Environmental Science Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Adv.*, 2024, **3**, 208

Quaternary ammonia compounds in disinfectant products: evaluating the potential for promoting antibiotic resistance and disrupting wastewater treatment plant performance

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Quaternary ammonium compounds (QACs) are a class of compounds that were widely used as disinfectants during the COVID-19 pandemic and continue to be used as disinfecting agents. After consumer usage, QAC concentrations are diluted in wastewater as they enter wastewater treatment plants. At sub-inhibitory concentrations, QACs may have unintended repercussions, including increased antibiotic resistance and inhibition of process performance in wastewater treatment plants. This review first summarizes how QACs inhibit bacteria and then highlights the mechanisms by which QACs can promote antibiotic resistance in general. Reported environmental concentrations of QACs are compared to concentrations that are suspected to impact antibiotic resistance, and the role QACs may have on antibiotic resistance proliferation in wastewater treatment is addressed. Finally, the specific impacts that QACs can have on biological wastewater processes (activated sludge and anaerobic digestion) are reviewed. We highlight key research gaps along with recommendations for future research. Of particular interest, research is needed to elucidate the relationship between the chemical structure of QACs and impacts on antibiotic resistance as well as process performance in wastewater treatment plants. Finally, the ability to mitigate (reverse) these impacts if QACs are removed needs to be determined.

Received 17th March 2023
Accepted 22nd November 2023

DOI: 10.1039/d3va00063j

rsc.li/esadvances

Environmental significance

Considering the expectation of increased loading of QACs due to the COVID-19 pandemic and long-term changes in consumer/business purchasing of products containing QACs, it is necessary to evaluate the potential effects of QACs on microbial communities, antibiotic resistance, and the performance of wastewater treatment. Understanding the impacts of specific QACs on environmental processes and antibiotic resistance can help protect public health while minimizing environmental impacts.

1. Introduction

Antibiotic resistance is a critical global health problem.[1,2] A report from the Organization for Economic Co-operation and Development (OECD) predicts that approximately 2.4 million people will die in Europe, North America, and Australia between 2015–2050 from infections by antibiotic resistant bacteria, costing up to USD 3.5 billion per year.[3] The actual situation, however, may be worse than estimated in the report. A recent predictive model revealed that a median of 1.27 million deaths in 2019 was directly attributable to antibiotic resistance, which is only less than the number of deaths due to SARS-CoV-2 (COVID-19) and tuberculosis.[1] Additionally, the Centers for Disease Control and Prevention (CDC) estimated over 2.8 million people in the United States are infected with antibiotic-resistant bacteria each year, which carries an economic burden of $20 billion.[4] In Europe, antibiotic resistance is estimated to be the annual cause of 33 000 fatalities and cost EU €1.5 billion for healthcare expenditures and lost productivity.[5] In spite of different actions taken to tackle this issue, the threat of antibiotic resistance is not only still present, but also has been exacerbated by the SARS-CoV-2 pandemic.[6,7] CDC data showed that antibiotic resistant infections and deaths both increased at least 15% from 2019 to 2020.[8] The reasons for the increasing prevalence of antibiotic resistance are complex. In addition to the overuse and misuse of antibiotics, the widespread use of biocides and disinfectants could be a significant driver of antibiotic resistance, particularly during the pandemic.[9–18]

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The use of disinfectants has risen dramatically in various settings as a result of the COVID-19 outbreak.[19] Among disinfecting products, quaternary ammonium compounds (QACs) are predominant in industrial, commercial, hospital, and consumer products due to their ability to inactivate enveloped viruses.[20–22] QACs are a class of organic, cationic chemicals that contain one or more positively charged quaternary amine groups and at least one hydrophobic alkyl chain.[23] The cationic charge is usually balanced by a chloride ion or bromide ion. The most commonly used and studied groups of QACs are monocationic compounds including benzyl alkyl dimethyl ammonium compounds (BACs, also known as benzalkonium compounds), dialkyl dimethyl ammonium compounds (DADMACs), and alkyl trimethyl ammonium compounds (ATMACs) (Fig. 1). Additionally, ethyl benzyl ammonium compounds (EtBACs) usage has been increasing in recent years.[24–26] Typically, these compounds are used as mixtures with varying chain lengths ranging from 8–20 carbon atoms. QACs are amphiphilic, due to their hydrophobic alkyl groups and hydrophilic positively charged N. In general, QACs are water soluble and stable, but their solubility diminishes as chain length increases.[23,27] Within the same class of QACs, as the alkyl chain length increases the octanol–water partition coefficient (K_{ow}) increases. QACs with longer alkyl chains are more readily sorbed by solid phases *via* electrostatic and hydrophobic interactions and are more likely to be found in biosolids, soil, and sediments.[28–32] This characteristic of QACs not only impacts the distribution and bioavailability of QAC in environments, but also restricts the biodegradation efficiency and toxicity of QACs.

Since the invention of BAC in 1935, QACs have been extensively used for a wide variety of purposes (Fig. 2).[34] In addition to being the main active ingredient in disinfectants, QACs can also be used as fabric softeners, surfactants, antistatic agents and wood preservatives. In the United States, BACs are used in applications on indoor and outdoor surfaces, humidifiers, decorative ponds, fountains, and more.[35] The global QACs market was valued at $963.7 million in 2019, and it will continue to grow at a rate of 6.8% after the COVID-19 pandemic to reach $1.63 billion by 2027 due to increased demand for disinfectant products from hospitals, clinics, the food industry, and households.[36] Currently over 200 disinfectants recommended by the US Environmental Protection Agency for use against COVID-19 contain QACs.[33] Thus, it should be anticipated that the amounts of QACs used and released to the environment will increase. Previous studies showed that QACs have been detected in a variety of natural and engineered environments, including drinking water, surface water, wastewater, biosolids, soil, house dust, and sediments, due to their widespread use.[37–42] It is estimated that approximately 75% of QACs used end up in sewers and wastewater treatment plants (WWTPs).[43] The majority of QACs (>90%) in influent wastewater can be removed *via* aerobic biodegradation and sorption to the activated sludge. The sludge is then transferred to solids handling processes such as anaerobic digestion where the solids are stabilized and then, in some cases, land applied.[28,44–46]

Pre-pandemic concentrations of QACs in WWTPs were typically hundreds μg L^{-1} in influents and ng L^{-1} in effluents, and mid to high μg g^{-1} range for sewage sludge.[34,40,47–50] While degradation of QACs could occur *via* biodegradation and photolysis, both degradation pathways are slow and thus QACs are persistent in various environments.[51] QACs tend to accumulate in anaerobic media such as anaerobic digesters and sediments.[28,43,52] Unfortunately, long-term QAC exposure not only negatively impacts the performance of microorganisms in industrial processes, but also may alter environmental microorganism community structure *via* enrichment of microorganisms with QAC and antibiotic resistance, leading to the dissemination of antibiotic resistance.[53–56] However, the impact of chronic QAC exposure on WWTP performance, *i.e.*, biological reactor treatment efficiency, and the development of antibiotic resistance are still major research gaps. Specifically, there is still a lack of sufficient direct evidence to elucidate whether, and how, environmental levels of QACs affect the activity and resistance of microbial communities in complex engineering environments. Thus, the broad objective of this review is to summarize the state of knowledge regarding QAC impacts on

