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Review

BACTERIAL RESISTANCE TO ANTISEPTICS AND DISINFECTANTS – MINIREVIEW

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Summary

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The appearance of bacterial resistance to disinfectants and antiseptics is an issue of substantial health concern, resulting in low efficiency of epidemic control activities and emergence of microorganisms with cross-resistance to antibiotics and biocides. A synopsis of the main mechanisms of development of resistance to biocides is presented. The emphasis is placed to health risks and impact on medical practice. The main methods for detection of resistance, and prevention measures of key importance for its control are outlined.

Key words: antiseptics, bacterial resistance, biocides

INTRODUCTION

During the past decades, numerous antibacterial substances – antibiotics, chemotherapeutics, antiseptics and disinfectants have been implemented in medical practice, which expanded substantial pathogen control arsenal and gave rise to optimistic prospects with regard to infectious diseases control. Unfortunately, a marked global tendency towards increase in microbial resistance to antibiotics is noted with resultant serious challenges to the treatment and control of infectious diseases in animals and men (Rossolini *et al.,* 2014). Improper use is outlined as the main cause for the emergence of resistance to antibiotics: abuse, insufficient doses, rather short or rather long treatment courses. Thus, natural selection stimulates microbial evolution with consequent continuous corrections in their genome and phenotype to make microorganisms more adaptable to the adverse environment (Wolff, 1993).

Apart towards antibiotics, microorganisms develop also resistance to various biocides, including disinfectants and antiseptics. It is established that some of mechanisms conferring resistance are common for both antibiotics and biocides. The number of evidence demonstrating that the frequent and repeated contact with some antiseptics and disinfectants leads to

development of resistance both to the specific substance and to some other biocides/antibiotics e.g. development of cross-resistance is continuously increasing (Gnanadhas *et al.,* 2012).

The selective pressure exerted by biocides could benefit the survival of resistant pathogens and lead to their broader spread with all ensuring health hazards. Unlike antibiotic resistance which is long known and extensively studied, the emergence of bacterial resistance to antiseptics and disinfectants is an often neglected problem whose health and practical impact is not fully recognised.

DISINFECTION

Disinfection is one of the basic epidemic control activities aimed at reduction of potential risk from onset and spread of infectious diseases among animals and men (Russell & Russell, 1995; Rutala, 1995; Karadzhov*,* 2013). The terms for chemical agents used in disinfection practice discriminate them with respect to the mode and site of action (Russell & Russell, 1995; Dvorak, 2008; Karadzhov*,* 2013): *biocides* – a more general term denoting chemical agents capable to destroy or irreversibly inactivate most pathogens or to inhibit their growth and development;; *disinfectants* – biocides applied on surfaces or other non-live objects; *antiseptics* – applied to living tissue e.g. skin, mucosae.

RESISTANCE

In general, resistance is the ability of microorganisms to survive the impact of harmful agents (Maillard*,* 2013). Biocide resistant microorganisms could be defined as: 1) strains that could not be killed or inhibited by concentrations of biocides that are usually used in the practice; 2) strains that could not be killed or inhibited by biocide concentrations, to which other strains of the same species are susceptible; 3) bacterial cells that could not be killed or inhibited by concentrations that are effective against most cells of the bacterial culture (SCENIHR, 2009b).

Microbial resistance could be inherent or acquired through mutation or exchange of mobile genetic elements (Poole, 2002). It is acknowledged that the natural resistance of microorganisms to environmental factors (high temperature, UV radiation, redox potential, ionising radiation etc.) and resistance/sensitivity to biocides are different. These variations are a manifestation of the inherent resistance of microorganisms, naturally encoded in the respective bacterial genome, expressed in all strains of a given species and due to different cell structure, composition and physiological features. Inherent resistance, this is the high level of resistance could be most accurately described as "insensitivity", as the specific microbial species has never been sensitive to the antimicrobial agent. For instance, non-enveloped viruses and bacterial spores are insensitive to the effect of quaternary ammonium compounds (QAC), alcohols and phenols (Karadzhov*,* 2013).

