Emergence of bacterial resistance to microbicides and antibiotics

Public awareness of the role of microorganisms in infection and spoilage and the role of the media in highlighting poor hygiene and the failure of healthcare settings in preventing hospital-acquired infections, have fuelled the use of products containing one or several microbicides in the healthcare environment but also at home. The number of such products with a microbicidal claim is increasing rapidly, although their impact on the microbial flora, notably in terms of emerging antimicrobial resistance has not been documented. With increasing evidence that microbicides can lead to bacterial resistance and cross-resistance to antibiotics, concerns have been expressed regarding the indiscriminate use of microbical products. This review aims to provide up-to-date information on the use of microbicides in the healthcare settings.

Bacterial resistance to chemotherapeutic antibiotics is highly topical and raises concerns about how to treat certain life-threatening bacterial infections. There is no doubt that the major contributor for emerging bacterial resistance to antibiotics is the use and misuse of these compounds. Recently, questions have been raised about the role of other antimicrobial agents, notably the microbicides, in the development and dissemination of antimicrobial resistance in bacteria (and other microorganisms). Microbicide-containing products are used widely in an increasing number of applications. The worldwide overall tonnage use per individual microbicide is difficult to estimate, but it is likely to increase, in accordance with their increased usage. In the healthcare industry, microbicalidal products have an important role to play for the control of microbial spoilage and infection. In this environment, microbicides are used mainly as preservatives in medicines and pharmaceutical products, for antisepsis and surface disinfection. However, they can also be found incorporated or impregnated in medical devices (for example, implants) and dressings, and are increasingly used in textiles (for example, bed linen, gowns) and in environmental surfaces (for example, bed rails, wall panels, door knobs, plastics). Owing to the increasing number of products containing a microbicide, it is pertinent to reflect on the risks associated with their usage, particularly in terms of emerging bacterial resistance, and potential dissemination of resistance determinants. This article reflects on the evidence supporting emerging bacterial resistance to microbicide resistance and its implication in practice.

Microbicide usage and healthcare

Microbicides are invaluable compounds in the healthcare settings where they are used for surface disinfection, hand disinfection, for the disinfection or sterilisation of specialised medical equipment (for example, endoscopes), and for the preservation of medicines and pharmaceutical products. Microbicides play an important role in preventing spoilage and infection in a wide range of applications, by decreasing the level of bacteria present, usually on surfaces, and as such they are a cornerstone of any effective prevention and control of healthcare-associated infections (HAI) program. At present there are two main schools of thought on how microbicides should be used in the healthcare environment. The first school advocates the indiscriminate use of microbicides for all surface disinfection, including floors and other non-critical surfaces, while the other school recommends a more prudent use, whereby microbicides should be restricted to the disinfection of critical and semi-critical surfaces. To date, there are insufficient data to favour one position over the other. It is clear, however, that the use of microbicides is increasing in healthcare settings, particularly following the use of these agents incorporated in various surfaces and textiles. Such a use, which is generally commercially motivated, has not been shown to have a beneficial impact in reducing HAI in practice, although, to be fair, such an impact is difficult to demonstrate in practice since a reduction in HAI depends on multiple factors. Nevertheless, there are not yet any published trial results on the effect of microbicalidal surfaces in controlling, for example, bioburden levels in healthcare settings.

An interesting practice is the use of ‘antimicrobial’ wipes on surfaces proximal to patients. Although the antimicrobial activity of such wipes might be questioned following stringent testing, their efficacy in removing bacterial contaminants from surfaces might be beneficial. The appropriate use of surface disinfectants and wipes is paramount in controlling microbial bioburden on surfaces. With that in mind, manufacturers have the responsibility to ensure that instructions for end users are clear,
Evidence of bacterial resistance to microbicides in practice

Bacterial resistance to microbicides used in practice has been reported since the 1950s, with the contamination of microbicide formulations including, for example, quaternary ammonium compounds (QACs), biguanides, and iodophors. More compelling evidence of bacterial resistance to a microbicide in practice has been reported with the use of glutaraldehyde for the high-level disinfection of medical devices. In the 1990s, Griffiths et al. isolated Mycobacterium chelonae strains resistant to the in-use 2% glutaraldehyde solution. These isolates were also shown to be cross-resistant to other microbicides such as sodium dichloroisocyanurate (1000 ppm), Virkon (1%) and Gigasept (10%). More recently, glutaraldehyde-resistant Mycobacterium massiliense were isolated from several hospitals in Brazil. These isolates were also resistant to a number of chemotherapeutic antibiotics including ciprofloxacin, cefoxitin and doxycycline, and were responsible for an HAI outbreak. In another study involving high-level disinfection, Martin et al. isolated several Gram-positive vegetative bacteria resistant to the in-use concentration of chlorine dioxide from an automated washer disinfector used for the sterilisation of medical devices. One Bacillus subtilis isolate (vegetative state) was also shown to be cross-resistant to hydrogen peroxide (7.5%). In most instances, the isolation of resistant bacteria to a specific microbicide follows the improper application of a microbicial product; the use of a lower concentration than the recommended one, the application of a shorter contact time, the lack of cleaning prior to disinfection, or the use of an incorrect regimen.

