

**TAB 11 FDA Reviewer's Literature Survey Regarding Antibiotic
and Biocide Cross-Resistance**



CONSUMER ANTISEPTIC DRUG PRODUCTS REVIEW

Office of Nonprescription Products (HFD-560)

Center for Drug Evaluation and Research • Food and Drug Administration

REVIEW DATE: July 25, 2005

FDA DOCKET NO.: 75N-0183H

PHARMACOLOGIC CATEGORY: Consumer Antiseptic Drug Products:
Antiseptic Handwash and Bodywash

REVIEWER: Colleen Kane Rogers, Ph.D.

I. Purpose

The purpose of this literature review is to provide a summary of published data that examines the association between the development of ‘resistance’ to biocides and cross-resistance to therapeutic antibiotics. To do this, a PubMed literature search was performed using combinations of the following search terms: “antibiotic,” “biocide,” “benzalkonium,” “benzethonium,” “chloroxylenol,” “consumer,” “cross-resistance,” “domestic,” “home,” “PCMX,” “resistance,” “trichlorocarbanilide,” “triclocarban,” and “triclosan.” In addition, pertinent references from the bibliographies of key articles were reviewed. The following review is limited to articles written in English and published between January 2000 and July 2005.

II. Overview and Summary

Since their discovery, antibiotics have been invaluable in treating bacterial infections. However, due to overuse and misuse, bacterial resistance to antibiotics has developed and become a worldwide problem. The recent proliferation of consumer products containing antibacterial biocides has raised concerns that overuse or misuse of these products may lead to bacterial resistance to biocides. More importantly, it is thought that bacteria with decreased susceptibility to biocides may also be less susceptible to antibiotics, possibly due to common resistance mechanisms. Consequently, in theory, the use of biocides in consumer products could select for bacterial strains which also are resistant to clinically important

antibiotics. This, in turn, could exacerbate the antibiotic resistance problem and make treatment of bacterial infections even more difficult.

Despite limited knowledge about biocide mechanisms of action and their role in cross-resistance to antibiotics, research in this area is increasing. The studies described below suggest that it is relatively easy for bacteria to become less susceptible to biocides after growth in amounts of the antimicrobial that are not lethal to bacteria (sublethal). Notably, resistance (i.e., nonsusceptibility) to moderate-to-high concentrations of triclosan and benzalkonium chloride occurred after exposure to sub-lethal doses. Many of the studies examined both clinical isolates and laboratory type strains. In general, there was no difference in the adaptive capabilities of clinical or type strains. Moreover, biocide nonsusceptibility was often stable. Taken together, this suggests that biocide nonsusceptibility can occur after exposure to small amounts of biocide and that biocide 'resistance' could occur outside of a laboratory setting.

The association between biocide nonsusceptibility and antibiotic resistance is still unclear. Most of the investigators were able to demonstrate cross-resistance between antibiotics and biocides. But, when cross-resistance was demonstrated, it was often shown for second-line drugs or drugs not usually used for therapy. In addition, nearly all of the articles describe laboratory experiments whose relationship to the real world situation is not defined. These studies only examined antibiotic and biocide sensitivities *in vitro*. Although bacterial susceptibilities to antibiotics are fairly well characterized, currently the relevance of a change in the minimum inhibitory concentration of an antiseptic is unknown. Even so, the fact that growing clinical isolates in sub-lethal biocide concentrations can lead to a change in the antibiotic susceptibility profile is something that can be related to the real world, especially if changes in biocide susceptibilities can be related to therapeutic levels of antibiotics.

Furthermore, several studies suggested that an efflux mechanism was involved in the biocide nonsusceptibility. Current knowledge of efflux mechanisms suggests that these pumps can utilize a variety of substrates, including both antibiotics and biocides, and therefore, may become a problem. Finally, data on antibiotic/biocide cross-resistance in domestic settings is very limited. Clearly, more research is needed to characterize the relationship between biocide nonsusceptibility and antibiotic resistance for consumer antiseptics.

III. Background

A. What is a biocide?

Biocide is a general term for a chemical or physical agent that kills all living organisms.¹ Biocides are further differentiated by the type of organism that they kill, for example bactericide, fungicide, or rodenticide. In the domestic setting, biocides may be found in antiseptics, disinfectants, preservatives, plastics, and textiles. The biocides used in antiseptics may be bactericides, virucides, or microbiocides; however, these chemicals are simply referred to as biocides in the literature. For this review, we will refer to the active ingredients in consumer antiseptics as biocides.

For the most part, the mechanisms of biocide action are poorly understood. Biocides are believed to have a non-specific mechanism of action and may act on multiple sites in bacteria. In contrast, an antibiotic is designed to affect a specific bacterial process.²⁵ However, recent evidence has shown that some biocides interact with specific bacterial targets just as antibiotics do.^{18, 19}

Even though biocides are chemically diverse, the damage inflicted on the bacterial cell may be similar. Many biocides affect the integrity of the bacterial cytoplasmic membrane in some way. The membrane may be physically disrupted, the proton motive force may be dissipated, or membrane-associated enzymes may be inhibited.¹⁶ Biocides may also increase the permeability of the outer membrane of gram-negative organisms or lead to coagulation of the bacterial cytosol.

