Non-antibiotic biocides: An updated review

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Non-antibiotic biocides are composed of heterogeneous groups of natural and synthetic substances which can deter, render harmless, or exert a controlling effect on microorganisms by biological or chemical means. Examples include disinfectants, preservatives, antiseptics, pesticides, herbicides, fungicides and insecticides. Use of biocides has wide applications in medicine, agriculture, forestry, and industry, but is generally limited to the external use due to their toxic characteristics to human cells.

This chapter will focus on updated revision on the classification of biocides, their mechanism of action, their current and future applications with emphasis on their clinical use, and development of antimicrobial resistance.

Keywords Biocides, classification, clinical application, antimicrobial resistance.

1. Definitions and classification of biocides

Non-antibiotic biocides are composed of heterogeneous groups of natural and synthetic substances which can deter, render harmless, or exert a controlling effect on microorganisms by biological or chemical means. Examples include disinfectants, preservatives, antiseptics, pesticides, herbicides, fungicides and insecticides.

Biocides could be classified according to their chemical structure or according to their clinical and non-clinical applications [1]. Within the Biocidal Products Directive 98/8/EC (BPD), which was adopted by the European Parliament in 1998, the classification of biocides, is broken down into 4 main groups (Disinfectants, Preservatives, Pest control, and others), and 23 product types according to their application categories, with several comprising multiple subgroups [1]. In this mini-review, the chemical structure is adopted for the classification of the biocides, focusing on the main groups of biocides that manage to establish applications with brief overview about their spectrum, mechanisms of action and clinical applications.

2. Main groups of biocides

2.1. Alcohols

Various alcohols have shown effective antimicrobial features including ethyl alcohol (ethanol), methyl alcohol (methanol), isopropyl alcohol (isopropanol), n-propanol, benzyl alcohol, phenylethanol (phenylethyl alcohol), pronopol[13] (2-bromo-2-nitro-1,3-diol), phenoxyethanol (phenoxetol), chlorbutanol (chlorbutol), and 2,4-dichlorobenzyl alcohol. However, the first three compounds are the most widely used as biocides [2]. Generally, the antimicrobial activity of alcohols is optimal in concentration of 60-95% range. Within this range, alcohols exhibit rapid broad-spectrum against vegetative bacteria (including *Mycobacteria spp.*), viruses, and fungi. Although they are not sporicidal, alcohols are known to have reversible features of inhibiting sporulation and spore germination. Therefore, alcohols are not recommended for sterilization but are still widely used for both hard-surface disinfection and skin antisepsis. Lower concentrations may also be used as preservatives in pharmaceutical and cosmetic products and to potentiate the activity of other biocides or other means of sterilization. Recent studies have shown that ethanol of low concentrations significantly enhances the sterilization effects of high-pressure thermal sterilization, which is used in keeping the qualities of foods [3]. To overcome their disadvantage of rapid evaporation, many commercially-available alcohol products include other biocides, such as chlorhexidine, which remain on the skin or surgical devices [4], or excipients which decrease the evaporation time and therefore increase the alcohol efficacy. Exact mechanism of action for alcohols as biocides is still to be known, but due to their increased efficacy in the presence of water, it is generally believed that they cause membrane damage, and therefore, inducing inhibition of cell wall synthesis, and rapid denaturation of proteins and inhibition of DNA and RNA synthesis, with subsequent interference with metabolism and cell lysis [2].

2.2. Aldehydes

Various aldehydes are currently in use as biocides including mono- and di-aldehydes such as formaldehyde, glutaraldehyde, formaldehyde-releasing agents and *ortho*-Phthalaldehyde.

2.2.1. Formaldehyde

Formaldehyde, the simplest aldehyde, is generally used in its aqueous solution or in combination with low-temperature steam as a disinfectant and sterilant. Formaldehyde has bactericidal, sporicidal, and virucidal properties, but it works more slowly than other aldehydes [5]. Formaldehyde solutions are topically applied in medicine, particularly in the treatment of warts. It is also used to inactivate bacterial products in the process of toxoid vaccines production. Formaldehyde has interactive, and cross-linking properties which is considered to play a considerable role in its mechanism of action. It is an extremely reactive chemical that interacts with protein, DNA and RNA in vitro. There are various formaldehyde-releasing agents that are claimed to be microbicidal on account of the release of formaldehyde and have found applications in the treatment of peritonitis [2]. Methenamine mandelate, a derivative of formaldehyde of a special interest, since it decomposes at an acidic pH to form formaldehyde and ammonia and was often used in treatment of urinary tract infection [6]. However, due to the carcinogenic effect of formaldehyde, this use has been minimized. Taurolidine has been investigated for the prevention of central venous catheter-related infections. While there is positive *in vivo* and *in vitro* evidence supporting such an application, it seems that the current evidence is "insufficient to warrant routine use of taurolidine [7].

