Triclosan is the active ingredient in a multitude of health care and consumer products with germicidal properties, which have flooded the market in recent years in response to the public’s fear of communicable bacteria. Although originally thought to kill bacteria by attacking multiple cellular targets, triclosan was recently shown to target a specific bacterial fatty acid biosynthetic enzyme, enoyl-[acyl-carrier protein] reductase, in Gram-negative and Gram-positive bacteria, as well as in the Mycobacteria. Triclosan resistance mechanisms include target mutations, increased target expression, active efflux from the cell, and enzymatic inactivation/degradation. These are the same types of mechanisms involved in antibiotic resistance and some of them account for the observed cross-resistance with antibiotics in laboratory isolates. Therefore, there is a link between triclosan and antibiotics, and the widespread use of triclosan-containing antiseptics and disinfectants may indeed aid in development of microbial resistance, in particular cross-resistance to antibiotics. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Biocide; Antiseptic; Disinfectant; Resistance; Antibiotic cross-resistance; Pseudomonas; Alcaligenes

1. Introduction

The bisphenols are a class of compounds that exhibit a broad spectrum of antimicrobial activity. The two most widely used members of this group are triclosan (2,4,4-trichloro-2'-hydroxydiphenylether) and hexachlorophene (2,2'-dihydroxy-3,5,6,3',5',6'-hexachlorodiphenylmethane) (Fig. 1). Because of toxicity concerns, the use of hexachlorophene in consumer products has been limited. Over the last 30 years, triclosan has become the most potent and widely used bisphenol [1]. Triclosan is used in many contemporary consumer and professional health care products. These include hand soaps, surgical scrubs, shower gels, deodorant soaps, health care personnel hand washes, hand lotions and creams, toothpastes, mouthwashes, and underarm deodorants [2]. Triclosan is also incorporated into fabrics and plastics, including children’s toys, toothbrush handles, cutting boards, pizza-cutter and mop handles, as well as surgical drapes and hospital over-the-bed table tops. Searches of patent databases further reveal a multitude of suggested or actual uses of triclosan-impregnated materials, ranging from concrete with antimicrobial properties to bowling ball finger inserts.

Triclosan has been extensively tested in human and animal studies through acute toxicity, chronic toxicity, mutagenicity, reproduction and teratology investigations which were recently reviewed [2]. Triclosan is a synthetic, non-ionic, broad-spectrum antimicrobial agent, possessing mostly antibacterial, but also some antifungal and antiviral properties [2]. Triclosan is fairly insoluble in aqueous solutions, unless the pH is alkaline, and readily soluble in most organic solvents. It is chemically stable and can be heated up to 200°C for up to 2 h [1]. This thermal stability makes it suitable for incorporation into various reinforced plastic materials which are distributed under the trademark Microban®. Depending on formulation and application, triclosan is recognized by the United States Food and Drug Administration (FDA) as either an over-the-counter or a prescription drug. In addition, it is FDA-accepted for use as an antimicrobial pesticide for fungicide/fungistat and bacteriostat applications [2]. Triclosan, provided under its trade name Irgasan CH3565, is the active ingredient in Bacto Pseudomonas isolation agar used for selection of Pseudomonas since these bacteria are naturally resistant to the concentration (25 μg ml⁻¹) of triclosan used in the formulation of this selective medium. Pseudomonas isolation agar is rec-
3. Triclosan resistance mechanisms

Bacterial antibiotic resistance as a result of antibiotic use is a long-established and widely studied problem. The finding of a specific triclosan target spurred renewed and increasing attention to studies of responses of bacteria to biocides. As rightfully pointed out by Russell [20], there is particularly a clear need to establish whether there is a link between antibiotic and biocide resistance, and whether biocides can select for antibiotic resistance. Despite the fact that most of the efforts geared towards elucidating bacterial triclosan resistance mechanisms were concentrated on the last 3–4 years, it is already evident that bacteria use multiple mechanisms to develop resistance to this biocide, including target mutations, increased target expression, active efflux and degradative enzymes (Table 1).