Acquired resistance is present when a given microorganism becomes resistant to a specific antimicrobial agent, to which it was previously sensitive. As s rule, multiple cell structures are targeted by biocides. Acquired resistance is usually associated to alterations of these target structures or structures that consequently impede the access of biocides to a specific target. Examples are modification of the cell envelope, changes in cell permeability, efflux pumps activity and conversion (biotransformation) of biocides into harm-

less metabolites (Davin-Regli *et al.,* 2008). Unlike inherent resistance, the acquired one is not a feature of the entire species, but of specific strains or subpopulation (Maillard*,* 2013).

Intrinsic resistance to disinfectants and antiseptics

To reach the target site of action, antiseptics and disinfectants should pass through the outer cellular layers. That is why, the specific structural features and functions of cell envelope determining its permeability are among the most important factors of inherent resistance (McDonnell & Russell, 1999). For instance the different cellular wall structure in Gr+ and Gr- bacteria is the reason for their different biocide resistance. The outer membrane of Gr- bacteria is a barrier restricting the entry of chemically different antimicrobial agents so as a rule, vegetative Gr- bacteria are more resilient than Gr+ (McDonnell & Russell, 1999). For example, the concentrations of benzalconium chloride (QAC) needed to inhibit the growth of *E.coli* and *P.aeruginosa* are 100 and 500 times higher, respectively compared to those for *S. aureus*. Similar relationships have been observed for other disinfectants – chlorhexidine, hexachlorophene and triclosan. The exceptionally high resistance of *P. aeruginosa* to these disinfectants is attributed to the higher amount of fat and cationic elements in its outer membrane, higher magnesium content and formation of stronger bonds among lipopolysaccharides as well as to lower size of porins, which does not allow biocides' simple diffusion (Brown, 1975, McDonnell & Russell, 1999; Chevalier *et al.,* 2017). At the same time, the higher amount of lipids in the cellular wall makes *P. aeruginosa* substantially more sensitive to *o*-phenylphenol (McDonnell & Russell, 1999). The knowledge and compliance with these specific features is essential for selection of an appropriate disinfectant in the practice and high efficacy of disinfection procedures.

Acquired resistance to disinfectants and antiseptics

It results from microbial alterations caused by mutation and/or changed expression of endogenous genes, as well as by transfer of exogenous genes (e.g. plasmids). These changes could be irreversible or transient, and as a rule are an expression of adaptation of microorganisms to changed environmental conditions (Wales & Davies, 2015).

Genetic changes in microorganisms could lead to several different mechanisms of resistance: altered target structure, reduced permeability of the cell envelope, microbial enzyme alterations with appearance of isoforms of higher activity or novel biocide-degrading enzyme synthesis, modification of efflux pumps resulting in enhanced biocide evacuation from microbial cells and others (Seier-Petersen, 2013).

One of the commonest adaptation responses from contact with sublethal concentrations of biocides, especially in Gr- bacteria, is the change in outer cell membrane permeability. It is mainly manifested with increased amount of lipids and lipopolysaccharides, reduction of porins, making the penetration of biocides more difficult (McDonnell & Russell, 1999; Denyer & Maillard, 2002; Sticker, 2004). It is found out that the decreased permeability of cell membrane could be due to modification of the composition of proteins, fatty acids and phospholipids with resultant change in membrane ultrastructure (Gandhi *et al.,* 1993; Méchin *et al.,* 1999; Winder *et al.,* 2000; Boeris *et al.,*

2007). Resistance to biocide could occur also secondary to modifications in other target cell structures. A classical example is resistance to triclosan (substance blocking the activity of bacterial and fungal enoyl reductase). Mutations in enzymatic structure decrease triclosan binding affinity and results in building of resistance, described in numerous microbial species (Maillard, 2013).