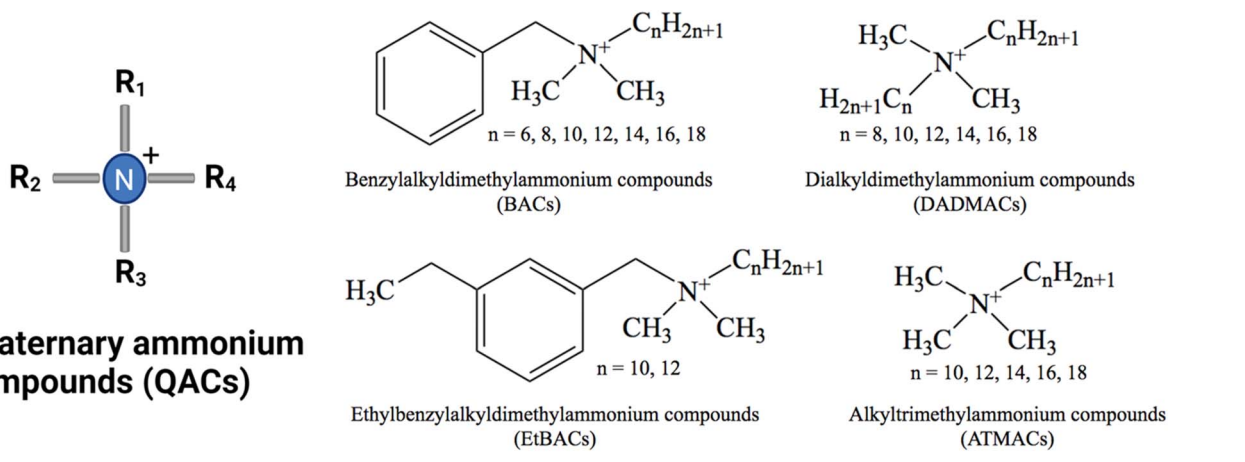

Fig. 1 General structure of the four main QAC chemical groups. The most commonly used QACs are C_8–C_{18} BAC, C_{12}–C_{14} EtBAC and C_8, $C_{8/10}$ and C_{10} DADMAC.[33]

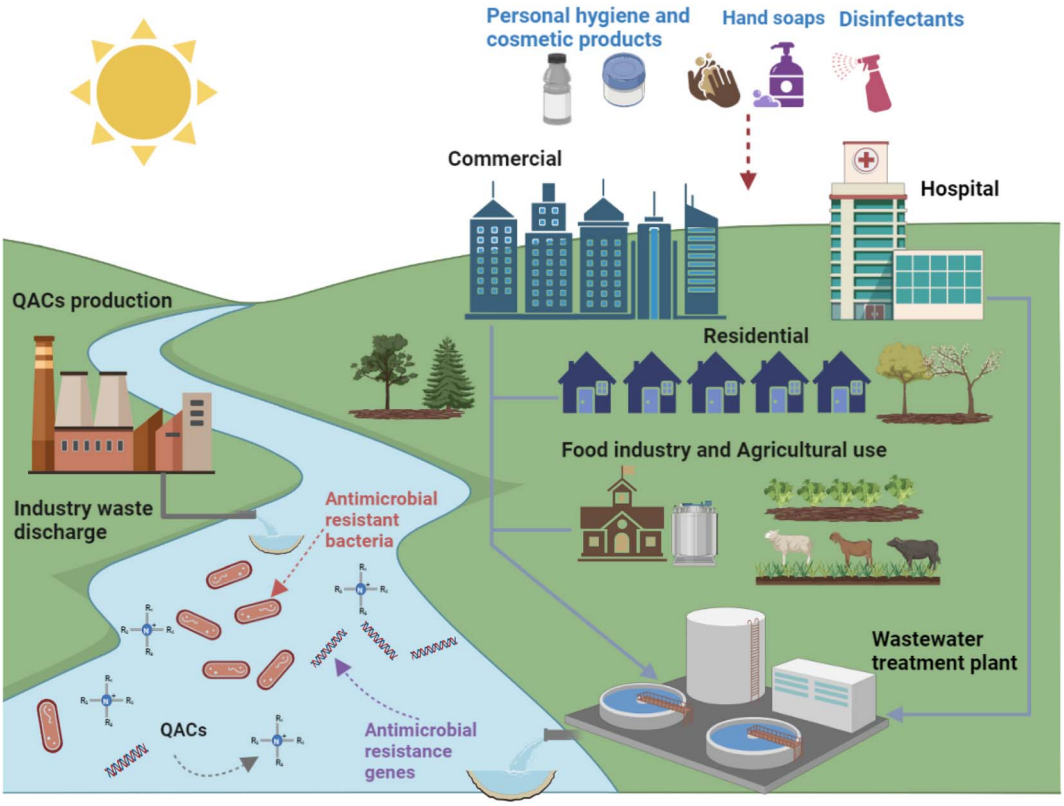

Fig. 2 Overview of the multiple potential sources of QACs in the environment. As described in this literature review, QACs can select for antibiotic resistant bacteria and increase the amount of antibiotic resistance genes in water environments.

antibiotic resistance and process performance in WWTPs. Specifically, this review covers the general antimicrobial activity of QACs as well as the mechanisms by which QACs impact antibiotic resistance, and then highlights how the environmental concentrations of QACs could impact antibiotic resistance and biological processes in WWTPs. Critical research gaps and proposed research plans to fill these gaps are presented. In general, this paper provides a comprehensive discussion on QACs as environmental stressors that could impact treatment process performance and antibiotic resistance.

2. Antimicrobial activity of QACs and resistance mechanisms of bacteria

2.1 Mode of action

QACs have antimicrobial properties that inhibit bacteria, yeasts, molds, and viruses, among other microorganisms.[35,57,58] The antimicrobial activity of individual QACs varies based on chemical properties including hydrophobicity, charge, and chemical functional groups.[23,32,59,60] In contrast to antibiotics which work on specific target sites, QACs exert their microbial inactivation by interacting with multiple target sites. The known modes of antimicrobial action include disruption of the cell membrane, inhibition of microbial aerobic respiratory system, precipitation of cytoplasmic material, and deactivation of the protective lipid coating of viruses.[9,61–63]

The interaction between QACs and the membranes of the target cells is the essential component of their antibacterial activity, which means QACs are broad-spectrum antimicrobials.[64–66] The positively charged quaternary nitrogen of QACs interacts with the negatively charged head groups of phospholipids in bacterial membranes *via* electrostatic interactions. The hydrophobic chain of QACs enters the bacterial lipid bilayer of the cell membrane.[67–69] In these processes, antimicrobial effects of QACs occur by disrupting membrane integrity, leading to leakage of cellular content.[70,71] Potassium ions, phosphates, and larger molecular weight molecules are released. Depending on the chain length, the effectiveness of QACs against bacteria varies, and this is one reason many commercial disinfectants contain multiple QACs with varying chain lengths. In general, alkyl chain lengths of C_{10} to C_{16} have higher antibacterial activity, while twin-chained compounds like DADMACs have superior bioactivity against Gram-positive bacteria compared to BACs.[68,72] When the concentration is below the minimum inhibitory concentration (MIC), QACs bind to anionic sites found on the membrane surface, which results in loss of membrane osmoregulation, inhibition of respiratory enzymes, dissipation of the proton motive force (which leads to decreased ATP production), and/or oxidative stress.[43,66,73]

