Chapter 25

Biocide Use and Antibiotic Resistance

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1. INTRODUCTION

Bacterial resistance to antibiotics is a well-known phenomenon, which has been extensively described. Despite the early optimism in the 1960s that the advent of the antibiotic era would eradicate bacterial diseases, drug resistance in bacteria has increased at an alarming rate, so that bacterial resistance has been described for most if not all available antibiotics. Multidrug resistance in bacteria is indeed a major clinical problem (Hawkey, 2001). This situation is further exacerbated by the slow pace at which new molecules are being produced and by the rapid microbial adaptation to new antimicrobials. Several reasons have been put forward for the emergence of bacterial resistance to antibiotics, among which the overuse and sometimes misuse of antibiotics have been the most important. More recently, biocides (i.e., disinfectants, antiseptics, and preservatives) commonly used in hospital settings and elsewhere, for example, domiciliary and industrial environments, have been implicated in the emergence of antibiotic resistance in bacteria. This has raised concerns in the scientific community (Bloomfield, 2002; Levy, 2000; Russell, 1999a, 2000, 2002a; Russell and Maillard, 2000; Schweizer, 2001) and among institutions (Anon, 1997) and prompted many investigations into the possible linkage between biocide and antibiotic resistance in bacteria.

This chapter aims to give a brief description of disinfectant usage, evidence of resistance to these agents with possible linkage between biocide and

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antibiotic resistance, and the possible role, or not, of disinfectants in selecting for drug resistance.

2. DISINFECTANTS: TYPES, ACTIONS, AND USAGES

2.1. Use of disinfectant in the hospital environment

Biocides have been used for centuries, originally for the preservation of foodstuff and water, then for antisepsis and more recently for disinfection purposes (Hugo, 1999a). The number and diversity of chemicals used as biocides have increased tremendously within the last 50 years. Likewise, the usage of biocides has diversified over the years and more recently has found a commercial if not practical niche in the home environment (Bloomfield, 2002) with public awareness of hygiene-related issues (Favero, 2002; Maillard, 2002).

The first recorded use of biocides for a "clinical" purpose was probably that of the burning of juniper branches in the 14th century to combat the episodic scourge of the plague (Hugo, 1999a). The use of biocides for wound healing, and notably the use of mercuric chloride, have been practiced since the Middle Ages. However, the use of biocides in the hospital environment really progressed in the 19th century with the work of Semmelweis and notably of Lister with his study on antiseptic surgery (Hugo, 1999a; Rotter, 1998, 2001). Since then the number of chemical biocides used in the hospital environment has increased tremendously with the development of cationic biocides such as the bisbiguanide chlorhexidine, quaternary ammonium compounds (QACs), phenolic compounds, and the aldehydes and peroxygen compounds (Russell, 1999b).

In the hospital environment, biocides fulfil several functions (Rutala and Weber, 1999): (1) disinfection of equipment and surfaces, (2) antisepsis (e.g., handwashing), and (3) preservation of medical and pharmaceutical products. The activity of biocides and their use often depends upon their activity against the different types of microorganisms (Maillard, 2002), but also on the type of equipment or surfaces to disinfect. Therefore, high-level disinfection is used for equipment that comes into contact with sterile parts of the body, intermediate-level for those that come into contact with broken skin, and low-level for equipment and surfaces that come into contact with intact skin (Maillard, 2002; Rutala and Weber, 1999).

2.2. Mechanisms of action of biocides

It is generally accepted that at "in-use" concentrations, biocides have multiple target sites on the bacterial cells, the inactivation/alteration/destruction of