Another common mechanism of resistance emergence is the modification (overexpression) of bacterial efflux pumps after sublethal contact with a biocide (Bailey *et al.,* 2009). Microbial efflux pumps comprise transport proteins that reduce intracellular content of toxic compounds, including biocides. Numerous chromosomally coded multidrug efflux pumps with enhanced activity have been described in Gr- bacteria (EmrE, MsfA, SugEE, PmpM etc.), responsible for the lower microbial sensitivity to QACs, triclosan and chlorhexidine (Seier-Petersen, 2013). In Gr+ bacteria, chromosomally encoded efflux pumps are less frequently encountered, but these are detected in *S. aureus* (Price *et al.,* 2012). An example for plasmid-encoded resistance to biocides and efflux pump alterations is methicillin-resistant *S. aureus* (MRSA) (Sasatsu *et al.,*1995). MRSA possess *qac* genes, plasmid DNA fragments encoding resistance to β-lactam antibiotics, heavy metals and some biocides as QACs, diamines, biguanides (chlorhexidine), as well as to some dyes e.g. acridines (McDonnell & Russell, 1999; Jaglic & Cervinkova, 2012). Multiple research reports have shown that MRSA exhibits a substantially higher resistance to cationic antiseptics and disinfectants than conventional strains (Al-Masaudi *et al.,* 1984; Townsend *et al.,* 1984; Brumfitt *et al.,* 1985; Gillespie *et al.,* 1989; Cookson *et al.,* 1991). In a study with 120 MRSA isolates, Irizarry *et al.* (1996) reported that MICs of QAC and chlorhexidine in MRSA were 5–10 times higher and suggested that residual amounts of antiseptics and disinfectants presented everywhere in hospital environments contributed to the selection and maintenance of MRSA strains in these facilities with all important clinical effects.

A less frequently encountered mechanism of emerging resistance is the synthesis of enzymes degrading and inactivating antimicrobial substances (Hugo, 1991; Ogase *et al.,* 1992; Bloomfield, 2000). An example is the development of resistance of aldehydes consequently to aldehyde dehydrogenase synthesis. In some clinical *Enterobacteriaceae* isolates and some *P. aeruginosa* and *P. putida* strains, formaldehyde is reduced by glutathione-dependent dehydrogenase, encoded by the *adhC* gene, part of plasmid DNA (Kummerle *et al.,* 1996; Seier-Petersen, 2013). In a similar manner, the active free radicals of disinfectants releasing chlorine and iodine could be inactivated by synthesis of catalases, superoxide dismutases or alkyl hydroperoxidases (Greenberg *et al.,* 1990; Demple, 1996).

RELATIONSHIPS BETWEEN BIOCIDE USE AND RESISTANCE TO ANTIBIOTICS AND DISNIFECTANTS

The antibacterial effect of biocides and antibiotics is realised through comparable mechanisms. Under the action of toxic stress exerted by one chemical agent, microorganisms react with adaptation response which could be also efficient against other chemically unrelated antimicrobial drugs – e.g. emergence of cross resistance to biocides and antibiotics (SCENIHR, 2009a). In most instances,

resistance is due to alterations in cellular efflux pumps (Levy, 2002), but also to changes in cell envelope (Denyer & Maillard, 2002). It is established that Gr- bacteria could decrease the amount of harmful intracellular agents by reduction of outer membrane permeability, through reduced synthesis of porins, increased amount of lipopolysaccharides and lipids or overexpression of efflux pump membrane proteins. This adaptation response incurs simultaneous resistance to antibiotics and biocides (Denyer & Maillard, 2002; SCENIHR, 2009a).

The risk from emergence of antibiotic resistance consequently to biocide use could be either direct or indirect. The direct risk is associated with selection of microorganisms resistance to both groups of antimicrobial drugs, under the pressure of biocides. The indirect risk comes from the transfer of mobile genetic elements encoding common mechanisms of resistance. A third option is possible – simultaneous presence of both risks which would result in exchange of resistance genes among already resistant bacteria, hence in resistance spectrum broadening (SCENIHR, 2009a). It is reported that the use of some biocides e.g. QACs, biguanides and phenols induces resistance more easily. It is affirmed that the use of cationic biocides like QAC and chlorhexidine is the exact cause for the wide spread of *qac* genes responsible for multidrug efflux pumps (Paulsen *et al.,* 1996). The application of triclosan also leads to development of the same type of resistance mechanisms (Schweizer, 2001). That is why the extensive use of the same biocides, were they triclosan or QAC, as disinfectants, sanitisers and preservatives in personal hygiene products has a cumulative effect and increases the risk from emergence of cross resistance (SCENIHR, 2009a).