QACs also act on intracellular targets such as bacterial DNA. A study indicated that nostocarboline (a bis-cationic QAC) exposure induced a SOS response following DNA damage in *Escherichia coli*, which may suggest some specific interaction

with DNA.[74] In another study, a mono-cationic QAC with aromatic rings displayed considerable inhibitory potencies to *Escherichia coli* DNA gyrase supercoiling assays.[75] Meanwhile, a newly synthesized mono-cationic QAC has been found to bind with DNA to block the synthesis of enzyme and receptor, and inhibit the migration of genomic DNA.[76,77]

2.2 Tolerance and resistance to QACs

Generally speaking, tolerance is the capacity of microorganisms to survive a transient exposure to antibiotics or disinfectants without a change in the mini MIC.[78] Understanding when microorganisms are more tolerant to QACs could mean that a greater exposure time to QACs, rather than a greater concentration of QACs, is required to produce the same extent of disinfection.[78] Mechanisms of tolerance to QACs is generally correlated with decreased growth rates, transcriptional modification of the density and structure of porins, and expression of efflux pumps.[79–81] In contrast to tolerance, resistance is the heritable ability of microorganisms to maintain biological activity at QAC concentrations above the MIC. QAC resistance is typically associated with modification of porins, regulatory hyperexpression of efflux pumps, enzymatic degradation, and acquisition of QAC resistance genes through horizontal genes transfer (HGT) (see Fig. 3).[43,45,73,82–88] It is important to highlight that effectiveness of QACs is decreased by mechanisms of bacterial resistance; specifically, a larger dose of the QACs is needed to have the same impact on a resistant strain as it does on a susceptible strain.[89,90] Unfortunately, current studies of QAC susceptibility do not distinguish between tolerance and resistance, which may result in the misclassification of tolerant microorganisms as resistant.

The structure of the cell wall and cell membrane directly influence the tolerance and resistance of microorganisms to QACs.[80] For instance, QACs are more effective at inhibiting Gram-positive bacteria and algae compared with Gram-negative bacteria and molds because the outer membrane of Gram-negative bacteria act as a permeability barrier to limit the entry of QACs into a cell.[73,95] For example, Gram-negative bacteria including *Escherichia coli*, *Salmonella typhimurium*, and *Proteus mirabilis* exhibit less susceptibility to QACs or other disinfectants.[63,91] Less acidic outer membrane lipopolysaccharides (LPS), small porins caused by strong LPS–LPS linkages, fewer porins, and a slime layer are physiological features that provide QAC tolerance.[73,87,91,96] In addition, mycobacteria have significant resistance to QACs due to their unusual cell wall made up of a hydrophobic mycolate layer and a peptidoglycan layer connected by the polysaccharide arabinogalactan.[97] Thus, the affinity of the outer cellular structure is one of the key determinants of QAC resistance.

2.2.1 Efflux pumps. Efflux pumps are proton, sodium, or ATP-dependent systems that actively remove the antibiotic and other compounds from inside the cell.[92] There are five families of membrane-spanning efflux proteins, including major facilitator superfamily (MFS), small multidrug resistance (SMR), resistance nodulation cell division (RND), ATP-binding cassette (ABC), and multidrug and toxic compound extrusion (MATE).[92,98] A number of proton-dependent efflux pumps (MFS, SMR, RND, MATE) effectively transfer mono-cationic QACs from the inside to the outside of the cell.[93,94,99–102] These efflux pumps can be encoded in both chromosomes and plasmids. For example, *cmeABC*/*cmeDEF* of *Campylobacter jejuni*, *sdeXY* of *Serratia marcescens*, *acrAB-TolC*/*yhiUV-TolC*/*emrE*/*mdfA*/*sugE* of *E. coli*, and *mexAB-OprM* of *P. aeruginosa* are important multidrug efflux determinants that confer intrinsic resistance to QACs.[99,103–108] These chromosome-encoded efflux pumps are usually non-specific and respond to a variety of other harmful chemicals in addition to QACs (Fig. 4). Additionally, a number of QAC efflux determinants are plasmid-encoded, and resistance arises from plasmid acquisition *via* horizontal gene transfer (HGT).[54,109–111] Most of these plasmid efflux pumps belong to the SMR family. SMR transporters are proton-dependent transporters of 100–140 amino acid residues.[112] Structural analysis showed that SMR genes have four hydrophobic transmembrane domains joined by flexible hydrophilic regions, which confers resistance to QACs *via* an electrochemical proton gradient.[103,112] Nine SMR genes (*qacC*/*smr*, *qacE*/*EΔ1*, *qacF*, *qacG*, *qacH*, *qacI, qacJ*, and *qacZ*) are mainly associated with mobile genetic elements (MGEs) such as plasmids and integrons, especially *qacE*/*EΔ1*.[94,112–115] The *qacEΔ1* gene (the attenuated variant of *qacE*) is widespread in Gram-negative bacteria (mainly in Enterobacteriaceae and *Pseudomonas* spp.) due to its presence in the 3′ conserved segment of most class I integrons.[116,117] Half of the environmental class I integrons carry *qac* cassettes.[118] Other plasmid-encoded efflux systems responsible for resistance to basic dyes, detergents, antibiotics (β-lactams, chloramphenicol, erythromycin, and tetracycline), as well as QACs, are derived from MFS (*qacA*, *qacB*) and RND (*oqxAB*) families.[91,92,103,119] The genetic determinants of MFS that confer QACs resistance include *qacA*/*B*, *norA*/*B*, *emeA*, *mdfA* and *mdeA* genes.[120] Among them, *qacA*/*B* genes are plasmid-encoded efflux pumps (frequently occur on the pSK1 and β-lactamase/heavy metal-resistance plasmids) and the most common QAC-resistant gene system found in Gram-positive bacteria (such as *S. aureus*) isolated from a variety of sources.[121,122] Under QAC-induced stress, the regulatory factor *qacR*, a DNA binding repressor protein, causes the overexpression of *qacA*/*B*.[123–125]

2.2.2 Enzymatic degradation. In 1977, it was first reported that there were microorganisms from sewage and soils that could degrade QACs.[84] Subsequently, more studies confirmed that QACs were biodegradable under aerobic conditions in engineered and natural systems.[44,83,129,130]

Microorganisms metabolize QACs under aerobic conditions *via* three pathways. The main difference between these pathways lies in the carbon location where the hydroxylation takes place. In the first pathway, hydroxylation of the alkyl chain at the terminal carbon takes place, followed by oxidation of the aldehyde group and then the acid group.[130] Subsequently, the compounds are processed by β-oxidation-generating acetyl-CoA. This strategy was proposed by Dean-Raymond and Aleksander in a study of the decyltrimethylammonium salt (C_{10} ATMAC) biodegradation carried out by *Xanthomonas*.[84] The results showed that decyl- and hexadecyltrimethylammonium

