Disinfectants as a double-edged sword: Are disinfectants promoting antimicrobial resistance?

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Abstract
The discovery of antibiotics has been a turning point in modern medicine, having saved countless lives. Antibiotics kill bacteria through different mechanisms; however, shortly after resistance to antibiotics started to emerge. Bacteria are able to acquire resistance to antibiotics via target alteration, efflux pump and enzymatic modification. Disinfectants are used to sterilize an environment and control the spread of dangerous pathogens. Nonetheless, the misuse of disinfectants can promote the rise of resistance via target alteration, impermeability and efflux pumps. These resistant strains may also be co-resistant to antibiotics and this superbug may be untreatable. It is evident that more research needs to be devoted to understanding if and how resistance emerges towards disinfectants and how it can be combatted.

Introduction
The Centre for Disease Control and Prevention (CDC) states that at least 2 million people are infected by resistant bacteria and 23,000 people die from these infections annually[1]. The breakthrough discovery of antibiotics is one of the turning points for modern medicine. Discovered in 1928, penicillin saved numerous lives however, only a few years later resistant strains started to emerge[2]. Resistance occurs when some bacteria are unsusceptible to antibiotics and are able proliferate in the presence of the antibiotic, leading to a rise in a resistant population (Figure 1).

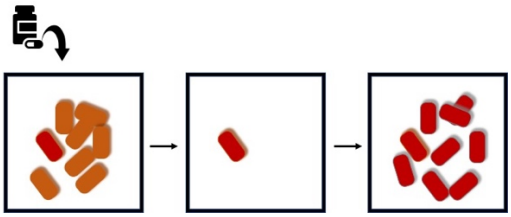

Figure 1. *How antibiotic resistance arises.* The antibiotic is given to a population of bacteria with a few that has resistance. The susceptible population is killed while the resistant one is unaffected. The resistant population is now able to proliferate.

Antibiotic resistance arises via many different mechanisms including target alteration, efflux pumps and enzymatic modification. Sterilization using disinfectants is of utmost importance to mitigate the spread of disease. However, when improperly used, this double-edged sword can lead to resistant strains. Bacteria may also confer resistance to disinfectants through different mechanisms including target alteration, impermeability and efflux pumps. Moreover, bacteria are able to produce biofilms which prevents the disinfectant from eradicating the microorganisms. The resistance that emerges due to the improper use of disinfectants may transfer as cross-resistance to antibiotics, making it a bigger concern[3]. These superbugs that do not respond to disinfectants which have many targets on the microorganism, may not respond to antibiotics with few targets. This harsh reality makes prior cleaning a necessity for appropriate sterilization as well as following the correct procedures when using the disinfectants. Time and money must be invested into research for new and improved antibiotics as well as for researching whether disinfectants are a large concern for the development of resistance.

Antibiotic Resistance
A report by de Kraker *et al.* (2016) states that antimicrobial resistance can kill up to 10 million people and cost $100 trillion annually by the year 2050 (Figure 2)[4].

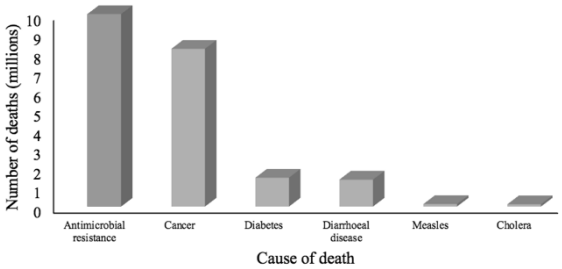

Figure 2. *The estimated number of deaths by 2050 caused by different diseases.* It is estimated that by the year 2050, antimicrobial resistant pathogens are going to be the main cause of death, killing 10 million annually. Following this is cancer at 8.2 million annually[4]. Adapted from Review on Antimicrobial Resistance.

Antimicrobials are compounds that inhibit the growth of or destroy harmful microorganisms without damaging the host. They are used to treat infections during complex surgeries such as organ transplants[5]. In addition, antibiotics are used in agriculture to promote growth and prevent infections in animals[5]. From their discovery, antibiotics have made significant contributions to modern medicine and have saved countless lives[5]. However, resistance to antibiotics is emerging at an alarming rate and is a source of growing burden on the healthcare system. Microorganisms continue to evolve and become unresponsive towards current interventions, through a variety of different mechanisms (Figure 3).