The concentration of biocides used to formulate consumer antiseptics is relatively low. For example, triclosan is used at concentrations of 0.1-0.5% and quaternary ammonium compounds are used at 0.2-0.3% in some popular consumer antiseptics. In comparison, healthcare antiseptic handwashes contain triclosan at concentrations up to 1%. It should be noted that the efficacy of biocides, especially in antiseptics, is formulation-dependent. One study has shown a 25-fold difference in killing activity between biocide alone and the same biocide in a soap formulation.¹³

B. How do bacteria become 'resistant' to biocides?

There are several ways to determine the antimicrobial susceptibilities of a bacterial isolate. Some, such as the microdilution method, provide a quantitative result that is expressed as a minimum inhibitory concentration (MIC). The MIC is the lowest concentration of antimicrobial that will inhibit the growth of an organism. MICs are often used to develop interpretive criteria (i.e., breakpoints) that delineate susceptible, intermediate, or resistant categories for a particular drug.²⁴ Furthermore, MICs provide an easy way to correlate bacterial sensitivity with drug levels achieved in blood or body fluids. The implication of a 'susceptible' result is that there is a high probability that the patient will respond to therapy with that drug.²⁴ Conversely, 'resistant' implies that treatment with the antimicrobial is likely to fail.

Bacterial resistance refers to a change in susceptibility such that a previously susceptible organism no longer responds to the antimicrobial. The term resistance is properly applied to therapeutic antibiotics where a change in susceptibility to the drug can lead to treatment failure. In contrast, since biocides may affect multiple bacterial targets, reduced susceptibility does not always correlate with treatment failure at use concentrations. For this reason, reduced susceptibility to a biocide will be noted as nonsusceptibility rather than resistance. Furthermore, it should be noted that currently there is no correlation of biocide MIC with bactericidal activity. Consequently, there is no standard definition for 'susceptible' or 'resistant' MIC values for biocides.

Bacterial nonsusceptibility to biocides, like antibiotic resistance, can be either intrinsic or acquired. Antimicrobial resistance can occur through mutation or amplification of a chromosomal gene, or by acquiring resistance determinants on extra-chromosomal pieces of

DNA (e.g., plasmids).²¹ Other mechanisms of biocide nonsusceptibility include a decrease in membrane permeability, active efflux, changes in bacterial target sites, or growth in biofilms.

Gram-negative organisms have a more complex membrane structure than gram-positive organisms. The low permeability of the gram-negative outer membrane helps prevent antibacterial agents from reaching their intracellular targets. However some antimicrobials, such as polycationic antibiotics and quaternary ammonium compounds, can destabilize the outer membrane and promote their own uptake. Thus, changes in the bacterial membrane can lead to antimicrobial resistance. Some examples of membrane changes that may lead to decreased susceptibility include alteration of the lipopolysaccharide, changes in membrane fatty acid composition, and loss of transmembrane proteins.⁶

A common resistance mechanism employed by both gram-positive and gram-negative organisms is antimicrobial removal via efflux pumps. Efflux pumps are transporter proteins that transmit toxic substances out of the bacterial cell. Efflux systems can be either inherent or acquired, and can be activated in response to a wide variety of environmental stimuli or through mutation of a regulatory gene.¹⁴ One well-known example of an energy-driven efflux system is the multiple antibiotic resistance (*mar*) regulon in *Escherichia coli*. Activation of the *mar* locus alters the expression of at least 60 genes and upregulates the AcrAB multidrug efflux pump.¹⁴ The AcrAB system can efflux antibiotics, triclosan, chlorhexidine, quaternary ammonium compounds, and pine oils. In addition, a combination of decreased membrane permeability with active efflux systems may lead to higher levels of resistance than from either mechanism alone. For example, *Pseudomonas aeruginosa* is intrinsically resistant to some antibiotics and biocides due to the makeup of its outer membrane and endogenous multidrug efflux pumps.²⁰

Antibiotic resistance also may arise as a consequence of drug target alteration. Until recently, this mechanism was not thought to occur with biocides. However, McMurry and colleagues have shown triclosan nonsusceptibility in *E. coli* is due to mutations in, or overexpression of, the *fabI* gene.¹⁹ This gene encodes enoyl reductase, a bacterial enzyme involved in fatty acid synthesis.

MICs assess the effects of antibiotics against planktonic, or free-floating, bacteria in the exponential phase of growth.⁸ However, many infections are caused by bacteria growing in biofilms. A biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.⁷ Bacteria growing in biofilms are much less susceptible to antimicrobials than planktonic bacteria. There are several reasons for this reduced susceptibility: delayed penetration of the antimicrobial through the biofilm matrix, slower growth rate of some biofilm organisms, and physiological changes (e.g., oxygen limitation, upregulation of efflux pumps).⁷

More information on mechanisms of biocide nonsusceptibility can be found at **TAB 10** in the background package.

