



Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology

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Disinfectants play an important role in maintaining acceptable health standards by significantly reducing microbial loads as well as reducing, if not eliminating, pathogens. This review focuses on quaternary ammonium compounds (QACs), a widely used class of organic disinfectants. Specifically, it reviews the occurrence, microbial adaptation, and degradation of QACs, focusing on recent reports on the ecology of QAC-degraders, the pathways and mechanisms of microbial adaptation which lead to resistance to QACs, as well as to antibiotics. With the help of culture-dependent and nonculture-dependent tools, as well as advanced analytical techniques, a better understanding of the fate and effect of QACs and their biotransformation products is emerging. Understanding the underlying mechanisms and conditions that result in QAC resistance and biodegradation will be instrumental in the prudent use of existing QAC formulations and foster the development of safer disinfectants. Development and implementation of (bio)technologies for the elimination of QACs from treated wastewater effluents will lessen adverse impacts to both humans and the environment.

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Introduction

Disinfectants are extensively used and their formulations contain active ingredients at levels well above the minimum inhibitory concentration (MIC) of targeted microorganisms. Inappropriate application of disinfectants, dilution in the environment after discharge and biodegradation result in biocide concentration gradients. Thus,

microorganisms are frequently exposed to non-lethal (i.e., sub-inhibitory) concentrations of biocides. Recent studies suggest that exposure to sub-inhibitory biocide concentrations facilitates the evolution of resistance to the biocide, and may also lead to co-resistance and cross-resistance to other antimicrobial agents such as antibiotics [1*,2].

Quaternary ammonium compounds (QACs) are cationic surfactants introduced in the late 1930s along with the first antibiotics, sulfonamides. They are classified as ‘high production volume’ chemicals [3]. The chemical structure of QACs depends on the four aliphatic or aromatic moieties attached to the central nitrogen atom ($R_1R_2N^+R_3R_4$). QACs are mainly used in disinfectant and antiseptic formulations utilized in homes, human and animal healthcare facilities, agriculture and industry. They are effective against a variety of bacteria, fungi and viruses at very low concentrations. When QACs are used as disinfectants, the applied concentration is typically between 400 and 500 ppm and almost always below 1000 ppm (e.g., 0.1% w/v in Lysol®) [3]. Domestic, hospital and industrial use of QACs results in QACs-bearing waste/wastewater. Because typical wastewater treatment plants are designed to remove major, easily degradable organics, most trace contaminants, including QACs, pass through wastewater treatment plants and are released into the environment. About 75% of QACs utilized annually are released into wastewater treatment systems, whereas the rest are discharged directly into the environment. The mean concentration of QACs in domestic wastewater, treated effluent wastewater, sewage sludge and surface water is reported to be around 0.5 mg/L, 0.05 mg/L, 5000 mg/kg dry weight, and 0.04 mg/L, respectively, which is significantly below applied concentrations [3–5].

Because QACs are biodegradable under aerobic conditions, their concentrations in indoor and outdoor environments continuously fluctuate. As a result, microorganisms are exposed to QACs dynamically over a wide range of concentrations (i.e., non-inhibitory, sub-inhibitory, over-inhibitory concentrations). In general, environmental concentrations of QACs are well below the MIC values. Sewage, biological wastewater treatment units, surface waters and sediments are environments where QACs are present at sub-inhibitory concentrations. When the high microbial diversity in these environments is coupled to sub-inhibitory QAC concentrations, such environments become selective, resulting in the emergence and dissemination of QAC resistance among different bacterial

genera, which may also include clinically important pathogens. Because many of the QAC resistance pathways and mechanisms are similar to those involved in antibiotic resistance, understanding QAC resistance and dissemination is very important in the context of the global antibiotic resistance problem. Although some suggest that there is no clear relationship between antibiotic resistance and exposure of microorganisms to QACs [6], many studies have shown that, for instance, exposure to QACs results in dissemination of integrons (i.e., promoterless mobile recombinational elements) which harbor resistance genes [7,8]. Evidence that soil bacteria and human pathogens share similar resistomes within integrons [9] suggests that there is a link between antibiotic resistance in nature and clinical settings, which is favored by exposure to QACs.