There is little doubt that a microbicide contributes to add a selective pressure in the environment. This is nicely illustrated with studies of long-term microbicide exposure in a complex microcosm. In such investigations a change in bacterial population has been observed; where susceptible bacteria disappear and the more resistant ones persist and/or thrive. While the infection control team should ensure that microbicidal products are used appropriately, the use of an incorrect regimen might still be susceptible to the in-use concentration of the microbicide. For example, Thomas et al. observed that P. aeruginosa with an elevated MIC to a cationic microbicide were nevertheless as susceptible as the parent strain to the in-use concentration of the microbicide. A similar conclusion was drawn by Lear et al. with the study of industrial bacterial isolates showing high triclosan or chloroxylenol MIC values. There have been relatively few studies that have looked at emerging bacterial resistance using in-use concentration of a microbicide and test conditions reflecting the use of the microbical product in practice. Interestingly, Cheeseman et al. observed that Staphylococcus aureus isolates showed different susceptibility to alcoholic hand rubs when in-use conditions were applied to the test; some formulations failed to produce a significant reduction in bacterial numbers in certain isolates even within a five-minute contact time. This investigation illustrated that the perception of bacterial resistance is complex, and that the descriptions of parameters and of the test protocol used are necessary to understand how resistance is defined within a specific study.

However, the multitude of studies based on elevated MIC as a measurement of resistance allowed the isolation and characterisation of bacteria expressing or displaying mechanisms, enabling them to survive a selective pressure caused by a microbicide, and contributed to our understanding of the mechanisms involved in bacterial resistance.

Mechanisms of bacterial resistance to microbicides

One of the problems associated with bacterial resistance to microbicide is that there is no rule-of-thumb and thus the predictability of a particular microbicide to induce resistance in a specific bacterium is difficult to establish. There have been many articles in the literature describing a number of bacterial resistance mechanisms. It is safe to say that bacteria possess and can express a number of diverse mechanisms enabling their survival to microbicide exposure (Figure 1). It is also likely that bacterial resistance to a microbicide results from the expression or presence of a combination of mechanisms.

While the bulk of evidence for bacterial resistance to microbicides comes from laboratory-based studies. The data are, however, often difficult to analyse as the type of microbicide, bacteria (including growth conditions and inoculum preparation), test conditions and test protocols differ widely between studies. Measurement of the minimum inhibitory concentration (MIC) is often used as a marker for resistance. This has been criticised since this type of measurement does not reflect the use of the microbicide in practice, and that bacteria with an elevated MIC might still be susceptible to the in-use concentration of the microbicide. While the infection control team should ensure that microbicidal products are used appropriately, the use of an incorrect regimen.