IV. Literature Review

A. Do biocide-nonsusceptible bacteria exhibit cross-resistance to antibiotics?

1. Triclosan

Braoudaki and Hilton investigated whether triclosan-adapted *Escherichia coli* strains were also resistant to antibiotics and other biocides.³ Several laboratory strains and clinical isolates of *E. coli* were adapted to grow in triclosan by serial passage through sub-inhibitory concentrations of the biocide. The following strains were tested: K-12, O157:H7, O55:H7, O55:H29, and O111:H24. *E. coli* O157:H7 became nonsusceptible to 2048 mg/L (i.e., 0.2%) triclosan after a single sub-lethal exposure. The remaining strains became nonsusceptible to the same concentration of triclosan after four serial passages.

When cross-resistance was examined, triclosan-adapted *E. coli* O157:H7 was less susceptible to seven of 12 antibiotics tested, including trimethoprim, compared to the parental strain. In contrast, triclosan-adapted O55:H7 exhibited a decreased sensitivity to just trimethoprim, and triclosan-adapted K-12 demonstrated decreased susceptibility only to chloramphenicol. These differences in drug susceptibility profiles suggest that the mechanisms of resistance are strain specific, rather than general. In addition, triclosan-adapted O157:H7 was nonsusceptible to benzalkonium chloride and chlorhexidine, while the other strains were not.

Overall, *E. coli* easily became nonsusceptible to triclosan. The authors conclude that *E. coli* O157:H7 has an “increased capacity to become resistant to the activity of triclosan and other antimicrobial agents.” This is somewhat troubling since O157:H7 is a verotoxin-producing serotype and may cause hemorrhagic colitis or hemolytic uremic syndrome. It is worth noting that at least two of the triclosan-adapted strains were cross-resistant to trimethoprim, an antibiotic used in the treatment of *E. coli* infections. Furthermore, the triclosan-adapted strains were nonsusceptible to triclosan concentrations up to 0.2%, which is higher than the amount of this biocide in many consumer products. However, other authors suggest that 0.2% is beyond the limit of solubility for this biocide.⁴

Chuanchuen and colleagues determined the antibiotic and triclosan susceptibilities of various *Pseudomonas aeruginosa* efflux mutants.⁴ *P. aeruginosa* is intrinsically resistant to triclosan due to one of its efflux pumps (MexAB-OprM). Therefore, the authors obtained or constructed *P. aeruginosa* mutants which expressed one or none of the four characterized efflux systems. The panel of mutants was then tested for MIC against triclosan and tetracycline, ciprofloxacin, trimethoprim, erythromycin, and gentamicin.

Triclosan was a substrate for all the efflux pumps tested except one. Mutants which expressed at least one of the other three efflux pumps were nonsusceptible to >128 µg/mL triclosan (i.e., 0.013%), which the authors state is the limit of solubility for triclosan in aqueous solutions. In contrast, mutants that expressed none of the efflux systems were susceptible to triclosan at 20-24 µg/mL.

Triclosan-susceptible efflux pump mutants readily became nonsusceptible to triclosan at a frequency of 10^{-6} . The authors also report that the mutants were resistant to all the tested

antibiotics with the exception of gentamicin. There was a 500-fold decrease in susceptibility to tetracycline, a 128-fold decrease in susceptibility to erythromycin, a 94-fold decrease in susceptibility to ciprofloxacin, and a 32-fold decrease in susceptibility to trimethoprim compared to the parental strain.

The authors demonstrated cross-resistance between triclosan and several antibiotics. They also showed that loss of one efflux system can lead to overexpression of alternate multidrug efflux systems. They conclude that exposing *P. aeruginosa* to triclosan efficiently selects for multidrug resistant (MDR) mutants, including resistance to a clinically important antibiotic. While ciprofloxacin is an alternative treatment for *P. aeruginosa* infections, none of the triclosan-nonsusceptible mutants showed a reduced susceptibility to gentamicin, a preferred drug for this organism.

Sanchez, Moreno, and Martinez determined the antibiotic susceptibilities of triclosan-nonsusceptible mutants of *Stenotrophomonas maltophilia*, an opportunistic pathogen.²² *S. maltophilia* (formerly *Xanthomonas maltophilia*) was adapted to grow in triclosan by plating on 64 µg/mL of the biocide. Triclosan MICs were determined for the mutants and 12 isolates with MICs >256 µg/mL (0.025%) were tested for tetracycline sensitivity. Five of the 12 triclosan-adapted mutants displayed reduced susceptibility to tetracycline compared to the parental strain and were further characterized. These mutants were less susceptible to ciprofloxacin and chloramphenicol compared to the parent strain, whereas the tobramycin MIC was lower or did not change.

