Journal of Applied Microbiology Symposium Supplement 2002, **92**, 144S–157S

Significance of biocide usage and antimicrobial resistance in domiciliary environments

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1. SUMMARY

Recent events have raised awareness of the need for effective hygiene in the home. Not least is the requirement to reduce antibiotic resistance by reducing the need for antibiotic prescribing. Current evidence suggests that improved hygiene in the domestic setting could have a significant impact. Recently, it has been suggested that widespread biocide usage, particularly in consumer products, may be a contributory factor in antibiotic resistance. In developing home hygiene policies, however, it is important that biocide use as an integral part of good hygiene practice is not discouraged in situations where there is real benefit. Although laboratory data indicate possible links, it is necessary to assess whether and to what extent biocide exposure could contribute to antibiotic resistance in clinical practice. The extent to which reduced susceptibility to biocides resulting from biocide exposure could compromise their 'in-use' effectiveness must also be considered. Equally, it is important that changes in susceptibility induced by biocide exposure are assessed relative to those induced by antibiotic exposure or the phenotypic changes induced by 'normal' environmental 'stresses'. It is proposed that to be effective, home hygiene policy should be based on the concept of risk assessment and risk prevention. Using this approach, critical risk situations are identified and appropriate hygiene procedures applied to reduce risks. This may involve either soap and water cleaning, or cleaning combined with a disinfection process. A 'targeted' hygiene approach not only provides the most effective means of preventing infectious disease, it also offers a means of addressing concerns about 'too much hygiene' and 'too many antibacterials' amongst a public who have lost confidence regarding appropriate hygiene for their home environment.

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2. INTRODUCTION

A range of events over the past few years has raised awareness of the importance of the domestic setting in the chain of infection transmission through the community. A key concern is infectious intestinal disease (IID) which remains at unacceptably high levels. A recent UK study estimated that the number of cases of IID in the community may be up to 10 million each year (Wheeler *et al*. 1999). The fact that many of these infections are viral in origin reinforces the importance of prevention through hygiene. Schmidt (1998) concluded that most of the IID which is food-borne occurs in the home. Also, demographic and social changes now mean that increasingly, the home is a place where those with reduced immunity to infection are cared for, and these home carers may also be family

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members. This includes the elderly, those with chronic and degenerative illness, neonates, immune-compromised patients discharged from hospital, patients taking immunosuppressive drugs or using invasive/inhalation systems, and carriers of Human Immunodeficiency Virus (HIV) or methicillin-resistant *Staphylococcus aureus* (MRSA). Bloomfield (2001) estimates that up to one in six people in the UK belong to an 'at risk group'. Other concerns include changing infectious disease patterns, escalating treatment costs, the problem of antibiotic resistance and the need to reduce the prescribing of antibiotics. Globalization of food supplies, travel and refugee movements all serve to introduce pathogens into communities where there may be little innate resistance. Although many of the respiratory and intestinal infections occurring in the home are non-serious and self-limiting, they still represent a significant economic burden. For 'at risk' groups, such infections may be more serious and require hospitalization with attendant costs. The potential for clinical sequelae of respiratory and enteric infections is also a concern; Lindsay *et al.* (1997) estimated that up to 2–3% of food-borne infections may be a factor in the development of subsequent clinical conditions, including malignant, inflammatory and other chronic degenerative diseases.

Taken together, these factors suggest that if infectious disease and the social and financial burden which it represents is to be contained in a sustainable manner, a commitment to shared responsibility by government and the public is essential. Unfortunately, however, at a time when there is a need to re-emphasize the importance of infection prevention through good hygiene, in the community as well as in hospitals and other settings, a range of other issues related to infectious disease and its prevention has been publicized through the professional and lay press which, although requiring serious consideration, has created a dilemma in the minds of the public. While on the one hand publicity about emergent pathogens, antibiotic resistance, vaccination, food poisoning etc. has increased concern about how best to protect the family from infection, on the other hand, the public is now told that too little exposure to germs could be weakening our immunity to infection and leaving us more exposed to allergic diseases (Rook and Stanford 1998).

In the past few years, research studies (as reviewed by Scott 1999 and Barker *et al.* 2001), focusing specifically on the home have not only provided a better understanding of how infectious diseases are spread in this environment, but have also indicated that a significant proportion of these infections are preventable. Such studies suggest, however, that home hygiene is still largely seen as a process of 'getting rid of household germs' in the belief that a 'germ-free' home is the appropriate means to protect the family against infection (Curtis 1998). Criticism of the growing numbers of household cleaning and other products containing anti-bacterials, which seem to offer consumers a 'modern' easy way of achieving this, has further increased the confusion. If we are to change attitudes, motivate better hygiene standards and promote rational use of biocidal products within this environment, the public must be better informed with messages which are consistent and make rational sense.

Although it is generally agreed that the cause of antibiotic resistance in clinical practice is the over-prescribing of antibiotics, some scientists have suggested that widespread use of biocides, particularly in consumer products, may be a contributory factor (Levy 1998; McMurry *et al.* 1998; Russell *et al.* 1998, 1999a). Some laboratory studies have demonstrated links between biocide and antibiotic resistance, but there is currently no evidence that it is a significant factor in the development of antibiotic resistance in clinical practice. The current view is that if biocides have a contributory role, it is likely to be a minor one. It is agreed, however, that this aspect requires constant review.

Concern about bacterial resistance has led to calls for increased education on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection (Anon 1999a; 2000). From another perspective, it is argued that if reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide use, as an integral part of good hygiene practice, is not discouraged in situations where there is real benefit in terms of preventing infection transmission. This means that there should also be concern about the possibility that indiscriminate use of biocides contributing to the development of biocide resistance, which could compromise their in-use effectiveness.

In this paper, studies of the mechanisms of antibiotic and biocide resistance, and the possible links between them, are reviewed. These data have also been reviewed by Russell (1998), McDonnell and Russell (1999), Russell *et al.* (1999a, b), Levy (2000), Beumer *et al.* (2000, http://www.ifh-homehygiene.org/public/micro00.htm) and Russell and Maillard (2000). One particular aim is to provide a better understanding of the practical implications of the available data in relation to prevention of infectious disease through effective hygiene.

