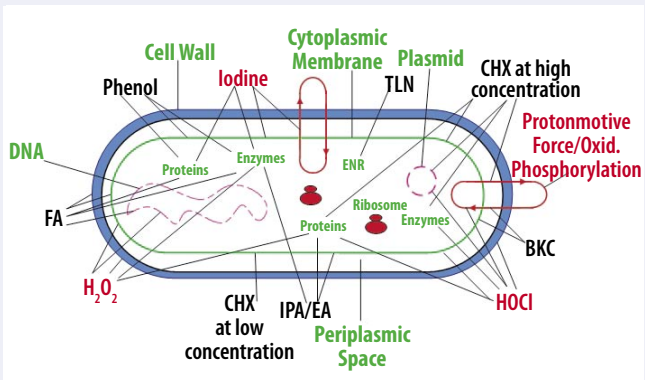
Mode of Action and Development of Resistance to Disinfectants, Part 2

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Last month, Part 1 of this article examined modes of action for oxidizing disinfectants after discussing terminology as it relates to the cleaning and sanitation of equipment and utensils required by GMP regulations. This month, Part 2 examines the modes of action for nonoxidizing disinfectants before discussing resistance and what these mean in the context of those regulations.



Bacterial target sites (green) of the disinfectants discussed in Part 1 (red) and in Part 2 (black)

Nonoxidizing Disinfectants

Isopropyl Alcohol (Isopropanol) and Ethyl Alcohol (Ethanol) are commonly used nonoxidizing disinfectants. Both are biocidal against vegetative bacteria (including mycobacteria), fungal spores, and enveloped viruses (**28–32**). Alcohols are not effective against bacterial spores and nonenveloped viruses, and they do not penetrate organic material. They do inhibit sporulation and spore germination (**33**), but that effect is reversible (**34**)

Isopropyl alcohol (IPA) and ethyl alcohol (EA) are excellent disinfectants when used at 60–90% (v/v) concentration in water. Their biocidal activity decreases quickly when they are diluted to below 50% concentration (**28**). Low concentrations are used as preservatives and to potentiate the activity of other biocides. The MIC for *Bacillus (Geobacillus) stearothermophilus, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae,* and *Escherichia coli* is 87.5 g/L (8.75%) for both alcohols (**35**). The same effect was produced for *Serratia marcescens* and *Acinetobacter calcoaceticus* but at a lower MIC of 43.75 g/L (4.37%) (**35**).

The biocidal activity of alcohols can be attributed to their ability to denature proteins. Therefore, concentrations above 90% are less effective because the action of denaturing proteins is inhibited without water present (**36**). Few studies have focused on the specific mode of action for IPA and EA. However, based on their increased activity in the presence of water, it is understood that they cause membrane damage and breakage, denaturation of proteins, and cell lysis (**37, 38**).

Reduced susceptibility to IPA and EA at use concentrations has not been demonstrated in any sensitive microorganisms. Increase of the MIC at below use concentrations has been documented for some gram-negative bacteria such as *E. coli* and *Salmonella* species due to the oxidative-stress response (SOS response). It promotes production of neutralizing enzymes to prevent cellular damage and repair DNA injuries (**39**). Most field failures of alcohol disinfection are due to the presence of biofilms, poor cleaning methods, or inadequate contact times. IPA and EA are not effective in penetrating organic materials and tissue, so biofilms are intrinsically resistant to alcohols. However, the most common culprit when efficacy is lost is evaporation, which translates to short contact times.

Formaldehyde (FA) is a monoaldehyde that exists as a freely water-soluble gas. The aqueous formaldehyde solution formalin contains 34–38% (wt/wt) FA along with methanol to delay polymerization. Although structurally written as CH_2O, it occurs at that configuration only in the anhydrous or gaseous form. In water it becomes hydrated

Product Focus: All biologicals

Process Focus: Manufacturing

Who Should Read: Manufacturing and process development, project managers, and technicians

Keywords: Cleaning, disinfection, sanitization, resistance, tolerance, susceptibility

Level: Basic to intermediate

to methylene glycol, $CH_2(OH)_2$ (**40**). Bactericidal, sporicidal, and virucidal, FA is used as a preservative, disinfectant, and sterilant (**41–45**). Low concentrations inhibit germination of spores. FA inactivates microorganisms by alkylating amines, amides, purines, pyridimines, and the sulfhydryl groups of proteins (**40, 46**). Its biocidal activity is thus due to interactions with proteins and nucleic acids.