The authors felt that this drug susceptibility profile was similar to previously characterized SmeDEF efflux pump mutants. Further analysis revealed that all five triclosan-adapted mutants overexpressed both *smeD* and SmeF. The authors conclude that triclosan can select for SmeDEF efflux pump overexpressing mutants of *S. maltophilia*. Furthermore, triclosan-selected efflux pump mutants demonstrate cross-resistance to antibiotics. Of the drugs tested, the mutants were least susceptible to ciprofloxacin. Ciprofloxacin is an alternative drug used to treat *S. maltophilia* infections. However, it would be of interest to compare these findings with the susceptibility profile for trimethoprim, which is a drug of choice for this organism.

Suller and Russell measured the susceptibility of 32 *Staphylococcus aureus* clinical isolates, including methicillin-resistant *S. aureus* (MRSA), to triclosan and a panel of 13 antibiotics.²³ The levels of triclosan susceptibility and antibiotic resistance profiles varied widely for the strains. The authors only reported strains as 'resistant' to a particular drug; no MICs were provided. Individual strains were resistant to between zero and seven drugs, and triclosan MICs varied from 0.025 to 1 mg/L (0.0001%). In general, the MRSA isolates were resistant to more drugs than the methicillin-sensitive isolates. However, there was no apparent correlation between drug resistance and triclosan MIC. It is interesting to note that the three MRSA strains that each were resistant to seven drugs, including mupirocin, were also the least susceptible to triclosan with MICs of 0.5-1 mg/L.

In addition, the authors isolated triclosan-adapted *S. aureus* mutants using the disk diffusion method. Triclosan nonsusceptibility was stable; the majority of the triclosan-adapted mutants

retained their nonsusceptible phenotype after culturing in the absence of triclosan for 14 days. Four of the triclosan-adapted mutants were tested for cross-resistance to a panel of antibiotics: vancomycin, methicillin, penicillin, gentamicin, erythromycin, and tetracycline. Both the parental strain and the mutants displayed equivalent MICs for all of the tested drugs.

Overall, the authors saw no correlation between the MIC for triclosan and the ability of triclosan to inhibit the organism. Moreover, exponentially growing organisms were not more susceptible to the effects of triclosan than those in stationary phase. The authors conclude that large-scale testing of bacterial isolates would be necessary to demonstrate cross-resistance between triclosan and antibiotics.

In summary, we reviewed several studies that examined bacterial susceptibilities to triclosan and various antibiotics. In all but one of these studies, the triclosan-nonsusceptible organisms also were considered resistant to clinically important antibiotics. However, the majority of the isolates were laboratory strains and the antibiotic resistance profiles were often against antibiotics that are not the preferred drug for the organism. Notably, the authors of two of the studies suggest that overexpression of an efflux pump system contributed to the antibiotic cross-resistance. Efflux pumps have many substrates, including antibiotics, so this is not a surprising finding, but may be a concern. Collectively, there is not enough information to determine the effect of triclosan nonsusceptibility on antibiotic resistance patterns.

2. Quarternary Ammonium Compounds (benzalkonium chloride)

Langsrud, Sundheim, and Holck studied the susceptibility of benzalkonium chloride (BKC)-adapted *E. coli* to various antibiotics.¹² Two *E. coli* type strains were adapted to grow in 150 µg/mL (0.015%) BKC by serial passage in stepwise higher concentrations of the biocide. The MICs of nine antibiotics were then determined for the adapted strains. Both adapted strains demonstrated greatly reduced susceptibility to chloramphenicol (12- and 24-fold higher MICs). However, the reciprocal was not true; chloramphenicol-adapted *E. coli* only exhibited approximately a 3-fold increase in the MIC for BKC. BKC-adapted organisms were moderately less susceptible to ampicillin and nalidixic acid (4- to 8-fold higher MICs), and slightly less susceptible to penicillin, norfloxacin, kanamycin, gentamicin, tetracycline, and erythromycin (1.5- to 4-fold higher MICs) than the parental strains.

Next, a range of experiments was performed to try to identify the mechanism of BKC nonsusceptibility. *E. coli* was exposed to a variety of stress-inducing compounds (e.g., salicylate) prior to determining the MIC for BKC. Pre-exposure to stress-inducers resulted in only slight increases in MIC to BKC. Ethidium bromide (EtBr) was transported out of the BKC-adapted *E. coli* at a faster rate after the addition of glucose than in parental cells, which suggests an efflux mechanism. Finally, increased amounts of a 27-kilodalton outer membrane protein were detected in the BKC-adapted strains. This protein may be part of the efflux mechanism.

The cross-resistance between BKC and chloramphenicol suggests a common resistance mechanism. It is unlikely that both BKC and chloramphenicol would be inactivated by the

same enzyme, and the EtBr data argues against changes in membrane permeability. The authors conclude that the main mechanism involved in BKC nonsusceptibility in *E. coli* is enhanced efflux. Furthermore, the authors conclude that exposure to biocides can enhance antibiotic tolerance. But, with the exception of chloramphenicol, antibiotic insusceptibilities were modest.