3. REDUCED SUSCEPTIBILITY TO BIOCIDES AND ANTIBIOTIC RESISTANCE

When assessing the significance of antimicrobial resistance it is important to bear in mind that the term 'resistance' is a relative word. Antibiotics often exert their effect through growth inhibition caused by inactivation of a single target, and they achieve bacterial eradication in conjunction with

the humoral and cellular defence mechanisms of the host. Their efficacy is assessed using minimum inhibitory concentration (MIC), which has direct relevance because it can be related to blood and serum levels. A change in susceptibility rendering an agent ineffective against an infection previously treatable by that agent is usually referred to as 'resistance'. Biocides, on the other hand, most often have multiple cell targets, and their efficacy is dependent upon producing effects that cause rapid kill, expressed as the log reduction in bacterial numbers. In many cases, conclusions about biocide 'resistance' have been based on MICs, which reflect their activity against the most sensitive target site but have little relevance to their efficacy as a disinfectant when used at bactericidal concentrations. It is thus suggested that the term 'reduced susceptibility' may be more appropriate in this context.

The significant number of studies investigating the relationship between development of resistance to biocides and antibiotics, and the mechanisms involved, are reviewed in detail by Russell (1998), McDonnell and Russell (1999) and Beumer *et al*. (2000, http://www.ifh-homehygiene.org/public/micro00.htm). Some of these studies show increased MICs for methicillin-resistant (MRSA) and other antibiotic-resistant strains of *Staphylococcus aureus* against biocides such as chlorhexidine, cetrimide, benzalkonium chloride (BAC), hypochlorite, triclosan, parahydroxybenzoates and betadine. In some cases, there is evidence of decreased susceptibility to bactericidal concentrations while in others, strains showing increased MICs compared with control strains show equal susceptibility in bactericidal tests. While some laboratory findings with Gram-negative spp. such as *Pseudomonas*, *Proteus* etc. also suggest a relationship between biocide and antibiotic resistance, other studies involving both Gram-negative and Gram-positive species indicate no such link. Interestingly, a study of 67 ciprofloxacin-resistant isolates of *Ps. aeruginosa* yielded four isolates which were hypersensitive to chlorhexidine (MIC 5 mg l^{-1}), while none were found amongst 179 ciprofloxacin-sensitive isolates (Baillie *et al*. 1993).

Overall, these studies indicate no consistent pattern, the observations suggesting or refuting such a link varying according to the nature of the biocide and the antibiotic, the conditions under which the evaluation was carried out, and the parameters assessed (MIC or bactericidal effects). In many cases, there is no indication of whether susceptibility changes were stable or reversible. In fact, it would be unbelievable if links between biocide and antibiotic resistance were not observed, since changes in the outer layers of the cell, particularly of Gram-negative species, are likely to affect resistance to both biocides and antibiotics. The variable response suggests that there is no single underlying cause.

4. PRACTICAL IMPLICATIONS

In assessing the potential implications of the observed links between reduced susceptibility to antibiotics and biocides, a number of questions require consideration. With regard to biocide usage and antibiotic resistance, it is necessary to assess whether it is possible that biocide use could contribute to the emergence of antibiotic multi-resistant populations in clinical practice and if so, to what extent this is a contributing factor relative to other factors such as antibiotic over-use. With regard to biocide usage, it is also necessary to assess the extent to which exposure of microbial populations to biocides results in reduced biocide susceptibility, and whether this could correlate with a failure to achieve 'hygiene' in practical situations. In making these assessments, it must be borne in mind that conclusions about antibiotics do not automatically apply to biocides; the two issues must be dealt with separately. This is often not the case and therefore a cause of confusion. A further aspect commonly not considered is the significance of changes in antimicrobial susceptibility induced by biocide exposure as compared with reduced susceptibility induced by 'normal' environmental 'stresses'. These questions are considered in the following sections, based on an evaluation of various studies of the physiological mechanisms responsible for changes in susceptibility to biocides and antibiotics.

4.1. Biocide usage and biocide resistance

In answer to the question could biocide use encourage the spread of biocide resistance, the consensus view is most probably no. Biocides, unlike antibiotics for which action against specific cell targets is fundamental to their clinical value, may act at one or several sites within the cell wall, membrane or cytoplasm. Mode of action studies, as reviewed by Russell and Chopra (1996) and Russell *et al*. (1999b), indicate that some biocides at the high concentrations used in practice to achieve microbiocidal action, produce generalized effects such as disruption of the cell membrane or inactivation of a broad range of enzymes. On the other hand, at lower, growth-inhibitory concentrations, these compounds can act in the same way as antibiotics, specifically affecting one or two cellular targets. For biocides which affect multiple targets, the susceptibility of each target is variable and dependant on the concentration of the biocide.

Although there are studies showing that exposure to low levels of biocides can be associated with reduced biocide susceptibility, the important issue is whether this is commensurate with loss of efficacy *in vivo* since, in practice, disinfectants and antiseptics are used at much higher concentrations which have a rapid microbicidal action. Thus, for example, McDonnell and Pretzer (1998) found

that whereas the MIC for triclosan against *FabI* mutants of *Escherichia coli* was 25–50 μg ml^{-1} compared with 0·1 μg ml^{-1} for a wild-type strain, triclosan-containing products showed no differences in the rate of kill of the mutants and wild-type strains. This is consistent with the hypothesis that triclosan has multiple target sites within the bacterial cell, such that mutations affecting the growth inhibitory properties of triclosan are unlikely to have any impact on susceptibility to biocidal concentrations. Recently, studies have shown that in *E. coli* and *Mycobacterium smegmatis*, triclosan has a specific action on the enzyme enoyl reductase, which is essential for fatty acid synthesis at growth-inhibitory concentrations (McMurry and Levy 1998; McMurry *et al*. 1999).