which lead to an inhibitory or lethal effect (Hugo, 1999b; Maillard, 2002). These agents can be divided according to their reactivity with the bacterial cells. Highly reactive biocides such as alkylating (e.g., glutaraldehyde, *ortho*-phthalaldehyde) and oxidising agents (e.g., hydrogen peroxide, peracetic acid, chlorine dioxide) are often used for high-level disinfection. Because of their nature and their interaction with the target cell (McDonnell and Russell, 1999; Russell, 1999b; Russell *et al*., 1997), the emergence of bacterial resistance to these compounds is uncommon, but not impossible as observed with glutaraldehyde (see Section 3.1). Halogen-releasing agents such as chlorine and iodine-based products have a broad spectrum of activity. Some of these agents find an important role in clinical settings. Povidone-iodine is widely used for antisepsis and notably in surgical soap, whereas chlorine-releasing agents such as sodium hypochlorite and sodium dichloroisocyanurate (NaDCC) are used to disinfect blood spillages and other contaminated materials (Russell, 1999b). Less reactive biocides used for intermediate- to low-level disinfection encompass membrane-active agents such as bisbiguanides (e.g., chlorhexidine), QACs (e.g., benzalkonium chloride), and the phenolics (e.g., triclosan). These agents act primarily by disrupting the bacterial membranes before altering/inactivating other target sites within the bacterial cytoplasmic membrane and cytosol. They have also a broad spectrum of activity. Their efficacy is often limited (if any) against spores, some mycobacteria, and certain viruses, particularly non-enveloped ones (Maillard, 2001; Russell *et al*., 1997). As for most biocides, the antimicrobial activity of these agents can be altered by several factors, such as concentration, pH, organic load, and temperature (McDonnell and Russell, 1999). Among these factors, concentration of biocides is of paramount importance (Russell and McDonnell, 2000), particularly for the emergence of bacterial resistance as described below. Indeed, although it is well recognised that biocides have multiple target sites on the microbial cells, some biocides, notably, the cationic and phenolic compounds, might have a "primary" target site, at a lower concentration (Maillard, 2002). This is particularly pertinent since these cationic and phenolic compounds have been implicated in a possible linkage between biocide and antibiotic resistance. Furthermore, there are sometimes similarities between biocide and antibiotic antimicrobial action. For example, triclosan and isoniazid both target the enoyl-acyl reductase carrier protein in mycobacteria whereas acridines, isothiazolones, chloracetamide, phenylethanol, β-lactams, fluoroquinolones, and novobiocin induce the formation of filaments in Gram-negative bacteria, although no cross-resistance between these biocides and antibiotics was observed (Ng *et al*., 2002). Further useful information on the types and mechanisms of action of biocides can be found in the papers by Russell (1995, 1999b, 2002b), Russell and Russell (1995), Russell *et al*. (1997), Hugo (1999b), and Maillard (2002).

3. BACTERIAL RESISTANCE TO BIOCIDES

Bacterial resistance to biocides has been reported since the 1950s, although the number of cases describing such resistance has increased steadily within the last two decades. This might reflect the increased use of biocides in the food, pharmaceutical industries and in the hospital and domiciliary environments. Biocides employed for intermediate- and low-level disinfection, antisepsis, and preservation have been particularly implicated in the emergence of bacterial resistance to biocides but also in the selection of bacterial strains with low-level antibiotic resistance. The mechanisms underlying such bacterial resistance are now better understood and it is emerging that bacterial cells have developed an arsenal of measures, which used together or not allow the cells to survive biocide exposure.

3.1. Evidence of bacterial resistance to biocides

For the last 50 years, there have been multiple reports on bacterial resistance to various biocides. Generally, bacterial resistance to biocides has been well reported in the literature (Poole, 2002), particularly for these biocides that are used for low- and intermediate-level disinfection, or antisepsis, such as the cationic biocides, QACs, chlorhexidine, and phenolics. In most cases, low-level resistance has been reported, often based on MIC determination, although in some instances high-level resistance has been described, for example, with triclosan (Heath *et al*., 1998, 2000a; Sasatsu *et al*., 1993). Microbial contamination of cationic biocide formulations (QACs or chlorhexidine) by Gram-negative microorganisms has been reported as early as the 1950s and has been amply described since then (see Russell [2002c] for a comprehensive list of references). However, it has to be noted that in many instances, the emergence of resistance to these biocides often resulted from an improper usage/storage of these products often implying a decrease in the active "in-use" concentration (Russell, 2002c). Anderson *et al*. (1984) reported the contamination of a commercial iodophor solution with *Pseudomonas aeruginosa*, which was probably linked to the formation of a biofilm in the manufacturing plant. Kahan (1984) reported the contamination of a 0.05% chlorhexidine wound irrigation solution with *Burkholderia picketti*, which lead to septicemia in six patients. Likewise, contamination of chlorhexidine with *Burkholderia cepacia* (Speller *et al*., 1971) and of a QAC by *Serratia marcescens* (Ehrenkranz *et al*., 1980) has been reported. Contamination of such products often results from a poor appreciation of the importance of the concentration exponent of a particular agent (Russell and McDonnell, 2000). For example, the inappropriate use of disinfectants (Centers for Disease Control, 1974; Curie *et al*., 1978; Sanford, 1970), the use of weak solutions (Prince and

Ayliffe, 1972), and the "topping-up" of containers lead to the observations of bacterial resistance to a biocide. The emergence of bacterial resistance in clinical practice is more of a concern. For example, Stickler (1974) reported the isolation of chlorhexidine-resistant *Proteus mirabilis* from patients with long-term indwelling catheterization after the extensive use of the biguanide to treat urinary tract infections. It has to be remembered that some microorganisms, notably spores, some mycobacteria, and Gram-negative bacteria such as *P. aeruginosa* are intrinsically resistant to some disinfectants, and therefore their presence where "in-use" or below "in-use" concentration of a biocide is used is not exceptional. Furthermore, the expectation to encounter such microorganisms should dictate the use of a more active biocidal formulation, even though, there have been some reports where resistant bacteria to high-level disinfectants have been isolated. For example, Griffiths *et al*. (1997) isolated two *Mycobacterium chelonae* resistant to the high-level disinfectant glutaraldehyde from endoscope washer disinfectors.

