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Review

Microorganism' response to disinfectants: A review

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Abstract

Hospital-acquired infections (HAIs) remain a pressing global health issue, contributing significantly to patient morbidity and mortality while imposing severe economic and logistical pressures on healthcare systems. These infections are often caused by multidrug-resistant (MDR) pathogens, complicating both prevention and treatment. Surface disinfectants are critical in mitigating pathogen transmission and curbing antimicrobial resistance within healthcare environments. However, the increasing prevalence of biocide resistance among pathogens necessitates a deeper understanding of the complex mechanisms that counteract the effects of disinfectants. This review explores the various responses of microorganisms to disinfectants and delves into current known bacterial strategies that overcome disinfectants activity. Understanting these microbial responses is essential for the development of more effective disinfection protocols in order to ensure an efficient control of hospital-acquired infections.

Keywords hospital-acquired infections, resistance, disinfectants, biocide, cross-resistance

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Introduction

Disinfectants are a class of chemical biocides with diverse structures and mechanisms of action, used in various settings, such as food processing, animal husbandry, and healthcare, to prevent and control the transmission of pathogens via contaminated environmental surfaces [1]. Resistance to antimicrobial agents in hospitals has increased steadily over the past decade. For example, the excessive usage of disinfectants containing quaternary ammonium compounds (QACs) in healthcare facilities during COVID-19 pandemic, increased the concern that bacteria can develop resistance to QACs or even contribute to antibiotic resistance [2].

However, the terminology used in the literature for defining the microbial adaptation to biocides remains unclear. Microorganisms that exhibit an increased Minimum Inhibitory Concentration (MIC) to a biocide have been described as "resistant" although they are still killed by the biocide at in-use recommended concentration. Terms like "reduced susceptibility" or "increased tolerance" are considered more accurate [3, 4, 5]. Bacterial responses to chemical biocides depend on several factors, including the biocide's chemical structure, the microorganism type, and several external factors such as pH, temperature, organic matter, concentration, exposure time and surface type [6, 7].

The emergence of increased bacterial tolerance to disinfectants is a significant challenge to life safety, health and the rational management of resources, due to the low efficiency of disinfectants often resulting from excessive and inappropriate use of disinfectants [8, 9]. In order to prevent the adaptation of microorganisms to the use of disinfectants, it is essential to precisely identify the mechanisms underlying their resistance [10].

Over the last decade, significant progress has been made for a better understanding of the responses of different types of bacteria to antimicrobial agents, and especially biocides. Various methods have been used for the purpose of studying tolerance and resistance to disinfectants, including evaluation of bacteria recovered either from surfaces which were disinfected previously, or directly from contaminated disinfectants [2, 11]. However, the most common method to study the adaptive behavior is to expose the bacteria in vitro to sublethal concentrations of disinfectants [12, 13, 14].

The mechanisms behind increased tolerance to disinfectants are various and may arise from bacterial genotypic and phenotypic adaptation [8]. Increasingly conclusive evidence from in vitro studies has demonstrated that bacteria have a remarkable ability to respond to chemical stress caused by biocides, through a variety of mechanisms, intrinsic or acquired, including the expression of efflux systems, reduces permeability of the cell wall, enzymatic degradation and the development of biofilms [8, 15, 16].

The mode of action of disinfectants is another essential factor involved in bacterial susceptibility to disinfectants. Biocides present a wider spectrum of activity, having various targets, unlike antibiotics which tend towards certain intracellular targets. However, regarding biocides that have a specific antimicrobial action mechanism (e.g., quaternary ammonium compounds) the development of antimicrobial resistance to disinfectants as well as cross-resistance to antibiotics is well documented [8, 18, 19, 20].

Natural tolerance

Natural tolerance, or intrinsic tolerance to disinfectants, can be genetically encoded within the species and can determine the fundamental spectrum of the effects of an antimicrobial compound, as well as phenotypic tolerance. Those mechanisms are particularly evident in Gram-negative bacteria, bacterial spores and mycobacteria [8]. Opposite of that,

Table 1. Disinfectants, their cellular targets and mechanisms of action (adapted after Basiry et al., 2022)

Biocide	Cellular target	Mechanism of action
Alcohols	Membrane and cytoplasmic proteins	Cell membrane alterations, enzyme inhibition, coagulation of cell components.
Aldehydes	Cell wall, outer membrane	Denaturation of nucleic acids and various proteins.
Anilides	Lipid biosynthesis, cell membrane	Inhibition of enoyl-(acyl scavenging protein) reductase
Biguanides	Cell wall, cell membrane	Damage to the integrity of cell membrane
Chelating agents	Cell membrane	Loss of membrane integrity and protein coagulation
halophenols	Lipid biosynthesis, cell membrane	Inhibition of enoyl-(acyl carrier protein) reductase
Heavy metals derivatives	The thiol groups of proteins, the nitrogenous bases of nucleic acids	Cytological changes and potassium losses
Phenols and cresols	Cell membrane and proteins	Loss of membrane integrity and protein coagulation
Peroxigens	The thyol groups of proteins	Oxidation to disulphides causing protein inhibition and structural modifications
Quaternary ammonium compounds	Lipids from the structure of the cell membrane and cell wall	Loss of celullar components as a result of cell membrane damage