Braoudaki and Hilton investigated the link between adaptive nonsusceptibility to biocides and cross-resistance to antibiotics in *E. coli* O157 and *Salmonella enterica*.² Four bacterial strains were adapted to grow in erythromycin, BKC, or other biocides by serial passage in subinhibitory concentrations of the antimicrobial. The following strains were tested: *E. coli* O157, *Salmonella enterica* serovar Enteritidis (a clinical isolate), *Salmonella* serovar Typhimurium, and *Salmonella* serovar Virchow (a food isolate).

No antibiotic cross-resistance was seen with BKC-adapted *Salmonella* serovar Enteritidis or Typhimurium. However, erythromycin-adapted *Salmonella* serovar Typhimurium was nonsusceptible to both chlorhexidine and triclosan. In contrast, the authors describe a high degree of cross-resistance between antibiotics and biocides for both *E. coli* and *Salmonella* serovar Virchow. BKC-adapted *E. coli* and *Salmonella* serovar Virchow demonstrated cross-resistance to amoxicillin, amoxicillin + clavulanic acid, chloramphenicol, chlorhexidine, imipenem, triclosan, and trimethoprim. BKC-adapted *E. coli* also was less susceptible to tetracycline, and triclosan-adapted *E. coli* demonstrated decreased susceptibility to tetracycline and erythromycin. Interestingly, cross-resistance was not seen for ciprofloxacin or gentamicin. Finally, the adaptive resistance phenotype was stable and remained even after culturing in the absence of drug or biocide for 30 days.

Overall, *E. coli* acquired biocide nonsusceptibility more rapidly than the salmonellae. The authors remark, “The speed and extent to which *E. coli* O157 becomes resistant to BKC and triclosan are of particular concern.” In this study, *E. coli* was adapted to grow in approximately 0.1% BKC or triclosan, concentrations similar to that found in consumer antiseptics. Furthermore, the authors report that these biocide-nonsusceptible strains were cross-resistant to several therapeutic antibiotics, including alternative drugs for treating *E. coli* infections (trimethoprim and imipenem).

Lambert, Joynson, and Forbes measured the susceptibility of 55 industrial, laboratory, and clinical isolates of *Pseudomonas aeruginosa* to BKC, chlorhexidine, and a panel of eight antibiotics.¹¹ The industrial isolates (n=19) were obtained from detergent factory swabs, detergent products (e.g., soap and shampoo), and animal sources. Clinical isolates (n=20) were divided by their aminoglycoside resistance profiles: sensitive, resistant due to membrane impermeability, or resistant due to production of aminoglycoside modifying enzymes. Isolates were designated sensitive or resistant to an antimicrobial based on the distribution of the log MIC values for the samples. Log MIC values greater than plus or minus 1 standard deviation were defined as being resistant or susceptible, respectively. It should be noted that the Clinical and Laboratory Standards Institute (i.e., NCCLS) definitions for drug resistance are higher than the definitions used here.

In general, the clinical isolates were the least susceptible to the antimicrobials and the industrial isolates were the most susceptible. No biocide/antibiotic cross-resistance was seen with the industrial isolates. For laboratory strains, there was a significant correlation between nonsusceptibility to chlorhexidine and imipenem. When the clinical isolates were considered together, significant correlations were seen between nonsusceptibility to BKC and gentamicin, and between chlorhexidine and polymixin B or ticarcillin. However, for all isolates, there was a significant correlation between chlorhexidine and five antibiotics. The authors conclude that biocide/antibiotic cross-resistance does occur, especially with clinical strains of *P. aeruginosa*. Furthermore, they suggest that the clinical environment, in particular the selective pressure of antibiotic use, is responsible for the positive correlation.

Loughlin, Jones, and Lambert looked at the ability of BKC-adapted *P. aeruginosa* to become cross-resistant to antibiotics and other biocides.¹⁵ The authors generated 16 stable *P. aeruginosa* BKC mutants by serial passage in the biocide up to 0.025%. Two of the strains were chosen for further examination. MICs were determined for each of the mutants and the parental strain at each passage step. Other biocides (including triclosan and chlorhexidine) and six antibiotics were tested.

One of the BKC-adapted *P. aeruginosa* strains showed decreased susceptibility to cetrimide, chloramphenicol, and polymixin B. In contrast, this organism became more susceptible to ceftazidime. The other BKC-adapted strain showed decreased susceptibility to cetrimide and increased susceptibility to tobramycin. The investigators examined other characteristics of the two BKC-adapted strains to determine the mechanism of resistance. Various tests of the bacterial outer membranes revealed a reduced amount of an uncharacterized fatty acid. The authors speculate that this fatty acid may be responsible for rigidification of the membrane, which has been suggested as a mechanism of resistance against membrane-active agents.

One of the BKC-adapted strains showed increased susceptibility to all the quaternary compounds tested, which suggests a common site of action. The other strain showed limited changes, suggesting that under the same environmental pressures, the potential resistance profile is strain dependent. Overall, BKC nonsusceptibility was easily acquired after passage in sub-MIC concentrations of the biocide. Moreover, the mutants were stable when grown in the absence of BKC. The authors conclude that cross-resistance to antibiotics is likely a result of a general decrease in membrane permeability, which is unlikely to affect patient therapy with these agents.