Studies reviewed by Sundheim *et al*. (1998) and Beumer *et al*. (2000a) using clinical, environmental and food isolates of Gram-positive (mainly MRSA and other strains of staphylococci) and Gram-negative spp. (mainly *Pseudomonas*, *Proteus*, *Providencia* and *Klebsiella* spp.) showed significant increases in MICs with biocides such as chlorhexidine, BAC, cetylpyridinium chloride (CPC) and triclosan. In some cases, reduced susceptibility was also demonstrated in bactericidal assays, but sensitivity to use dilutions was not affected. Heir *et al*. (1999) showed that *Staph. aureus* cells with a survival advantage (reduced log kill) in environments containing low concentrations of BAC compared with quaternary ammonium compound (QAC)-sensitive controls, also expressed a plasmid-borne *qacG*. Suller and Russell (1999) showed that step-wise exposure to increasing concentrations of chlorhexidine, CPC and triclosan produced some increase in the MIC but the resistance was unstable, indicating that it was intrinsic rather than acquired in nature. Attempts to transfer chlorhexidine resistance from *Ps. stutzeri* to *Ps. aeruginosa* by conjugation were not successful, and the authors suggest that a likely reason for the co-resistance is a non-specific decrease in cell permeability (Tattawasart *et al*. 1999). The fact that the resistance of *Ps. stutzeri* was stable through 15 sub-cultures suggests that the decrease in cell permeability arose by selection of mutants with altered cell wall structure (Tattawasart *et al*. 2000a,b).

In some instances, however, clinical or environmental isolates have been obtained from environments where biocides were in constant use and where insusceptibility was demonstrated at or above use concentration. Bolton *et al*. (1988) and Sundheim *et al*. (1998) investigated isolates of *Staph. aureus*, lactobacilli and *Pseudomonas* spp. associated with disinfection failures involving the use of chlorine and BAC-based disinfectants in cattle and poultry farming. Sundheim *et al*. (1998) suggested that the resistance could be related to selection of intrinsically-resistant strains, but both workers agreed that it was most likely the result of regulated phenotypic adaptation induced by low level biocide exposure, which for lactobacilli and staphylococci probably involved synthesis of protective exopolysaccharide. Willingham *et al*. (1996) showed that 5% of isolates (including *Serratia marcescens*, *Bacillus* spp., *Enterococcus* spp. and *Ps. stutzeri*) from two of three chicken hatcheries were resistant to use concentrations of commercial disinfectants. Some isolates were multi-resistant, but only three were resistant to QACs compared with seven for phenol and 15 for glutaraldehyde. The authors suggested that this may be correlated with the usage of glutaraldehyde in US hatcheries over many years. No attempt was made to determine whether the resistance was reversible.

The overall conclusion based on current evidence, also expressed by Russell (1997), Russell *et al*. (1999a) and Gilbert *et al*. (2002), is that although development of reduced susceptibility to biocides in response to biocide exposure can occur, it is not a significant consideration in practical terms, since the level of resistance expressed is low and unlikely to compromise practical effectiveness where much higher concentrations are used. Populations of biocide-resistant bacteria do sometimes appear in practice, but most often this is the result of their capacity for phenotypic adaptation and survival in their constantly changing environment where conditions are frequently stressful and growth-limiting. This aspect is further discussed in section 3.3.2. It has also been found that when the biocide is withdrawn, susceptibility is restored.

4.2. Biocide usage and antibiotic resistance

In answer to the question could biocide usage contribute to antibiotic resistance, the consensus view is that it is possible but there is currently no evidence that it does. Where simultaneous changes in susceptibility to antibiotics and to the types of biocides used as disinfectants and antiseptics have been investigated, the resistance determinants mostly involved are genes encoding for multi-drug efflux pumps, either plasmid-borne in Gram-positive species or chromosomally-encoded in Gram-negatives. Some recent studies have shown that this could also arise where a selective target site for biocides is shared by a therapeutic agent or agents. Since there are fundamental differences between these aspects, they will be considered separately.

4.2.1. Chromosomally-encoded multi-drug efflux pumps in Gram-negative bacteria. Chromosomally-encoded multi-drug efflux pumps, such as MexAB, MexCD and MexEF in *Ps. aeruginosa*, and AcrAB in *E. coli*, are key in defining the intrinsic susceptibility of Gram-negative bacteria to both biocides and antibiotics; they also play a role in the development of multi-resistance to these agents. Up-regulation of *acrAB* is largely, but not exclusively, controlled by the regulator MarA. Phenotypic adaptation in

response to environmental stimuli, or mutations that increase expression of efflux genes or MarA, result in elevated levels of resistance. Multi-drug efflux pumps identified in these and other bacterial species, such as *Neisseria gonorrhoea*, *Haemophilus influenza*e and *Burkholdia cepacia*, are reviewed in more detail by Nikaido (1998a, b) and Paulsen *et al*. (1996).

Schweizer (1998) has shown that the biocide, triclosan, can also be a substrate for the MexAB-OprM multi-drug efflux pump in *Ps. aeruginosa*. Chuanchuen *et al*. (2000) showed that exposure to triclosan selected a multi-drug-resistant strain that hyper-expressed the *MexCD* efflux system genes from a susceptible population of *Ps. aeruginosa* mutants in which *MexAB* was deleted. This strain showed a marked decrease in susceptibility, as assessed by MIC, not only to triclosan but also to several antibiotics. The Acr system in *E. coli* is known to act as a transporter for several antibiotics and also to certain biocides, such as acriflavine, phenylethylalcohol, sodium dodecyl sulphate and deoxycholate. Nikaido (1998a,b), Paulsen *et al*. (1996), Moken *et al*. (1997) and McMurry *et al*. (1998) have shown that mutations causing over-expression of *marA* or *acrAB* are associated with reduced susceptibility to triclosan, thus indicating that *Acr* can also act as an efflux system for triclosan, chloroxylenol and quaternary amines.

