EDITORIAL

Do antiseptics and disinfectants select for antibiotic resistance?

The development of bacterial resistance is a major world-wide problem complicating the use of chemotherapeutic agents and the control of infectious diseases [1]. The mechanisms of resistance can generally be considered as being intrinsic or acquired [2]. Intrinsic resistance (intrinsic insusceptibility) is a natural property of an organism and usually manifests itself as an impaired uptake of chemically unrelated drugs so that fewer molecules can reach their target site. Acquired resistance is usually demonstrated by mutations at a normally sensitive target site, by plasmid- or transposon-mediated antibiotic inactivation or by intracellular removal of antibiotics by efflux-mediated systems. The widespread and often indiscriminate use of antibiotics can lead to the selection of resistant cells [1].

Resistance to antiseptics, disinfectants and preservatives has been less extensively studied [3]. An early conclusion [4] was that plasmid-mediated resistance to inorganic mercury compounds and organomercurials, but not to other biocidal agents, was often found. Since then, the responses of bacteria to antiseptics and disinfectants have been more widely investigated, but have posed several unanswered questions. As with antibiotics, bacterial resistance to antiseptics and disinfectants may be acquired, but is more commonly intrinsic: associated with the outer membrane in gram-negative bacteria; the spore coats and to some extent the cortex in bacterial spores; and the mycolarabinogalactylpeptidoglycan complex in mycobacteria [3].

The apparent similarity between mechanisms of resistance to biocides and to antibiotics presumes that both groups have similar mechanisms of action on non-sporulating bacteria. By their very nature, antibiotics must be selectively toxic, whereas biocides may have harmful effects on both bacterial and human cells and application of antiseptics to human skin should be at concentrations that are non-toxic. Antibiotics are generally considered as having a single target site in bacteria, thereby inhibiting a specific biosynthetic process. However, aminoglycosides affect cytoplasmic membrane function as well as protein synthesis [2]. β-Lactam antibiotics bind to penicillin-binding proteins and inhibit peptidoglycan synthesis; rifamycins bind to RNA polymerase; 4-quinolones bind to DNA gyrase, although their lethality is related to a sequential cascade of events; and a wide range of antibiotics inhibit various stages of bacterial protein synthesis [2]. Biocidal agents tend to have multiple target sites in the bacterial cell [5]. Although there might be a primary or major effect on the cell, other 'secondary' effects contribute to the overall lethal effect, thereby explaining how uptake into the bacterial cell, bacteriostasis and bacterial cell death arise [6]. This is, of course, also true of aminoglycosides, in which uptake, bacteriostasis and bactericidal action are also separate. A recent study of the effect of triclosan on Escherichia coli showed that low concentrations of the bisphenol inhibited an enoyl-reductase involved in fatty acid synthesis and that the enzyme was not inhibited in resistant cells [7]. It was proposed that triclosan had a single target site in E. coli with the clear implication that resistance could arise by a single mutation at this site. However, the minimum bactericidal concentration (MBC) of triclosan for sensitive strains was much higher than the minimum inhibitory concentration (MIC), although the MBCs of triclosan for a triclosan-resistant mutant (high MIC) and the triclosan-sensitive parent strain were similar [8]. Clearly, additional cellular changes are needed to produce a bactericidal effect by triclosan in both strains. Therefore, a key aspect is the concentration at which an antiseptic or disinfectant is used.

To what extent are bacterial resistance mechanisms common to antiseptics, disinfectants and antibiotics? Mutations at appropriate target sites (penicillin-binding proteins, ribosomal subunits, RNA polymerase, DNA gyrase) are obviously associated with resistance to specific antibiotics, as are antibiotic-inactivating enzymes, such as β-lactamases, chloramphenicol acetyltransferase and aminoglycoside-modifying enzymes. Impermeability in gram-negative bacteria – and probably in mycobacteria – is non-specific and is associated generally with the physicochemical properties of the drug or biocide, such as hydrophobicity or hydrophilicity and mol.wt [2]. However, cationic disinfectants, like quaternary ammonium compounds (QACs) and chlorhexidine, as well as some antibiotics (polymyxins) probably cause outer membrane damage in gram-negative bacteria thereby promoting their own entry into the cells [3], where other, lethal, effects occur.
Another major resistance mechanism in both gram-positive and gram-negative bacteria is efflux, whereby molecules taken up by the cells are pumped out by specific membrane proteins [9,10]; this occurs with tetracyclines, fluoroquinolones and macrolides. Some antiseptics and disinfectants are also known to be subject to efflux, but the clinical relevance is less clear-cuts. Many studies have examined plasmid-associated qac genes in staphylococci. At least five of these genes (qacA-E) are known, with A and B, and C and D sharing homology. The qacA/B system is the most important: qacB specifies resistance to QACs, acridines, diamidines (pentamidine, propamidine, dibromopropamidine) and ethidium bromide, all of which are cationic compounds, whereas qacA additionally encodes resistance to chlorhexidine and is often carried on penicillinase plasmids. Resistance has usually been measured in terms of MIC increases, which in resistant strains are 2-8-fold higher than in sensitive strains. Nevertheless, MBCs of cationic agents for sensitive and resistant strains are closely similar, although our investigations indicate that resistant strains may be killed more slowly. Antibiotic-sensitive Staphylococcus aureus and other staphylococci are usually antiseptic-sensitive, whereas strains for which the MICs of antiseptics indicate intermediate or high resistance are also more resistant to a wide range of antibiotics [11].