Joynson, Forbes, and Lambert produced mutants of *P. aeruginosa* that were adapted to grow in BKC, amikacin, or tobramycin.⁹ The authors then looked for cross-resistance between these antimicrobials. A laboratory strain of *P. aeruginosa* was repeatedly subcultured in increasing concentrations of BKC, amikacin, or tobramycin. Organisms were adapted to grow in 0.45 mg/mL BKC (0.045%) or 0.06 mg/mL amikacin or tobramycin. In addition, several clinical isolates were adapted to grow in BKC. The susceptibility profile for amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, ticarcillin, and tobramycin then was determined for the adapted strains.

The MICs for all tested drugs were either the same or lower than the parent strain for both the BKC-adapted laboratory and clinical strains. In contrast, the amikacin- and tobramycin-adapted strains were much less susceptible to amikacin, tobramycin, and gentamicin than the parent. The authors conclude that nonsusceptibility to BKC does not confer cross-resistance to other antibiotics. On the other hand, amikacin and tobramycin-adapted organisms displayed an increased MIC to BKC, but only slightly. In other words, adaptation to amikacin or tobramycin results in high-level cross-resistance to other aminoglycosides, but only low-level cross-resistance to BKC.

Furthermore, the BKC-adapted strain was cultured in nonselective broth to examine the stability of this phenotype. The MIC to BKC decreased rapidly when grown in the absence of biocide, but did not return to original levels, suggesting that BKC nonsusceptibility is somewhat stable. Moreover, electron microscopy of the BKC-adapted strain grown in the presence of BKC revealed blebbing of the outer cell membrane. The investigators speculate that the mechanism of BKC nonsusceptibility involves loss of outer membrane lipids via blebbing.

Lambert measured the susceptibility of 256 clinical isolates of *S. aureus*, including MRSA, and 111 clinical isolates of *P. aeruginosa*, to a panel of eight biocides and 14 antibiotics.¹⁰ The tested biocides included BKC, benzethonium chloride, bleach, chlorhexidine, PCMX, and triclosan. Population mean MICs were compared and MIC data also were transformed into \log_{10} values and used to calculate the degree of cross-resistance using the Spearman-Rho nonparametric method.

When mean MIC values were compared, the MRSA isolates were significantly less susceptible than methicillin-sensitive *S. aureus* (MSSA) isolates to benzethonium chloride, bleach, PCMX, chlorhexidine, and all of the antibiotics except vancomycin. There was no statistical difference between mean MICs for a majority of the antibiotics when *S. aureus* strains were compared based on year of isolation (1989 vs. 2000). For the biocides, MSSA was less susceptible to benzethonium chloride, chlorhexidine, PCMX, and triclosan in 2000 compared to 1989; however, changes in mean MICs were modest. In contrast, the BKC MIC was significantly lower in 2000 compared to 1989 for both MSSA and MRSA. There was a significant difference in mean MIC for *P. aeruginosa* in 2000 compared to 1989 for benzethonium chloride and ciprofloxacin. For the other antimicrobials, mean MICs were not significantly different or were lower in 2000 than in 1989.

Pairwise analysis revealed a significant correlation between nonsusceptibility to erythromycin and triclosan for MSSA. In other words, increases in erythromycin MIC were associated with increases in triclosan MIC. In contrast, MRSA demonstrated a negative correlation between erythromycin and triclosan. Furthermore, MRSA also demonstrated a significant correlation between quaternary ammonium compounds (BKC and benzethonium chloride) and ciprofloxacin, clindamycin, cefazolin, erythromycin, amoxicillin/clavulanic acid, and oxacillin. The pairwise correlation analysis for *P. aeruginosa* revealed a significant positive correlation between gentamicin and PCMX, and a negative correlation between ciprofloxacin and BKC, PCMX, and triclosan. Overall, the data suggests a link between increased MICs to quaternary ammonium compounds and antibiotics in *S. aureus*; however,

this is not the case for *P. aeruginosa*. The author concludes that the data does not support the hypothesis that increased biocide resistance is a cause of increased antibiotic resistance, either in *S. aureus* or *P. aeruginosa*.

In summary, several gram-negative organisms (*E. coli*, *Salmonella*, and *Pseudomonas*) readily adapt to grow in the presence of BKC. Most of the studies demonstrated cross-resistance between BKC and at least one antibiotic. However, there was no specific correlation between BKC nonsusceptibility and resistance to a particular antibiotic. Both enhanced efflux and outer membrane changes were proposed to explain the decreased susceptibility to BKC. These are common mechanisms that may play a part in decreased susceptibility to antibiotics and other biocides as well.

Finally, the study by Lambert, Joynson, and Forbes is especially interesting because they compared the biocide and antibiotic susceptibilities of clinical, laboratory, and industrial isolates. These investigators found that the clinical isolates were the least susceptible to the antibiotics and biocides, and the industrial isolates were the most susceptible. They suggested that the clinical environment may be a factor contributing to the development of reduced biocide susceptibility in addition to antibiotic resistance.