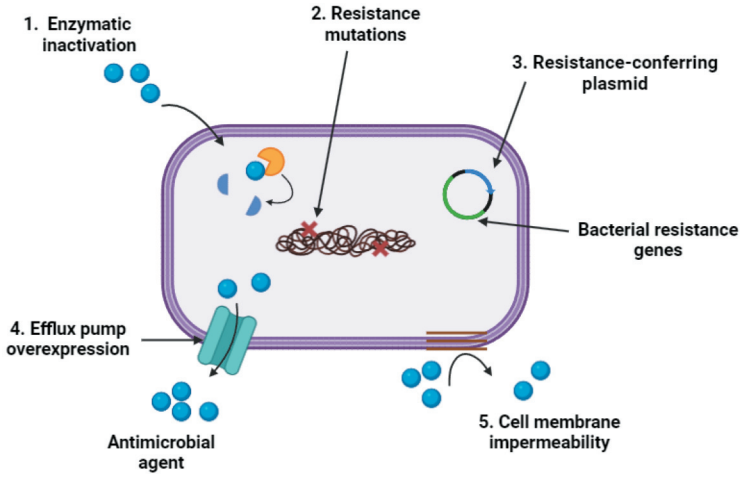

Fig. 1. Bacterial mechanisms of resistance to antimicrobial agents (adapted after De Gaetano et al., 2023)

Gram-positive bacteria lack an outer membrane, making it more difficult for biocides to reach their target site [2].

The impermeability of the cell membrane

To ensure the effectiveness of a disinfectant product, it is necessary for it to cross the outer layers of the microorganism's cell. The composition and nature of these outer coatings can vary depending on the microorganism type and can directly impact on the effectiveness of disinfectants.

Gram-negative bacteria are often more tolerant to antimicrobial substances than Gram-positive bacteria, mostly due to the structure of the outer membrane [22, 23]. In Gram-negative bacteria, the outer membrane has an asymmetric structure of lipopolysaccharides causing a lower permeability of the membrane. In contrast, Gram-positive bacteria present a cell wall mainly made up of a thick layer of peptidoglycans, teichoic and lipoteichoic acids, which are less effective in preventing disinfectant action [23, 24]. In mycobacteria, increased tolerance to disinfectants was observed, most likely due to the complex structure of the cell walls comprised of peptidoglycan layers and hydrophobic mycolic acid, interconnected by arabinogalactan, which constitute an effective barrier and prevent the penetration of antimicrobial agents into the cell [25].

Bacterial spore production

The bacterial ability to form spores is a key mechanism of natural resistance. Numerous bacteria including, *Bacillus spp.* and *Clostridium spp.*, have the capability to form spores in response to unfavorable conditions, such as high temperatures or exposure to chemical agents [26, 27].

The structure of a typical bacterial spore is complex, consisting in a spore protoplast, spore cortex, outer spore coats (intine and exine), exospore and spore appendages. The spore coats act as a physical barrier, and the resistance become evident during the sporulation [27, 28]. Studies have shown that bacterial spores from the genera *Bacillus* and *Clostridium* show a very high resistance, unlike spores from other bacterial species [29, 30, 31, 32]. The tolerance of bacterial spores to disinfectants is corelated with both the outer coats and the spore cortex [33].

The activity of efflux pumps

The activity of efflux pumps plays an important role in bacterial resistance [16]. Efflux pumps are transmembrane proteins that can perform important physiological functions and have the ability to expel harmful molecules, thus conferring resistance to various compounds, including disinfectants and other antimicrobial substances [16, 34]. Efflux pumps encoded by plasmid genes represent key mechanisms of resistance to metals, antibiotics, disinfectants and antiseptics, such as chlorhexidine, CCA, acridines and diamidines [22, 35, 36].

A study by Rozman et al. (2021) further indicates the increased activity of efflux pumps can decrease the efficiency of various disinfectants, including hydrogen peroxide, iodinated compounds, quaternary ammonium compounds, benzalkonium chloride and triclosan. Wand and Sutton (2022) also mention that efflux pumps associated with biocide tolerance often include pumps such as AcrAB-TolC that tolerated benzalkonium-chloride or chlorhexidine (found in *Escherichia coli* and *Klebsiella pneumoniae*) or MexCD-OprJ that tolerates chlorhexidine (in *Pseudomonas aeruginosa*).

