface antibacterial agents. A comprehensive survey by Cole *et al.*³⁸ could find no relationship between the use of triclosan and other biocides and antibiotic resistance in homes where biocidal products were or were not being used.

What, then, of the incorporation of triclosan into oral products? Will the use of triclosan in dental hygiene products result in the development of triclosan-resistant bacteria with reduced susceptibility to important antibiotics? An Expert Panel review concluded in 2000 that there was no evidence of resistant, opportunistic or pathogenic microorganisms developing.³⁹ The short-term use of triclosan had no major impact on normal oral microflora or on the streptococcal susceptibility to antibiotics.⁴⁰ Chronic exposure to triclosan did not demonstrate significant decreases in antibiotic susceptibility in dental bacteria.⁴¹ In general terms, the use of antimicrobial agents in dental care products in order to reduce plaque is considered to be justified.⁴²

Overall, there is no convincing evidence to support the contention that triclosan usage has resulted in the clinical development of antibiotic-resistant Gram-negative bacteria, antibiotic-resistant cocci or isoniazid-resistant *M. tuberculosis*.¹⁶ Nevertheless, it would be wise to restrict the use of triclosan to areas where it has been shown to be effective.⁴³

Uses of triclosan

There remain concerns about the unnecessary use of triclosan and other biocides in the home and in clinical settings.^{44,45} Furthermore, triclosan has been found in human milk and in the aquatic environment in Sweden.⁴⁶ Triclosan has been used in skin-care products for some 30 years. It has also been employed as surgical scrubs, hand-washes, body washes to control MRSA and in dental hygiene products. More recent uses include the suggestion that it could be employed to control encrustation and blockage of Foley catheters caused by *Proteus mirabilis*, an organism that is highly susceptible to triclosan.⁴⁷ In addition, novel inhibitors of FabI are being developed.⁴⁸ Clearly, in view of the concerns expressed about the phenylether, the future aim should be to retain its important and valuable applications whilst eliminating the more frivolous and unnecessary ones.

Finally, although it is sometimes stated that resistance to triclosan and other biocides is increasing,⁴⁴ this conclusion is generally based upon MICs rather than bactericidal estimations. Bacterial resistance to disinfectants in general is most certainly not a new phenomenon, and there are known examples of reduced susceptibility being described over a century ago.⁴⁹ Triclosan, of course, is of more recent vintage. Consequently, it is necessary to continue to monitor whether reduced susceptibility to it and to antibiotics occurs.

References

1. Parfitt, K. (1999). In *Martindale, The Complete Drug Reference*, p. 1127. Pharmaceutical Press, London, UK.
2. Jones, R. D., Jampani, H. B., Neman, J. L. *et al.* (2000). Triclosan: a review of effectiveness and safety in health care settings. *American Journal of Infection Control* **28**, 184–96.
3. Schweizer, H. P. (2001). Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiology Letters* **202**, 1–7.
4. McLeod, R., Muench, S. P., Rafferty, J. B. *et al.* (2001). Triclosan inhibits the growth of *Plasmodium falciparum* and *Toxoplasma gondii* by inhibition of apicomplexan FabI. *International Journal of Parasitology* **31**, 109–13.