Evidence of resistance from laboratory settings

While the bulk of evidence for bacterial resistance to microbicides comes from laboratory-based studies. The data are, however, often difficult to analyse as the type of microbicide, bacteria (including growth conditions and inoculum preparation), test conditions and test protocols differ widely between studies. Measurement of the minimum inhibitory concentration (MIC) is often used as a marker for resistance. This has been criticised since this type of measurement does not reflect the use of the microbicide in practice, and that bacteria with an elevated MIC might still be susceptible to the in-use concentration of the microbicide. For example, Thomas et al. observed that P. aeruginosa with an elevated MIC to a cationic microbicide were nevertheless as susceptible as the parent strain to the in-use concentration of the microbicide. A similar conclusion was drawn by Lear et al. with the study of industrial bacterial isolates showing high triclosan or chloroxylenol MIC values. There have been relatively few studies that have looked at emerging bacterial resistance using in-use concentration of a microbicide and test conditions reflecting the use of the microbical product in practice. Interestingly, Cheeseman et al. observed that Staphylococcus aureus isolates showed different susceptibility to alcoholic hand rubs when in-use conditions were applied to the test; some formulations failed to produce a significant reduction in bacterial numbers in certain isolates even within a five-minute contact time. This investigation illustrated that the perception of bacterial resistance is complex, and that the descriptions of parameters and of the test protocol used are necessary to understand how resistance is defined within a specific study.
One of the most described mechanisms is a barrier to microbicide penetration conferred, for example, by the exosporium and spore coats in bacterial endospores, the outer membrane in Gram-negative bacteria, and the lipid-rich structure of the outer cell wall in mycobacteria. Extensive work has also been conducted to characterise the role of efflux pump systems as a mechanism of resistance in a wide range of bacteria. The expression of efflux systems might not be able to confer resistance to a high microbicide concentration; although it does confer resistance to a clinical concentration of chemotherapeutic antibiotics; but it is sufficient to enable bacteria to survive lower concentrations which may be found in a number of formulations. The enzymatic transformation of a microbicide, which effectively inactivates its activity, has been comparatively poorly studied. A number of microbicides, including metallic salts, parabens, aldehydes and oxidising agents have been shown to be degraded by various bacterial enzymes. It is, however, unclear whether or not such a mechanism can confer resistance to a high microbicide concentration. It is more likely that enzymatic transformation contributes to a small decrease in a microbicide concentration, enabling the bacteria to survive exposure to a low concentration of the product.

The modification of bacterial targets (by mutation) conferring resistance to a microbicide is a rare event and has only been described in depth with triclosan. Triclosan interacts specifically with an enoyl-acyl reductase carrier protein involved in fatty acid synthesis, and the modification of this enzyme has been shown to confer an increase in bacterial MIC.

### Bacterial changes following exposure to microbicides

The mechanisms described above are often intrinsic bacterial mechanisms. The contribution of microbicide exposure in changing bacterial structure, or in inducing the expression of specific mechanisms or of a stress-response is a more worrying aspect. A number of studies have described changes in bacterial membrane ultrastructure, including proteins, fatty acid composition, phospholipids, and membrane charge as a result of microbicide exposure. Microbicide exposure can also induce the expression and overexpression of multidrug efflux systems. The effect of bacterial mutation in conferring resistance to a microbicide has been little studied. Interestingly, a bypass of metabolic activity conferring resistance to the microbicide triclosan has been recently described.

It is interesting to note that the recent use of transcriptome analysis highlighted that the presence of microbicide induces the expression of multiple genes involved in drug efflux, resistance (for example, marA), oxidative stress response (for example, soxS) and membrane permeability (for example, ompR).

### Genetic dissemination of resistance genes

The maintenance and dissemination of bacterial resistance genes following microbicide exposure have not been particularly well-studied. Cationic microbicides have been implicated in the dissemination and maintenance of genetic mobile elements in various bacteria (staphylococci, mycobacteria, Escherichia coli). It has been speculated that horizontal gene transfer is the most likely mechanism for selecting and increasing antibiotic resistance. Extra chromosomal elements encoding for both microbicide and antibiotic resistance genes have also been reported in bacterial isolates. The dissemination of these mobile elements following the selective pressure from a microbicide may represent a significant risk owing to the intensive use of microbicides.

### Common mechanisms of bacterial resistance to microbicides and antibiotics

The role of microbicides in emerging bacterial resistance to chemotherapeutic antibiotics remains controversial. Evidence supporting such a claim mainly derived from the observation that bacteria with an increased insusceptibility to a microbicide might also show a decreased susceptibility to a number of antibiotics. While the amount of evidence is increasing, observations in situ remain scarce. Carson et al. highlighted a significant relationship between high MICs to QAC and triclosan and resistance to one or more antibiotics in a study performed in the community. Duarte et al. described the first in situ investigations linking failure of a microbicide, cross-resistance to chemotherapeutic antibiotics and an HAI outbreak. However, such findings are not universal. Other in vitro studies have also failed to make a direct link between a microbicide exposure and emerging clinical resistance to antibiotics; although the antibiotic susceptibility profile of microbicide-resistant mutants was often altered.

A number of mechanisms providing bacterial resistance to both microbicides and antibiotics have been described (Table 1), including the expression of multidrug efflux pumps, the overexpression of multigene components or operons (for example, mar, soxRS and oxyR), a change in outer cell wall permeability and, in the case of triclosan, an alteration of the target site.