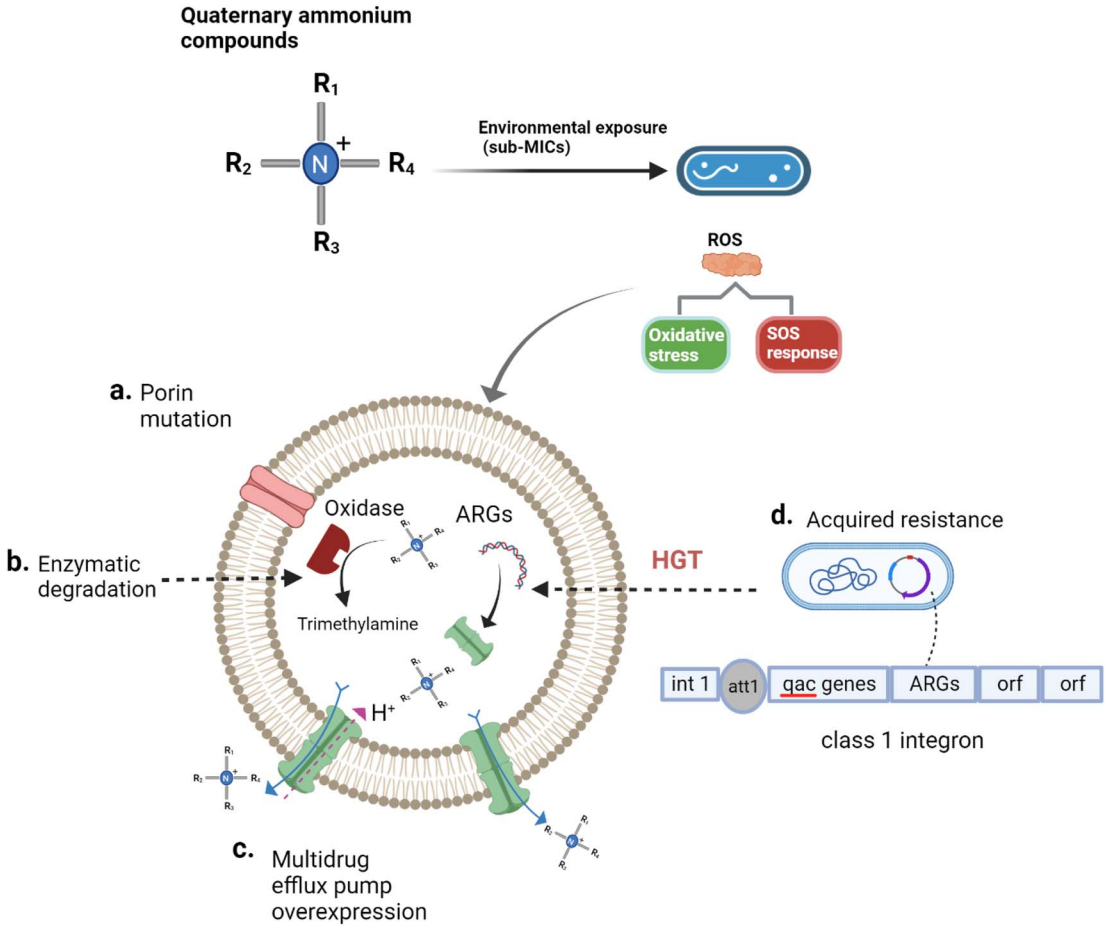

Fig. 3 Mechanisms of QACs tolerance and resistance.[35,66,86–88,91–94] QACs stimulate microorganisms to produce reactive oxygen species (ROS). ROS impact the expression of efflux pumps, promote porin mutation, and frequency of horizontal gene transfer (HGT) *via* oxidative stress and SOS response. (a) QACs exposure reduces the number of porins on the cell surface and alters the porin structure to decrease the permeability of cell surface which could prevent the passage of QACs into the cell, (b) the hydroxylation and oxidation of QACs *via* enzymes, (c) QACs activate the regulatory system of multidrug efflux pumps leading to their overexpression and QAC removal using proton gradients, (d) QACs promote HGT *via* an increase in the conjugation rate.

bromides were decomposed by organisms derived from sewage and soil. A mixture consisting of individual strains of *Pseudomonas* and *Xanthomonas* grew in solutions containing C_{10} ATMAC as the sole carbon source.[84] The second mechanism is the hydroxylation at the carbon adjacent to the central nitrogen followed by the central fission of the molecule resulting in separation of the hydrophobic and hydrophilic moieties.[131] It was proposed that the hexadecyltrimethylammonium chloride (C_{16} ATMAC) degradation carried out by *Pseudomonas* began with *N*-dealkylation catalyzed by an oxygen/NADH-dependent monooxygenase and yielded trimethylamine.[131] The first intermediate in the biodegradation pathway of C_{16} ATMAC was hexadecanol, which was oxidized to hexadecenoic acid by an alkanol dehydrogenase. The acid was further metabolized through β-oxidation. The third aerobic QAC degradation pathway involves hydroxylation at methyl C attached to the central N, followed by demethylation. This scheme was proposed for the degradation of dodecyltrimethyl ammonium chloride (C_{12} ATMAC) by *Pseudomonas* sp. strain 7-6 isolated from a WWTP.[85]

Aerobic biodegradation of QACs has been attributed mainly to bacterial species in the genera of *Xanthomonas*, *Aeromonas*, and *Pseudomonas*. Several microorganisms have been identified that are resistant to QACs and capable of QACs degradation including *Xanthomonas*,[84] *Pseudomonas B1*,[131] *Pseudomonas Fluorescens TN4*,[132] *Pseudomonas* spp. *Strain 7-6*,[85] *Pseudomonas putida A ATCC 12633*,[129] *Pseudomonas nitroreducens B* and *DB*,[83] and *Pseudomonas* sp. *BIOMIG1*.[133] The ability to aerobically degrade QACs depends largely on the presence of monooxygenases, dioxygenases, amine dehydrogenases, and/or amine oxidases. An amine oxidase can reduce the BAC toxicity to a bacteria 500 fold.[83] In addition, Ertekin *et al.* characterized a gene cluster encoding for transporters; an integrase and a dioxygenase were involved in BAC biotransformation under aerobic conditions.[133] Multi-drug efflux pump genes such as *sugE*, *PmpM*, *mexAB-oprM* and *mexEF-oprN* were enriched in a BAC-degrading community, which indicates that the efflux pumps and oxidase reinforce microbial resistance to QACs.[38,83,134] The impact of BAC degradation products should also be investigated to determine if degradation is beneficial, or

