THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
Dec 2023 3(11) 4050-4054

Popular Article

Biocide Resistance in Environmental Bacteria – Lesson from Triclosan Use

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https://doi.org/10.5281/zenodo.10259678

1. Introduction

Prior to the discovery of microbes, various food preservation methods were employed, often involving the use of biocides, without a clear understanding of their specific targets. Then, biocides were applied to treat wounds and eliminate contaminating microorganisms. There are several chemicals that are categorized as biocides, and these chemicals are applied in various industries like food, pharmaceuticals, hospitals, water treatment plants, personal care, textile, aquaculture, veterinary, and refineries. Recently, greater awareness and conciseness for health among individuals has led to a rise in biocide use. Contrarily, uncontrolled and excessive use of biocides may contribute to the spread of antimicrobial resistance (AMR). AMR is a condition in which microorganisms develop resistance against antimicrobial treatments, including biocides. It has become a major threat to human health and poses a challenge to the development process of new drugs.

Consequently, AMR makes it harder to treat diseases and elevates the death rate. Investigations reveal that nearly 1.27 million individuals died due to AMR, surpassing the death tolls caused by HIV and malaria. Moreover, AMR is projected to kill 10 million people by the end of 2050 (Pires *et al.*, 2017). There are different factors involved in AMR, including natural and anthropogenic factors. Naturally, AMR develops via spontaneous mutation or through genetic

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exchange. However, in recent times, the excessive use of antibiotics and other medical products has become the primary driver of antimicrobial resistance. Additionally, antimicrobials like triclosan (TCS) and similar biocides provide a suitable condition for the microbes to develop AMR.

Triclosan is a biocide with a broad-spectrum bisphenol and is widely used in several consumer products, especially personal care products (PCPs). TCS is an antimicrobial agent in PCPs, where it kills microorganisms at higher levels, while at lower levels, it inhibits the growth of microorganisms, which in the longer term can develop antimicrobial resistance among the exposed microbial community. It is known that TCS is effective against gram-positive and gram-negative bacteria, fungi, protozoans, and viruses. However, unregulated use is a cause of concern. Similarly, various biocides contribute to the development of AMR. Thus, biocides gained substantial attention in the scientific investigations that evaluated the effect of biocides on the development of AMR.

2. Biocides in the environment and bacterial resistance

Biocides are an integral part of several consumer-oriented products used daily. The most common biocidal products are disinfectants, preservatives, anti-fouling agents, pest control agents, and corrosion inhibitors. Due to widespread use, biocides like TCS and N, N-diethyl-3-methylbenzamide are frequently found in wastewater treatment plants (WWTPs). These WWTPs can remove up to 80% of biocides, but a considerable amount still reaches nearby water bodies. For instance, TCS is reported at the rate of 750-1030 ng L^{-1} in Versova creek of Mumbai (Thilakan *et al.*, 2019).

Interestingly, biocides are accumulated in aquatic organisms such as algae and fish. For example, 4.99 ng g^{-1} Methylparaben was observed in fish muscle (Juksu *et al.*, 2019). Hence, biocide concentrations in the natural environment are rising and threaten all forms of life living in the respective environments.

Globally, AMR is primarily caused by the excessive use of antibiotics, but sanitation, pollution, and other associated factors also play a significant role in its development. The occurrence of resistant bacteria in aquatic systems was first recognized in the late 90s. In 1990, resistant gram-negative bacteria were found in the drinking water. Currently, due to high concentrations of biocides, aquatic systems are considered a reservoir of resistant microorganisms. Once microbes develop resistance to a particular biocide, resistant genes can spread by horizontal gene transfer (HGT) process across the bacterial flora of the aquatic ecosystem. Moreover, transferring mobile elements, such as plasmids, transposons, and integrons, between bacterial

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populations, might spread antimicrobial resistance genes (ARGs). Additionally, the release of naked DNA and transduction by bacteriophages contribute to the development of AMR. Together, biocides can facilitate the spread of ARGs among bacteria as well as across the aquatic ecosystem.

3. Development of bacterial resistance through biocides: Special reference with TCS

TCS residues enter the environment and interact with cell membranes similarly to other phenolic compounds. Consequently, the cytoplasm is lost, and oxidative phosphorylation is uncoupled from the respiratory cycle. TCS breaks cell walls at higher concentrations, but at sub-lethal concentrations, it acts on specific targets that result in the development of bacterial resistance (Karmakar *et al.*, 2019). TCS is often present in sub-lethal levels in the environment, which influence the mutation, co-resistance, and enzymatic degradation, paving the basis for the AMR to the specific biocide.