B. Has cross-resistance been demonstrated in domestic settings?

Cole and colleagues performed a randomized survey of bacteria collected from multiple sites in the homes of both antibacterial product users and nonusers.⁵ Households were recruited from three geographic locations: North Carolina, New Jersey, and England. For each location, ten antibacterial product users and ten nonusers were chosen to participate. Households were excluded if they had preschool children in day care over 20 hours per week, had recent water damage, or if any individual had been on antibiotic therapy within the past 30 days, worked in a healthcare occupation, or if a nonuser used more than one antibacterial product.

An inventory of cleaning and personal hygiene products was conducted to confirm user or nonuser status. Various areas of the kitchen (sink, drain, counter top, and floor) and bathroom (sink, drain, counter top, floor, toilet, bathtub, and tub drain) were swabbed for bacterial isolation. In addition, the hands and mouths of 1-2 individuals from the household were swabbed and a soil sample was collected from the yard. Swabs were eluted and plated for bacterial identification. One of each colony type was identified using biochemical tests.

Antibiotic and biocide susceptibilities were determined from a subset of the collected organisms (i.e., target bacteria). A total of 1238 target bacteria, which represent clinically important strains, were tested using one of three standard antibiotic panels: gram-positive, gram-negative, or *Enterobacteriaceae*-specific. The antibiotic susceptibility profiles for environmental isolates from user and nonuser homes were quite similar. The antibiotic susceptibility profiles from the clinical isolates (i.e., hand and mouth swabs) were also similar, with the exception of viridans *Streptococcus*. The authors report that viridans *Strep.* showed a greater measure of resistance in the user group (19.4% vs. 6.3%); however, MICs

were not provided. Overall, most bacteria were susceptible to preferred and alternative drugs of treatment.

Next, a subset of preferred antibiotic resistant (n=26) and sensitive (n=46) isolates was tested against triclosan, para-chloro-meta-xyleneol (PCMX), BKC, and pine oil. For gram-positive isolates, the biocide susceptibility patterns between user and nonuser homes were comparable. However, three isolates from nonuser homes were nonsusceptible to pine oil. There were essentially no differences in biocide MICs between user and nonuser homes for the gram-negative isolates. Not surprisingly, *Citrobacter* and *Pseudomonas* had the highest biocide MICs, although there was no difference between user and nonuser homes.

The authors conclude there is a lack of antibiotic and biocide cross-resistance in clinically important bacteria isolated from the homes of antibacterial product users and nonusers across different geographical locations. They also conclude that there is an increased prevalence of potential pathogens in the homes of nonusers. However, statistical significance for differences between users and nonusers was not provided. Furthermore, the authors do not describe the number or type of antibacterial products that were used in the households or the length of time the households used antibacterial products.

McBain and others investigated the effect of short- and long-term use of triclosan on the antibacterial resistance of domestic-drain biofilm ecosystems.¹⁷ An artificial domestic-drain microcosm was established in a constant-depth film fermenter by seeding with material taken from a kitchen drainpipe. Commercially available dishwashing soap containing triclosan (TCSD) was added to the fermenters at specific intervals.

In the short-term (14-day) experiments, increasing concentrations of TCSD were added to the fermenter every other day after day 5. TCSD was moderately bactericidal against biofilm organisms at concentrations of 10% and above. For long-term experiments, biofilms were allowed to stabilize for 6 months. Then, biofilms were treated with 0.2% TCSD for 10 minutes every 6 hours for 3 months, and likewise with 0.4% TCSD for another 3 months. This long-term exposure to low levels of triclosan did not affect the total culturable cell counts. However, there was a reduction in microbial diversity after triclosan exposure. This change in population included an increase in triclosan-degrading bacteria. The MICs for eight antibiotics were determined for bacteria taken from the fermenter before and after 6 months of triclosan exposure. There were no significant differences in MICs for any of the tested drugs.

The authors conclude that “long-term exposure of domestic-drain biofilms to sub-lethal levels of triclosan did not affect bacterial vitality or significantly alter antimicrobial susceptibility.” Yet, most of the bacteria in the biofilm ecosystem were innately tolerant to triclosan or antibiotics before triclosan treatment. They further conclude, “the emergence of antibiotic resistance through triclosan use in the kitchen is highly improbable.” However, this study was performed in a simulated kitchen environment and results from actual kitchen ecosystems may be different.

Overall, there is not enough data from studies performed in domestic settings to determine if antiseptic use by consumers influences bacterial antibiotic resistance profiles in the home. The only study that actually looked for antibiotic and biocide cross-resistance in the homes of antibacterial product users had several limitations. The investigators did not describe the number or type of antibacterial products that were used in the households, or the length of time those antibacterial products were used prior to study initiation. Furthermore, the statistical significance of their findings was not provided. Other investigators have developed alternative models to try to address the issue of antibiotic/biocide cross-resistance in the home, but the relevance of these models to the domestic environment is unclear. More research is needed to determine the relationship between biocide nonsusceptibility and antibiotic resistance patterns in the domestic environment.