Reduced susceptibility and/or resistance against FA seems to be found most often in gram-negative bacteria such as *Pseudomonas* species and *Enterobacteriaceae*. One strain of *P. aeruginosa* was acclimatized to grow at 0.075% FA concentration (**47**). A strain of *E. coli* was acclimatized to grow at a 0.02% FA concentration (**48**). The use concentrations are 1–8% for immersion and wetting disinfection and 0.05–0.2% for preservation. Data derived from the VU3695 strain of *E. coli* indicates that reduced susceptibility to FA depends on the function of a highly active glutathione-dependent FA dehydrogenase, which inactivates FA (**48**). That enzyme is known to work outside the cytoplasm, so it is logical to assume that it is overwhelmed at high FA concentrations. So even the two less susceptible bacteria strains will be killed by FA at use concentrations of 1–8%.

Based on the above information, the presence of FA at sublethal levels can be used as a substrate for bacteria such as *P. aeruginosa* and *E. coli* that have FA dehydrogenases. Thus, less susceptible microorganisms would provide protection to more susceptible microorganisms. The FA-degrading bacteria would actively lower the levels of FA-based biocides, which allows more susceptible microorganisms to survive. In biofilms, the rate of biocide diffusion is lowered because microorganisms are physically protected by a barrier (**47**). Both mechanisms further increase the odds for survival of microorganisms that are less susceptible to FA.

Regrowth observed in vitro for FA-based disinfectants used in preservation has multiple causes and occurs in stages. First, most bacteria are killed by FA available in their culture medium. Second, that available FA is exhausted by reacting with organic molecules present. Third, any bacteria that survived, protected by organic material (e.g., cellular debris), and received a sublethal dose may acclimatize to the residual FA and grow. Bacteria with FA dehydrogenase will degrade FA and keep growing. As FA is depleted from the medium, other bacteria will grow (**47, 50**). Failure of FA-based preservatives seems to be caused by poor selection of the biocide, the presence of biofilms and/or substrates that reduce FA activity, and exhaustion of available FA. FA-based preservatives are mainly used at concentrations of 0.05–0.20% (free FA).

At use concentration, even bacterial spores cannot survive exposure to FA. For example, the Chemical and Biological Defense Establishment of the United Kingdom used a solution of FA (5%) in seawater applied by surface spray to decontaminate some 40,000 m^2 of land from spores of *Bacillus anthracis*. The procedure was performed at a location that had been heavily contaminated with the spores of virulent *B. anthracis* during biological weapons trials (**51**).

Phenol: Among the nonoxidizing disinfectants, one of the most effective (and longest in use) is phenol. In the mid-19th century, it was used by Sir Joseph Lister in his pioneering technique of antiseptic surgery. At low concentrations, phenol induces leakage of intracellular components through the cytoplasmic membrane (CM) (**52**), and at high concentration it also induces coagulation of cytoplasmic constituents (**53**). Thus it has been known as a cytoplasmic poison. Normal use concentration is 0.5–3.0% w/v.

Some bacteria can survive in low concentrations of phenol. Even more, some can use it as a source of carbon and even thrive in low phenol concentrations (<0.3%). For example, a strain of *Micrococcus pyogenes* (variation *aureus*) was developed as resistant to the bacteriostatic action of phenol in the concentration range of 0.20–0.30% (**54**). And a strain of *Pseudomonas (Brevundimonas) putida* was able to metabolize phenol and the isomers of cresol at low concentrations (**55, 56**). At use concentrations, none of those bacteria would survive. In fact, tolerance has been demonstrated only to phenol concentrations of <5 mM (0.47%) (**57**). Reduced susceptibility is innate and/or plasmid mediated (**57**). So far, resistance or tolerance to phenol at use concentrations has not been substantiated.

Terminology

Antiseptic: a compound which destroys or inhibits the growth of microorganisms which is designed for application to living tissue (**1**).

Biocidal: refers to substances intended to destroy, inactivate, prevent the action of, or otherwise exert a controlling effect on any harmful or undesirable microorganism (**1**).