Do these findings imply that staphylococcal resistance to cationic biocides is likely to be a major clinical problem? Can the presence of a qac gene select for antibiotic-resistant bacteria? The concentrations of cationic antiseptics and disinfectants used clinically are many times higher than the MICs of resistant strains. Furthermore, stepwise development of stable resistance to chlorhexidine and other agents cannot be achieved in staphylococci [2]. It is unlikely, therefore, that resistance to such agents will be a clinical problem. The second question is more difficult to answer. A strain of S. aureus isolated some 50 years ago contained the qacB gene, whereas most strains isolated since the 1980s contain qacA [12]. From this it was inferred that qacA had evolved from qacB and that the widespread introduction of chlorhexidine into hospital practice in the early 1980s (earlier in the UK) was responsible for selecting for strains containing qacA. It was also stated, but has yet to be proven, that a QAC (benzalkonium chloride) induced the expression of both qacA and qacB, and that the chronological emergence of these genes on multi-resistance plasmids in clinical isolates of S. aureus mirrored the introduction and usage of cationic biocides (acridines, QACs, chlorhexidine). The qacA gene encodes a membrane protein, which is involved in an antiporter system whereby drugs and low concentrations of biocides are exported and protons (H+) are taken up. At high disinfectant concentrations, severe membrane damage occurs and the amount exported is very low and insufficient to prevent cell death. Investigation of the effects of high and low residual concentrations of QACs and chlorhexidine on membrane perturbation in sensitive and multiresistant S. aureus strains would provide some instructive information about membrane damage, loss of viability and efflux.

Strains of Pseudomonas aeruginosa, Providencia stuartii and Proteus spp. isolated from urinary tract infections in paraplegic patients were resistant not only to cationic disinfectants (QACs, chlorhexidine), but also to four or more chemically unrelated antibiotics [13]. It was suggested that the widespread usage of these disinfectants was selecting for the presence of antibiotic-resistant strains, although no plasmid link was found.

Chromosomally encoded efflux systems, such as marRAB, acrAB and soxRS, play an important role in conferring intrinsic resistance in gram-negative bacteria [10]. It has been proposed [14] that a small increase in resistance of E. coli to triclosan, from an MIC of 0.09 mg/L to 0.33 mg/L, was responsible for selecting for antibiotic resistance. The efflux resistance mechanism, apart from being chromosomal, appears to be analogous to the qacA/B systems in S. aureus. Pine oil has also been found to select for a low-level increase in resistance to antibiotics in E. coli [15]. However, the concentrations of triclosan and pine oil used in these studies were very low and did not equate to those used in clinical practice.

Concern has been expressed about the increased usage of non-antibiotic antimicrobial agents in the home, because of the possible development of biocide resistance and because of the possible selection for antibiotic-resistant bacteria. There is a need to examine in considerably more detail the effects of repeated bacterial exposure to low (residual) concentrations of disinfectants.

Increased resistance of bacteria to antiseptics and disinfectants is not a clinical problem at present. The issue as to whether low-level resistance to these agents is a selection factor for antibiotic-resistant strains in the clinical and domestic environments has yet to be settled [16]. However, mutations in the inhA gene of Mycobacterium smegmatis results in resistance to both triclosan and isoniazid [17] and this must be considered as being a matter for concern.

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References
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CONTENTS

**CLINICAL BACTERIOLOGY**
Brucellosis: an update The perspective from the Mediterranean basin
J. Ariza

Anaerobic infections
I. Brook

**MOLECULAR VIROLOGY**
Type III (contact-dependent) secretion in Gram-negative bacteria
J.R. Kerr

**EUKARYOTIC PARASITOLOGY**
*Trichomonas vaginalis, a model mucosal parasite*
J.F. Alderete

**SUSCEPTIBILITY TO INFECTIOUS DISEASE**
Host factors in genetic susceptibility to infectious diseases
R. Bellamy

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLINICAL BACTERIOLOGY</td>
<td>Brucellosis: an update The perspective from the Mediterranean basin</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Anaerobic infections</td>
<td>137</td>
</tr>
<tr>
<td>MOLECULAR VIROLOGY</td>
<td>Type III (contact-dependent) secretion in Gram-negative bacteria</td>
<td>155</td>
</tr>
<tr>
<td>EUKARYOTIC PARASITOLOGY</td>
<td><em>Trichomonas vaginalis, a model mucosal parasite</em></td>
<td>165</td>
</tr>
<tr>
<td>SUSCEPTIBILITY TO INFECTIOUS DISEASE</td>
<td>Host factors in genetic susceptibility to infectious diseases</td>
<td>175</td>
</tr>
</tbody>
</table>