2.2.2. Glutaraldehyde

Glutaraldehyde is widely used as a disinfectant at low temperatures and sterilant of endoscopes and surgical equipments. It has a wide spectrum of activity against bacteria and their spores, fungi, and viruses. Low concentrations inhibit germination while high concentrations are sporicidal [5]. The mechanism of action of glutaraldehyde involves a strong cross-linking of proteins in cell envelope, mainly in the outer layers, and a possible additional effect on the proteins of the inner membrane and elsewhere in the cell. The actions on bacterial spores, *Maycobacteria spp.*, viruses, protozoa are still not known but believed to be similar to the effects on bacterial cells [8]. Glutaraldehyde is more active at alkaline than at acidic pHs. As the external pH is altered from acidic to alkaline, more reactive sites will be formed at the cell surface, leading to a more rapid microbicidal effect. The cross-links thus obtained mean that the cell is then unable to undertake most, if not all, of its essential functions [2].

2.2.3. *Ortho*-phthalaldehyde

Ortho-phthalaldehyde (OPA) is a relatively new type of di-alhehydes, that is claimed to have potent bactericidal and sporicidal activity. OPA appears to be less cytotoxic than glutaraldehyde even though bactericidal effects were comparable [9]. Therefore, disinfection with OPA is indicated for semi-critical instruments that come into contact with mucous membranes or broken skin, such as specula, laryngeal mirrors, and internal ultrasound probes and has been suggested as a replacement for glutaraldehyde in endoscope disinfection. To date, the mechanism of its antimicrobial action has been little studied, but preliminary evidence suggests an action similar to that of glutaraldehyde. A sequence of events responsible for the antimicrobial action of OPA has been described: It binds to membrane receptors due to cross-linkage; impairs the membrane functions allowing the biocide to enter through the permeabilized membrane; interacts with intracellular reactive molecules, such as RNA, compromising the growth cycle of the cells and, at last, interacts with DNA [10].

2.3. Anilides

The anilides, such as salicylanilide, triclocarban and diphenylureas (carbanilides), are rarely used clinically. Triclocarban, the most studied of the anilides, is used mostly as antiseptic in soaps, deodorants and other household products. It is mainly active against Gram positive bacteria and significantly less active against Gram negative bacteria and fungi and lacks appreciable persistency for the skin [2]. The effectiveness of triclocarban antibacterial is maintained at very low concentrations ranging for various Gram positive resistant strains. The mechanism of action for anilides is probably occurring by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane, leading to cell death [11].

2.4. Biguanides

Various biguanides are currently in use as biocides including chlorhexidine, alexidine and polymeric biguanides.

2.4.1. Chlorhexidine

Chlorhexidine is probably one of the most commonly used biocide in antiseptic products, particularly in hand washing and dental and oral products. In addition, it can also be used as a disinfectant and preservative. Chlorhexidine is usually used as an active ingredient in mouthwash designed to reduce plaque, gingival inflammation and bleeding. It has been shown to have an immediate bactericidal action and a prolonged bacteriostatic action due to adsorption onto the pellicle-coated enamel surface. Its use is considered a powerful adjuvant to mechanical oral hygiene (brushing and flossing), especially in those cases in which it cannot be performed correctly [12]. It has an effect on Gram positive and

to a lesser extent on Gram negative bacteria, with both bactericidal and bacteriostatic mechanisms of action, that are mainly depending on membrane disruption. In addition, there are same reports linking its mechanism of action to the inhibition of membrane-bound ATPase [13]. Chlorhexidine has a biphasic effect depending on its concentration. It affects membrane integrity in low concentrations, and therefore lysis of the protoplasts and spheroplasts, while it causes congealing of the cytoplasm and precipitation of proteins and nucleic acids in high concentrations. Chlorhexidine is also useful against fungi and enveloped viruses, though this has not been extensively investigated but is expected to be similar to the effect on bacterial cells. Chlorhexidine is not sporicidal but prevents developments and outgrowth of spores without inhibiting the germination. Its common applications is attributed to its long-lasting broad-spectrum efficacy, and substantivity for the skin. In addition, it is considered a safe compound, with minimal and transitory local and systemic side effects. Despite its advantages, its activity is pH dependent and is greatly reduced in the presence of organic matter blood and pus [2].

2.4.2. Alexidine

Alexidine is more rapid bactericidal and produces a significantly faster alteration in bactericidal permeability than chlorhexidine. Application and mechanism of action are similar to those related to chlorhexidine.

2.4.3. Polymeric biguanides

Polymeric biguanides such as vantocil have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. Vantocil is active against Gram positive and most Gram negative bacteria, however, is not sporicidal.

2.5. Diamidines

The isethionate salts of two aromatic diamidine compounds, propamidine and dibromopropamidine, have been used as antibacterial agents. Clinically, diamidines are used for the topical treatment of wounds. The exact mechanism of action is unknown, but believed to inhibit oxygen uptake and induce leakage of amino acids [2].

2.6. Halogen-releasing agents

Examples of halogen-relaseing agents include chlorine-, iodine- and bromine- based compounds that have been traditionally used for both antiseptic and disinfectant purposes. However, Bromine-related compounds such as ammonium bromide and alkaline bromine derivative are rarely used.

2.6.1. Chlorine-releasing agents

The most important members of chlorine-releasing agents include sodium hypochlorite, chlorine dioxide, and the N - chloro compounds such as sodium dichloroisocyanurate (NaDCC), with chloramine-T being used to some extent [14]. It is worth mentioning that chloroform is forbidden in the European Union by the Biocidal Products Directive 98/8/EC (BPD) [1]. Sodium hypochlorite solutions are widely used for hard-surface disinfection in household bleach and can also be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. NaDCC can also be used for this purpose and has the advantages of providing a higher concentration of available chlorine and being less susceptible to inactivation by organic matter [15].