Acquired resistance is more of a concern, whereby a previously sensitive microorganism becomes resistant to a biocide. Staphylococci showing resistance to QACs have been isolated from healthcare facilities (Gillespie *et al*., 1986) but also from other sources such as food preparation (Heir *et al*., 1995). Biocide resistance in staphylococci has also been observed, particularly to cationic agents, acridines, and diamidines (reviewed by Russell, 2002a) and has been linked to the acquisition of multidrug resistance determinants (Section 4.2.4). Lear *et al*. (2000) isolated from biocide manufacturing sites, *Acinetobacter* and *Citrobacter* that showed high-level resistance to triclosan. Some staphylococci with low-level resistance to the bisphenol were also observed. Laboratory experiments also provide valuable information on the mechanism of the development of bacterial resistance to biocides. Tattawasart and colleagues (1999) were able to develop *Pseudomonas stutzeri* resistance to biguanides and other cationic compounds. Long-term and short-term exposure of *P. aeruginosa* to sub-inhibitory (residual) concentration of chlorhexidine showed some marked increase in resistance to the biguanide and to benzalkonium chloride (Thomas *et al*., 2000). Winder *et al*. (2000) observed that *P. aeruginosa* was able to develop resistance to isothiazolones as a result of a constant exposure to sub-inhibitory concentrations. Likewise, McMurry *et al*. (1998a) demonstrated that resistance of *Escherichia coli* to triclosan and other compounds (Walsh *et al*., 2003), and of *Mycobacterium smegmatis* (McMurry *et al*., 1999) to the bisphenol can be increased, although in most cases low-level resistance was observed as measured by MIC determination. As mentioned above, the emergence of bacterial resistance to highly reactive biocides has also been described. Two isolates of *M. chelonae* have been isolated from endoscope washer disinfectors (Griffiths *et al*., 1997; van Klingeren and Pullen, 1993). These two isolates were shown to be highly resistant to

glutaraldehyde (Fraud *et al*., 2001; Manzoor *et al*., 1999; Walsh *et al*., 2001). Resistance to hydrogen peroxide and peracetic acid has also been described, but in this case, microbial biofilms were responsible (see below).

Chapman (1998) and Chapman *et al*. (1998) described bacterial strains, which were virtually resistant to all known preservatives. Russell (2002a) pointed out that although the emergence of such strains would be expected, since preservatives have been used extensively for a long period of time, the number of cases concerning bacterial-resistance to "in-use" concentrations of biocides is low.

3.2. Mechanisms of bacterial resistance to biocides

In order to survive biocide exposure, the main objective of the microorganism is to decrease the toxic concentration of chemicals and thus several "resistance" mechanisms can be activated. The main mechanisms are probably based on decreased uptake/penetration of the agents through "reinforcing" an impermeability barrier, and to some extent the decrease of the intracellular concentration of the agents through efflux systems and also degradation. A selected decrease in the toxic effect of the agents can also take place through phenotypic alteration and target modification.

Some of these mechanisms are intrinsic to microorganisms, whereas other can be acquired. Acquired resistance can arise from one or several processes: mutation or amplification of an endogenous chromosomal gene, and the acquisition of resistance determinants from extra-chromosomal elements (i.e., plasmids and transposons) (Poole, 2002). Phenotypic variations that might lead to biocide resistance are dealt with later (Section 3.2.5) (Chapman, 2003). In addition, one preliminary investigation on the adaptation of *Staphylococcus aureus* to chlorhexidine demonstrated the possible role of bacterial "alarmones" (extracellular induction components), which confer the Gram-positive bacteria a 3-fold increase in MIC. However, no evidence was found that such a mechanism was involved with increased level of triclosan resistance or in *E. coli* (Davies and Maillard, 2001).

3.2.1. Impermeability

The most resistant microorganisms to disinfection are bacterial spores (Russell, 1990; Russell *et al*., 1997). The particular structure of bacterial spores (i.e., the presence of several envelopes), their low water content, and the presence of small soluble proteins (SASPs) account for their high resistance to antimicrobials (Russell, 1990). Intrinsic resistance of mycobacteria to biocides involves mainly the outer cell layer, which acts as an impermeability barrier (McNeil and Brennan, 1991; Russell, 1996; Russell *et al*., 1997). For example, Manzoor *et al*.