Biostatic: agents that inhibit growth of microorganisms (**1**). The MIC identifies the lowest concentration of biostatic agent or antibiotic at which growth for a specific organism is inhibited, and this reflects its "biostatic activity".

Minimum bactericidal concentration (MBC): the concentration of biocidal product that kills or inactivates >99.9% of microorganisms in an in-vitro test (**4**).

Minimum inhibitory concentration (MIC): the MIC is the lowest concentration of the antibacterial agent that results in inhibition of visible growth (i.e., colonies on a plate or turbidity in broth culture) under standard test conditions (**4**).

"Recommended use concentration" refers to disinfectant manufacturers' suggested methods because the international regulatory agencies and pharmacopoeias have not yet published disinfectant guidances.

Triclosan (TLN) is a halogenated phenolic derivative that was developed as an antiseptic: a chlorinated biphenyl ether in the bisphenol group of disinfectants. TLN is heavily exploited as a preservative and disinfectant. It is incorporated as an antibacterial in personal care products such as hand soaps, acne creams, foot deodorants, toothpastes, cosmetics, and even in printing inks and some plastics. Also, it has become the most widely used surgical antiseptic scrub.

TLN is poorly soluble in water, but it is fat-soluble and easily crosses cell membranes. Once inside a microorganism, it blocks the active site of the enzyme enoyl-acyl carrier protein reductase (ENR), which is used in the manufacture of fatty acids for building cell membranes and other vital cell functions (**58, 59**). One molecule of TLN poisons one ENR molecule, which explains why triclosan is so effective at very low concentrations on susceptible microorganisms.

The mechanism of action is the same for TLN as for antibiotics in its highly specific method of killing microorganisms. So it is very different from general-purpose disinfectants described elsewhere in this article. This is why many researchers are concerned about its potential role in promoting antibiotic-resistant strains of bacteria. Moreover, some have demonstrated that mutations in the bacterial gene FabI — which produces ENR — can produce TLN-resistant bacteria (**60–62**).

Generally, TLN is inactive against *P. aeruginosa* as well as some gram-negative bacteria and molds. Strains of *Klebsiella pneumoniae, E. cloacae, Acinetobacter baumannii, P. fluorescens,* and *E. coli* O157 have grown on media containing TLN at 1024 µg/mL, about 0.1% (**63, 64**) concentration — which is very near the concentrations found in some consumer products. Specifically, some soap bars contain about 0.25–1.50% TLN by weight (**64, 65**). For disinfection, the recommended use concentration is 0.5–1.0% w/v.

Reduced susceptibility to TLN has been found in strains of *S. aureus*. The typical triclosan MIC for *S. aureus* is acknowledged to be 0.016 µg/mL. Some mutant strains have a triclosan MIC of 2 µg/mL, a >50-fold increase. The same study revealed that a mutation in the FabI gene provides TLN resistance, and that the altered FabI must be overexpressed at three- to fivefold higher levels than it is expressed in TLN-sensitive strains (**60**).

Although wild-types of *P. aeruginosa* express the target molecule, ENR, they are intrinsically resistant to TLN by use of multiple resistance mechanisms, including efflux pumps and FabI target mutations (**66, 67**). Moreover, clinical and laboratory *E. coli* strains have reduced susceptibility to TLN through a multidrug efflux pump (**62, 64**). Efflux pumps are nothing more than conveyor systems dedicated to move substances such as waste products and harmful chemicals out of a cell.

Table 1: Oxidizing disinfectants

Disinfectant Class	Example	Mechanism of Action	Reference
Peroxides	Hydrogen peroxide*	Oxidation of essential cell components, including lipids, proteins, and DNA	8
Chlorine-based	Sodium hypochlorite (chlorine bleach)	Destroys the cellular activity of proteins, disrupts oxidative phosphorylation and other membrane-associated activity	2
Iodine-based	Iodine*, povidone-iodine*	Inactivation of proteins and enzymes	22–24
Alkalis (hydroxides)	Sodium hydroxide	Destroys cell membranes and inactivates proteins	49

* antiseptic

Chlorhexidine (CHX) is a cationic biguanide that comes in the form of diacetate, gluconate, and hydrochloride salts (**68**). The most commonly used is chlorhexidine gluconate, which is found in scrub soaps at 0.5–4.0 % w/v and is used as an antiseptic at 2–4% w/v (**69**). It has several desirable characteristics for use as an antiseptic: high antibacterial activity, low toxicity, and an affinity for binding to skin cells. CHX is frequently used in consumer products such as surgical scrubs, skin wound cleansers, germicidal hand washes, and antibacterial mouthwash.