The mechanism of action of chlorine-releasing agents is not fully understood, however it well known that they are highly active oxidizing agents and thereby may destroy the cellular activity of proteins. They are not sporecidal. Their effect is not due to DNA damage since spore coat seems to protect the spore from their effect. The effect seems, however, appears to be due to the damage to the spore's inner membrane during the germination process [16]. Although, they also possess virucidal activity, further studies are needed to explain the antiviral action.

2.6.2. Iodine-releasing agents

Iodine-releasing compounds including free iodine, iodophors (“iodine carriers”), and iodoform. Although less reactive than chlorine derivatives, iodine-releasing agents are rapidly bactericidal, fungicidal, virucidal, and sporicidal [15]. Although aqueous or alcoholic solutions have been used for centuries as antiseptics, their irritation and excessive staining were always considered as major disadvantage. In addition, aqueous solutions are generally stable for short period of time. These problems were overcome by the development of iodophors, such as povidone-iodine and poloxamer-iodine. Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures [2]. Similar to chlorine-related products, the antimicrobial action of iodine derivatives is rapid, even at low concentrations, but the exact mechanism of action is still unknown. Iodine derivatives rapidly penetrate into microorganisms and attacks thiol groups of proteins which follows in cell death. Little is known about its antiviral action.

2.7. Heavy metallic derivatives and salts

These derivatives include copper, mercury, titanium and silver compounds.

2.7.1. Mercury compounds

Mercury compounds include mercurochrome, nitromersol, thiomersal, phenylmercuric nitrate, and phenylmercuric acetate. Although they also have same mechanism as silver compounds, mercury compounds have only historical use.

2.7.2. Titanium compounds

The photocatalytic properties of titanium dioxide are well known and have many applications including the removal of organic contaminants. The mechanism of action involves degradation of the cell wall and cytoplasmic membrane due to the production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide. This initially leads to leakage of cellular contents then cell lysis and may be followed by complete mineralisation of the microorganism [17].

2.7.3. Silver Compounds

The most important silver compound that is currently in use is silver sulfadiazine, although silver acetate and silver nitrate have antimicrobial properties. Silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts. They are also used as preservative in pharmaceutical products [18]. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol groups [19]. Silver sulfadiazine is essentially a combination of two antibacterial agents, silver ions and sulfadiazine. The question whether the antibacterial effect of silver sulfadiazine arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. Silver sulfadiazine has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in bacteria. Silver sulfadiazine binds to cell components, including DNA. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription [20].

2.8. Peroxygens

2.8.1. Hydrogen peroxide

Hydrogen peroxide is widely used for disinfection, sterilization, and antisepsis, particularly in applications where its decomposition into non-toxic by-products is important. It is commercially available in a variety of concentrations ranging from 3-90%. Hydrogen peroxide demonstrates broad-spectrum efficacy against bacteria and thier spores, viruses, and fungi. In general, greater activity is seen against Gram positive than Gram negative bacteria; however, the ability of bacteria to produce catalase can increase tolerance when lower concentrations are used. Higher concentrations of hydrogen peroxide and longer contact times are required for sporicidal activity, although this activity is significantly increased in the gaseous phase. Hydrogen peroxide acts as an oxidizing agent by producing hydroxyl free radicals which attack essential cell components, including lipids, proteins and DNA [21]. It has been proposed that exposed sulfhydryl groups and double bonds are particularly targeted.

2.8.2. Ozone

Ozone gas has a high oxidation potential and is more powerful than chloride-releasing agents when used as an antimicrobial agent against bacteria, viruses, fungi, and protozoa [22]. Such features justify the current interest in its application in medicine and dentistry where it can be used for the treatment of alveolitis as a replacement for antibiotic therapy and as a mouthwash for reducing the oral microflora, as well as for the adherence of microorganisms to tooth surfaces. Ozone has been shown to stimulate remineralisation of recent caries-affected teeth after a period of about six to eight weeks [23-24].

2.8.3. Peracetic acid

Peracetic acid is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal, and fungicidal at low concentrations. Its main use as a disinfectant for wastewater effluents [25]. Peracetic acid has advantages of being free from decomposition by peroxidases, unlike hydrogen peroxide, and remaining active in the presence of organic loads. Its main application is as a low-temperature liquid sterilant for medical devices, flexible scopes, and hemodialyzers, but it is also used as an environmental surface sterilant [2]. Major disadvantage associated with peracetic acid disinfection is the increases of organic content due to acetic acid and thus in the potential microbial re-growth. Another drawback to the use of peracetic acid is its high cost, which is partly due to limited production capacity worldwide [25]. Similar to hydrogen peroxide, peracetic acid probably denatures proteins and enzymes and increases cell wall permeability by disrupting sulfhydryl and sulfur bonds.

2.9. Phenols

Phenolic-type antimicrobial agents have long been used for their antiseptic, disinfectant, or preservative properties, depending on the compound. This group is composed of cresols, non-coal tar phenols, halo- and nitrophenols, and bis-phenols.