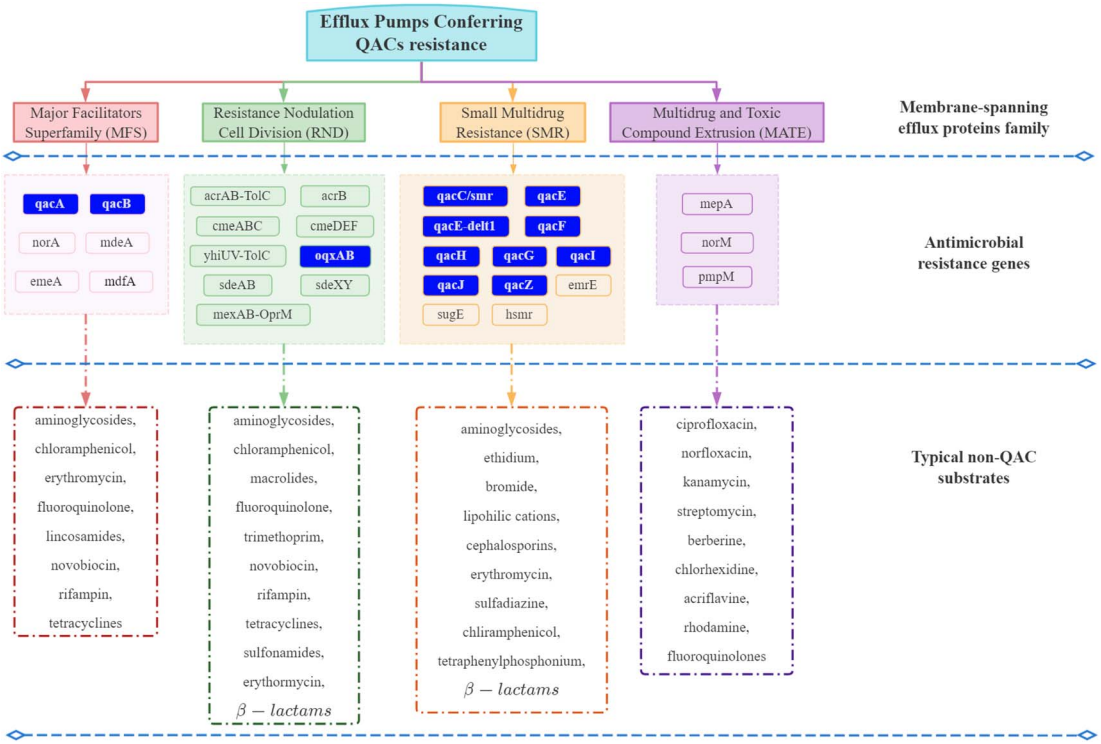

Fig. 4 Efflux pumps conferring QAC resistance. The efflux pumps in the blue box are often plasmid-encoded. Others are primarily chromosome-encoded.[92–94,99–101,103–108,119,126–128]

if potentially the degradation products impact antibiotic resistance as well.

3. How QACs can promote the spread of antibiotic resistance

Considering their biological activity and amphiphilic properties, QACs may play multiple roles in the development of antibiotic resistance. First, as biocides, QACs have potential to directly select for antibiotic resistant microorganisms. Secondly, QACs can stimulate horizontal gene transfer of antibiotic resistance genes (ARGs). Lastly, QACs could indirectly promote the transmission of ARGs by increasing the bioavailability of other stressors, thereby reducing the actual concentration of a stressor (*e.g.*, metal or antibiotic) required to trigger a response. Additionally, QACs could increase cell membrane permeability which would allow stressors to more readily have an effect.

3.1 Selection of antibiotic resistant microorganisms

Although QACs and antibiotics have different modes of action against microorganisms,[70,87,98] QAC exposure can promote the development of intrinsic non-specific cross-resistance to QACs and antibiotics through expression of multi-drug resistant efflux pump genes and a reduction in surface permeability.[54,105,119,135] For example, MFS and RND family efflux proteins have a broad range of substrates including nine kinds of antibiotics (Fig. 4).[98,103] *Pseudomonas* exposed to sub-inhibitory concentrations of mixed BACs (60% C_{12} BAC & 40% C_{14} BAC) had high levels of *mexCD-oprJ* multi-drug efflux pump gene expression, which could lead to resistance to BACs, chlorhexidine, quinolones, macrolides and six other antibiotics classes.[9,53] Another study also confirmed that the expression of the multi-drug efflux pumps *sugE-A*, *sugE-B*, and *acrB*, increased when BAC was added to the growth media.[136] Thus, QAC exposure can directly increase the expression of multi-drug resistant efflux pump genes. In addition, QAC resistance genes and ARGs are frequently located together (co-resistance) on mobile genetic elements, which could be another important reason for the development of antibiotic resistance due to QAC exposure.[137–140] The first identified *qac* gene (*qacA*) was found on a transmissible plasmid that also contained β-lactam and heavy metal resistance genes.[141] In that case, all three resistance genes may be co-selected and transmitted if the microorganism is exposed to one of these stressors. Co-selection caused by QAC exposure is usually associated with class I integrons. Approximately 1–5% of bacterial cells in soil, freshwater, and biofilms contain Class I integrons.[117] Class I integrons can acquire diverse gene cassettes that confer adaptive phenotypes and move between chromosomal locations, species, and lineages.[142] The *qac* cassette, which has potential to provide a survival advantage under the selective stress of QACs, is carried by approximately 50% of environmental class I integrons.[118] Ghaly *et al.* proposed a modified hypothesis for integron assembly based on the structure of class I integrons,[139] where one chromosomal class I integron carrying a *qac* gene cassette was captured by a Tn5090-like transposon to form Tn402. The

position of this complex mosaic component on a broad host range IncP plasmid would then have allowed its conjugative transfer into a diversity of bacterial hosts when the selective stress caused by QACs was present.[139] In Enterobacteriaceae, the *qac* genes have been frequently found in combination with ARGs to aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and β-lactams on large, transmissible plasmids (resistance plasmids) that carry integrons.[143] Thus, co-selection and transfer of QAC resistance genes and ARGs has the ability to occur from QAC exposure.

It is important to note, however, that even carrying QAC resistance genes does not necessarily confer a corresponding survival advantage to the microorganism. Cervinkova *et al.* investigated the adaptation of *Staphylococcus aureus* (SK982) carrying the QAC efflux pumps (*qacA* and *norA*) when exposed to BAC under various circumstances.[144] The results showed that the expression of *norA*, one of the efflux pumps conferring QAC resistance belonging to MFS family, was relatively low regardless of the BAC concentration. The *qacA* gene was only overexpressed in the exponential phase of growth. Several studies also proposed that the role of QAC resistance genes may be overstated.[89,145–147] It is crucial, therefore, to understand the practical significance of QAC resistance genes, while monitoring the residual concentration and persistence of QACs in environments to predict the fate of ARGs.

3.2 Promotion of horizontal gene transfer (HGT)

HGT is an important aspect affecting how microorganisms acquire antibiotic resistance and can be achieved through three pathways: conjugation, transformation, and transduction.[148–152] HGT can be affected by various environmental factors, such as antibiotics, disinfectants, oxidants, nanomaterials, metals, and microplastics.[153,154] QACs have also been confirmed to be HGT-promoting stressors. The low concentration exposure of different QACs (C_{10}ATMAC, C_{12}DADMAC, C_{12-16}BAC) promoted conjugation frequency of the RP4-resistant plasmids in *E. coli*.[54] C_{12}BAC had the most obvious effect on the transconjugative efficiency, with a 15-fold increase at a concentration of 0.1 mg L^{-1}. In other studies, the promotional effect of different QACs on conjugative transfer at sub-MIC concentrations exposure has also been demonstrated, and the facilitating ability is concentration and core structure dependent.[155,156] QACs facilitate plasmid conjugation transfer *via* multiple pathways including increasing cell membrane permeability, altering the composition and content of extracellular polymeric substances (EPS), and regulating gene expression associated with plasmid conjugation.[54,155,156] A recent study indicated that the ratio of extracellular polysaccharides and proteins increased with the exposure of QACs (C_{10} DADMAC and C_{12} BAC), which caused an increase in cellular hydrophobicity, thereby contributing to the intercellular contact between donors and recipients.[156] Additionally, QACs regulate the transcriptional expression levels of the genes that code for quorum sensing regulator (*luxS*), global regulators (*korA*, *korB*, *trbA*), DNA replication and translocation (*trbB*) and mating pairing formation (*trbB*).[156] Besides conjugation, QACs also have potential to facilitate the transformation of exogenous ARGs *via* multiple pathways. Jia *et al.* studied the effect of benzalkonium bromide (BB) and benzalkonium chloride (BC) on bacterial transformation at environmentally relevant concentrations (0–0.1 μg ml^{-1}).[157] BB and BC significantly enhanced the transformation of ARGs in *E. coli via* increased membrane permeability, improved bacterial flagellum motility and enhanced expression of genes related to secretion systems. These studies illustrate the potential risk associated with QAC exposure at environmental residual concentrations on the spread of antibiotic resistance. However, these findings are based on simple, artificially constructed HGT models. It is important to determine if QACs are equally effective in complex engineered environments, especially for antibiotic resistance development in WWTPs.