CHX compounds are active against both gram-positive and gram-negative bacteria, lipophilic viruses, and most fungi (**53, 70–72**). It is biostatic to mycobacteria and bacterial spores (**73, 74**). Best portrayed as a low- to intermediate-level disinfectant and antiseptic, CHX interacts with cell surfaces (**75**) and promotes membrane damage (**76**), which in turn causes irreversible loss of cytoplasmic contents. The biocidal action of CHX at relatively low concentrations (e.g., 2.0–2.5 µg/mL) involves a series of related cytologic and physiologic changes that lead to ion leakage from cytoplasmic membranes and cytoplasmic precipitation (**77**). In fact, the way CHX alters cell surfaces and cytoplasmic membranes at low concentrations is reminiscent of some antibiotics. At high concentrations (>20 µg/mL), it causes extensive cell damage, coagulation of cytoplasm, and precipitation of proteins and nucleic acids (**70**).

Reduced susceptibility to CHX among gram-positive bacteria is somewhat uncommon, but it has been reported in *S. aureus* (**78**). On the other hand, gram-negative bacteria — such as *E. coli, Salmonella enteritica* (**61**), *Proteus mirabilis* (**79**), *Providencia stuartii* (**80**), *P. aeruginosa* (**81**), *Burkholderia cepacia* (**82**), and *S. marcescens* (**83**) — have frequently been reported to show reduced susceptibility and in some cases resistance at use concentrations. MICs reported have been as high as 1600 µg/mL, especially with strains of *Providencia* (**84**).

Reduced susceptibility usually is only to CHX (**85**); however, "cross-resistance" to some antibiotics has been shown. For example, an association between resistance to antibiotics and reduced susceptibility to CHX has been documented in *P. aeruginosa* (**86**). Various mechanisms for reduced susceptibility are described. Acquired reduced susceptibility is almost certainly

Table 2: Nonoxidizing disinfectants

Disinfectant Class	Example	Mechanism of Action	Reference
Alcohols	Isopropyl alcohol*/ ethyl alcohol*	Precipitation of proteins and solubilization of lipids present in cell membranes	28–32
Biguanides	Chlorhexidine*	Damages the cellular membrane and coagulation of intracellular constituents.	78, 79
Quaternary ammonium compounds (QACs)	Benzalkonium chloride*	Damages the cellular membrane, disrupts the membrane potential and pH gradient across the membrane	100, 101
Phenolics	Phenol	General protoplasmic poison and damages the cellular membrane.	52, 53
Phenol derivatives	Triclosan*	Blockage of the active site of enoyl-acyl carrier protein reductase	58, 59
Aldehydes	Formaldehyde	Alkylation of amines, amides and sulfhydryl groups of proteins, and purines and pyridimines	40, 46

* antiseptic

connected to inner cell membranes (**81**), outer membranes (OMs) (**87**), cell surfaces (**88**), or cell walls (**89**). Plasmids are another source (**90**).

Some gram-negative bacteria can be acclimatized to grow at low concentrations of CHX — and in some outstanding cases to thrive at or near use concentrations. One example involved the exposure of *P. aeruginosa* to 5 µg/mL of CHX, with its MIC subsequently increasing within six days from <10 µg/mL to 70 µg/mL (**81**). *P. stutzeri* reached a MIC of 50 µg/mL after 12 days of exposure (**91**). Wild strains of *P. aeruginosa, K. pneumoniae,* and *A. baumannii* isolated from soap dispensers were found to grow in a half dilution of 2% CHX liquid soap (**92**): Those three bacterial strains grew in 1% CHX, which is within its recommended use concentration as an antibacterial agent.