3.1. Target mutations and increased target expression

In laboratory studies with triclosan, fabI mutations se-
lected by exposure to triclosan caused cross-resistance with other antimicrobial agents in *E. coli* [17]. This finding raised the fear that, in certain instances, biocides may share targets with antibiotics and imprudent and widespread use of this biocide may thus select resistance against clinically useful drugs. This notion has since been corroborated by several studies showing that some mutations affecting *InhA*, and leading to triclosan resistance in *M. smegmatis* [13] and *M. tuberculosis* [14,16], also caused resistance to isoniazid, a drug widely used for treatment of *M. tuberculosis* infections. Selection of spontaneous triclosan-resistant mutants in *M. smegmatis* caused mutations in the *inhA* gene which, like those in triclosan-resistant *fabI* mutants of *E. coli*, lie close to the NADH cofactor binding site [13,17]. In contrast, all triclosan-resistant mutants of *M. tuberculosis* examined to date contained a T to G point mutation in the putative ribosome binding site upstream of *mabA*, the gene located upstream of *inhA* [14]. This same mutation has been identified in isoniazid-resistant clinical isolates of *M. tuberculosis* and it results in an eight-fold increase in transcription and/or translation of a reporter fusion [21]. The frequency at which spontaneous triclosan-resistant mutants arose in *M. smegmatis* and *M. tuberculosis* was $\sim 1 \times 10^{-9}$.

### 3.2. Detoxification via efflux pumps

#### 3.2.1. Efflux pump families

Another, perhaps more widespread, mechanism of triclosan resistance is active efflux from the bacterial cell. Bacteria express diverse efflux pumps that are classified in mainly five families [22,23]. These include the resistance nodulation cell division (RND) family, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the ATP binding cassette (ABC) family and the multidrug and toxic compound extrusion (MATE) family. Well-characterized representatives of these families from Gram-positive and Gram-negative bacteria are shown in Fig. 2. All of these transporters catalyze active drug efflux and therefore require energy, mostly in the form of ATP.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Mechanisms of resistance</th>
<th>Antibiotic cross-resistance</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>E. coli</em></td>
<td><em>fabI</em> mutations; efflux</td>
<td>Yes*</td>
<td>[10,11,32]</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>multiple efflux systems: <em>fabI</em></td>
<td>Yes</td>
<td>[12,34]</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td><em>fabI</em> mutations</td>
<td>No</td>
<td>[15]</td>
</tr>
<tr>
<td><em>M. smegmatis</em></td>
<td><em>inhA</em> mutations</td>
<td>Yes</td>
<td>[13,14]</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td><em>inhA</em> upregulating mutations</td>
<td>Yes</td>
<td>[14,21]</td>
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<td><em>P. putida</em>, <em>A. xylosoxidans</em></td>
<td>degradation</td>
<td>NDc</td>
<td>[36]</td>
</tr>
</tbody>
</table>

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* *fabI* mutations cause cross-resistance to diazoborines which are not used as therapeutics.

*Although purified FabI is efficiently inhibited by triclosan, to date no in vivo triclosan resistance due to *fabI* mutations was observed; presumably, because efflux via multiple systems is the primary detoxification mechanism.

*ND, not determined. Although both species are resistant to multiple antibiotics, a link between the mechanisms responsible for triclosan resistance and antibiotic resistance has not yet been demonstrated.

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**Table 1**

Mechanisms of triclosan resistance in bacteria

<table>
<thead>
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**Fig. 2.** Schematic illustration of the main types of bacterial drug efflux pumps. Illustrated are NorA, a member of the MFS, MexAB-OprM, a member of the RND family, and LmrA, a member of the ABC family. All systems extrude drugs in an energy-dependent manner, using either proton motive force or ATP. The two other types of efflux systems found in bacteria, MATE and SMR, look structurally similar to the MFS but are designated as distinct families based on phylogenetic diversity (MATE) or size (SMR). To date, only the RND systems have been shown to efflux triclosan. In *P. aeruginosa*, triclosan is pumped by several different RND-type systems and there is evidence that an outer membrane channel is not always required for triclosan detoxification. M indicates the outer membrane protein channel OprM.
the form of proton motive force, but some also in the form of ATP. The structure of RND-type drug efflux systems is most complex since they mediate drug efflux across two membranes. They possess components that form a channel spanning the entire cell envelope, an inner membrane translocase, an outer membrane protein channel and a periplasmic membrane fusion protein [23]. Most bacterial RND-type efflux systems are chromosomally encoded and the numbers range from no RND efflux operons identified in the chromosome of M. tuberculosis to 12 RND-type efflux operons encoded by the chromosome of P. aeruginosa [24]. It is now recognized that synergy between a low-permeability outer membrane and active efflux is the main cause for the high intrinsic and acquired resistance of P. aeruginosa to many antibacterials, including many clinically relevant antibiotics.