3.3 Indirect promotion of antibiotic resistance through altered bioavailability

QACs as cationic surfactants have the potential to indirectly affect the transmission of ARGs *via* altering the bioavailability of poorly soluble antibiotics or other hydrophobic stressors in water.[158] Previous studies showed that the dissolution of hydrophobic substances (*i.e.*, antibiotics) in suspension requires a significant amount of energy to counteract the effect of increased area of liquid–solid interface and interfacial surface tension.[159–161] QACs can lower the interfacial tension to reduce surface energy and alter the zeta potential of particles in the suspension system due to their amphiphilic property from hydrophobic alkyl groups and hydrophilic positively charged N atom.[68,161–163] For instance, the solubility of erythromycin increased linearly with increasing ATMAC concentration.[158] Due to electrostatic attractions, C_{16} ATMAC showed the highest molar solubilization capacity for erythromycin. Therefore, QAC exposure can theoretically facilitate the dispersion of the hydrophobic antibiotics/stressors in an aqueous solution and increase the thermodynamic stability of the system, which enhances mass transfer of antibiotics and increases toxicity, thereby subsequently increasing the selective pressure. A recent study by Wang *et al.* revealed the roles and critical mechanisms of anionic surfactants (sodium dodecylbenzenesulfonate, SDBS) on sulfadiazine (SDZ) stressing for ARGs during anaerobic fermentation.[164] Evidently, the exogenous SDBS sped up the propagation of ARGs stressed by SDZ. In addition to enhancing SDZ solubility in the waste activated sludge fermentation system, the presence of SDBS also increased cell permeability and destroyed EPS, which hastened the SDZ interaction with ARG hosts and aided in the spread of ARGs. Although this is about the role of anionic surfactants (SDBS), previous studies have demonstrated that QAC cationic surfactants (ATMAC) had a higher solubilization capability in comparison to nonionic (Brij) and anionic surfactants (SDBS).[158] Thus, QAC exposure may have a similar or stronger effect, especially in some important wastewater treatment processes where various contaminants co-occur, such as aerobic biological oxidation, nitrification/denitrification, and anaerobic digestion. However, there is still a lack of relevant studies about the role of QACs on antibiotic selective pressure in WWTPs,

making it difficult to estimate the selection pressure of QACs in real-world settings.

4. Linking QACs to antibiotic resistance in WWTPs

WWTPs are reservoirs for three key factors in antibiotic resistance development: selective stressors (such as QACs and antibiotics), resistance donors, and resistance recipients.[40,165–168] While concentrations of QACs in consumer and industrial products are typically several orders of magnitude greater than necessary to kill bacteria, when QACs enter WWTPs their concentrations are diluted, exposing bacteria to sub-inhibitory concentrations of QACs (Fig. 5).[19,40,50] Levels of QACs were reported in mg L^{-1} range in influent wastewater and μg L^{-1} range in effluent.[34] As mentioned in Section 3, these sub-inhibitory concentrations of QACs can still lead to the spread of resistance. Some unit operations may have elevated QAC levels compared to other unit operations, particularly in solid treatment process like anaerobic digestion where QACs levels are as high as 500 mg kg^{-1}.[22] Due to the high densities and metabolic activities of microbes in biological processes (activated sludge tanks or anaerobic digesters), QACs may play a role in the spread of antibiotic resistance during wastewater treatment.

In recent years, genes that encode for multi-drug efflux pumps and ARGs were frequently detected in WWTPs.[186–190] Pärnänen *et al.* investigated the prevalence of ARGs in 12 WWTPs in seven European countries. Results showed that *qacEΔ1* and *sul1* (sulfonamide resistance gene) were core wastewater ARGs that were present in all influent and effluent samples. Another QAC resistance gene, *qacH*, persisted after treatment in 92.1% of the analyzed samples.[191] Meanwhile, *qacH* was also detected in foam and activated sludge in wastewater treatment plants.[192] In a recent study, Tan *et al.* investigated the abundance of ARGs and mobile genetic elements (*e.g.*, integrases) in aerobic composting systems for sewage sludge, finding a high relative abundance of QACs resistance genes (*qacEΔ1*, *qacH*, *sugE*, *emrE*).[165] The co-occurrence of QAC resistance genes, ARGs and MGEs was also detected in industrial wastewater.[94,193,194] However, in most studies, no statistically significant correlation could be established between antibiotic