Benzalkonium chloride (BKC) is a mixture of alkylbenzyl dimethylammonium chlorides. Biochemically speaking, it is a cationic quaternary ammonium surface-acting agent. BKC is biostatic and biocidal against both gram-negative and gram-positive bacteria as well as certain viruses, fungi, yeasts, and protozoa depending on concentration (**93**). Bacterial spores are considered resistant, and gram-positive bacteria are more susceptible than gram-negative bacteria. BKC has plenty of uses in consumer and ethical products as a disinfectant, preservative, and antiseptic. It is primarily used in hand scrubs, face washes, as a preservative in many ophthalmic solutions, and as a spermicide. Typical use concentrations are ≤0.1% as a preservative (in ethical and consumer products) up to 3% in rinse-off products and 5% as a disinfectant.

An early report on BKC's mechanism of action was published in 1976 by Richards and Cavill, documenting that it stripped the outer cell membrane of a sensitive *P. aeruginosa* strain (**94**). The bacterial cells retained their shape after their OMs were removed, and their peptidoglycan layers remained intact. Removal of the OM was accomplished by excluding boundary-divalent cations such as Mg_2^+ and inducing disorder in the cell membranes (**87**). At low concentrations in gram-positive bacteria (e.g., *Listeria monocitogenes*), BKC works by disrupting the membrane potential and the pH gradient across the membrane. At high concentrations, BKC causes complete inhibition of acidification and respiration and a total depletion of ATP pools (**95**). By its mode of action, therefore, BKC resembles more an antibiotic than a general disinfectant.

Reduced susceptibility to BKC has been reported in several bacteria, either through intrinsic tolerance or adaptation. Current research indicates that efflux pumps are the major mechanism of adaptation in gram-positive bacteria like *S. aureus, L. monocitogenes,* and other species of staphylococci. In general, staphylococcal QAC resistance genes are plasmid borne, normally harbored by small plasmids, and they encode efflux proteins belonging to the small multidrug resistance (SMR) family (**90, 96–98**). The efflux proteins push out hydrophobic drugs including QACs, the intercalating dye ethidium bromide (EBR), and some other cationic biocides (**99, 100**). In gram-negative bacteria, however, plasmid-mediated resistance to BKC has not been reported. Some *L. monocitogenes* strains have shown intrinsic resistance, but the responsible mechanism is unknown. It seems that a shift in the fatty acid profile of the CM may be the source (**101**).

A *P. aeruginosa* strain with adapted resistance to *N*-dodecylpyridinium iodide, whose structure is similar to cetylpyridinium chloride (a QAC), showed cross-resistance to BKC. Analysis of the OM protein of this resistant strain showed a significant increase in the expression level of a protein called OprR (**102**). Hydrophilic molecules of low molecular weight can usually go inside microorganisms by means of porins. It appears that *P. aeruginosa* may possess porins that may not function as they do in other bacteria. Several studies have found that its resistance to BKC is acquired by passage in sub-MIC concentrations. Even worse, such resistance was retained even in the absence of the disinfectant, and in some cases cross-resistance occurred with the membrane-active antibiotic polymyxin B.

The lipoprotein content on the cell surface was reported to be increased significantly in *S. marcescens* intrinsically resistant to BKC (**103**). Another study showed an increase of lipid content in BKC-resistant *E. cloacae* (**104**). Thus, quantitative and qualitative changes in phospholipids (PLs) in the outside layer of the OM on gram-negative bacteria are related to susceptibility and resistance to BKC and other QACs. Additionally, rigidification of the OM has been observed along with the changes in PL contents. It could be hypothesized that a more rigid OM would present a stronger barrier to membrane-active agents like BKC. Reduced susceptibility of *P. aeruginosa* to hydrophilic chemicals (e.g., phenols