(1999) observed that the reduction in susceptibility of *M. chelonae* isolates to glutaraldehyde was associated with a change in cell wall polysaccharides. In Gram-negative bacteria, the outer envelope is responsible for resistance to both antibiotics and biocides. It has been suggested that Gram-negative bacterial susceptibility to biocides could be decreased with a change in cell permeability (McDonnell and Russell, 1999), for example, by changes to overall cell hydrophobicity (Tattawasart *et al*., 1999), but particularly outer membrane ultrastructure (Tattawasart *et al*., 2000a, b), protein composition (Brözel and Cloete, 1994; Gandhi *et al*., 1993; Winder *et al*., 2000), and fatty acid composition (Guérin-Méchin *et al*., 1999, 2000; Jones *et al*., 1989; Méchin *et al*., 1999).

The importance of the outer membrane impermeability of Gram-negatives in decreasing biocide susceptibility has been exemplified by the use of permeabilizers such as ethylene diamine tetraacetic acid (EDTA), which induces a release of lipopolysaccharides (LPS) (Ayres *et al*., 1998; McDonnell and Russell, 1999). Likewise, the use of permeabilizers in mycobacteria has been shown to reduce the intrinsic resistance of these microorganisms to biocides (Broadley *et al*., 1995). The investigation of bacterial spheroplasts can also provide information on the role of the outer cell layer in bacterial resistance to biocides (Fraud *et al*., 2003; Munton and Russell, 1970).

3.2.2. Multidrug efflux pumps

Multidrug efflux pumps have been shown to play a role in bacterial resistance to a wide range of antimicrobials (Nikaido, 1996; Paulsen *et al*., 1996a). Multidrug efflux determinants have now been found to be widespread in Gram-negative and -positive bacteria. They can be separated into five main classes: (1) the small multidrug resistance (SMR) family, which is now described as part of the drug/metabolite transporter (DMT) superfamily, (2) the major facilitator superfamily (MFS), (3) the ATP-binding cassette (ABC) family, (4) the resistance-nodulation-division (RND) family, and (5) the multidrug and toxic compound extrusion (MATE) family (Borges-Walmsley and Walmsley, 2001; Brown *et al*., 1999; McKeegan *et al*., 2003; Poole, 2001, 2002; Putman *et al*., 2000).

Bacterial resistance to QACs has been particularly studied and in many cases active-efflux systems have been involved (Heir *et al*., 1995, 1999; Leelaporn *et al*., 1994; Littlejohn *et al*., 1992; Lomovskaya and Lewis, 1992; Sundheim *et al*., 1998; Tennent *et al*., 1989). In *S. aureus*, several genes have been characterised; *qacA* and *qacB*, which confer high-level resistance, and *qacC* and *qacD*, which confer low-level resistance (Littlejohn *et al*., 1990; Rouche *et al*., 1990), *smr* (Lyon and Skurray, 1987), *qacG* (Heir *et al*., 1999), and *qacH* (Heir *et al*., 1998). In addition, *qacC* and *qacD* are similar to the *ebr* gene encoding for resistance to ethidium bromide in *S. aureus.* As a result,

resistance to QACs is often concurrent with resistance to ethidium bromide. In Gram-negative bacteria, similar efflux systems have been described. In *P. aeruginosa*, the MexAB-OprM, MexCD-OprJ, and MexEF-OprN (Schweizer, 1998), in *E. coli, emrA* (Lomovskaya and Lewis, 1992), *qacE* and *qacEΔ1* (Kazama *et al*., 1998). The role of efflux pumps in antimicrobial resistance is discussed further in Section 4.2.2.

3.2.3. Degradation

The degradation of biocides has been described in several investigations. Resistance to heavy metals (including mercury, copper, and silver) is usually associated with enzymatic reduction of the cation to the metal (Cloete, 2003). Likewise, the presence of aldehyde dehydrogenase in some strains has been responsible for the bacterial resistance to aldehyde and notably formaldehyde (Kummerle *et al*., 1996). The mechanism of action of oxidising agents such as peroxygens is via the production of free radicals. Microorganisms have developed a system to prevent and repair radical-induced damage, particularly with the synthesis of proteins such as catalases, superoxide dismutase, and alkyl hydroperoxidases (Demple, 1996). In *E. coli*, these enzymes are encoded by multigene systems such as *soxRS* and *oxyR*, which can be induced by biocides (Dukan and Touati, 1996). In addition, such multigene systems can confer cross-resistance to other oxidants (Greenberg and Demple, 1989; Greenberg *et al*., 1990). For example, induction by hydrogen peroxide confers resistance to hypochlorous acid and vice versa (Dukan and Touati, 1996). Degradation of the bisphenol triclosan has been described among environmental isolates (Hundt *et al*., 2000), although there is no evidence that degradation of bisphenol takes place in clinical isolates.