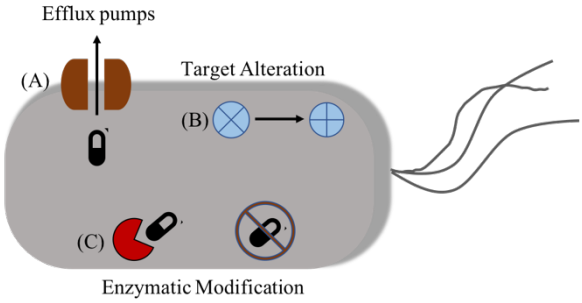

Figure 3. *The different mechanisms of resistance to antibiotics.* (A) Bacteria can pump the antibiotic to the external environment via efflux pumps making antibiotics ineffective. (B) They can have an altered target resulting in the antibiotic not being able to exert its effect on the target. (C) Bacteria possess enzymes that are capable of inactivating the antibiotic.

Additionally, there is a shortage of novel antibiotics entering the market and there are several reasons for this. Due to the fact that antibiotics are taken for about two weeks and are relatively cheap compared to other drugs, pharmaceutical companies have little incentive to research and develop new antibiotics. Their return on investment is simply too low[6]. Furthermore, there is an initiative to decrease the amount of antibiotics prescribed since its overuse may lead to resistance. This impacts sales and consequently research towards finding new antibiotics decreases as companies focus on more profitable drugs[6]. In addition to creating new antibiotics, molecular modifications of current antibiotics could also be useful. For example, Dale Boger and his team at The Scripps Research Institute were able to chemically modify vancomycin and improve its antimicrobial potency against resistant strains[7]. Nonetheless, the startling rise in resistant microorganisms cannot be ignored and makes research towards finding new and improved antimicrobials vital.

Mechanisms of Antibiotic Resistance

Antibiotics are able to induce cell death via four main targets: DNA, RNA, the cell wall and proteins[8]. Target alteration is a mechanism that bacteria use to develop resistance through alteration of an area normally targeted by the antibiotic. For example, bacteria resistant to fluoroquinolones have been reported to have altered DNA gyrase and topoisomerases that the antibiotic cannot target, allowing the bacteria to proliferate[9]. Another mechanism that they use are efflux pumps that transport toxic molecules out of the bacteria[10]. Efflux pumps are able to transport antibiotics to the external environment, protecting the bacteria from death and they confer medium to high level resistance to tetracyclines, macrolides and fluoroquinolones[11]. Finally, enzymatic modification is another mechanism of antibiotic resistance and occurs via two main pathways; hydrolysis or group transfer[12]. Many of these drugs possess bonds that are essential to their activity and bacteria

have evolved enzymes that can destroy these bonds and consequently halt its activity. Hydrolysis is a main resistance pathway against β-lactams due to β-lactamases which are enzymes that hydrolyze the β-lactam ring making them ineffective at destroying microorganisms[13]. Furthermore, group transferases are enzymes that covalently alter the antibiotic and weaken its activity[12]. Acetyltransferases modify hydroxyl or amine groups found on antibiotics and a well classified acetyltransferase is chloramphenicol acetyltransferases[12]. These enzymes possess active sites that deprotonate the nucleophilic hydroxyl group on the antibiotic, inactivating it[12]. It is also important to note that resistance can develop as a result of a combination of the above mechanisms.

Biocide Resistance

Biocides are compounds that include disinfectants, antiseptics and preservatives and its main purpose is to sterilize the area of concern and prevent microbial growth[14]. This review will focus mostly on disinfectants and how misuse and overuse could possibly result in selective pressure and consequently resistance. Disinfectants are classified into three levels: high, intermediate and low-level[2]. High level disinfectants are able to kill all microorganisms except a high number of bacterial spores and examples include hydrogen peroxide and glutaraldehyde. Intermediate level is effective against vegetative bacteria, mycobacteria, most viruses and fungi but not bacterial spores and examples include alcohol and hypochlorite. Finally, low-level disinfectants such as phenolics cannot destroy mycobacteria or spores[2]. Disinfectants are often advertised as an essential safety measure for homes but there is no research to support these claims[14]. In addition, these misleading advertisements encourages the overuse of disinfectants resulting in selective pressure and possible resistance. Sterilizing instruments in healthcare environments are an essential component of ensuring that infections do not spread which is why disinfectants are of utmost importance. However, when these areas are not properly sterilized, or the microorganisms confer resistance by other means, resistant bacteria can spread at a very fast rate. It is clear that disinfectants are crucial for stopping the spread of diseases however, while doing so, we may be selecting for superbugs and the spread of these superbugs are more concerning. Researching the means of resistance against disinfectants can help better understand their mechanism of action and how resistance can be combatted.