Table 3: Reported "resistance" concentration to disinfectants

Disinfectant	Microorganism(s)	"Resistance" Concentration	Normal Use Concentration	Reference
Hydrogen peroxide	*B. subtilis*	0.05%	Disinfectant: 3–10% v/v Antiseptic: 2–4% v/v	10
	P. aeruginosa	1.7%		11
Formaldehyde	*E. coli*	0.02%	Preservative: 0.05–0.2% w/v Disinfectant: 1–8% w/v	48
	P. aeruginosa	0.075%		47
Phenol	*B. putida*	0.05–0.20%	Disinfectant: 1.0–3.0% w/v	55, 56
	M. pyogenes (var. aureus)	0.20–0.30%		54
Iodophor	*B. cepacia*	1.0%*	Disinfectant: 1–2% w/v	25
Chlorine (HOCl + OCl-)	*Legionella spp.*	0.5–1.0 PPM	Potable water: 0.5–1.0 PPM Disinfectant: 200–5000 PPM	20
	Bacillus, micrococci	1–10 PPM		18
	E. coli (AB1157)	0.1 PPM		19
Triclosan	*K. pneumoniae, E. cloacae, A. baumannii, P. fluorescens, E. coli (O157)*	0.1%	Soaps: 0.25–1.5% w/w Disinfectant: 0.5–1.0% w/v Preservative: ≤0.3% w/v Antiseptic: 0.1–1.0% w/v	63, 64
	S. marcescens	0.5%		196
	B. putida, A. xylosoxidans	1%		197
Benzalkonium chloride	*B. cepacia*	16%	Preservative: 0.01–0.1% w/v Rinse-off products: ≤3% w/w Antiseptic: 0.1–0.2% w/v Disinfectant: 5% w/v	114
	Ps. aeruginosa	0.05%		195
	Ps. fluorescens	10%		198
Chlorhexidine	*E. coli, S. enteritica, P. mirabilis, P. stuartii, P. aeruginosa, B. cepacia, S. marcescens*	≤1600 µg/mL (0.16%)	Scrub Soap: 0.5–4% w/w Antiseptic/disinfectant: 2–4% w/v Preservative: 0.0035% w/v	82–87
	S. marcescens	2%		194
Isopropyl alcohol/ ethyl alcohol	*G. stearothermophilus, B. subtilis, S. aureus, E. cloacae, E. coli*	≤8.6%	Disinfectant/Antiseptic: 60–90 % (v/v) Preservative: 15% v/v	35
	S. marcescens, A. calcoaceticus	≤4.3%		

* protected by organic material

and dyes) has been linked to the OM lipopolysaccharide. Overall, reduced susceptibility and resistance in gram-negative bacteria to hydrophobic detergents and some hydrophilic chemicals are intrinsically mediated (**105, 106**).

A strain of *B. cepacia* has taken BKC tolerance to a higher level. This strain was originally isolated from a 0.15% *N*-alkyl dimethyl benzyl ammonium chloride solution used as a cleansing agent in catheters and preserved with 0.05% BKC. The *B. cepacia* strain survived for 14 years in that solution. Its tolerance was increased stepwise up to 16% BKC by increments of 0.5%. The strain was not only tolerant to BKC, but it was also able to use it as substrate for growth (**107**).

Discussion

Antibiotics typically have a single target and a very specific mode of action, interacting with bacteria as a lock and key to accomplish a biocidal or biostatic action. Most disinfectants, however, have multiple targets and modes of action. Antibiotics and disinfectants are as dissimilar from one another as trying to open a door with a key or a shotgun, respectively.

Resistance to antibiotics can arise from one mechanism or by the interaction of several mechanisms. Generally, there are four main mechanisms of resistance: altered permeability to an antibiotic, inactivation of it, alteration of its target site, and replacement of a sensitive metabolic pathway. Those mechanisms can be intrinsic or acquired (by mutation or through plasmids). Resistance that appears after introduction of an antibiotic frequently results from a discriminatory process. For example, antibiotics select for survival of those bacterial strains with intrinsic resistance or those that acquire a gene providing resistance. From an evolutionary standpoint, this is nothing new; bacteria have competed for nutrients and survival this way for billions of years. They have developed ways to compete with each other, and many secrete their own antibiotics to kill other bacteria. Microorganisms have been and always will be in a perpetual war, and their weapons are biochemical agents.

Recent concern has been expressed that the problem of antibiotic resistance might also show itself with disinfectants. This is indeed a concern for certain disinfectants. As presented herein, some have modes of action that are basically the same as those of antibiotics. In this group we find BKC, CHX, and TLN. Those three disinfectants, along with other similar chemical agents, have a closer relationship with antibiotics than they do to general-purpose disinfectants. So it is not surprising that bacteria can develop resistance or tolerance to them.