In assessing the importance of multi-drug efflux pumps, it is necessary to establish whether, and to what extent, they may be responsible for failures in antibiotic therapy in clinical practice. Evidence for this comes mainly from studies with clinical isolates of *Ps. aeruginosa*. Rella and Haas (1982) showed that mutations in the repressor gene of the *MexAB* operon leads to over-production of this efflux system and raises the MICs of ciprofloxacin and carbenicillin eight and 32 times, respectively, as compared with the wild-type. Among carbenicillin-resistant clinical isolates of *Ps. aeruginosa* collected in a UK study, almost 80% did not produce carbenicillin-hydrolysing β-lactamase and appeared to belong to the elevated efflux type (Williams *et al*. 1984). A study in France found that about one third of ticarcillin-resistant *Ps. aeruginosa* clinical isolates had a resistance pattern characteristic of 'intrinsic resistance' (Bert and Lambert-Zechovsky 1996).

For *E. coli* and *Salmonella*, evidence for a role of efflux in clinical resistance is less convincing. Ma *et al*. (1994) suggest that although Acr and MexAB have similar substrates, only MexAB is likely to produce clinically-significant levels of resistance to small lipophilic agents, such as tetracycline, chloramphenicol and fluoroquinolones, because entry through the narrow *Ps. aeruginosa* porin channels is significantly slower than through *E. coli* porin channels. A survey of 28 quinolone-resistant clinical strains of *E. coli* showed that elevated *Mar*A transcription did occur in three strains (Manneewannakul and Levy 1996). Mazzariol *et al*. (2000) showed over-expression of *acrA* in nine out of 10 clinical isolates of *E. coli* with high-level ciprofloxacin resistance (MIC > 32 μg ml^{-1}), but not in 15 isolates for which the MIC was < 1 μg ml^{-1}. Over-expression of *MarA*, however, also reduces levels of the porin, OmpF, which probably contributes to the resistance of such strains (Gutman *et al*. 1985).

However, even for *Ps. aeruginosa*, it is not clear what fraction of the resistant isolates of clinical origin correspond to efflux mutants, and opinions differ as to their importance in clinical practice. In a recent review, Nikaido (1998a) conceded that multi-drug efflux is probably not yet the most frequent mechanism of resistance amongst clinical isolates, but noted that reports suggest efflux-based resistance now occurs with increasing frequency.

If antibiotic exposure is a factor in the emergence of multi-drug efflux mutants conferring clinically-significant antibiotic resistance, it is possible that biocide exposure could also have the same effect. Levy and co-workers (Moken *et al*. 1997) have shown that *E. coli* mutants selected for reduced susceptibility to pine oil disinfectant also have reduced susceptibility to multiple antibiotics, including tetracycline, chloramphenicol, ampicillin and nalidixic acid, which is mediated via the *mar* and *acr* operons. Importantly, however, the level of antibiotic resistance which develops is relatively low and unlikely to compromise effectiveness in clinical use.

In assessing the possible impact of biocide usage on efflux-mediated antibiotic resistance, it is important that it is considered in relation to other inimical agents which can elicit this effect. Miller and Sulavick (1996) review studies showing that not only low levels of antibiotics, such as chloramphenicol and tetracycline, act as weak inducers of the *MarRAB* operon in *E. coli*; *MarRAB* also responds to a range of inducers reflecting a range of environmental conditions, including the weak acid salicylate. Tetracycline and chloramphenicol are less effective than salicylate (albeit at higher concentrations) in *mar* up-regulation, suggesting that salicylate is a 'normal' substrate for this pump.

From their review, Miller and Sulavick (1996) suggest that efflux pumps are a natural defence mechanism against toxic compounds in the environment. They suggest that the wide substrate specificity of these pumps and, for the AcrAB pump, regulation by global stress signals rather than specific substrates, make them well suited for a defensive role. If efflux pumps evolved as a defence against antimicrobial agents occurring in the environment, then it is to be expected that a wide range of 'natural' antimicrobial agents, including materials such as pine oils, would produce these effects. The *marRAB* regulon is also induced by the positive regulator, SoxS, which is produced by transcription of *soxRS* in response to exposure to free radicals. Recently, it has been postulated (Bloomfield *et al*. 1998; Dodd *et al*.

1998) that if bacterial cells are growth-arrested by treatment with an inimical agent, the imbalance between anabolism and catabolism causes a burst of free radical production which results in loss of viability in addition to damage produced by the inimical process. If this hypothesis is correct, it is possible that any chemical substance which produces a sudden decrease in growth rate could cause increased expression of *acr* multi-drug efflux pumps. In line with this, Whyte *et al.* (2001) showed that a significant proportion of a wide range of food, household and personal products acted as inducers of *mar* and *acr* when tested against *lacz* fusions of *E. coli*. These inducers included food substances such as mustard, chilli and garlic, and household products, none of which made hygiene claims.

Miller and Sulavick (1996) suggest that although the wide substrate specificity of efflux pumps may render them well suited for a defensive role, it also means that cells may 'accidentally' pump out key metabolites. Thus, it is likely (as suggested in recent studies by Gilbert, personal communication) that if the primary function is to facilitate continuous 'modulation' of efflux activity in response to their ever-changing environment, mutant populations which express efflux systems constitutively will decline in favour of wild-type populations when the selective pressure is removed; however, Levy (2000) suggests that this may not be the case.

4.2.2. Plasmid-mediated efflux mechanisms in Gram-positive species. Plasmid-mediated antibiotic resistance in Gram-positive bacteria is well established as a significant clinical problem. Since resistance to some biocides is also plasmid-mediated, this raises concern that biocide exposure could contribute to spread of antibiotic resistance by selection and dispersal of plasmids, producing resistance to both antibiotics and biocides. This could be a plasmid bearing a resistance determinant for a common target site shared by the antibiotic and one or more biocides, or a biocide resistance determinant alongside structurally-unrelated determinants for antibiotic resistance, e.g. penicillin-binding proteins.