Mechanisms of Biocide Resistance

Target Alteration

Disinfectants target multiple aspects of a microorganism making the rise of resistance less common when compared to antibiotics which have one or few targets[15]. However, several outbreaks of resistant bacteria have been reported related to disinfectant use. Target alteration is a mechanism that bacteria use to confer resistance to biocides and occurs when the normal target is distorted, resulting in the biocide being unable to exert its full effect. For example, triclosan is an antimicrobial chemical found in many household items such as soaps and lotions[16] Triclosan exerts its effect by blocking the active site of enoyl reductase, an essential enzyme involved in fatty acid synthesis. Bacteria undergo fatty acid synthesis via the type II fatty acid biosynthetic system[17]. Without fatty acids, the bacteria are unable to build its cell membrane or reproduce leading to cell death. Since this enzyme is absent in humans, it makes it an attractive target for antimicrobial agents. An example of resistance to triclosan has been reported in *Escherichia coli (E. coli)* and emerges due to a mutation in the *fabI* gene. The gene *fabI* encodes for enoyl reductase and a point mutation at codon 93 substitutes a glycine to a valine. Enoyl reductase catalyzes the last step in each cycle of elongation of the type II fatty acid biosynthetic system. Glycine at position 93 is a part of the binding groove and a valine substitution alters the binding groove making triclosan unable to bind[17,18]. This mutation makes the bacteria 300-fold more resistant to triclosan

than wild-type bacteria[19].

Impermeability

Bacteria reduce the ability of the biocide to collect inside by preventing the entry and this is another mechanism of resistance. Gram-negative bacteria have a higher level of resistance than Gram-positive bacteria due to its outer membrane composition. The outer membrane contains liposaccharides which makes it more impermeable to biocides and accordingly, destroying the outer membrane makes it more susceptible[15,20]. For example, as a result of its outer membrane composition, *P. aeruginosa* is less susceptible to quaternary ammonium compounds (QACs) and chlorhexidine diacetate (CHA)[21]. The outer membrane of *P. aeruginosa* is a significant barrier for large molecules and it slows the rate of entry of small hydrophilic molecules making *P. aeruginosa* less susceptible to antibiotics such as β-lactams and quinolones. These antibiotics cross the cell membrane via porins, mainly oprF, that usually form trimers[22]. Loss of porins has been associated with antibiotic resistance and it is hypothesized that it will also lead to biocide resistance since it may prevent or slow the entry of biocides[22]. Addtionally, the outer membrane hydrophobicity is important, as the mycobacterial cell wall is hydrophobic and highly complex which makes it unsusceptible to many biocides[20].

Efflux Pumps

Transporting the biocide out of the microorganism is another mode of conferring resistance and is accomplished by efflux pumps. Efflux pumps, as opposed to the other mechanisms of resistance, is an active method of inactivating the biocide and do so by pumping the biocide out of the cell. While some are specific, others possess a wide range of substrate specificity meaning that they can affect a wide range of biocides. These are called multidrug transporters and are divided into two categories: secondary multidrug transporters and ATP-binding cassette (ABC)[23]. Secondary multidrug transporters use the gradient of protons or sodium ions to pump the toxic molecule out. While protons or sodium ions enter, the toxic molecule exits. Likewise, ABCs use ATP hydrolysis to drive the extrusion of the biocide[23]. For example, methicillin-resistant *S. aureus* is reported to have less susceptibility to the disinfectant chlorhexidine gluconate due to the efflux pumps encoded by *qacA, qacB and qacC* from the major facilitator superfamily[24]. The pump encoded by *qacA* confers resistance to biguanides such as chlorhexidine and diamidine such as pentamidine[25]. The pump encoded by *qacB* is different from *qacA* only by one amino acid at position 323 and has less resistance to biguanides and diamidine[25]. Finally, the pump encoded by *qacC* is accountable for resistance to quaternary ammonium compounds[25].

Biofilms

Biofilms may also promote the development of resistance. Biofilm is a layer of protein and polysaccharide produced by the bacteria and is a film that acts as shelter. The National Institute of Health states that about 65% of all infections are caused by biofilm formation[26]. They are commonly found on medical devices and tissue and are a major source of concern for diseases such as periodontitis, osteomyelitis and cystic fibrosis[27]. However, we will direct our attention to biofilms found on medical devices since this review is focused on disinfectants. For example, biofilms can form on central venous catheters either on the external surface or the lumen where Gram-negative bacteria can grow in intravenous fluids[27]. It has been shown that bacteria in biofilms are 10 to 100 fold more resistant to disinfectants than suspended bacteria[28]. There are a couple of mechanisms to explain resistance due to biofilm formation. First, due to this physiological protection, the biocide may be unable to penetrate the layers and reach the bacteria[29,30]. It was previously shown that chlorine was unable to reach more than 20% of a mixture of *Klebsiella pneumoniae* and *P. aeruginosa* biofilm, making it an ineffective disinfectant for biofilms[31]. Furthermore, when bacteria are starved, they enter a slow growth phase which is

associated with increased resistance. Biofilms are composed of slow growing bacteria and this may be the reason for higher resistance to biocides[31]. Additionally, biofilm environment is different for every strain since the surroundings change for each cell. This is known as heterogeneity which can result in varying responses to disinfectants and the rise of possible resistance[31]. Finally, bacteria in biofilms are in a high-density environment which activates the general stress response. RpoS is a specialized sigma factor that is expressed when the cell is undergoing stress. It was shown that RpoS is expressed by bacteria in cystic fibrosis patients with chronic *P. aeruginosa* biofilm infections which may be contributing to the increased resistance. RpoS activates genes required for the cell to sustain growth during the stationary phase and since they have also been observed in biofilms, it may be mediating protection against biocides[31,32]. It is evident that new measures need to be taken in order to eradicate the formation of biofilms on medical devices. If left untreated, these bacteria can proliferate and cause epidemics while being resistant to current interventions.