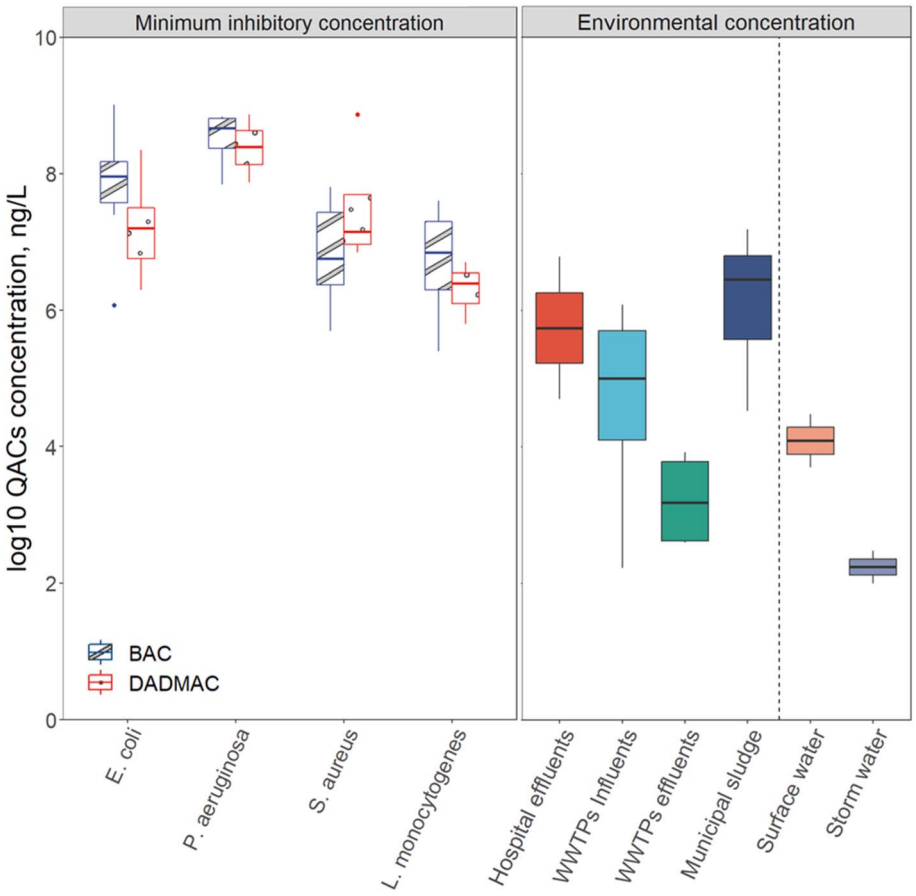

Fig. 5 The QACs MIC of various isolates and total concentration of QACs in various environments. Left boxes are the MIC of BAC and DADMAC for different sensitive strains. Right boxes are range of total QACs concentrations found in each environmental setting. Biosolids (municipal sludge) concentrations were converted from mg kg^{-1} to mg L^{-1} by assuming 3% total solids in reactors that produce biosolids. The line extending above the box is the maximum and the line extending from the bottom of the box is the minimum. The box's up side represents the first quartile of the data, the line in the middle of the box represents the median of the data, and the box's bottom side represents the third quartile.[46–50,73,106,169–185]

consumption, antibiotic residues, and abundance of ARGs.[195,196] This raised the speculation that other potential stressors in wastewater, as well as conditions and processes of wastewater treatment, may play an important role in the fate of ARGs. Of all the possible interfering factors, QAC concentration is one that cannot be ignored considering that residual concentrations are generally higher than those of antibiotics.[96]

Moreover, QAC resistance genes, specifically *qacF*, *qacEΔ1*, and *qacH*, were identified on the resistance plasmid pSp1 isolated from activated sludge bacteria residing in a municipal wastewater treatment plant and linked with other ARGs.[197] As noted in Fig. 4, these genes confer resistance to QACs and antibiotics. IncP-1 resistance plasmids isolated from WWTP bacteria also have been found to encode QAC efflux pumps linked to ARGs such as streptomycin, β-lactam, and sulfonamide resistance genes.[113] Similar associations were confirmed in three Swedish sewage treatment plants;[198] *qacEΔ1*, *sul1*, and the aminoglycoside resistance gene *ant(3″)-la* were located with an *int1* integrase gene in microorganisms in the influent and primary sludge. The most recent studies showed chronic exposure to QACs (C_{16} ATMAC, benzethonium chloride) significantly enriched QAC resistance genes (*qacEΔ1* and *qacH*) and ARGs (*aadA*, *aac(6′)-Ib* and *blaTEM*) in a partial nitrification/anammox system.[199–201] Thus, WWTPs may be a hotspot for the development and spread of QAC resistance genes and ARGs.[166,202]

Genes encoding efflux-driven QAC resistance are also abundant in natural aquatic environments, especially in rivers or lakes located downstream of WWTPs and livestock farms.[203–207] A study by Chen investigated the prevalence, abundance, and distribution of the RND family efflux pump genes (*acrA/B*), which is responsible for resistance to QACs, and 20 other ARGs conferring resistance to five major classes of antibiotics including tetracycline, sulfonamide, quinolone, aminoglycoside, and macrolide in wastewater from 12 livestock farms and their downstream surface water.[208] The results showed that the detected frequency of QAC resistance genes (*arcA* and *arcB*) were above 90%, although livestock farms are not an application scenario for QACs, indicating promiscuity of the enzymes or selection on other traits in the genome where the QAC resistance genes reside. The relative abundance of these QAC resistance genes is less than tetracycline and sulfonamide resistance genes, which are the two primary veterinary antibiotics used in animal husbandry. Moreover, *arcA* and *arcB* were also detected in drinking water sources with presumably no or little exposure to QACs.[205] Canonical correlation analysis between antibiotic concentration and ARG subtype abundance revealed that ARG abundance was closely related with the total concentration of quinolones. Thus, exposure to antibiotics may lead to QAC resistance through efflux and, similarly, exposure to QACs could provide resistance to antibiotics. Likewise, a large abundance of QAC resistance genes (*qacEΔ1*, *qacH*) was reported in an estuary from China,[155] indicating that estuary sediments are a reservoir of QAC resistance genes. The detection frequencies of QAC resistance genes (*qacEΔ1* and *qacH*) were 100% in estuary sediments. Meanwhile, *qacEΔ1*, *qacH-01*, and *oprD* were the top three most abundant genes among the fifteen detected multidrug resistance genes in the studied estuary area. In addition to the above-mentioned environments that are highly disturbed by anthropogenic activities, 250 ARGs were subtypes found in both remote lakes (7.25×10^{-2} copies/16*S* rRNA) and marine environments (2.70×10^{-2} copies/16*S* rRNA), four multi-drug resistance genes (*qacA*, *acrA*, *acrB*, *emrE*) that confer QAC resistance were found,[209] which may indirectly confirm that ARGs and QAC resistance genes are naturally present.

5. The potential impact of QACs on process performance in wastewater treatment plants

Due to the extensive use of QACs in medical, industrial, and personal consumer products, most of these compounds are released into WWTPs and then are dispersed into natural environments *via* liquid effluent discharges and land application of biosolids.[34,96] Both the activated sludge process and anaerobic digestion serve as core technologies to treat wastewater, have a high solids content, and are therefore potentially impacted by QACs that enter the reactor sorbed to solids. A previous study showed that mixed BACs (40% C_{12} BAC, 50% C_{14} BAC, 10% C_{16} BAC) inhibited oxygen uptake and utilization in the activated sludge process, which hindered COD removal.[45] Chen and Zhang also found that exposure to high BAC concentration affected aerobic respiratory behaviors and the tricarboxylic acid (TCA) cycle of microorganisms in membrane bioreactors, resulting in decreased ATP.[210] Zhang proposed that the decrease in ATP production may be caused by damage to energy-production enzymes (*e.g.*, adenylate kinase, ADK).[79] These studies indicate that BACs can impact bulk carbon removal (BOD_5), but they can also impact nutrient removal.

Nutrient (nitrogen and phosphorus) removal is a key goal for wastwater treatment. Different biological processes can be employed to remove nitrogen from wastewater including nitrification, denitrification, and anammox.[211–213] Nitrification is a two-step process. Ammonia oxidizing bacteria (AOB) convert ammonia to nitrite. Then, nitrite oxidizing bacteria (NOB) convert nitrite to nitrate. Denitrification occurs in the absence of oxygen under anoxic conditions using heterotrophic bacteria (usually *Pseudomonas*) to reduce nitrate to nitric oxide, nitrous oxide and nitrogen gas.[214] Anammox is another nitrogen-removal technique for treating nitrogen-rich wastewater.[215] Autotrophic anammox bacteria get energy from converting ammonium and nitrite simultaneously into nitrogen gas in the absence of oxygen. A recent study indicated that chronic exposure of benzethonium chloride (BEC) at low concentration (1–2 mg L^{-1}) significantly inhibited the nitrification capacity of a lab-scale reactor.[200] During the initial phase of exposure, the ammonia oxidation was inhibited, but recovered completely within 3 days with nitrite accumulation, which means ammonia oxidizing bacteria have excellent adaptability to BEC. In contrast, the activity of nitrite oxidizing bacteria (NOB) severely decreased with BEC exposure. BEC exposure converted nitrification to partial nitrification, but this effect was reversible. Other studies also showed BEC can selectively eliminate NOB in