For staphylococci, reduced biocide susceptibility is commonly associated with plasmid-encoded efflux proteins. Studies of some *Staph. aureus* strains, as reviewed by McDonnell and Russell (1999), show that *qacA*, *B*, *C* and *D* genes encoding multi-drug efflux in conjunction with antibiotic resistance determinants on multi-resistance plasmids, are associated with reduced susceptibility (assessed by MICs) to biocides such as acriflavine, cetrimide, BAC, chlorhexidine and propamide isethionate. Heir *et al.* (1995) have shown that *qac* genes are widely distributed in clinical and food isolates of *Staph. aureus*. Of concern is the finding that the *qacA/B* family of genes shows significant homology to other energy-dependant transporters, such as tetracycline transporters found in tetracycline-resistant strains, while *qacA/B* can be borne on penicillinase plasmids (Rouche *et al.* 1990; Russell *et al.* 1999a). Although the physiological basis of reduced susceptibility to QACs has not been elucidated for other plasmid-carrying MRSA strains which have been identified, it is quite likely that qac efflux proteins are involved.

Paulsen *et al.* (1996) suggest that staphylococcal *qac* genes may have evolved before the introduction and use of topical antimicrobials and disinfectants and, although they may once have played other physiological roles, they have subsequently been acquired by clinical pathogens for protection against hydrophobic antimicrobial agents and become widely disseminated due to the selective pressure imposed by the use of biocides in antiseptic and disinfectant formulations. Paulsen *et al.* (1999) have presented evidence based on DNA homology that *qacA* has evolved from *qacB*, and have proposed that emergence of the *qacA* determinant among *Staph. aureus* clinical isolates during the 1980s may have resulted from the extensive usage of divalent cationic compounds, such as chlorhexidine in hospital environments, although prospective studies of the comparative resistance of hospital isolates obtained during this period are needed to substantiate the proposal. Bacquero *et al.* (1991) found no evidence of any association between prolonged chlorhexidine usage in the hospital setting and reduced susceptibility to chlorhexidine.

For Gram-negative bacteria, changes in susceptibility to antibiotics and biocides mediated through *AcrAB* and *mexAB* multi-drug efflux genes are thought unlikely to be transferable, since they are chromosomally-mediated. However, Saier *et al.* (1998) have shown that genes for divalent cation efflux pumps of similar construction already exist on plasmids, and suggest that there is no reason to expect that drug efflux genes of this type could never be transferred to resistance plasmids.

The possibility that biocides might facilitate the spread of plasmid-mediated antibiotic resistance also depends on the stability and transferability of the relevant plasmids. Russell (1997) concluded that although plasmid-borne transfer of mercury resistance is a common property of clinical isolates, for biocides commonly used as disinfectants, plasmid-mediated biocide resistance is relatively uncommon; some biocides, such as cationic agents, can actually reduce plasmid transfer in *Staph. aureus*. Pearce *et al.* (1999) have studied the effect of sub-MICs of chlorhexidine, povidone iodine and cetrimide on acquisition of antibiotic resistance genes. At low concentrations, cetrimide reduced plasmid transfer efficiency while the other biocides had no effect. Low concentrations of povidone iodine and chlorhexidine reduced phage transfer efficiency, while cetrimide caused some increase, probably by an effect on the recipient strains.

4.2.3. Other relevant studies involving biocides with specific target sites. As stated in section 3.1, many or most biocides attack several targets with differing susceptibilities depending on concentration. Although under use conditions bactericidal effects at higher concentrations may result from generalized cell disruption, growth inhibition at lower concentrations may occur through interaction with specific target sites. Where such sites are shared by a therapeutic agent, selection of a population bearing that target site by exposure to low level biocide would have no effect on susceptibility to the biocide, but it could render the population clinically resistant to the therapeutic agent. Also of concern is the possibility (but no evidence) that if the resistance determinants were transferred to plasmids also bearing determinants for one or unrelated targets conferring antibiotic resistance, e.g. penicillin-binding proteins, then persistent low-level biocide exposure could select for antibiotic populations through selective pressure and plasmid transfer.

The recent finding that the enoyl reductase enzyme in *Myco. smegmatis* (McMurry *et al*. 1999) is the target not only for triclosan but also for the chemotherapeutic agent, isoniazid, is of concern. Deletion of *InhA* resulted in a 1·2–8·5-fold increase in the MIC of isoniazid, and a 4–6·3-fold increase in the MIC of triclosan. Despite earlier studies (Cookson *et al*. 1991b) which identified co-transfer of low-level triclosan resistance and mupirocin resistance from MRSA strains to sensitive *Staph. aureus* recipients, recent studies with *Staph. aureus* (Suller and Russell 2000) have shown that acquisition of a plasmid encoding mupirocin resistance was not associated with changes in triclosan MICs. Suller and Russell (2000) showed that although enhanced triclosan resistance was, in several cases, stably inherited in the absence of triclosan, the mutants were not less sensitive to a range of antibiotics or to the lethal effects of triclosan than the parent strain.

Studies on MGRSA strains (*Staph. aureus* strains resistant to both methicillin and gentamicin) suggest that the plasmids they contain (termed GNAB plasmids) code for a nucleic acid binding (NAB) site which is a common target for gentamicin and chlorhexidine. MGRSA strains without NAB plasmids were more sensitive to chlorhexidine than methicillin-sensitive strains, whereas MRSA isolates with GNAB plasmids encoding resistance to gentamicin were more resistant to the bisbiguanide (Cookson *et al*. 1991a). Although plasmid-borne and/or stable resistance has been reported for some other biocides (for example, Tattawasart *et al*. (1999) showed development of stable resistance to chlorhexidine and cetylpyridinium chloride (CPC) in *Ps. stutzeri*), for plasmid-carrying MRSA strains (as described in section 3) showing reduced susceptibility to biocides, no specific target site has been identified for these agents.