Mechanisms of phenotypic adaptation

Bacterial biofilms represent a crucial survival mechanism and are ubiquitous in the natural environment [38]. A biofilm represents a complex microbial community consisting of cells irreversibly attached to an interface, to a substrate or to each other, embedded in a matrix of extracellular polymeric substances (polysaccharides, proteins, eDNA [38, 39].

In the clinical setting, biofilms often lead to chronic healthcare-associated infections, being involved in 70% of

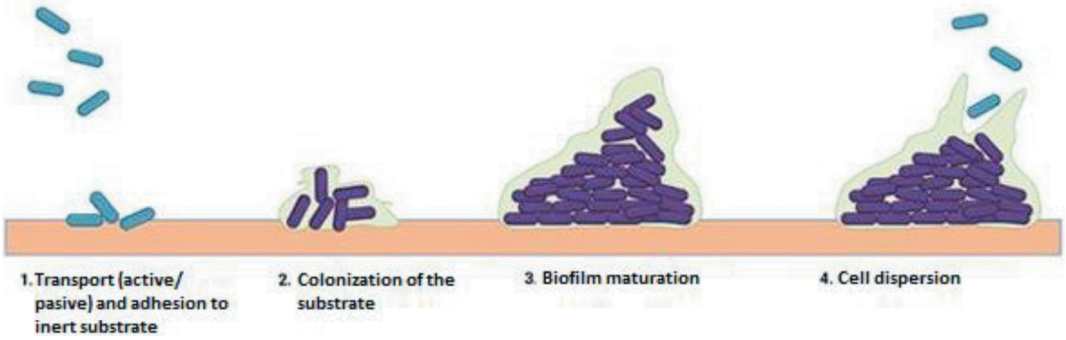

Fig. 2. Stages of biofilm development: 1. Bacterial celss reversibly adhere to an inert substrate; 2. Irreversible attachement of the bacterial cells to the substrate through the synthesis of exopolysaccharides and the formation of microcolonies; 3. The maturation of biofilm architecture; 4. The rupture of the biofilm and the dispersion of bacterial cells (adapted from Ma et al., 2022)

infections caused by microorganisms [40, 41, 42]. The development of the three-dimensional structure of the biofilm includes distinct stages like adsorption, adhesion, formation of microcolonies, maturation and dispersion [43].

Biofilms can develop on various surfaces such as medical implants like cardiovascular implants, gastrointestinal implants, orthopedic implants, urological implants, intravascular devices, dental implants, contact lenses and even breast implants [45]. These infections are often treatable only by removing the implant, which increases patient trauma and treatment costs. Most nosocomial infections are associated with bacterial species that form resistant biofilms, such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [46, 47].

Li et al. (2021) highlights that multispecies biofilms show a higher resistance to disinfectants compared to single-species biofilms. Disinfectants such as chlorine, QACs, peracetic acid or hydrogen peroxide are less effective most likely due to complex microbial interactions. However, the mechanisms of tolerance are not fully understood. In order to develop new effective control strategies, it is essential to have a better knowledge of the bacterial biofilm, which will contribute more effective to patient management approaches [41].

Mechanisms of acquired tolerance

Acquired tolerance is achieved through mutations or the integration of mobile genetic elements, such as horizontal genetic transfer, transferable plasmids and other cellular components. The acquired resistance is genetically determined and usually stable, unlike phenotypic adaptation, which is reversible once the exposure to biocides ends [8, 16].

The bacterial susceptibility to disinfectants can be triggered by changes in the outer membrane of proteins and the high expression of cellular structures. For example, lipopolysaccharides is associated with the impermeability of the external membrane appears, which facilitates the penetration of disinfectants, changes the size and expression of the pores, thus preventing entry and decreasing sensitivity [8]. Zhang et al. (2019) showed evidence that prolonged exposure of *Klebsiella pneumoniae* to chlorhexidine led to acquired resistance, which might had an effect on bacterial growth as well.

Chromosomal mutations

The interactions between different mobile genetic elements have favored the rapid development of bacteria that are tolerant to numerous biocides. Rozman et al., (2021) highlights the bacterial tolerance to various disinfectants, including triclosan, peracetic acid, hydrogen peroxide, alcohols, glutaraldehyde and formaldehyde, chlorhexidine and QACs. This tolerance was associated with mutations at the genetic level.

The highest tolerance was observed in case of triclosan was associated with mutations in fabl, marR, ycjD (in *Escherichia coli*), fabV (in *Pseudomonas aeruginosa*), and sa-Fabl (in *Staphylococcus aureus*), as well as for chlorhexidine with mutations in efrA, efrB (in Enterococcus), qacA, qacB, smr, norA (in *Staphylococcus aureus*) [8, 50].