2.9.1. Halo- and nitrophenols

Chloroxylenol is the key halophenol used in antiseptic or disinfectant formulations [26]. Chloroxylenol is bactericidal, but *Pseudomonas aeruginosa* and many fungi are highly resistant. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. However, because of its phenolic nature, it would be expected to have an effect on microbial membranes. Phenol induces progressive leakage of intracellular constituents through its effect on cytoplasmic membrane, including the release of potassium ions. It was also proposed that halophenols acts only at the point of separation of pairs of daughter cells, with young bacterial cells being more sensitive than older cells towards phenol [27].

2.9.2. Bis-phenols

Bis-phenols include derivatives of dihydroxydiphenylmethane, hydroxydiphenylether, diphenylsulphide. Triclosan and hexachlorophane are the most widely used biocides from this group, especially in antiseptic soaps and hand rinses. They exhibit broad spectrum efficacy but have little activity against *Pseudomonas aeruginosa* and fungi and are sporostatic towards bacterial spores. Triclosan exhibits particular activity against Gram positive bacteria. Its efficacy against Gram negative bacteria and fungi can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA causes increased permeability of the outer membrane [28]. Although originally thought to kill bacteria by attacking multiple cellular targets, triclosan was recently shown to target a specific bacterial fatty acid biosynthetic enzyme, enoyl-[acyl-carrier protein] reductase, in Gram negative and Gram positive bacteria, as well as in the *Mycobacteria spp*. [29]. Despite the broad spectrum efficacy of hexachlorophene, concerns about toxicity, in particular in neonates, have meant that its use in antiseptic products has been limited [2].

2.10. Surface active agents

Surface active agents or surfactants are amphiphilic molecules consisting of at least two parts: an apolar hydrophobic tail and a polar hydrophobic head. They are often classified on the basis of the charge of the polar head group. According to this, they are divided into non-ionic (without net charge), zwiterionic or amphoteric (having both positive and negative charges), anionic (negative charge) and cationic (positive charge) [30]. Anionic, amphoteric and non-ionic agents are used in household products and in pharmaceutical and cosmetic products as preservatives.

2.10.1. Cationic agents

The cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants. QACs are widely used as adjuncts to hygiene in domestic cleaning products. They have been used for a variety of clinical purposes. Examples include their use as preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces. In addition of having antimicrobial properties, QACs are also excellent for hard-surface cleaning and deodorization [31]. It has been known that QACs are membrane-active agents targeting predominantly the cytoplasmic membrane in bacteria or the plasma membrane in fungi. The cationic agents react with phospholipid components in the cytoplasmic membrane, thereby producing membrane distortion and protoplast lysis under osmotic stress. QACs are sporostatic; they inhibit the outgrowth of spores but not the actual germination processes by an unknown mechanism. Likewise, the QACs are not mycobactericidal but have a mycobacteriostatic action, although the actual effects on mycobacteria have been little studied. The QACs have an effect on lipid, enveloped but not non-enveloped viruses [2].

3. Microbial resistance against biocides

Microbial resistance towards biocides has long been reported in the health care settings. In most instances, cases emerge following the improper use or storage of the formulations, resulting in a decrease in the effective concentration of the biocide [32]. However, most of the evidence on microbial resistance towards biocides comes from laboratory based experiments which investigated a wide range of agents [33].

The concentration of a biocide has been considered the most important factor that affects its efficacy. In the case of nosocomial pathogens growing as biofilms, the biocide concentration and consequently the microbial susceptibility are strongly affected by the reduced diffusion of active molecules through the biofilm [34]. Various methodologies that have been used to measure microbial resistance to biocides are based on the determination of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and bacterial growth kinetics [33]. However,

determination of critical micelle concentration (CMC) has been recently used as a reference concentration for surface active agents due to the fact that CMC could be considered an approximate measure of the partition coefficient of the surfactant between the aqueous and apolar media [35].

Although the basis of microbial resistance to antibiotics is well-defined, the knowledge about insusceptibility mechanisms towards biocides is still scarce. As mentioned earlier, biocides have multiple target sites against microbial cells. Thus, the emergence of general resistance is unlikely to be caused either by a specific modification of a target site or by a by-pass of a metabolic process. It is more likely that these mechanisms operate synergistically. Insusceptibility to biocides can be intrinsic or acquired [36].

3.1. Intrinsic insusceptibility to biocides

Different groups or forms of bacteria vary in their intrinsic insusceptibility to biocides, with bacterial spores being the most resistant, followed by *Mycobacterium spp.*, then Gram negative organisms, with Gram positive bacteria generally being the most susceptible. However, There are wide discrepancy within this general classification [37]. The mechanisms beyond the intrinsic insusceptibility towards biocides include impermeability of the cell envelope, existence of active efflux pumps, ability to form biofilms, and enzymatic transformation of biocides.

3.1.1. Cellular impermeability

The cell envelop/wall plays a vital role in microbial growth when they are in the form of vegetative cells and and also in the survival in hostile environments when the microbes are in the spore form. Cellular impermeability is not only restricted to spores but can be also noticed in vegetative bacteria such as *Mycobacterium spp.* and to a lesser extent in Gram negative bacteria.