In P. aeruginosa, the expression of all but the MexAB-OprM-encoding efflux operon is tightly regulated and the other efflux systems are only expressed in regulatory mutants obtained by antibiotic exposure in vitro or in vivo (Fig. 3) [25–28]. Pump-expressing mutants isolated in the laboratory or obtained from clinical posttherapy isolates often contain mutations in adjacent regulatory genes. However, other negative regulatory factors must also be involved since many clinical isolates constitutively express various efflux pumps in the absence of identifiable up-stream regulatory mutations, suggesting regulation by hitherto unidentified mechanisms [27,29,30]. In addition, the expression of some efflux pumps may also be subject to positive regulation, including at least one efflux operon whose transcription is quorum sensing-regulated [31].

The efflux pumps implicated in triclosan resistance thus far all belong to the RND family. In E. coli clinical and laboratory strains, triclosan is a substrate of the AcrAB-ToIC multidrug efflux pump [32]. Although it has been known for quite some time that P. aeruginosa is extremely resistant to triclosan, this was mostly attributed to the relative impermeability of the outer membrane to antibacterial agents [33] and to its high cell wall lipid content. However, it has recently been shown that clinical and environmental P. aeruginosa isolates tested to date are intrinsically resistant to triclosan by virtue of constitutive expression of the MexAB-OprM efflux pump [34]. Mutants containing a deletion of the entire mexAB-oprM operon are triclosan-susceptible. Of the 12 RND-type efflux systems encoded by the P. aeruginosa genome, only four, i.e., MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM (reviewed in [23]), have been characterized. Triclosan is a substrate of all but the MexXY-OprM system [34].

3.2.2. Triclosan selects for regulatory mutants expressing efflux pumps

Since triclosan is a substrate of most of the RND efflux pumps, exposure of bacterial cells to triclosan could select for regulatory mutants expressing these efflux pumps. If this were the case, then triclosan would simultaneously select for multiple antibiotic resistance since these efflux pumps are multidrug transporters. As an experimental model system, a triclosan-susceptible P. aeruginosa mutant was chosen which contains a Δ(mexAB-oprM) mutation. This strain does not express MexAB-OprM and, presumably, none of the other known efflux pumps. It does not grow in triclosan-containing medium but triclosan-resistant variants outgrow the culture in less than 24 h. These triclosan-resistant variants were obtained at relatively high frequencies (1 × 10⁻⁶) and three out of three isolates tested expressed high levels of OprJ, the outer membrane channel of the MexCD-OprJ efflux pump. Expression of the MexCD-OprJ system was the only mechanism responsible for high-level triclosan resistance in these mutants since deletion of this efflux system resulted in triclosan-susceptible strains. All three mutants analyzed contained mutations in the upstream nfxB regulatory gene [34]. These mutations were similar to the types obtained by exposure to fluoroquinolone antibiotics (Table 2). These results confirmed that exposure of a susceptible P. aeruginosa strain to triclosan selects for multidrug-resistant variants, including resistance to clinically useful antibiotics. Triclosan is an excellent substrate for multidrug efflux pumps and indeed a tool for selecting pump regulatory mutations. Using triclosan, another mutant derivative expressing a hith-
could be found in the literature. The many recent connections exist between triclosan and antibiotic resistance. The link between triclosan and antibiotics modification mechanisms may also exist in bacteria. Regulation in this area will reveal additional bacterial triclosan resistance mechanisms. Two root fungi were recently shown to transform triclosan by either glucosylation or xylosylation of the hydroxyl group (Fig. 1). Similar modification mechanisms may also exist in bacteria.