3.2.4. Alteration of target sites

The alteration of a specific target site is a well-established mechanism of bacterial resistance to antibiotics (Chopra *et al*., 2002), but not to biocidal agents, with the exception of the bisphenol triclosan. As previously mentioned, biocides interact with the bacterial cell by targeting multiple nonspecific sites (Maillard, 2002). Triclosan has been found to interact with an enoyl-acyl reductase carrier protein in a range of microorganisms including *E. coli* (Heath *et al*., 1998; McMurry *et al*., 1998b), *P. aeruginosa* (Hoang and Schweizer 1999), *Haemophilus influenzae* (Marcinkeviciene *et al*., 2001), *Bacillus subtilis* (Heath *et al*., 2000b), *M. smegmatis* (McMurry *et al*., 1999), *Mycobacterium tuberculosis* (Parikh *et al*., 2000), and *S. aureus* (Heath *et al*., 2000a; Slater-Radosti *et al*., 2001). Indeed, crystallographic studies have shown that triclosan binds specifically with FabI (Heath *et al*., 1999; Levy *et al*., 1999, Roujeinikova *et al*., 1999; Stewart *et al*., 1999). Mutations in the

M. smegmatis inhA and *E. coli fabI* genes have been associated with resistance of these microorganisms to triclosan (Heath *et al*., 2000a; McMurry *et al*., 1999; Parikh *et al*., 2000). However, while initially mutation in the *fabI* gene was described as responsible for triclosan resistance (McMurry *et al*., 1998a), other mechanisms of resistance have been described since (Gilbert and McBain, 2003), notably involving efflux pumps (Schweizer, 2001).

3.2.5. Biofilms

Biofilms are responsible for infections in the hospital environment by growing on implants, catheters, and other medical devices (Costerton and Lashen, 1984; Costerton *et al*., 1987; Gilbert *et al.*, 2003; Salzman and Rubin, 1995), but also by colonising various surfaces such as air conditioning systems and cooling towers. Biofilms are notoriously more resistant to biocide action that their planktonic cells (Allison *et al*., 2000). The involvement of biofilms in the resistance to biocides is complex and involves several mechanisms that work together towards decreasing or inhibiting the detrimental effect of a biocide (Gilbert *et al*., 2003). The emergence of bacterial resistance following phenotypic adaptation upon attachment to surfaces and within the biofilms is an important mechanism (Brown and Gilbert, 1993; Das *et al*., 1998), although impairment of biocide penetration into the biofilm matrix, quenching by the exopolysaccharides, and enzymatic inactivation have a role to play (Gilbert *et al*., 2003; Gilbert and Allison, 1999). For example, some biocides, such as iodine and povidone-iodine (Favero *et al*., 1983), chlorine, and peroxygens (Huang *et al*., 1995) have been shown to be inactivated to some extent by bacterial biofilm as a result of an interaction with the glycocalyx. Certain degradative enzymes, for example, formaldehyde dehydrogenase, can become concentrated within the glycocalyx and can lead to a decrease of biocidal activity, by hindering the penetration of biocide (Sondossi *et al*., 1985). Such mechanisms might lead to the formation of a sublethal gradient-concentration, which in turn might induce further resistance mechanism such as efflux (Gilbert *et al*., 2003).

4. EVIDENCE OF CROSS-RESISTANCE BETWEEN BIOCIDES AND ANTIBIOTICS

4.1. Evidence of bacterial resistance to biocides and antibiotics

Possible linkage between biocide and antibiotic resistance in bacteria has been discussed in the literature, particularly within the last few years

(Bloomfield, 2002; Levy, 2000; Russell, 2000, 2002a; Russell and Maillard, 2000; Russell *et al*., 1999; Schweizer, 2001).

In *S. aureus*, β-lactam resistance has been associated with QAC resistance (Akimitsu *et al*., 1999). Chuanchuen and colleagues (2001) observed that triclosan-resistant *P. aeruginosa* possessed elevated MICs to several antibiotics. Chlorhexidine-resistant *P. stutzeri* have also been found to be resistant to some antibiotics (Russell *et al*., 1998; Tattawasart *et al*., 1999). Cross-resistance in *E. coli* has also been observed between pine oil, triclosan, and multiple antibiotics (Cottell *et al*., 2003; Moken *et al*., 1997), and diverse biocides (e.g., QACs, amine oxide) and multiple antibiotics (Walsh *et al*., 2003), although in these studies, only a low-level resistance was observed. Triclosan exposure of triclosan-sensitive mutants of *P. aeruginosa* produced an important increase in resistance to the bisphenol and ciprofloxacin (Chuanchuen *et al*., 2001). However, other studies showed that the isolation of laboratory triclosan-resistant *S. aureus* was not necessarily correlated with an increase in antibiotic resistance (Slater-Radosti *et al*., 2001; Suller and Russell, 1999).

Methicillin-resistant *S. aureus* (MRSA) strains exhibiting low-level resistance to triclosan (MICs 2–4 μg/ml) and resistance to mupirocin (MIC > 512 μg/ml) have been isolated from patients treated with nasal mupirocin and daily triclosan baths (Cookson *et al*., 1991a). However, Suller and Russell (2000) did not find changes in triclosan MICs in MRSA strains after the acquisition of a plasmid encoding for mupirocin resistance. Likewise, chlorhexidine appeared to be as effective against MRSA strains as against their sensitive counterparts (Cookson *et al*., 1991b).