A primary risk factor for spread of resistance to antibiotics and biocides is the bacterial predisposition to acquire and transfer it through genetic exchange. Three mechanisms of horizontal genetic transfer are acknowledged: 1) transduction: via bacteriophages, 2) transformation: through diffusion of genetic elements to the cell and their incorporation in the genome, and 3) conjugation. The latter is perhaps the most efficient transfer realised through direct contact between two cells, allowing for transfer of plasmids, transposons and other genetic determinants (Dzidic & Bedeković, 2003). The members of the genus *Enterococcus* and family *Enterobacteriaceae* are the most prone to genetic exchange (Courvalin, 1994).

The extensive utilisation of biocides, for instance in agriculture, livestock husbandry and household and industrial products could lead to spread of mobile genetic elements encoding for mechanisms of resistance and selective replication and accumulation of resistance bacteria in the environment.

METHODS FOR DETECTION OF RESISTANCE TO BIOCIDES

Regardless that so far, there is no standardised method for detection and evaluation of microbial resistance to biocides, as well as cross-resistance to biocides and antibiotics, several laboratory techniques could be applied to this end. They are the determination of the minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC) of the biocide in bacterial isolates, suspension or surface tests for evaluation of the efficiency of disinfectants and antiseptics, microbiological antibiotic sensitivity tests, and molecular-genetic techniques for detection of genes determining resistance

(Sekiguchi *et al.,* 2004; Knapp, 2014; Knapp *et al.*, 2015).

MEASURES FOR BIOCIDE RESISTANCE CONTROL

Biocides are broadly spread chemical agents and ingredients of various products – disinfectants, antiseptics, preservatives in cosmetics, pesticides etc. The authorisation for placing on the market and use of biocides at the territory of the European Union is regulated by Regulation (EU) No 528/2012 of the European Parliament and of the Council (Anonymous, 2012). It includes also a requirement to manufacturers of biocides to provide information and evaluation of the probability for emergence of resistance among targeted microorganisms. Regulations associated to the use of biocides in the EC and the USA are continuously developing. With regard to the increasing use of biocides and the aggravation of the problem with resistance to antibiotics, additional data and implementation of procedures for risk assessment and control are necessary (SCENIHR, 2009a). Data for the environmental impact of disinfectant and antiseptic residues should be expanded. In the soil and wastewater, biocides are already diluted, e.g. at sublethal concentrations which could give rise to development of resistance among commensal microbial species and further, through genetic exchange, to transfer resistance to pathogens of public health importance. That is why, the collection and purification of wastewater containing traces of biocides is an important step to decrease this specific risk (Nuñez & Moretton, 2007).

The initial stage of the global control on resistance should include implementation of monitoring programmes for surveillance of the of biocide resistance level and development of cross resistance in all fields involving biocide application (SCENIHR, 2009a). In our belief, a primary measure should imply the introduction of microbiological monitoring on the sensitivity of target isolates from hospitals to prescribed biocidal concentrations on a periodical basis. It is demonstrated that one of the possible and highly hazardous sites for occurrence of resistant strains are containers where solutions of disinfectants or antiseptics are stored (Weber *et al.,* 2007). That is why, the periodical tests of microbiological purity of working solutions has been advised.

To decrease the risk from emergence of resistance in the practice, it is important that biocides are applied at an efficient concentration and exposure that would decrease the probability from sublethal exposure of bacteria and occurrence of adaptation responses (Russell & McDonnell, 2000). Another relevant recommendation is biocide use at a rotational basis e.g. periodical change of the used active substance. The frequent changes of used disinfectants could reduce the probability from development of resistance and limit the accumulation of resistant microorganisms in the environment (Murtough *et al.,* 2001). There is no specific answer to the questions about biocides' rotation frequency – it depends on the type of disinfectant, the site and conditions for its application, as well as the extent of microbial contamination. The frequency should correspond to the risk and probability of development of resistance to the respective chemical agent and the "more frequent is better" principle. The most commonly recommended period for alternation of active substances of disinfectants in the practice is one month (Murtough *et al.,* 2002). The efficient rotation of disinfectants however requires detailed knowl-

edge on their mechanisms of action – rotation would be efficient only if the disinfectant that would be used next has a different mechanism of action and acts on different microbial target structure. Thus, the replacement of a QAC disinfectant with a product from the biguianide group e.g. chlorhexidine would be irrational and erroneous due to the similar target and mechanism of action (Karadzhov, 2013).

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