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5. Ciba-Geigy. (1988). Document: Irgasan DP 300. Ciba, Wyhlen, Germany.
6. Al-Doori, Z., Morrison, D., Edwards, G. *et al.* (2003). Susceptibility of MRSA to triclosan. *Journal of Antimicrobial Chemotherapy* **51**, 185–6.
7. Suller, M. T. E. & Russell, A. D. (1999). Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococcus. *Journal of Hospital Infection* **43**, 281–91.
8. Suller, M. T. E. & Russell, A. D. (2000). Triclosan and antibiotic resistance in *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **46**, 11–8.
9. McMurry, L. M., Oethinger, M. & Levy, S. B. (1998). Triclosan targets lipid synthesis. *Nature* **394**, 531–2.
10. McMurry, L. M., Oethinger, M. & Levy, S. B. (1998). Over-expression of *marA*, *soxS* or *acrAB* produces resistance to triclosan in *Escherichia coli*. *FEMS Microbiology Letters* **186**, 305–9.
11. Russell, A. D. (2003). Similarities and differences in the responses of microorganisms to biocides. *Journal of Antimicrobial Chemotherapy* **52**, 750–63.
12. Levy, C. W., Roujeinikova, A., Sedelnikova, S. *et al.* (1999). Molecular basis of triclosan activity. *Nature* **398**, 383–4.
13. Heath, R. J. & Rock, C. O. (2000). A triclosan-resistant bacterial enzyme. *Nature* **406**, 145–6.
14. McMurry, L. M., McDermott, P. F. & Levy, S. B. (1999). Genetic evidence that *InhA* of *Mycobacterium smegmatis* is a target for triclosan. *Antimicrobial Agents and Chemotherapy* **43**, 711–3.
15. Russell, A. D. (2002). Mechanisms of antimicrobial action of anti-septics and disinfectants: an increasingly important area of investigation. *Journal of Antimicrobial Chemotherapy* **49**, 597–9.
16. Russell, A. D. (2003). Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infectious Diseases* **3**, 794–803.
17. McDonnell, G. & Pretzer, D. (1998). Action and targets of triclosan. *ASM News* **84**, 670–1.
18. Villalain, J., Mateo, C. R., Aranda, F. J. *et al.* (2001). Membranotropic effects of the antibacterial agent triclosan. *Archives of Biochemistry and Biophysics* **390**, 128–36.
19. Denyer, S. P. & Maillard, J.-Y. (2002). Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. *Journal of Applied Microbiology* **92**, 35S–45S.
20. Heath, R. J., White, S. W. & Rock, C. O. (2001). Lipid biosynthesis as a target for antibacterial agents. *Progress in Lipid Research* **40**, 467–97.
21. Lear, J. C., Maillard, J.-Y., Dettmar, P. W. *et al.* (2002). Chloroxylenol- and triclosan-tolerant bacteria from industrial sources. *Journal of Industrial Microbiology and Biotechnology* **29**, 238–42.
22. Chuanchen, R., Beinlich, K., Hoang, T. T. *et al.* (2001). Cross-resistance between triclosan and antibiotics is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects *nfxB* mutants overexpressing MexCD-OprJ. *Antimicrobial Agents and Chemotherapy* **45**, 428–32.
23. Beinlich, K. I., Chuanchen, R. & Schweizer, H. P. (2001). Contribution of multidrug efflux pumps to multiple antibiotic resistance in veterinary isolates of *Pseudomonas aeruginosa*. *FEMS Microbiology Letters* **198**, 129–34.
24. Chuanchen, R., Karkhoff-Schweizer, R. R. & Schweizer, H. P. (2003). High-level triclosan resistance in *Pseudomonas aeruginosa* is solely a result of efflux. *American Journal of Infection Control* **31**, 124–7.
25. Bamber, A. I. & Neal, T. J. (1999). An assessment of triclosan susceptibility in methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *Journal of Hospital Infection* **41**, 107–9.
26. Fan, F., Yan, K., Wallis, G. S. *et al.* (2002). Defining and combating the mechanisms of triclosan resistance in clinical isolates of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **46**, 3343–7.
27. Parikh, S. I., Xiao, G. & Tonge, P. J. (2000). Inhibition of *InhA*, the enoyl reductase from *Mycobacterium tuberculosis*, by triclosan and isoniazid. *Biochemistry* **39**, 7645–50.