4.2. Possible mechanisms involved in bacterial resistance to biocides and antibiotics

The main difference between antibiotic and biocide is their mechanism of action at their "in use" concentration. Whereas antibiotics have (generally) a unique target site within the bacterial cell (Chopra, 1998; Chopra *et al*., 2002), biocides are different in having multiple target sites, the inactivation/ alteration/ destruction of which lead to an inhibitory or lethal effect (Hugo, 1999b; Maillard, 2002). Microorganisms have developed several mechanisms to survive the lethal or static effects of antibiotics (Poole, 2002) and biocides (see Section 3.2). The main difference between these mechanisms is that the alteration of target sites and the degradation of drugs are far more common against antibiotics than against biocides. Nevertheless, some mechanisms bear some similarities, particularly, impermeability and efflux systems and can lead potentially to a linkage in resistance to both antibiotics and biocides. In addition, the induction of some mechanisms (e.g., efflux and change in

permeability properties) and the acquisition of genetic elements play an important role in the dissemination of bacterial resistance to both antimicrobials.

4.2.1. Changes in membrane permeability

Changes in the outer membrane have been shown to confer resistance to both antibiotics and biocides in some investigations. For example, Tattawasart *et al*. (1999) showed that *P. stutzeri*, which developed cationic resistance, also presented an altered antibiotic susceptibility profile.

4.2.2. Induction of multidrug efflux systems

Multidrug efflux systems have been particularly involved in bacterial resistance to both biocides and antibiotics. The structure and function of these pumps have been reviewed recently by Borges-Walmsley and Walmsley (2001), Poole (2001), and McKeegan *et al*. (2003). In *P. aeruginosa*, the MexAB-OprM has been involved in bacterial resistance to several unrelated antibiotics (Schweizer, 1998), but also to some biocides. In *S. aureus*, several proteins involved in efflux mechanisms have been described: QacA, QacB, Smr (QacC, QacD, Ebr), SepA, QacEΔ1, QacG, and QacH (Borges-Walmsley and Walmsley, 2001; McKeegan *et al*., 2003; Narui *et al*., 2002). NorA is another efflux pump which has been linked to the low-level resistance to some antiseptics and to fluoroquinolones in *S. aureus* (Noguchi *et al*., 2002). In *E. coli*, the Acr-AB-TolC multidrug efflux pump exhibits a broad-spectrum activity, targeting antibiotics and biocides such as QACs and phenolics (George and Levy, 1983; McMurry *et al*., 1998b; Moken *et al*., 1997; Zgurskaya and Nikaido, 2000).

Bacterial exposure to sub-effective concentration of some biocides, such as phenolics (Levy, 1992), has been shown to upregulate the expression of multidrug resistance operons and efflux pumps. In particular, the induction of the *mar* phenotype and its relevance to cross-resistance between triclosan and pine oil, and multiple antibiotic resistance has been well studied (Moken *et al*., 1997). Overexpression of MarA leads to an increase in MIC triclosan and to antibiotics (McMurry *et al*., 1998a). A similar phenomenon has been observed with the overexpression of SoxS and AcrAB efflux pumps (McMurry *et al*., 1998a; Wang *et al*., 2001).

4.2.3. Alteration of target sites

As mentioned previously, the mechanism of action of triclosan at a low concentration is (at the moment) unique among biocides, since the bisphenol has been shown to target, specifically, a bacterial enzyme. In *M. smegmatis*,

mutation in *inhA* confers resistance to not only triclosan but also to isoniazid (McMurry *et al*., 1999).

4.2.4. Acquisition of genetic determinants

The presence of resistance determinants on mobile genetic elements favors the spread of resistance via horizontal gene transfer. The presence of genes encoding for resistance determinants have been detected on genetic mobile elements such as conjugative plasmids, transposons (Lyon and Skurray, 1987), and integrons (Paulsen *et al*., 1993). For example, the *qacA/B* genes have been found on plasmids encoding β-lactamases and heavy metal resistance determinants. Likewise, the *smr* gene has been detected on large conjugative plasmids with multiple-resistance determinants (Bjorland *et al*., 2001; Lyon and Skurray, 1987). In *S. aureus*, the plasmid pSK01 also carries trimethoprim and aminoglycosides resistance. Paulsen and colleagues (1993) found the *qacE* and *qacEΔ1* genes to be located on the 3′ conserved sequence of integrons in Gram-negative bacteria. These genes have been associated with multiple resistance to biocides and antibiotics in clinical isolates of Gram-negative bacteria (Kücken *et al*., 2000). Lemaitre and colleagues (1998) showed that QACs resistance in listeriae resulted from the transfer of plasmids, which presented high frequency of transfer. Inorganic (Hg^{2+}) and organomercurial resistance can be carried on plasmids encoding for penicillinase in clinical isolates of *S. aureus* (Shalita *et al*., 1980). Plasmids that carry genes encoding for antibiotic resistance but also metal ion resistance are not uncommon in Gram-negative bacteria (Silver *et al*., 1989). Foster (1983) recognised that mercury resistance was inducible and transferable by conjugation and transduction.