Cross-resistance among disinfectants and antibiotics has been proven only for the antibiotic-like disinfectants. Mutant strains of *S. aureus* resistant to BKC showed a higher resistance than their parent

strains to various ß-lactam antibiotics and to ofloxacin, a quinolone (**108**). One strain of *Salmonella typhimurium* that developed resistance to CHX also became resistant to erythromycin and BKC. In the same study, another strain of *Salmonella* species developed a high degree of cross-resistance between antibiotics and biocides (between erythromycin and TLN and between erythromycin and CHX) as well as among biocides (between BKC and TLN and between CHX and TLN). The study showed cross-resistance between BKC and amoxilin, chloramphenicol, imipenen, and trimethoprim, and between CHX and tetracycline, among others (**61**).

In addition, adaptive cross-resistance between BKC, amikacin, and tobramycin has been documented (**109**). Exposure of a *P. aeruginosa* strain susceptible to TLN selected multidrug-resistant bacteria at high frequencies. Those strains of bacteria hyperexpressed an efflux pump system due to regulatory gene mutations. The MICs of tetracycline, trimethoprim, erythromycin, and gentamicin for the mutants were increased up to 500-fold. The MIC of ciprofloxacin (a quinolone) was increased 94-fold (**110**). Resistance of all those bacteria to both disinfectants and antibiotics developed from changes in the OM and/or cell wall that increased their barrier properties and prevented access to the sites of action (**61, 108–110**). However, other factors may also be involved.

Those studies demonstrated that bacteria developed resistance or have intrinsic resistance to antibiotic-like disinfectants. The reason for this is that antibiotic-like disinfectants have specific target sites within bacteria. In this sense, chemical agents such as BKC, CHK, and TLN can be considered antibiotics. A note of caution here regarding TLN: Although some studies have repeatedly shown inhibited enoyl-acil protein carrier reductase, others in recent years have demonstrated that other events are involved in TLN bacterial inactivation (**111, 112**).

Rotation of disinfectants in hospitals and in the pharmaceutical and food industries has lately been promoted to prevent the development of bacterial resistance. It's argued that one disinfectant should be replaced by another having an unrelated mode of action. This recommendation shows little knowledge of the ways in which such agents work. In fact, the recommendation is derived from the experience with antibiotics, which does not apply directly to general-purpose disinfectants. Table 3 shows that bacteria develop resistance (or tolerance) only to antibiotic-like disinfectants. The so-called "resistance" reported to general-purpose disinfectants is not at use concentrations, and it is actully reduced susceptibility (**113, 114**).

General-purpose disinfectants oxidize, denature, or strike in other broad ways, making the development of resistance extremely difficult. Moreover, because they are used on inanimate objects rather than in/on persons, general-purpose disinfectants are used at concentrations exceeding the MIC or MBC by several factors. Consequently, a decrease in susceptibility by a factor of two or more, which is important to an antibiotic, has no relevance to the effectiveness of a general-purpose disinfectant. Keeping with the metaphor of key and shotgun, changing a lock will not prevent a shotgun from blasting through the door. Furthermore, chlorine and phenol were in use before the 20th century; there has been plenty of time for bacteria to adapt to them. But true resistance or tolerance at disinfection use concentrations has not yet been documented for either one.

Beware Extrapolation

Unfortunately, experiences with the development of resistance to antibiotics in the clinical environment have been extrapolated both to general-purpose disinfectants and the general environment. But the mechanisms of action, in most cases, are very different for general-purpose disinfectants and antibiotics. A common vocabulary relating to disinfectant and antibiotic susceptibility tests seems to be the justification for extrapolation. And it is often done without considering the fundamental differences between their mechanisms of action and the methods used to evaluate their efficacy. Extreme care should be taken in using as a base the common terminology for interpreting studies because it could lead to misleading interpretations.

Development of resistance to antibiotics is a natural outcome of using them. The same applies to antibiotic-like disinfectants. Nevertheless, development of resistance or tolerance to use concentrations of general-purpose disinfectants (e.g., phenol, H_2O_2, chlorine, iodine, IPA/EA, and formaldehyde) is not supported by current research and data. Development of resistance or tolerance to them is unlikely because of their modes of action; therefore, the rotation of such disinfectants should be unnecessary (**113, 114**). Disinfectants that leave no residues, however, should be preferred over those that do.

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