McMurry *et al*. (1998) analysed 29 antibiotic-resistant clinical *E. coli* isolates and found that three showed reduced susceptibility to triclosan, two of which were *mar* mutants. It was not known whether the antibiotic resistance was due to multi-drug efflux, whether the triclosan had selected for the antibiotic resistance, or whether the antibiotic resistance had selected for the triclosan resistance.

Several other studies suggest the possibility of shared target sites. Agents such as the aminoglycosides, for example, are known to gain access to the cell through a self-promoted mechanism (Hancock 1981; Taber *et al*. 1987) whereby the agent de-stabilizes cations associated with the cell envelope, causing re-organization of the LPS and facilitating antibiotic entry. It is notable that many biocides, particularly polymeric biguanides (Wilkinson and Gilbert 1987), share this mechanism of cellular uptake. Similarly, phenylethanol (Silver and Wendt 1967) is known to inhibit the initiation of DNA replication and to cause a similar filamentation to that of the isothiazolones. Should these sublethal effects be directed towards a DNA gyrase, it is not difficult to imagine how the action of quinolone antibiotics might also be affected.

4.2.4. Conclusions based on the results of these studies. The data reviewed in sections 4.2.1–4.2.3 suggest that some types of biocides do have the potential to encourage the emergence of antibiotic-resistant populations, either by selection of multi-drug-resistant populations or by transfer of multi-resistant plasmids. In assessing the practical implications of these largely laboratory-based data, two criteria need to be assessed: first, how extensively these mechanisms might occur in the environment or in clinical practice and secondly, whether the level of antibiotic insusceptibility is sufficient to compromise the clinical response.

Current evidence suggests that for Gram-negative multi-drug efflux pumps, the level of antibiotic resistance induced or acquired through biocide exposure is relatively low and unlikely to compromise clinical effectiveness. The significance of multi-resistant plasmid transfer in Gram-positive species, particularly in *Staphylococcus* species, requires further investigation, as does the possibility of mutation in shared target sites in Gram-negative and Gram-positive species. Although biocides are normally used at concentrations which are rapidly bactericidal, in any environment (or downstream of that environment) there is likely to be a continuum of biocide concentration ranging from treatment concentration to nil. Theoretically, sublethal concentrations of biocide for any given cellular target will occur at some point along this concentration gradient, providing a selection pressure for mutations in a multiplicity of cellular targets. Biofilm communities also provide highly selective environments where sharp gradients of antimicrobial agents will

prevail and selective pressures will be greatest (McBain and Gilbert 2002).

However, if acquisition of multi-target plasmids encoding reduced biocide susceptibility alongside antibiotic resistance is a real possibility, it is interesting to speculate to what extent this might occur outside the hospital environment. Logically, the sequential addition of antibiotic resistance determinants onto plasmids conferring reduced susceptibility to biocides should only occur where bacterial populations are exposed not only to a persistent low level of biocides, but also regularly subjected to the selective pressure from antibiotics. This situation is less likely to occur in the home than in the hospital, where antibiotic usage is much less. Jarvinen *et al*. (1993) reported a study of clinical isolates of *Streptococcus mutans* from the mouths of 114 school children and students from families in which about 70% used oral preparations containing chlorhexidine on a regular basis. MIC determinations showed no evidence of increased resistance to chlorhexidine or to a range of antibiotics. Rutala *et al*. (2000) found that the frequency occurrence of antibiotic resistance in environmental isolates from homes was much lower than for clinical isolates from a hospital intensive care unit and an outpatient setting. A few isolates of *Enterococcus* and *Enterobacter* showed resistance to vancomycin and cefotaxime, respectively, but no evidence of resistance was seen in isolates of *Staph. aureus*, *Ps. aeruginosa*, *E. coli* or *Klebsiella pneumoniae*.

At present, however, as also concluded by other reviewers (Russell *et al*. 1999a; Russell 2000; Gilbert *et al*. 2002), there is no equivocal evidence that biocide usage contributes to the development of antibiotic resistance, either in clinical practice or in the general environment. This is supported by the fact that the history and pattern of biocide use does not correlate with emergence of antibiotic resistance; in the UK, biocide use in hospitals has declined over the last 30 years whereas antibiotic resistance has steadily increased.

It must also be borne in mind that if selection of co-resistance to biocides and antibiotics through shared targets depends on the existence of specific biocide targets, this is unlikely (although not impossible) to apply to chemically-reactive agents, such as chlorine or oxygen-releasing agents, or solvent molecules, such as alcohols. The likelihood is further reduced by the fact that these agents are unstable or volatile and thus, do not persist in the environment in an active form. It is therefore perhaps surprising that Murray *et al*. (1984) reported that chlorination of sewage produced an increase in the proportion of antibiotic-resistant isolates. A suggested explanation was that chlorination caused selection of stress-tolerant strains which were also antibiotic-resistant, or stimulated transfer of resistance plasmids, but these aspects were not investigated.

4.3. Antimicrobial resistance caused by exposure to environmental stresses

As pointed out in section 4.2.1, conditions which could facilitate development of antibiotic resistance include not only biocide exposure, but also the whole series of environmental stresses to which microbial populations are continuously subjected. Bacteria are intermittently or continuously under much stress of both the general type (e.g. paucity of nutrients) and the specific type (presence of superoxide, peroxide and antibacterial substances both endogenous and exogenous). Any sudden change in the environment to which cells cannot immediately respond (e.g. plenty as well as starvation) may be seen by a microbial population as a 'stress'.

4.3.1. Intrinsic resistance to antibiotics and biocides through phenotypic adaptation. As recently reviewed by Brown and Barker (1999), whereas studies using populations grown under optimal laboratory conditions give valuable information, in reality, such conditions rarely occur *in situ*. In the domestic environment as in all environments, microbes typically exist in a nutrient-depleted, slow-growing or non-growing state, either in suspension as biomasses or as adherent biofilms. Biofilms occur on the surface of meat in the domestic kitchen, or on the toilet bowl surface, or on the surface of cleaning cloths. In these environments, protozoa can also act as reservoirs of microbes.