4. The link between triclosan and antibiotics

Less than two years ago, statements such as “no connection exists between triclosan and antibiotic resistance” [39] could be found in the literature. The many recent findings documented earlier in this review clearly link triclosan and antibiotics, and establish that triclosan can select for antibiotic resistance. This is best illustrated by two key findings. First, triclosan and antibiotics not only share multidrug efflux systems as a common mechanism of resistance but triclosan and antibiotics also cause expression of these efflux pumps by selecting similar mutations in the respective regulatory loci. Although P. aeruginosa is already triclosan-resistant, its resistance could theoretically be further increased by turning on additional efflux pumps. Clinical isolates that are resistant to high levels of antibiotics quite often express more than one efflux pump [30,40]. Second, in M. tuberculosis the same up-regulating mutation leading toisoniazid resistance in clinical isolates was also obtained by selecting triclosan resistance in the laboratory. While the connection between triclosan and antibiotics has clearly been established, we still do not know how this relates to the real world, i.e., we do not know how triclosan affects the microbial flora in all environments in which it is intensively used, from hospital to household settings. For example, widespread use of triclosan could lead to environments where bacteria like Pseudomonas and other triclosan-resistant bacteria thrive. While this still has to be proven, some anecdotal evidence supporting such a notion exists. Although after introduction of a new hand wash disinfectant containing 1% triclosan at a neonatal intensive care unit methicillin-resistant S. aureus was eliminated within 12 months, and the total number of multiply resistant Gram-negative organisms did not significantly change, P. aeruginosa was singled out as being reported three times more often within 14 months after the introduction of triclosan [41]. While the clinical significance of these findings was not further investigated, the data nonetheless suggest that some bacteria can compete, survive and propagate in an environment in which triclosan is routinely used. It has been argued that the low-level resistance observed in vitro selected triclosan-resistant mutants does not compare well to the real world since most commercially available products contain triclosan ranging from 2 to 20 mg ml⁻¹, far exceeding the minimal inhibitory and minimal bacteriocidal concentrations observed in these mutants. Therefore, it was reasoned, development of resis-
rurance would be unlikely. While it may be true that germicidal products contain triclosan concentrations that are considerably higher than those used in laboratory experiments, several observations argue against the resulting conclusion that bacteria are unlikely to develop resistance because of these higher concentrations. First, some bacteria can tolerate and survive the triclosan concentrations found in most commercially available products, i.e., they are resistant to these high triclosan concentrations, and one can speculate that some of these resistance mechanisms may be transferable to other bacteria. Second, triclosan is extremely stable in the environment and low residual levels might encourage preferential survival of triclosan-resistant mutants, especially those expressing efflux pumps. This may be particularly important in environments where bacteria encounter materials designed to slowly release triclosan, e.g., around plastics and fabrics, or after dermal adsorption in the bloodstream and various organs, where triclosan has been found at concentrations that were only a fraction of those applied topically [42]. Many bacteria show minimal inhibitory concentrations high enough to be able to cope with such residual triclosan concentrations. P. aeruginosa expressing a single efflux pump exhibited a triclosan minimal inhibitory concentration of 0.13 mg ml⁻¹. This was measured in an aqueous environment at pH ~ 7 and the values are probably even higher in the presence of well-tolerated organic solvents or more alkaline pH values.

Given similar resistance mechanisms for triclosan and antibiotics, including target mutations, enzymatic inactivation/modification (degradation), increased target expression and, perhaps most importantly, widespread multidrug efflux pumps, it would be a surprise if the overuse of triclosan would not select for resistant strains. Since triclosan and antibiotics are linked, it is therefore quite possible that widespread use of triclosan may indeed compound antibiotic resistance. Clearly, the time has come to depart from the ‘wait and see’ attitude when considering a link between triclosan and antibiotics, and actively engage in a policy of increasing public awareness concerning the possible consequences of triclosan overuse, and to teach and heed the lessons we hopefully learned from antibiotic overuse.

Acknowledgements

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References


READER: The reference list contains many publications related to the use of triclosan and its potential resistance, as well as the effects on bacteria in various environments. The text emphasizes the importance of recognizing the possible consequences of triclosan overuse and the need for increased public awareness. The conclusion of the text suggests that widespread use of triclosan may indeed compound antibiotic resistance, highlighting the need for continued research and policy development.