References

1. Block SS. Definition of terms. In: Block SS, ed. *Disinfection, Sterilization, and Preservation*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:19-28.
2. Braoudaki M and Hilton AC. Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol*. 2004;42(1):73-78.
3. Braoudaki M and Hilton AC. Low level of cross-resistance between triclosan and antibiotics in *Escherichia coli* K-12 and *E. coli* O55 compared to *E. coli* O157. *FEMS Microbiol Lett*. 2004;235(2):305-309.
4. Chuanchuen R, Beinlich K, Hoang TT, Becher A, Karkhoff-Schweizer RR, and Schweizer HP. Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects *nfxB* mutants overexpressing MexCD-OprJ. *Antimicrob Agents Chemother*. 2001;45(2):428-432.
5. Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, Newell MS, Wilkins J, Gaber DJ, Wineinger T, and Criger DA. Investigation of antibiotic and antibacterial agent cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *J Appl Microbiol*. 2003;95(4):664-676.
6. Denyer SP and Maillard JY. Cellular impermeability and uptake of biocides and antibiotics in gram-negative bacteria. *J Appl Microbiol*. 2002;92 Suppl:35S-45S.
7. Donlan RM and Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*. 2002;15(2):167-193.
8. Fux CA, Costerton JW, Stewart PS, and Stoodley P. Survival strategies of infectious biofilms. *Trends Microbiol*. 2005;13(1):34-40.
9. Joynson JA, Forbes B, and Lambert RJ. Adaptive resistance to benzalkonium chloride, amikacin and tobramycin: the effect on susceptibility to other antimicrobials. *J Appl Microbiol*. 2002;93(1):96-107.
10. Lambert RJ. Comparative analysis of antibiotic and antimicrobial biocide susceptibility data in clinical isolates of methicillin-sensitive *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* between 1989 and 2000. *J Appl Microbiol*. 2004;97(4):699-711.
11. Lambert RJ, Joynson J, and Forbes B. The relationships and susceptibilities of some industrial, laboratory and clinical isolates of *Pseudomonas aeruginosa* to some antibiotics and biocides. *J Appl Microbiol*. 2001;91(6):972-984.

12. Langsrud S, Sundheim G, and Holck AL. Cross-resistance to antibiotics of *Escherichia coli* adapted to benzalkonium chloride or exposed to stress-inducers. *J Appl Microbiol.* 2004;96(1):201-208.
13. Levy SB. Antibacterial household products: cause for concern. *Emerg Infect Dis.* 2001;7(3 Suppl):512-515.
14. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *J Appl Microbiol.* 2002;92 Suppl:65S-71S.
15. Loughlin MF, Jones MV, and Lambert PA. *Pseudomonas aeruginosa* cells adapted to benzalkonium chloride show resistance to other membrane-active agents but not to clinically relevant antibiotics. *J Antimicrob Chemother.* 2002;49(4):631-639.
16. Maillard JY. Bacterial target sites for biocide action. *J Appl Microbiol.* 2002;92 Suppl:16S-27S.
17. McBain AJ, Bartolo RG, Catrenich CE, Charbonneau D, Ledder RG, Price BB, and Gilbert P. Exposure of sink drain microcosms to triclosan: population dynamics and antimicrobial susceptibility. *Appl Environ Microbiol.* 2003;69(9):5433-5442.
18. McMurry LM, McDermott PF, and Levy SB. Genetic evidence that InhA of *Mycobacterium smegmatis* is a target for triclosan. *Antimicrob Agents Chemother.* 1999;43(3):711-713.
19. McMurry LM, Oethinger M, and Levy SB. Triclosan targets lipid synthesis. *Nature.* 1998;394(6693):531-532.
20. Nikaido H. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. *Semin Cell Dev Biol.* 2001;12(3):215-223.
21. Poole K. Mechanisms of bacterial biocide and antibiotic resistance. *J Appl Microbiol.* 2002;92 Suppl:55S-64S.
22. Sanchez P, Moreno E, and Martinez JL. The biocide triclosan selects *Stenotrophomonas maltophilia* mutants that overproduce the SmeDEF multidrug efflux pump. *Antimicrob Agents Chemother.* 2005;49(2):781-782.
23. Suller MT and Russell AD. Triclosan and antibiotic resistance in *Staphylococcus aureus*. *J Antimicrob Chemother.* 2000;46(1):11-18.
24. Turnidge JD, Ferrarro MJ, and Jorgensen JH. Susceptibility test methods: general considerations. In: Jorgensen JH, ed. *Manual of Clinical Microbiology.* Vol 1. 8th ed. Washington DC: ASM Press; 2003:1102-1107.

25. Walsh C. Antibiotics: Initial concepts. *Antibiotics: Actions, Origins, Resistance*. Washington DC: ASM Press; 2003:3-9.