In clinical situations, cells may grow, again under nutrient-depleted conditions, as biofilms on epithelial surfaces, or intracellularly in macrophage or gut cells. Intracellular growth and growth under biofilm or planktonic conditions in nutrient-depleted, starved or stressed conditions gives rise to distinct phenotypes. The phenotypes, expressed by growth under these conditions, are typically significantly more resistant to biocides and antibiotics; the clinical or therapeutic impact of this reduced susceptibility may also be as great, if not greater, than that associated with biocide exposure. Brown *et al*. (1990), Gilbert *et al*. (1990) and Brown and Gilbert (1993) review laboratory data which show that the resistance to many antibiotics and biocides of bacteria grown in nutrient-limited conditions as biofilms, or in the stationary phase of growth, may be orders of magnitude higher than that of log-phase cells growing in laboratory media. A typical laboratory study simulating in-use conditions using *Ps. aeruginosa* and *Staph. aureus* showed that whereas the bactericidal concentration of BAC (producing a 5 log reduction in 5 min) against laboratory-grown suspensions was 10–20 μg l^{-1}, the concentration required to produce a similar effect against simple biofilms grown for 24 h on stainless steel discs was as much as 2000 μg l^{-1} (Bloomfield and Sims 1996). Interestingly, however, under the same conditions, the bactericidal concentration of

alcohol was little affected. For *Legionella pneumophila*, replication in macrophages results in morphological and biochemical changes, and 1000-fold increases in resistance to antibiotics and biocides, compared with cells grown in conventional media (Barker *et al*. 1995).

Recent studies (Foley *et al*. 1999) suggest that the response to stress may be a critical event in chronic infection, resulting in at least a sub-population of cells contributing to the characteristic antibiotic resistance of chronic infections. These workers have produced evidence of strong expression of *rpo*S, the major regulator of the general stress response, in sputum from cystic fibrosis patients with chronic *Ps. aeruginosa* lung infection, and suggest that this may explain, at least partially, the exceptional antibiotic resistance of biofilms. It may also be of critical importance in the *in vivo* response of environmental strains not only to biocides but also the whole range of food, personal and other products in daily use which have an affect on microbial survival and growth. Whyte *et al*. (2001) showed that a high proportion of a range of food, household and personal products which, as described previously, act as inducers of Mar and Acr when tested against *lacz* fusions of *E. coli*, also induce strong expression of *rpoS*. Again, this study included food substances and household products, none of which made hygiene claims. Greenaway and England (1999a, b) have recently shown that the stringent response may also be a factor in determining increased intrinsic resistance to biocides and antibiotics.

It is concluded that although the practical implications of reduced susceptibility to antimicrobial agents associated with exposure to sublethal concentrations of biocides must not be underestimated, the impact must be weighed up in relation to the changes in antimicrobial susceptibility induced by other agents (physical or chemical) which have an inimical effect on the cells and/or the 'stressful' environmental pressures to which microbial populations are continually exposed.

4.3.2. Acquisition of resistance to antibiotics through persistence in the environment. Although there is no specific evidence available, various workers (George 1996; Nikaido 1998a, b) have raised concern that the acquisition of low-level resistance through persistent low-level exposure to biocides as well as antibiotics could facilitate persistence of 'low-level-resistant' populations until a clinically-significant and stable level of antibiotic resistance is achieved through plasmid accumulation or mutation of target sites. It must be borne in mind, however, that, as discussed in section 3.2.1, low-level resistance resulting from expression of efflux pumps such *acr* or *mex* can equally well occur in response to agents, such as salicylate and paraquat, as well as to a whole range of antimicrobial substances and other chemical compounds in daily use. It is also known that other global 'stress' conditions, such as addition of 0·5 mol l^{-1} NaCl or 4% ethanol or entry into the stationary phase (many of which represent the 'typical' environment for microbial cells in the real world), increase expression of the *AcrAB* operon (Ma *et al*. 1995) via other mechanisms not involving *MarRAB* or *SoxRS*.

Recently, Levy (2000) has proposed that over-use of biocides could also have a negative impact by providing a selective environment for less dominant organisms such as MRSA. In line with this, Akimitsu *et al*. (1999) showed that selection of MRSA with decreased susceptibility to BAC produced strains which were cross-resistant to a variety of B-lactam antibiotics but not to other antibiotics. This correlates with a report of four cases in different geographical locations in which children with limited resistance to lactam antibiotics died with MRSA (Anon 1999b).

5. A RISK APPROACH TO HOME HYGIENE

Working parties across Europe and elsewhere engaged in implementing strategies to reduce prescribing of antibiotics in humans acknowledge the need for improved hygiene as a vital component of these strategies (Anon 1999a, 2000). The benefits of this approach have been demonstrated in clinical settings where good hygiene has contributed to reduced antibiotic resistance through reduced prescribing (Schmitz *et al*. 1998).

Research studies over the past few years specifically focusing on the home and reviewed by Scott (1999) and Barker *et al*. (2001), are now providing a better understanding of how infectious diseases are spread in this environment and how, through improved hygiene, a significant proportion of these infections could be prevented. The data show the extent to which bacteria and viruses can be shed or spread from infected food, people, pets etc. They also shows how bacteria and viruses can survive and be transferred in the environment in significant numbers and for significant time periods, and, particularly for viruses, that the infectious doses can be very small. An evaluation of UK surveillance data for 1992–1998 indicated that while inadequate cooking and storage of food accounted for about 61% of outbreaks occurring in the domestic setting, poor hygiene involving the hands and other surfaces was a contributory factor in up to 39% of outbreaks (Ryan *et al*. 1996). Evidence reviewed by Evans *et al*. (1998), Goldmann (2000) and Eccles (2000, http://www.ifh-homehygiene.org/newspage/new05.htm) shows that person to person contact via hands and other surfaces is a significant factor in the spread of enteric viruses, such as rotavirus and SRSV, and also respiratory viral infections such as those caused by rhinovirus and respiratory syncytial virus. Poor home hygiene contributes to the spread of other bacterial infections, e.g. those caused by *Shigella sonnei*.