Since it is well established that some mechanisms can confer resistance to both antibiotics and microbicides (at an in-use or therapeutic level), the main question is whether or not microbicides can induce the expression of these mechanisms and facilitate the transfer of resistance between bacteria in situ. A number of studies have implicated the use of cationic microbicides for the spread of qac genes and thus the occurrence of multidrug efflux pumps. The presence of conjugative plasmids involved in the co-transfer of genes encoding for resistance mechanisms to a specific microbicide (for example, enzymatic transformation, efflux pump) and antibiotic gene(s) has also been described with cationic microbicides and metallic salts.

### Biofilms and microbicide resistance

Bacteria are usually closely associated with surfaces and grow as a community (that is, biofilm). The mechanisms of resistance and cross-resistance described so far concerned individual bacterial
cells. When a bacterial community is considered, additional mechanisms contribute to bacterial survival when exposed to microbicides. As a result, bacteria in biofilms are usually less susceptible to antimicrobials than individual cells. Several mechanisms have been associated with a biofilm-resistant phenotype including a decrease in metabolism, quiescence and reduced penetration due to the presence of an extracellular polymeric matrix. In addition, bacteria within a biofilm can also regulate their membrane permeability, express efflux systems and secrete degradative enzymes and express (overexpress) efflux systems.

**Measuring bacterial resistance**

Protocols used to measure the bactericidal efficacy of microbicides are essential to provide reliable information on microbicide efficacy. Several standardised efficacy test protocols are available to test the lethal efficacy of a microbicide against planktonic or surface-attached bacteria, but there are no standard tests to measure bacterial resistance to microbicides or cross-resistance between microbicides and antibiotics. The determination of a MIC might be an indicator of microbial sensitivity change to a microbicide at best but it cannot measure the development of bacterial resistance. The measurement of growth kinetics can also be indicative of the interaction between a microbicidal microbicide and a bacterial population. The detrimental effect (stress) caused by the microbicide can be associated with a longer lag phase and a slower culture doubling time. However, to assess whether or not bacteria have developed resistance to a microbicide, the use of bactericidal efficacy tests or the determination of the minimum bactericidal concentrations (MBC) are the most appropriate methods, since they enable the comparison of susceptibility between resistant bacteria and their susceptible counterpart. Such methods also reflect the use of microbicide in practice.

One important criticism of using bactericidal methods to measure the level of bacterial survival and possibly bacterial resistance following exposure to a microbicide is that tests are performed in the laboratory usually under set conditions that do not necessarily reflect upon the conditions found in practice. Indeed, a number of factors will affect the efficacy of a microbicide (Table 2) and hence the presence of bacterial survivors. The discrepancies in activity observed between results obtained in vivo and in vitro can often be explained by the use of different parameters. In terms of using these tests to measure the development of bacterial resistance, the presence of bacterial survivors might be explained by a decrease in microbical activity rather than the presence of resistant bacteria, if the parameters chosen are inappropriate or not controlled. Thus the microbicide in-use concentration and the concentration left following usage, together with regimen and conditions at the point of use (for example, temperature, soiling, contact time, type of surface) should be reflected in the test.

The measurement of cross-resistance following microbicide exposure is usually measured indirectly, whereby the susceptibility to the microbicide is evaluated first followed by the assessment of the antibiotic susceptibility. A change in the antibiotic susceptibility profile based on reporting zone of inhibition differences has very little impact in practice. More meaningful measurement of antibiotic resistance based on standardised tests such as those recommended by the British Society for Antimicrobial Chemotherapy (BSAC) or Clinical and Laboratory Standards Institute (CLSI) are needed as these can measure antibiotic failure in practice.

**Table 1. Principles for emerging cross-resistance between microbicides and chemotherapeutic antibiotics in bacteria.**

<table>
<thead>
<tr>
<th>Principles</th>
<th>Examples of mechanisms conferring microbicide resistance</th>
<th>Level of resistance to microbicides</th>
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<tr>
<td>Expression of genes encoding resistance to both the microbicide and one or more therapeutic antibiotic classes</td>
<td>Efflux</td>
<td>Low-medium</td>
</tr>
<tr>
<td>Change in the physiological response of the bacterium following microbicide exposure</td>
<td>Decrease in metabolism</td>
<td>Unknown</td>
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<tr>
<td>Transfer and expression of resistance genes carried on the same extra chromosomal elements (co-resistance)</td>
<td>Degradative enzymes</td>
<td>Low</td>
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<tr>
<td>Indirect selection for bacterial sub-population resistant to both microbicides and antibiotics</td>
<td>Change in outer cell wall permeability</td>
<td>High</td>
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<tr>
<td>Enhanced DNA repair</td>
<td>Activation of an SOS response</td>
<td>Unknown</td>
</tr>
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</table>

The determination of the “minimum selecting concentration” is an interesting concept, which would measure the minimum microbicide concentration required to select for, or induce/trigger, the expression of bacterial resistance mechanisms that will lead to bacterial resistance to biocides and/or antibiotics in practice. The measurement of such an indicator must combine a standardised bactericidal test, a standardised antibiotic susceptibility test and robust molecular protocols to measure mechanisms conveying bacterial resistance (for example, change in outer membrane protein, efflux expression).