The structure and chemical composition of bacterial spores differ noticeably from those of vegetative cells. These differences largely account for the unique resistance properties of the spore to environmental stresses, including biocides, resulting in the emergence of spore-forming bacteria as major pathogens. The exosporium if exists, the outer and the inner coat layers, and to some extent the cortex of the spores, present a barrier to the intracellular penetration of many biocides [38]. Experimentally, the removal of coat and cortex layers leads to a marked increase in biocides susceptibility [39]. It essential to mention that different outer structures are found within spores belonging to different bacterial species, which lead to various degrees permeability to biocides.

Mycobacterial cells are characterized by a complex cell wall, with a high lipid content which confers to the bacilli resistance. In particular, presence of a mycoylacylarabinogalactan and arabinogalactan/arabinomannan of the cell wall of the mycobacterial cell wall limit the concentration of active biocide that can reach the target sites in these bacterial cells [34, 41].

Regarding Gram negative bacteria, presence of lipopolysaccharides can act in a similar way as the lipid in mycobacterial cells [42]. In addition, Gram negative bacteria can regulate the permeability of their membranes by decreasing the synthesis of porins; membrane pore-forming proteins involved in antibiotic and biocides uptake, and modifying the lipopolysaccharide structure [36,43]. Other elements may also participate in developing cellular impermeability. Examples include *Pseudomonas aeruginosa* which has high Mg^{2+} content in the outer membrane that helps in producing strong lipopolysaccharide links that can restrict the uptake of biocides. The charge property of the cell surface also plays a role in bacterial resistance mechanisms to positively charged biocides such as QACs [2].

On the other side, Gram positive bacteria seem to possess a permeable cell envelop that does not restrict the penetration of biocides in general. For example, biocides of small molecules such as alcohols, aldehydes, phenols, QACs and biguanides has no difficulties in penetrating the wall of Gram positive bacteria, which are generally sensitive to all these classes of biocides. However, evidence to resistance towards antibiotics due to restricted penetration can occur, as illustrated by vancomycin-intermediate resistant *Staphylococcus aureus* which produces increased amounts of nonamidated glutamine residues in the peptidoglycan layer, forming, therefore, a markedly thickened cell wall [40]. More research is required to study the effect of these structural modifications of Gram positive cell wall on the penetration of biocides.

3.1.2. Over-expressing the efflux pumps

Gaining recent attention, over-expressing the efflux pumps (membrane proteinous complexes involved in antibiotic expulsion) has been well described and is increasingly implicated as a resistance mechanism towards biocides. Efflux pumps come in a variety of structures, but a single protein may act alone to perform efflux [44]. Five major classes of efflux pump systems can be differentiated, most of which have been shown to be involved in biocide efflux [45]. Efflux pumps decrease the intracellular concentration of toxic compounds, including biocides, and therefore, they have been shown to reduce the efficacy of a number of biocides including QACs, biguanides, phenols and diamidine. However, bacterial resistance induced by efflux pump has yet to be confirmed against alcohols, aldehydes, peroxides and chlorine compounds and their derivates [46]. There is evidence that *Pseudomonas aeruginosa* can efflux triclosan representing

an important intrinsic susceptibility mechanism towards this biocide [47]. Moreover, there is noticeable reduced susceptibility towards chlorhexidine in *Staphylococci* which seem to obtain efflux-mediated resistance genes [48].

3.1.3. Formation of biofilms

Most bacteria are associated with surfaces and usually grow as biofilm rather than as planktonic cells. A biofilm can be defined as a cluster of microorganisms adhering irreversibly to a surface and surrounded by a complex matrix of extracellular polymeric substances. Biofilms can be formed by a single bacterial strain, however, most natural biofilms are actually formed by multiple bacterial species. Bacteria within biofilms differ phenotypically from planktonic bacterial cells and have been consistently described as being more resistant to biocides and antibiotics [49-51]. Types of surfaces within clinical settings that may be prone to biofilm development are numerous and include wounds, teeth, the gastrointestinal tract, indwelling medical devices and prostheses, water systems within hospitals and dental units, dialysis equipment and endoscopes [52]. Conventional methods for bacterial removal, such as applications disinfectants, are often ineffective for biofilm populations due to their special physiology and physical matrix barrier. It has been estimated that billions of dollars are spent every year worldwide to deal with damage to equipment, contaminations of products, energy losses, and infections in human beings resulted from microbial biofilms [53]. Recently, it was reported that although biocides may be effective against populations of highly antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, some biocides currently used in hospitals are ineffective against microbes growing as biofilms attached to surfaces and fail to control this reservoir for hospital-acquired infections [54]. Although several factors are associated with this response, notably nutrient limitation, decreased metabolism, quiescence, reduced penetration due to the extracellular polymeric matrix and diffusibility of biocide across a biofilm [55], the full story has yet to emerge. Nonsusceptibility associated with biofilm-forming bacterial cells can be considered an intrinsic nonsusceptibility mechanism resulting from physiological (phenotypic) adaptation cells. Several biofilm control strategies have been proposed focusing mainly on preventing initial microbial attachment to surfaces, minimizing biofilms formations, and targeting the production of extracellular polymeric substances. However, a greater understanding of biofilm processes should lead to novel, effective strategies for biofilm control and improvement in patient care and management.