3.1. Efflux Pump

The efflux pump is a process utilized by microorganisms to eliminate toxic substances like antimicrobial agents, metabolites, and quorum indicator molecules. Efflux pumps are highly effective in gram-negative bacteria, as efflux pumps in gram-negative bacteria may be selective or transport antibiotics of various classes, thereby acquiring multiple drug resistance (MDR). Specifically, the functioning of different efflux pumps varies. For instance, AcrAB efflux pump contributes to the development of resistance to TCS in *E. coli* and *Salmonella enterica* (Webber *et al.*, 2008). On the other hand, the majority of the non-specific efflux pumps activated by TCS are potentially effective against several therapeutically significant antimicrobials. Therefore, the microorganisms acquired antibiotic resistance in advance, enhancing the risk of disease transmission.

3.2. Modification of Target Site

Modification of the target site is another mechanism by which bacteria can develop resistance to biocides. Primarily, these modifications are spontaneous mutations that promote resistance against the drug. In a similar way, microbes develop TCS resistance through the mutations in the FabI gene, which modulates the target protein FabI. Similarly, TCS modifies FabV, FabK, and FabL genes to promote AMR in *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Bacillus subtilis* (Heath and Rock, 2010). Interestingly, certain bacteria have naturally occurred isozymes that, when activated under particular conditions, drive the development of drug resistance. Collectively, these modifications facilitate cross-resistance between antibiotics with similar target sites.

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3.3. Co-resistance

Cross-resistance is the resistance of bacteria to multiple antimicrobial agents acquired by a single molecular mechanism. Here, multiple genes located in the same genetic region are coupled to facilitate the development of resistance. This type of modification may cause resistance to completely unrelated antibiotics, indicating resistance not only to a single antibiotic but to the entire class of biocides. In support, the outer membrane of bacteria is nonspecific, allowing them to engage in cross-resistance. Alternatively, bacteria can develop biocide resistance through the action of enzymes such as β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferases (Dockrell *et al.,* 2004). Together, cross-resistance is potentially induced by both genetic makeup and enzyme activity.

4. Multi-Drug Resistance (MDR) through TCS

TCS mode of action is similar to other commercially available antibiotics, which act against microbes by breaking the cell wall. Similarly, microbes acquire resistance against TCS via cross-resistance. In addition to cross-resistance, various efflux pumps do not recognize a broad range of substrates, allowing bacteria to discharge various unrelated compounds. In particular, the AcrAB gene, which encodes the efflux pump, can give resistance to TCS, chloramphenicol, and tetracycline. Similarly, the SmeDEF gene encodes an efflux pump when TCS binds to its repressor SmeT resulting in microbial resistance against ciprofloxacin, norfloxacin, nalidixic, and ofloxacin (Hernández *et al.,* 2011). However, the efficacy of these biocides may vary depending on the species and biocide combination used. For example, TCS exposure stimulates the efficiency of chloramphenicol and tetracycline by 10-fold and may reach up to 500-fold. This suggests that biocides have a synergistic effect on microorganisms (Carey and McNamara, 2015).

TCS has been widely used in PCPs, and its concentrations in these products range from 0.1% to 0.3% (Sabaliunas *et al.,* 2003). Additionally, the use of medical and healthcare products has multiplied by 10 to 20 due to COVID-19, which has increased the environmental concentration of TCS. About 96% of TCS is predicted to originate from products and reach aquatic systems through sewage treatment plants (STPs). Generally, TCS disrupts fatty acid synthesis and protein synthesis in lower organisms. However, these modifications work nonspecifically and prevent antibiotics from breaking the cell wall. For example, TCS exerts its inhibitory effects on the fabI gene, but frequent exposure to TCS causes overexpression/mutation of the fabI gene, which stops TCS from interfering with fatty acid synthesis and thereby promoting drug resistance. Additionally, frequent TCS exposures increase the MIC for oxytetracycline 2-3 fold (Karmakar *et al.,* 2019). Furthermore, frequent TCS exposures disseminate ARGs via HGT.

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5. Conclusion

The excessive use of antibiotics promotes the development of bacterial resistance. Biocides such as TCS are widely used in personal care products. After entering into the environment, they can take part in the AMR development process through mechanisms similar to those of antibiotics. Additionally, biocides are bioaccumulative, and little is known about their biotransformation. Widespread, unregulated application of TCS and identical biocides poses challenges for the future. Thus, it is essential to understand the in-depth mechanism of action of biocides along with strict monitoring of current usage to regulate the AMR. Extensive research needs to be carried out from all possible perspectives, and stringent frameworks must be established to monitor the usage and regulate the negative implications of biocides.

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