The role of plasmids in bacterial resistance

Plasmids have been studied for their ability to encode disinfectants tolerance, and while they were not normally responsible for increased levels of disinfectant tolerance observed in different species or strains, several studies associated the presence of plasmids in bacteria to high tolerance of disinfectants [7, 16, 51].

Eight different pSK1-like plasmids were identified on *methicillin-resistant Staphylococcus aureus* (MRSA) alone [52], while *Staphylococcus spp* usually have multiple plasmids that contribute to resistance to antimicrobials such as antibiotics, disinfectants or heavy metals [16].

Mechanisms of cross-resistance

Cross-resistance occurs when a microorganism develops resistance to a certain antimicrobial substance but also be-

Table 2. Biocide and antibiotic cross-resistance in various bacterial species of interest

Microorganism	Cross-resistance between biocides and antibiotics	Mechanism of cross-resistance	References
Escherichia coli	QACs- ceftazidime, cexotaxime; Triclosan- chloramphenicol, nitrofurantonin, ciprofloxacin; PHMB- trimethoprim-sulfamethoxazole, ciprofloxacin.	Enhanced efflux system (AcrAB efflux pump)	[55, 56].
Pseudomonas aeruginosa	Triclosan- ciprofloxacin; Benzalkonium chloride-ciprofloxacin, fluoroquinolones; Chlorhexidine- cefepime, meropenem.	Mutation in nfxG gene and in gyrA, activity of efflux pumps (MexED-OprN)	[3, 55, 57, 58, 59].
Staphylococcus aureus	Triclosan- ciprofloxacin Chlorhexidine; Benzalkonium chloride-norfloxacin, ciprofloxacin; Chlorexidine-digluconate-gentamicin, penicillin, tetracycline.	Alteration in cell membrane structure and function	[55, 57, 60].
Mycobacterium sp.	Triclosan- isoniazid.	InhA mutations	[18, 55].
Salmonella sp.	Triclosan-chloramphenicol, erythromycin, imipenem, tetracycline; Chlorine- tetracycline, ciprofloxacin, florfenicol.	Active efflux pumps	[55, 59].
Citrobacter freundii	Triclosan-erythromycin.	Outer membrane adaptation	[55].
Campylobacter jejuni	Triclosan-erythromycin, ciprofloxacin.	Efflux pumps	[55].
Listeria monocytogenes	Heavy metals- macrolides, cefotaxime; Cationic Quaternary Ammonium Compounds- ciprofloxacin, kanamicin, novobiocin, penicilin.	Mutations in fepR	[57, 61].

comes resistant to other antimicrobial substances. For example, a microorganism that is resistant to a certain class of antibiotics may become tolerant to certain disinfectants or biocides, and vice versa. Cross-resistance between antibiotics and disinfectants resistance mechanisms may be associated with bacterial adaptation to adverse environmental conditions, such as changes in membrane permeability, efflux pumps or biofilm formation [21].

Numerous studies highlight this phenomenon in countless examples. Coombs et al., (2023) investigated this phenomenon with the hypothesis that exposure to subinhibitory levels of biocides may induce antibiotic resistance. While some of the studies found that bacteria can adapt to biocides and also develop antibiotic resistance, like in the case of QACs, chlorhexidine or triclosan, other studies found that biocides such as alcohols, azoles or iodine were not directly associated with cross-resistance [18, 53, 54].

The mechanism of cross-resistance was also observed in various species including *Salmonella enterica, Escherichia coli, Bacillus subtilis* and *Mycobacterium chelonae* [7, 20]. The expression of multidrug efflux pumps has been considered as a major mechanism responsible for cross-resistance in many studies [18, 19].

Conclusion

The persistent challenge of bacterial resistance to antimicrobial agents, especially disinfectants, highlights how complex the bacterial survival mechanisms are. The persistence of bacteria in different environments is attributed to both natural and acquired resistance. When it comes to disinfectants, natural tolerance mechanisms such as cell membrane impermeability, spore formation, or efflux pumps activity have an essential role in bacterial survival. Additionally, phenotypic adaptation enables bacteria to be more tolerant to disinfectants, particularly through biofilm formation. Chromosomal mutations and the transfer of resistance genes via plasmids, cause a rapid development of multidrug-resistant strains, further complicating treatment strategies and infections control. Furthermore, the occurrence of cross-resistance highlights the interdependence of resistance to various antimicrobial agents, where resistance to one class of antimicrobial resistance may confer resistance to others.

To develop effective disinfection strategies, it is crucial to first understand the diverse resistance mechanisms. More research is needed when it comes to genetic and molecular bases of disinfectant tolerance, as well as what role environmental factors play in the selection of resistant strains. However, mitigating the impact of bacterial resistance on public health require continuous research and innovative approaches and strategies that may offer a counterattack for the emerging threat of antimicrobial resistance.

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