Many reviews exist on the fate and effect of QACs in the environment, QAC-related antimicrobial resistance, and implications of QAC resistance to human health [3,10,11]. In this review, we discuss the ecology of QACs-exposed microbial communities, as well as the pathways and mechanisms of microbial adaptation to sub-inhibitory QAC concentrations following the context of a recent review by Andersson and Huges by linking the similarities of antibiotic and disinfectant resistance [12[•]]. In addition, we consider the role of QAC biodegradation on the evolution and dissemination of QAC resistance by creating microenvironments with sub-inhibitory QAC concentrations. Moreover, technologies utilizing novel QAC-degraders for the alleviation of QAC contamination are discussed.

Pathways and mechanisms of QAC resistance at sub-inhibitory concentrations

The mode of action of QACs above MIC in bacteria is disruption of the cell membrane's physical and ionic stability [13]. For example, benzalkonium chlorides (BACs) bind to the cell membrane of *Pseudomonas fluorescens* by ionic and hydrophobic interactions, bringing about changes of membrane properties and function, followed by cellular disruption, loss of membrane integrity, ultimately resulting in leakage of essential intracellular constituents [14,15]. Above MIC, bacteria with durable cell membrane are selected and proliferate. On the other hand, the mode of action of QACs at sub-MICs is complicated and always includes multiple processes such as loss of membrane osmoregulation, inhibition of respiratory enzymes, the dissipation of proton motive force and oxidative stress, which triggers SOS response, inducing error-prone DNA replication leading to mutations and gene transfers [16,17]. Adaptation to QACs at sub-MICs is achieved by modification of the outer membrane, cell membrane, density and structure of porins, regulatory hyperexpression of efflux pumps, and acquisition of QAC-specific efflux pumps through mobile recombinational elements, such as plasmids and integrons

upon oxidative stress or (followed by) stress-induced mutagenesis [3].

Major pathways in the evolution of resistance at sub-inhibitory antibiotic concentrations have been recently reviewed [12[•],18]. Bacteria follow similar pathways while adapting to QACs. Exposure to QACs at sub-MIC creates (oxidative) stress. For instance, exposure of *E. coli* to cetyltrimethylammonium bromide resulted in intracellular production of superoxide and hydrogen peroxide [19]. Bacteria compensate for oxidative stress by SOS-response and induction of stress-response sigma factors rpoS, promoting cell survival by DNA repair, while nucleotide polymorphism may occur, resulting in mutations. Oxidative stress responses also boost gene transfer and recombination events via prophages, transposons, integrons and integrative-conjugative elements (ICE) (Figure 1). As a result, resistant sub-populations evolve and dominate in a microbial community upon exposure to any antimicrobial agent [20]. Major mechanisms of adaptation to QACs include modification of cell membrane structure and composition, enhanced biofilm formation, acquisition of efflux genes, overexpression of efflux pump systems, and biodegradation. Generally, multiple mechanisms co-develop during adaptation of bacteria to QACs [21].

Exposure to QACs at sub-MICs enhances biofilm formation [22]. QAC resistant strains of bacteria form biofilms faster and these species are less susceptible to QACs than planktonic species [23]. Presence of multiple species in biofilms increases QAC resistance [24^{••},25]. Expression of certain genes enhanced biofilm formation by QAC resistant *Listeria monocytogenes* [26,27].

Several mutations result in selection of bacteria with reduced cell permeability. Resistant cells have modified cell membrane fatty acids, phospholipids, and outer membrane lipopolysaccharides [28], resulting in a more anionic and hydrophobic cell surface, thus restricting easy passage of the QACs through the cell surface. Other cell modifications in response to exposure to QACs are density reduction and composition change of the porins [29^{••},30], as well as change of the outer membrane protein composition [31].

Efflux-mediated QAC resistance has received significant interest because it has a genetic origin, confers co-resistance to antibiotics and is transferable among species through horizontal gene transfer. Multidrug efflux pumps mediate the transfer of biocides from the inside to the outside of the cell through an energy or proton-dependent mechanism. Efflux determinants, which confer resistance to QACs, are given in Table 1. QAC resistance via efflux pumps follows two mechanisms. First, QAC resistance is induced by overexpression of efflux pumps upon exposure to QAC or as a result of QAC-induced stress. Such stress either triggers a regulatory system that controls the

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