3.1.4. Enzymatic transformation of biocides

The enzymatic transformation of biocides has been described as an intrinsic resistance mechanism in bacteria towards various biocides. Environmental bio-degradation of different compounds has been well-described among various microbial communities. Examples include enzymatic reduction of the cationic parts of heavy metallic derivatives of silver and copper compounds [56], production of formaldehyde dehydrogenase to inactivate aldehydes [57], and production of catalase, super oxide dismutase and alkyl hydroperoxidases which remove free radicals from peroxygens [58]. However, the importance of degradation as a bacterial resistance mechanism towards high concentrations of biocides remains unclear. As for efflux, increased resistance following degradation of biocides has been measured as an increase in MICs but not necessarily as a decreased in lethal activity of the microbe.

3.2. Acquired insusceptibility towards biocides

As towards antibiotics, microbes can develop resistance against biocides through acquired mechanisms such as development of mutation or acquisition of resistant determinants or mobile DNA (transposon, plasmids) coding for resistant elements (enzyme, transporter, or modification of target sites) [59-61]. The acquired resistance towards biocides is of great concern since a bacterium that was previously susceptible can become insusceptible to a compound or a group of compounds. The possession of resistant genes has been well described and it is predominantly important to consider this as it might provide occasional cross or co-resistance. However, there is little information on the effect of biocides on the transfer of genetic determinants. For example, acquired modification of target sites after the use of biocides been described on rare occasions and does not seem to be widespread among microbes.

3.3. Final thoughts about microbial resistance against biocides

Resistance to biocides can be observed in all health settings, however, unlike antibiotic resistance, the issues relating to biocide resistance are considered to have a relatively low priority [62]. It is important to note that antibiotic and biocide antibacterial actions share many similarities in terms of target, mechanism of action, behaviour and clinical aspects and development of resistance. Due to these similarities, there is an increasing possibility that resistance towards biocides can be invested by microorganisms to confer resistance towards antibiotics. Use of a biocide may not only select for resistance against the agent itself, but may at the same time co-select for resistance against other biocides and antibiotics if resistance genes are linked on a determinant or mobile DNA such as a transposon or a plasmid.

Although the link between the use of biocides against microbes and potential emerging antibiotic resistance is not straightforward, certain biocides and antibiotics co-resistance is evident under experimental conditions. Examples include that the widespread use of triclosan-containing antiseptics and disinfectants may indeed aid in development of

microbial resistance, in particular cross-resistance to antibiotics. In the last decade or so, a number of studies have verified the occurrence of triclosan resistance with a possibility that triclosan resistance may contribute to reduced susceptibility to clinically important antibiotics, due to either cross-resistance or co-resistance mechanisms. Although the number of studies elucidating the association between triclosan resistance and resistance to other antimicrobials in clinical isolates has been limited, recent laboratory studies have confirmed the potential for such association in *Escherichia coli* and *Salmonella enterica*. Thus, widespread use of triclosan may represent a potential public health risk in the emergence of concomitant resistance to antibiotics currently in use [63]. Another example include the resistance towards QACs that is widespread among a diverse range of microorganisms and is facilitated by several mechanisms. Development of resistance in both pathogenic and non-pathogenic bacteria has been related to the wide application of QACs in human medicine and the food industry. Use of QACs in cosmetic products will inevitably come into intimate contact with the skin or mucosal linings in the mouth and thus are likely to add to the selection pressure toward more QAC-resistant microorganisms among the skin or mouth flora. There is increasing evidence of co-resistance and cross-resistance between QACs and a range of other clinically important antibiotics and disinfectants. More indiscriminate use of QACs such as in cosmetic products may drive the selection of further new genetic elements that will aid in the persistence and spread of antimicrobial resistance and thus in limiting our treatment options for microbial infections [64]. Resistance plasmids may also carry genes mediating resistance against heavy metals derivatives. Therefore, their abundant use as disinfectants or for growth promotion in animals used for food, may also co-select for antibiotic resistance [65].

A key question is whether the use of biocides facilitates the induction of antibiotic resistance remains to be answered. There is a need to establish conclusively and comprehensively whether there is a clear-cut linkage between antibiotic and biocide resistance and whether biocides can induce for antibiotic resistance. Additionally, the responses to biocides of new and emerging pathogens must be assessed. Further research is needed to establish a correlation between biocide exposures and development of antibiotic resistance [66].

4. Conclusions

Biocides are without doubt an important set of compounds in our lives. New compounds are consistently finding their way to the markets and their use is widening in various clinical aspects. Although there is scientific evidence that biocides select for microbial resistance towards them, there is, so far, no conclusive evidence that this also determined or will determine an increase in resistance against antibiotics; a group of compounds that remains essential for our survival. Further research is required to clarify the possibility of transferring microbial resistance mechanisms towards biocides to use them against antibiotics.