Possible Cross-Resistance

The misuse of disinfectants in households and in healthcare settings raises the question of whether this resistance will translate over to antibiotic resistance. It is an alarming reality that if a microorganism develops insusceptibility or resistance to a certain disinfectant, it may also be unresponsive towards antibiotics[3]. Cross-resistance occurs when the biocides induce cell death via the same pathway or target and since disinfectants have many targets, occurrence of cross-resistance to antibiotics is possible[3]. Moken *et al.* (1997) observed that pine oil, which is used as a disinfectant, may be selecting for *E. coli* that overexpresses the MarA protein and confers resistance prompted by certain antibiotics[33,34]. The MarA protein is a transcriptional activator of antibiotic and superoxide resistance promoters and provides *E. coli* with resistance to some antibiotics as well as superoxide-generating reagents[35,36]. In addition, they were able to show that low levels of cross-resistance does indeed occur[20]. Another example of cross resistance occurs when bacteria are exposed to hydrogen peroxide and hypochlorous acid. They undergo oxidative stress and turn on its oxyR radical defense systems. OxyR is involved in the expression of efflux pumps and detoxifying enzymes. The outcome is bacteria that are resistant to both hydrogen peroxide and hypochlorous acid as well as some antibiotics[3]. Furthermore, the inappropriate use of disinfectants may be promoting cross-resistance. The concentration of disinfectant, the contact time as well as the frequency of application is very important for proper sterilization. If sub-inhibitory concentrations are used, it will exert a selective pressure on the microorganism leading to the activation of stress responses. The end result of this would be a change in gene expression which will lead to superbugs that are resistant to disinfectants, while being unresponsive to antibiotics[37]. Finally, the biofilms that form as a result of improper disinfectant use may be creating bacteria that cannot be treated via antibiotics if infection in humans does occur[37].

A Step in Avoiding Resistance: Prior-Cleaning

A critical barrier that affects the efficiency of disinfectants is biofilm formation and other dirty materials because it hinders the biocide's ability to reach the microorganism. This fact makes prior cleaning crucial for proper sterilization[2]. Prior cleaning is the mechanical removal of inorganic or organic materials on a surface before the application of a disinfectant. For example, many medical devices such as surgical instruments require proper sterilization to avoid contamination and disease transmission. Surgical instruments are presoaked or rinsed before disinfection as prior cleaning. Disinfectants are unable to exert their full lethal effect when these inorganic or organic compounds are blocking it from reaching the bacteria and when they are exposed, the disinfectants are more effective. Since the disinfectant will be applied at full inhibitory concentration, as opposed to sub-inhibitory concentration, the chances of a

selective pressure driving resistance will also be lower[2]. Other measures that could decreases chances of resistance include using FDA-approved disinfectants at the proper concentration for the right amount of time and following the proper procedure for preparing the solutions[2]. Finally, it is crucial to avoid diluting the disinfectant too much as this may lower its efficacy[2].

Conclusion

Antibiotic resistance and its mechanisms have been extensively studied and there is a plethora of evidence for the alarming rise of resistance. The ability of bacteria to adapt to its changing environment and still sustain growth is the basis of this resistance. It is evident that action must be taken to avoid a disaster. Unfortunately, not enough antibiotics are entering the market and the current ones are becoming ineffective. Furthermore, while disinfectants are crucial for sterilization, they may also be selecting for resistant strains and therefore contributing to the rise of resistant strains. Disinfectants promote resistance in bacteria via target alteration by the microorganism, efflux pumps which pump the disinfectant out of the cell and impermeability of the bacterial cell wall. Biofilms are another concern for the emergence of resistance that should be taken into consideration.

It is evident that more research needs to be devoted to defining resistance to biocides as well as a better understanding of its mechanisms. Additionally, if biocides are properly used, the emergence of resistance can be also be avoided. Before biocides are marketed, a comprehensive study should be completed to determine if and how microorganisms confer resistance so that a better protocol for its use can be developed. There are still many questions left to be answered in determining if biocides are a real danger for our health. For example, can biocide resistance truly transfer as antibiotic resistance and what is the mechanism of this cross resistance? Understanding biocide resistance is key to controlling superbugs before they cause a catastrophe.

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