Pearce *et al*. (1999) studied the effects of sub-inhibitory concentrations of several biocides in the transfer of antibiotic resistant genes in *S. aureus.* Povidone-iodine at a low concentration (0.005%) reduced the conjugation efficiency of the pWG613 plasmid, while other biocides (at sub-inhibitory concentrations), namely cetrimide and chlorhexidine, had no effect. However, both the biguanide and povidone-iodine effectively inhibited transduction, whereas cetrimide (0.0001%) increased the transduction efficiency.

4.2.5. Biofilms

The multicomponent resistance response of a biofilm accounts for its resistance to both biocides and antibiotics (Gilbert *et al*., 2003). It was previously mentioned that the concentration of degradative enzymes within the glycocalyx might play a role in biocidal resistance by enhancing the barrier activity through degradation of molecules. Enzymes such as β-lactamase have also been found within the glycocalyx (Giwercman *et al*., 1991) and might

play a role in the bacterial biofilm resistance to antibiotics (Stewart, 1996). Other mechanisms such as the ones described in Section 3.2.5, namely the presence of a "biofilm-associated, drug-resistant phenotype" (Ashby *et al*., 1994), the induction of multidrug resistance operons, and efflux pumps, also play an important role (Maira-Litrán *et al*., 2000).

5. DISINFECTANT USAGE AND ANTIBIOTIC RESISTANCE

Following the evidence of a possible linkage between biocide and antibiotic resistance in bacteria, several authors have claimed that the usage of biocides was deemed to cause the emergence of antibiotic resistance.

There are a number of cases whereby the use of a biocide was associated with emerging antibiotic resistance in bacteria. The use of silver sulphadiazine for the treatment of burn infection which was somewhat successful (Lowbury *et al*., 1976) in decreasing patient mortality, was also linked to the emergence of plasmid- mediated sulphonamide resistance (Bridges and Lowbury, 1977). Newsom *et al*. (1990) observed that although the use of chlorhexidine scrub-based preoperative showers effectively decreased skin flora, it might also promote the growth of staphylococci post-surgery, and notably those presenting high-resistance to methicillin. However, similar observations were made when antibiotic prophylaxis was used instead of biocide (Archer and Armstrong, 1983).

Triclosan has been particularly investigated since the number of products containing the bisphenols (and notably household products) has increased dramatically over the last few years. Although bacterial resistance to triclosan has raised concerns (Chuanchuen *et al*., 2001; Heath and Rock, 2000; Lear *et al*., 2000; Levy, 2001), emergence of bacterial resistance to other biocides commonly used in the hospital environment have been described, notably to chlorhexidine. Gram-negative bacteria with a significant increase in chlorhexidine resistance and to several antibiotics have been isolated from patients with long-term indwelling catheterization after the extensive use of the biguanide to treat urinary tract infections (Stickler 1974; Stickler and Chawla, 1988; Stickler *et al*., 1983). However, no plasmid-linked association between antibiotic and chlorhexidine resistance was isolated. Tattawasart *et al*. (1999, 2000a, b) observed an increase in chlorhexidine insusceptibility in *P. stutzeri* and several antibiotics after repeated exposures to the biguanide. However, Thomas *et al*. (2000) noted that although exposure to low residual concentration of the biguanide resulted in an increased insusceptibility of *P. aeruginosa* to the biocide, there was no cross-resistance to any of the antibiotics tested. In staphylococci, chlorhexidine resistance has almost always been associated with antibiotic resistance. Reverdy *et al*. (1992) concluded that the spread of

resistant staphylococci in the hospital environment was increased by the use of either antibiotics or biocides.

The selective pressure exerted by the use of cationic biocides has been deemed to play a role in the dissemination of *qac* genes and thus the widespread occurrence of multidrug efflux pumps (Heir *et al*., 1998, 1999; Mitchell *et al*., 1998; Paulsen *et al*., 1996b, c; Sundheim *et al*., 1998). However, Russell (2002c) pointed out that although a link between the introduction of cationic biocides and the spread of resistance determinants in staphylococci is conceivable, further evidence and investigations were needed to warrant this possibility.

Other biocides have also been involved in the emergence of antibiotic resistance in bacteria. Armstrong *et al*. (1981, 1982) isolated multiple-antibiotic resistant bacteria from drinking water. Disinfection and purification of water, and notably chlorination, have been suggested to increase the occurrence of antibiotic-resistant bacteria (Murray *et al*., 1984).