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References

[1] The European Parliament and of the Council of the European Union. Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. *Official Journal of the European Community*. 1998.
[2] McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev*. 1999;12:147-179.
[3] Zhang Z, Jiang B, Liao X, Yi J, Hu X, Zhang Y. Inactivation of Bacillus subtilis spores by combining high-pressure thermal sterilization and ethanol. *Int J Food Microbiol*. 2012;160:99-104.
[4] Yeung LL, Grewal S, Bullock A, Lai HH, Brandes SB. A comparison of chlorhexidine-alcohol versus povidone-iodine for eliminating skin flora before genitourinary prosthetic surgery: a randomized controlled trial. *J Urol*. 2013;189:136-140.
[5] Power EGM. Aldehydes as biocides. *Prog Med Chem*. 1995;34:149-201.
[6] Kevorkian CG, Merritt JL, Ilstrup DM. Methenamine mandelate with acidification: an effective urinary antiseptic in patients with neurogenic bladder. *Mayo Clin Proc*. 1984;59:523-529.
[7] Bradshaw JH, Puntis JW. Taurolidine and catheter-related bloodstream infection: a systematic review of the literature. *J Pediatr Gastroenterol. Nutr.* 2008;47:179-186.
[8] Abbott L. The use and effects of glutaraldehyde: a review. *Occup Health (Lond).* 1995;47:23823-23829.
[9] Iwasawa A, Niwano Y, Kohno M, Ayaki M. Bactericidal effects and cytotoxicity of new aromatic dialdehyde disinfectants (ortho-phthalaldehyde). *Biocontrol Sci*. 2011;16:165-170.
[10] Simões M, Simões LC, Cleto S, Machado I, Pereira MO, Vieira MJ. Antimicrobial mechanisms of ortho-phthalaldehyde action. *J Basic Microbiol*. 2007;47:230-242.
[11] Drugeon HB, Rouveix B, Michaud-Nerard A. Triclocarban antibacterial activity on resistant staphylococci, streptococci, and enterococci. *Med Mal Infect*. 2012;42:276-279.
[12] Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. *Minerva Stomatol*. 2012;61:399-419.
[13] Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. *Journal of Clinical Periodontology*. 1988;15:415-424.

[14] Dychdala GR. Chlorine and chlorine compounds. *Block SS (ed.), Disinfection, sterilization, and preservation, 4th ed. Lea & Febiger, Philadelphia, Pa.* 1991;131-151.
[15] Bloomfield SF**.** 1996. Chlorine and iodine formulations. *Ascenzi JM (ed.), Handbook of disinfectants and antiseptics. Marcel Dekker, Inc., New York, N.Y.* 1996;133-158.
[16] Young SB, Setlow P. Mechanisms of killing of Bacillus subtilis spores by hypochlorite and chlorine dioxide. *J Appl Microbiol*. 2003;95:54-67.
[17] Foster HA, Ditta IB, Varghese S, Steele A. Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. *Appl Microbiol Biotechnol*. 2011;90:1847-1868.
[18] Politano AD, Campbell KT, Rosenberger LH, Sawyer RG. Use of silver in the prevention and treatment of infections: silver review. *Surg Infect (Larchmt)*. 2013;14:8-20.
[19] Lansdown AB. Silver in health care: antimicrobial effects and safety in use. *Curr Probl Dermatol*. 2006;33:17-34.
[20] Hoffmann S. Silver sulfadiazine: an antibacterial agent for topical use in burns. A review of the literature. *Scand J Plast Reconstr Surg*. 1984;18:119-126.
[21] Linley E, Denyer SP, McDonnell G, Simons C, Maillard JY. Use of hydrogen peroxide as a biocide: new consideration of its mechanisms of biocidal action. *J Antimicrob Chemother*. 2012;67:1589-1596.
[22] Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. *J Contemp Dent Pract*. 2008;9:75-84.
[23] Saini R. Ozone therapy in dentistry: A strategic review. *J Nat Sci Biol Med*. 2011;2:151-153.
[24] Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent*. 2008;36:104-116.
[25] Kitis M. Disinfection of wastewater with peracetic acid: a review. *Environ Int*. 2004;30:47-55.
[26] Wilson M, Mowad C. Chloroxylenol. *Dermatitis*. 2007;18:120-121.
[27] Rayne S, Forest K, Friesen KJ. Mechanistic aspects regarding the direct aqueous environmental photochemistry of phenol and its simple halogenated derivatives. A review. *Environ Int*. 2009;35:425-437.
[28] Saleh S, Haddadin RN, Baillie S, Collier PJ. Triclosan - an update. *Lett Appl Microbiol*. 2011;52:87-95.
[29] Schweizer HP. Triclosan: a widely used biocide and its link to antibiotics. *FEMS Microbiol Lett*. 2001;202:1-7.
[30] Holmberg K, Jönsson B, Kronberg B, Lindman B. *Surfactants and polymers in aqueous solution. John Wiley & Sons, Ltd.* 2002.
[31] McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P. Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility. *Appl Environ Microbiol*. 2004;70:3449-3456.
[32] Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol*. 2002;92:121-135.
[33] The European Parliament and of the Council of the European Union. Scientific Committee on Emerging and Newly Identified Health Risks - Assessment of the Antibiotic Resistance Effects of Biocides. *Official Journal of the European Community*. 2009.
[34] Anderson GG, O'Toole GA. Innate and induced resistance mechanisms of bacterial biofilms. *Curr Top Microbiol Immunol*. 2008; 322:85-105.
[35] Vieira OV, Hartmann DO, Cardoso CM, Oberdoerfer D, Baptista M, Santos MA, Almeida L, Ramalho-Santos J, Vaz WL. Surfactants as microbicides and contraceptive agents: a systematic in vitro study. *PLoS One*. 2008;3:e2913.
[36] Poole K. Mechanisms of bacterial biocide and antibiotic resistance. *J Appl Microbiol*. 2002;92:55-64.
[37] Russell AD. Bacterial resistance to disinfectants: present knowledge and future problems. *J Hosp Infect.* 1998;43:S57-S68.
[38] Leggett MJ, McDonnell G, Denyer SP, Setlow P, Maillard JY. Bacterial spore structures and their protective role in biocide resistance. *J Appl Microbiol*. 2012;113:485-498.
[39] Bloomfield SF. Bacterial sensitivity and resistance: Resistance of bacterial spores to chemicals. *Russell AD, Hugo WB, Ayliffe GAJ, (eds.), Principles and Practice of Disinfection, Preservation and Sterilization, 3rd Ed. Oxford: Blackwell Science.* 1999;303-320.
[40] Lambert PA. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *J Appl Microbiol*. 2002;92:S46-S55.
[41] Hawkey PM. Mycobactericidal agents. *Fraise AP, Lambert PA, Maillard J-Y, (eds), Principles and practice of disinfection, preservation and sterilization, 4th ed. Oxford: Blackwell Scientific Publication*. 2004;191-204.
[42] Denyer SP, Maillard J-Y. Cellular impermeability and uptake of biocides and antibiotics in Gram negative bacteria. *J Appl Microbiol*. 2002;92:35S-45S.
[43] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev*. 2003;67:593-656.
[44] Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. *Journal of Applied Microbiology Symposium Supplement.* 2002;92:65S-71S.
[45] Putman M, van Veen HW, Konings WN. Molecular properties of bacterial multidrug transporters. *Microbiology and Molecular Biology Reviews*. 2000;64:672–693.
[46] Chojecka A, Jakubiec K, Jakimiak B, Röhm-Rodowald E, Kanclerski K. Significance of the efflux phenomenon as a mechanism of bacterial resistance on active substances of biocide. *Przegl Epidemiol*. 2012;66:39-44.
[47] Poole K. Efflux pumps as antimicrobial resistance mechanisms. *Ann Med*. 2007;39:162-176.
[48] Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? *J Antimicrob Chemother*. 2012;67:2547-2559.
[49] Gilbert P, McBain AJ, Rickard AH. Formation of microbial biofilm in hygienic situations: a problem of control. *Int Biodeter Biodegrad*. 2003;51:245-248.
[50] Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F. Resistance of bacterial biofilms to disinfectants: A review. Biofouling. 2011;27:1017-32.
[51] Maira-Litrán T, Allison DG, Gilbert P. Expression of the multiple antibiotic resistance operon (mar) during growth of *Escherichia coli* as a biofilm. *J Appl Microbiol*. 2000;88:243-247.