**Conclusions**

Microbicides are valuable compounds to control or eliminate the spread of HAI in the healthcare environment. The indiscriminate
use of these agents, notably in new applications such as antimicrobial surfaces and textiles, might limit the number of microbicides available for use in the future, mainly because of environmental toxicity issues, but possibly following bacterial resistance concerns.

Despite the widespread use of disinfectants and antiseptics in healthcare settings, emerging bacterial resistance to microbicides and cross-resistance to antibiotics has become a significant concern. Therefore, there is a need for developing new classes of antimicrobials with novel mechanisms of action.

Finally, it should be mentioned that exposure to sub-lethal concentrations of cationic deconjugated compounds and triclosan has recently been found to induce virulence gene expression in Listeria monocytogenes. This study raised an interesting new development about the effect of microbicides on bacterial resistance.

The lack of standardised protocols to measure microbicide resistance and antibiotic cross-resistance in bacteria, together with the discrepancies associated with testing conditions between laboratory studies and the practice do not help in assessing bacterial resistance in situ, nor in comparing results between studies. A number of investigations have, however, clearly pointed out that following exposure to a specific microbicide, bacterial isolates from the healthcare environment can survive and become resistant to several unrelated microbicide and/or antibiotics. However, only one study to date linked the use of a microbicide with antibiotic resistance and an HA outbreak. It would be pertinent to note that since microbicide-resistant bacteria have been successfully isolated from healthcare settings, surveillance studies would be beneficial.

Finally, it should be mentioned that exposure to sub-lethal concentrations of quaternary ammonium compounds and triclosan has recently been found to induce virulence gene expression in Listeria monocytogenes. This study raised an interesting new development about the effect of microbicides on bacterial resistance. It is important that the role of microbicides is maintained in the healthcare environment and as such their efficacy must be maintained. The indiscriminate use of microbicides in an increasing number of applications might go against a prudent use of these antimicrobials.

References
1. SCENHR (Scientific Committee on Emerging and Newly Identified Health Risks), Assessment of the Antibiotic Resistance Effects of Biocides, 19 January 2009.

Table 2. Factors affecting the efficacy of microbicides.

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<thead>
<tr>
<th>MICROORGANISMS</th>
<th>Test genera/species/strain/isolate</th>
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<tr>
<td></td>
<td>Preparation of bacterial inoculum</td>
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<tr>
<td></td>
<td>Concentration of bacteria</td>
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<td></td>
<td>Age of bacterial inoculum</td>
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<td>Growth phase of bacteria</td>
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<td></td>
<td>Association of inoculum with fomites</td>
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<td></td>
<td>Biofilm</td>
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<td>Quenching of antimicrobial activity</td>
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<td>Detection and enumeration of survivors</td>
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<th>TEST PARAMETERS</th>
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1. SCENHR (Scientific Committee on Emerging and Newly Identified Health Risks), Assessment of the Antibiotic Resistance Effects of Biocides, 19 January 2009.


Biography

Jean-Yves Maillard is a Reader in Pharmaceutical Microbiology at the Welsh School of Pharmacy (Cardiff University, Wales). He graduated (BSc Hons) in 1991 and obtained his PhD in 1994 from Cardiff University. He is the recipient of the FEMS Fellowship award (1998), the WH Pierce Memorial prize (2003) and British Pharmaceutical Conference Science Medal (2003). His field of expertise concerns biocides; their activity, mechanisms of action, the emergence of microbial resistance and cross-resistance to antimicrobials, and the design of efficacy test protocols. He has published over 96 scientific articles in peer-reviewed journals, 11 book chapters (mainly on antimicrobial biocides) and 2 books and over 80 refereed conference communications. He is also working as a consultant for the biocide/pharmaceutical industry and the European Commission on matters related to biocides and particularly to microbial resistance. He is the Chief Editor of Letters in Applied Microbiology and a member of the editorial board for the Journal of Antimicrobial Chemotherapy.