28. Slayden, R. A., Lee, R. E. & Barry, C. E., 3rd (2000). Isoniazid affects multiple components of the type II fatty acid synthase system of *Mycobacterium*. *Molecular Microbiology* **38**, 514–25.
29. Russell, A. D. (2000). Do biocides select for antibiotic resistance? *Journal of Pharmacy and Pharmacology* **52**, 227–33.
30. Poole, K. (2002). Mechanisms of bacterial biocide and antibiotic resistance. *Journal of Applied Microbiology* **92**, 55S–64S.
31. Levy, S. B. (2001). Antibacterial household products: cause for concern. *Emerging Infectious Diseases* **7**, Suppl. 3, 512–5.
32. White, D. G. & McDermott, P. F. (2001). Biocides, drug resistance and microbial evolution. *Current Opinions in Microbiology* **4**, 313–7.
33. Levy, S. B. (2002). Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **49**, 25–30.
34. Gilbert, P. & McBain, A. J. (2001). Biocide usage in the domestic setting and concern about antibacterial and antibiotic resistance. *Journal of Infection* **43**, 85–91.
35. McBain, A. J., Rickard, A. H. & Gilbert, P. (2002). Possible implications of biocide accumulation in the environment on the prevalence of bacterial antibiotic resistance. *Journal of Industrial Microbiology and Biotechnology* **29**, 326–30.
36. Lambert, R. J. W., Graf, J. F. & Sedlak, R. I. (2002). Antimicrobial resistance and cross-resistance in several bacterial species between 1989 and 2000. In *Program and Abstracts of the Forty-second Inter-science Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, 2002*. Abstract C2-1127, p. 103. American Society for Microbiology, Washington, DC, USA.
37. Marshall, B. M., Robleto, E., Dumont, T. *et al.* (2003). The frequency of bacteria and antibiotic resistance in homes that use and do not use surface antibacterial agents. In *103rd General Meeting of the American Society for Microbiology, Washington, DC, USA, 2003*. Abstract A-147. American Society for Microbiology, Washington, DC, USA.
38. Cole, E. C., Addison, R. M., Rubino, J. R. *et al.* (2003). Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *Journal of Applied Microbiology* **95**, 664–76.
39. Expert Panel. (2000). Laboratory and clinical evidence documenting the microbiologic safety of Colgate Total®. *Biological Therapies in Dentistry* **16**, 17–20.
40. Sullivan, A., Wretling, B. & Nord, C. E. (2003). Will triclosan in toothpaste select for resistant oral streptococci? *Clinical Microbiology & Infection* **9**, 306–9.
41. McBain, A. J., Ledder, R. G., Sreenivasan, P. *et al.* (2004). Selection for high-level resistance by chronic triclosan exposure is not universal. *Journal of Antimicrobial Chemotherapy* **53**, DOI: 10.1093/jac/dkh168.
42. Health Council of The Netherlands. (2001). *Disinfectants in Consumer Products*. Publication No. 2001/05E. Health Council of The Netherlands, The Hague, The Netherlands.
43. Cozad, A. & Jones, R. D. (2003). Disinfection and the prevention of infectious disease. *American Journal of Infection Control* **31**, 243–54.
44. Tan, L., Nielsen, N. H., Young, D. C. *et al.* (2002). Use of antimicrobial agents in consumer products. *Archives of Dermatology* **138**, 1082–6.
45. Dixon, B. (2004). Biocides in the kitchen. *ASM News* **70**, 4–5.
46. Adolfsson-Erici, M., Pettersson, M., Parkkonen, J. *et al.* (2002). Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* **46**, 1485–9.
47. Stickler, D. J., Jones, G. L. & Russell, A. D. (2003). Control of encrustation and blockage of Foley catheters. *Lancet* **361**, 1435–7.
48. McDevitt, D., Payne, D. J., Holmes, D. J. *et al.* (2002). Novel targets for the future development of antibacterial agents. *Journal of Applied Microbiology* **92**, 28S–34S.
49. Russell, A. D. (2004). Bacterial adaptation and resistance to anti-septics, disinfectants and preservatives is not a new phenomenon. *Journal of Hospital Infection*, in press.