[52] Lindsay D, von Holy A. Bacterial biofilms within the clinical setting: what healthcare professionals should know. *J Hosp Infect.* 2006;64:313-25.
[53] Yang L, Liu Y, Wu H, Hóiby N, Molin S, Song ZJ. Current understanding of multi-species biofilms. *Int J Oral Sci*. 2011;3:74-81.
[54] Smith K, Hunter IS. Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. *J Med Microbiol*. 2008;57:966-973.
[55] Pan Y, Breidt F, Kathariou S. Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. *Appl Environ Microbiol*. 2006;72:7711-7717.
[56] Cloete TE. Resistance mechanisms of bacteria to antimicrobial compounds. *Int Biodet Biodegrad*. 2003;51:277-282.
[57] Kummerle N, Feucht HH, Kaulfers PM. Plasmid-mediated formaldehyde resistance in Escherichia coli: characterization of resistance gene. *Antimicrob Agents Chemother*. 1996;40:2276-2279.
[58] Demple B. Redox signaling and gene control in the Escherichia coli soxRS oxidative stress regulon- a review. *Gene*. 1996;179:53-57.
[59] White DG, McDermott PF. Biocides, drug resistance and microbial evolution. *Curr Opin Microbiol*. 2001;4:313-317.
[60] Chapman JS. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeter Biodegrad.* 2003;51:271-276.
[61] Poole K. Acquired resistance. *Fraise AP, Lambert PA, Maillard J-Y, (eds), Principles and practice of disinfection, preservation and sterilization. 4th ed. Oxford: Blackwell Scientific Publication.* 2004;170-83.
[62] Cookson B.Clinical significance of emergence of bacterial antimicrobial resistance in the hospital environment. *J Appl Microbiol*. 2005;99:989-996.
[63] Yazdankhah SP, Scheie AA, Høiby EA, Lunestad BT, Heir E, Fotland TØ, Naterstad K, Kruse H. Triclosan and antimicrobial resistance in bacteria: an overview. *Microb Drug Resist*. 2006;12:83-90.
[64] Hegstad K, Langsrud S, Lunestad BT, Scheie AA, Sunde M, Yazdankhah SP. Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? *Microb Drug Resist*. 2010;16:91-104.
[65] Frimodt-Møller N, Kolmos HJ. Selection and spreading of antibiotic resistance in bacteria. *Ugeskr Laeger*. 2011;173:2885-2888.
[66] Oggioni MR, Furi L, Coelho JR, Maillard JY, Martínez JL. Recent advances in the potential interconnection between antimicrobial resistance to biocides and antibiotics. *Expert Rev Anti Infect Ther*. 2013;11:363-366.