activated sludge at high levels (70 mg L^{-1}).[199,216] Meanwhile, BEC has potential to increase EPS secretion and alter protein secondary structure, while inhibiting the dominant NOB-*Nitrospira*.[199] C_{16} ATMAC has also been found to affect the nitrogen removal performance *via* inhibiting the anammox (*hzsB*) gene at environmental concentrations (0.5 mg L^{-1}).[201] C_{16} ATMAC exposure enriched two types of anammox bacteria (*Candidatus Jettenia* and *Candidatus Kuenenia*) to maintain a good nitrogen removal performance in a partial nitrification/anammox system. In addition, QACs (C_{12} BAC, C_{12} ATMAC, C_{12} DADMAC) inhibited denitrification.[217] With the increase of QACs concentration, the abundance of *Thauera*, a denitrifying genera, decreased significantly. At the time of this review, no papers were found that discussed how QACs impact biological phosphorus removal treatment processes. This lack of knowledge represents a key research gap that would be important to WWTPs that employ biological phosphorus removal.

The strong sorption capacity of activated sludge for QACs (C_{12} & C_{16} ATMAC, and C_{12} & C_{16} BAC) results in QACs entering solids handling processes such as anaerobic digestion.[28] During anaerobic digestion, complex organic matter is converted to methane and carbon dioxide through hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[218] Under the stress of C_{12}–C_{16} BAC and C_8 and C_{10} DADMAC, both methanogenesis and acidogenesis were inhibited at concentrations $\geq$25 mg L^{-1} meaning that the overall anaerobic digestion process was inhibited and methane production was decreased.[219] Another study indicated that BACs (C_{12}, C_{14}, and C_{16}) caused release of soluble polysaccharides and proteins, which were positively related to the residual levels of BACs in an anaerobic digester.[220] Higher concentrations or shorter alkyl chain lengths caused more inhibition of methane production and the accumulation of short chain fatty acids.[221] The long-term stress of BACs (C_{12} BAC, C_{14} BAC, and C_{16} BAC) reduced microbial diversity and decreased the relative abundance of aceticlastic methanogens and increased the relative abundance of hydrogenotrophic methanogens.[90,221] C_{12} BAC, with a shorter alkyl chain length than the other BACs in this study, had more significant effects on microbial diversity and shifted the main acid-producing bacteria from *Proteiniclasticum* and *Intestinibacter* to *Candidatus Microthrix* and *Lutispora*. Meanwhile, the proportion of *Firmicutes*, *Bacteroidetes* and *Chloroflexi* in the community decreased significantly under the impact of mixed BACs (69% C_{12}, 23% C_{14}, 3% C_{16}), while *Proteobacteria* and *Actinobacteria* were increased. The effect of BAC (C_{12}–C_{16}) on Archaea in anaerobic digestion is mainly reflected in the screening of methanogenic trophic types.[220] BACs led to a shift in methanogens from *Methanosaeta*, a strictly aceticlastic methanogen group, to hydrogenotrophic methanogens, such as *Methanobacterium* and *Methanobrevibacter*.[220,221] Overall more volatile fatty acids were formed and less methane was produced. It is still unclear, however, which microorganisms responsible for anaerobic digestion are most significantly affected by QACs. Minor impacts could include changes to the microbial community structure with no loss in treatment efficiency. If there is functional redundancy between QAC-sensitive microorganisms and QAC-tolerant microorganisms, then QAC impacts would be expected to be minor. Conversely, major impacts could include loss of process function at concentrations not-yet-seen in an anaerobic digester. Furthermore, it is important to determine if and how microbial communities respond after QAC stressors are removed to know if the impacts are reversible.[222]

6. Conclusions and research gaps

QACs are commonly used cationic disinfectants and surfactants, and a vast amount of QACs enter WWTPs following usage by consumers. QACs disrupt microbial cell membranes leading to gradual leakage of cytoplasmic components out of the cell, thereby inhibiting microbial growth. As a result, QAC resistant microorganisms and QAC resistance genes may be selected and enriched under specific conditions in WWTPs. Due to the mechanism of QAC resistance and the fact that QAC resistance genes are often encoded on mobile genetic elements with other ARGs, persistent residues of QACs may lead to co-transformation and co-accumulation of QAC resistance genes and ARGs in WWTPs, further exacerbating the growing risk of antibiotic resistance. Meanwhile, microbial community structure and process performance of WWTPs could be affected by long-term exposure to QACs.

Against the backdrop of increased use of disinfectants such as QACs due to the COVID-19 pandemic, there are still many knowledge gaps that need to be filled to clarify the role of QACs on the development of antibiotic resistance. First, it is not known if exposure to QACs contributes to the rise of antibiotic resistant pathogens in complex engineered environments. Although previous studies revealed that QAC exposure can promote the development of antibiotic resistance,[155,156,223,224] evidence is lacking about how QACs impact clinical antibiotic resistance. The impact of the coexistence of QACs and other stressors on antibiotic resistance is also an important knowledge gap. Antibiotics, disinfectants, and heavy metals are common selective pressures leading to antibiotic resistance in wastewater. Although they have different modes of action on microorganisms, these chemicals may trigger the same resistance mechanisms, such as upregulation of multi-drug efflux pumps. In addition, QACs as surfactants have potential to alter the bioavailability of other organic compounds. Thus, the simultaneous presence of multiple stressors may significantly reduce the threshold of effect concentration of a single stressor, leading to an easier transfer of resistance genes. Additionally, the synergistic effect of multiple contaminants may create oxidative stress, thereby enhancing the capture efficiency of mobile genetic elements to antibiotic resistance cassettes, and the frequency of HGT. Studies pursuing these issues could further elucidate the molecular mechanisms of cross-resistance and co-resistance between QACs and other stressors.

Complex microbial environments may be conducive to the spread and dissemination of antibiotic resistance. The microbial community structure in wastewater and different unit operations such as aerobic nitrification reactors or anaerobic digesters can vary widely. The sensitivity of different microbial communities to QACs also varies. It is important, therefore, to

understand which microbial communities are most susceptible to changes induced by QACs. The conventional physicochemical characteristics of environmental systems, such as temperature, ionic strength, pH, and organic matter content may also play a role in QACs affecting the susceptibility of specific microbial communities to antibiotic resistance.

In addition, it is not known how the core chemical structure of QACs is linked to impacts on biological wastewater treatment process performance, including nitrification, denitrification, annamox, biological phosphorus removal, and anaerobic digestion. Although previous studies have revealed the acute impacts of select BACs, ATMACs and DADMACs on the activated sludge process and anaerobic digestion, structural differences in QACs may significantly affect bioavailability and toxicity. Furthermore, the impacts of EtBACs on any of these biological wastewater treatment processes are yet to be studied. Overall, as the amount, and types, of QACs in consumer usage continues to grow, so too must our understanding of environmental impacts. Only then can we make the best-informed decisions to protect public health and ensure stability of environmental engineering treatment systems.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was funded by the National Science Foundation through projects CBET 2051336 and 2051313.

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