It has been observed that clinical isolates of Gram-negative bacteria tend to be more resistant to biocides than their culture collection counterparts (Hammond *et al*., 1987; Higgins *et al*., 2001). This might be explained by the repeated exposures to the selective pressure provided by disinfection (Russell, 2002c). It was also proposed that the overuse of biocides might provide a selective environment for less susceptible microorganisms such as MRSA (Levy, 2000). However, at an "in-use" concentration of a biocide, antibiotic resistant microorganisms are not necessarily more resistant to biocides than their sensitive counterparts (Russell 1999b, 2000). It is pertinent to note that selective pressure from biocide exposure is not necessarily correlated with an increase in bacterial resistance to biocides and antibiotics. Lear *et al*. (2000, 2002) isolated only two microorganisms (*Acinetobacter johnsonii* and *Citrobacter freundii*) showing elevated resistance to triclosan from industrial sites where biocides are manufactured (apart from a multitude of intrinsically resistant pseudomonads). However, the authors were not able to correlate increased triclosan resistance with a change in antibiotic susceptibility profile (unpublished data). For further information on this subject, Russell (2002c) comprehensively reviewed the current evidence of biocide usage and the emergence of antibiotic resistance in hospital.

Disinfectants are used in general at a very high concentration, often exceeding 1,000 times that of their MIC, in order to achieve a rapid rate of kill. At a high concentration, a biocide will interact with multiple target sites on the bacterial cell, producing multiple damages, which ultimately will account for cell death. In addition, at these concentrations, it is unlikely that bacteria will become resistant through adaptation or other mechanisms. However, it has to be noted that some bacteria might be intrinsically resistant to a biocide, as already mentioned. Hence, one has to take into account the type of microorganism likely to be encountered and the level of disinfection that needs to be achieved.

Furthermore, the concentration of biocides and the conditions of use (i.e., presence of organic load, temperature, bioburden, etc.) are of paramount importance to achieve an overall lethal activity (Russell and McDonnell, 2000).

6. CONCLUSION

The introduction of biocides into the hospital environment and the selective pressure exerted by their extensive use might confer some level of, or select for, biocide-resistant bacteria, which might impact ultimately on antibiotic resistance. However, it is pertinent to remember that the selective pressure exerted by the extensive use, and sometimes misuse, of antibiotics themselves bears a far greater significance in the emergence of antibiotic resistant microorganisms (WHO, 2000). The development of antibiotic resistance caused by overuse is particularly well documented (French and Phillips, 1999; Gould and MacKenzie, 2002; Hossein *et al*., 2002). However, there is an increasing body of evidence of shared bacterial resistance mechanisms between biocides and antibiotics. Although the historical use of biocides for hospital disinfection is unlikely to have caused the emergence of antibiotic resistance in hospitals, the improper use of biocides or the use of low sub-inhibitory concentration of a biocide might lead to the development of bacterial resistance to biocide (at last at the MIC level) and to some low-level resistance to antibiotics.

One of the main questions is the clinical relevance of such resistance. Several authors have expressed their doubts as to whether the bacterial resistance described in the literature (notably evidence from *in vitro* investigations) has relevance in practice. It has been pointed out that genes encoding for efflux pumps do not necessarily predominate in MRSA when compared to their sensitive counterparts (methicillin-sensitive *S. aureus*, MSSA) (Bamber and Neal 1999; Suller and Russell, 1999). Furthermore, biocides such as triclosan are still effective in killing hospital strains such as MRSA (Webster 1992; Webster *et al*., 1994; Zafar *et al*., 1995). It has been suggested that although a low-level biocide resistance might be produced by efflux systems, the concentration of cationic biocides used in practice is much higher. At these concentrations, where multiple target sites are attacked, it is unlikely that efflux mechanisms will operate efficiently (Favero, 2002; Russell and Maillard, 2000). Finally, some authors and institutions have advocated the rotation of biocide used for low-level disinfection (Murtough *et al*., 2001).

When employed correctly, biocides have an important role to play in combating infection in the hospital environment (Larson *et al*., 2000; Russell, 2002a). Therefore, it is important to ensure that biocides are used appropriately, which implies a compliance with disinfection and antisepsis (notably handwashing) regimens and possibly, staff training programs. Overall, more

information is needed to establish whether the use of biocides in the clinical context induces the emergence of antibiotic resistance. Bacterial resistance to low (residual) concentration of biocide, notably to cationic and phenolic compounds, might need to be monitored.

Monitoring and understanding the mechanism(s) involved in the emergence of bacterial resistance to biocides are important, since they can indicate whether a disinfection failure results from an operator (no compliance with manufacturer instructions), a product, or the formation of bacterial biofilm. They might further indicate a possible risk for cross-resistance with antibiotics.

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