Growing interest about home hygiene has led an international group of experts to form the International Scientific Forum on Home Hygiene (IFH, http://www.ifh-homehygiene.org). The aim of this organization is to raise awareness of the role of home hygiene in preventing infectious diseases and to promote understanding of practice based on evaluation of the scientific principles. As part of this work, the IFH has produced guidelines for home hygiene and recommendations on suitable hygiene procedures to reduce the infection risk (Beumer *et al.* 1998, 2000, http://www.ifh-homehygiene.org/2public/2pub00.htm). The key feature of the guidelines and recommendations is that they are based on the concept of risk assessment and risk prevention. The guidelines start from the premise that homes always contain potentially harmful microbes (people, pets, food etc.), and that good hygiene is not about eradication but about targeting measures in the places and at the times that matter in order to limit risks of exposure. The risk assessment approach, already well accepted and enforced in public health, food manufacturing etc., is now being adopted as the most cost-effective means of infection control in hospital and community settings, including the home (Bloomfield and Scott 1997; Jones 1998). Since targeted hygiene focuses on preventing cross contamination, major target sites in the home are obviously the hands, and hand and food contact surfaces in the kitchen, bathroom and toilet. Cleaning cloths are also critical factors. Intervention at the appropriate time (i.e. during raw food handling, rather than as part of daily routine cleaning) is an equally fundamental part of targeted hygiene.

Within a targeted approach to home hygiene, it is necessary to identify safe and effective procedures which can be used to reduce the risks of infection transmission. In general, it is assumed that hygiene of cooking and eating utensils, and the hands, can be achieved using detergent and hot water. Since this process relies on mechanical removal of microbes, to be effective it must be applied in conjunction with a rinsing process. Recent studies suggest that an assumption that 'soap and water' cleaning is sufficient to achieve hygiene in all domestic situations is questionable. Working with groups of 20 participants, Cogan *et al.* (1999) demonstrated that following preparation of contaminated chickens in domestic kitchens, *Salmonella* and *Campylobacter* could be isolated from 17·3% of the hands and hand and food contact surfaces sampled. Results suggest that not only hands but also cloths were responsible for dissemination. When participants were asked to carry out a typical washing-up routine with detergent and hot water in a washing-up bowl and then use the cloth to wipe the surfaces, there was some decrease in the frequency occurrence of pathogens but overall, 15·3% of the surfaces sampled showed evidence of contamination. It is likely that *Campylobacter* and *Salmonella* spp. were being spread by the cloth to kitchen surfaces during the cleaning process. In kitchens where hypochlorite was used in addition to the prescribed cleaning regime, a significant reduction in the number of contaminated sites was observed, with only 2·6% of sites showing evidence of contamination. In a further study (Cogan *et al.* 2002) involving a limited number of sites (hands, cloths, chopping board, utensils, tap handles), the numbers of bacteria disseminated were evaluated. The results show that for isolates taken immediately after meal preparation, on 8·3 and 50% of occasions, respectively, the counts of *Salmonella* and *Campylobacter* exceeded 100 cfu per sample area, with 4·8 and 35% of sites showing counts of > 1000. A separate study involving the hands, cloths and chopping board showed that when surfaces were cleaned using the typical bowl-washing routine followed by thorough rinsing under running water, a more significant reduction in the risk of contamination could be achieved. However, 23% of 60 sites sampled still showed *Salmonella* contamination, with 3·3% showing counts greater than 100 cfu.

In a recent study, Rheinbaben *et al.* (2000) investigated transfer of viruses in a student living environment using a bacteriophage, ϕX174, as a model. Following contact (hand-shaking) with a volunteer whose hands were contaminated with the virus, and a door handle also contaminated with the phage, the virus could be isolated with high frequency from hands of contacts. Successive transmission from one person to another could be followed up to the sixth person. Further studies showed that virus transmission was reduced but not prevented by hand-washing with bar soap. The limited efficacy of soap and water in preventing spread of contamination via cloths and washing-up bowls etc. in the kitchen and elsewhere has also been demonstrated by other workers (DeWit *et al.* 1979; Scott *et al.* 1984; Scott and Bloomfield 1990; Humphrey *et al.* 1994).

While inappropriate promotion of antimicrobial products must not be allowed to promote a false sense of security, in some situations they can give an important margin of safety. Although direct epidemiological evidence for the impact of disinfectants on domestic infection rates is not available, a range of *in vivo* studies shows the effectiveness of disinfectants in interrupting the chain of bacterial and viral transmission. Kitchen studies show that a significant reduction in contamination of surfaces and cloths can be achieved by ensuring that contaminated cloths are not used during food preparation (Scott and Bloomfield 1993), while other studies (Ward *et al.* 1991; Sattar *et al.* 1993, 1994) have shown that infection by transfer of rotavirus from surface to hand to mouth, and of rhinovirus from surfaces via the hands to the nasal mucosa, can be prevented by disinfecting surfaces. In devising home hygiene policy, it must be borne in mind that the domestic setting is fundamentally different from hospital, manufacturing and other settings. Although infection risk may be lower, the

level of understanding of hygiene may be poor. In addition, facilities for maintaining hygiene, e.g. inadequate kitchen facilities and over-crowding, may be a problem. For this reason, it may be advisable in certain situations to recommend hygiene procedures which carry a higher margin of safety, such as those involving a disinfectant product.

Good home hygiene also has a role in preventing the spread of antibiotic-resistant strains. Masterton *et al.* (1995) reported a case of MRSA carriage in a nurse associated with a hospital outbreak. Several attempts were made to eliminate carriage using accepted regimes. The home environment was then investigated and MRSA was cultured from a number of surfaces. Topical and systemic anti-staphylococcal treatment was only effective after MRSA had been eradicated from the domestic environment. Herold *et al.* (1998), Zylke (1998) and Dancer and Crawford (1999) report increases in MRSA infections acquired in the community amongst hospitalized cases without predisposing risk factors. A number of reports have identified cases of antibiotic-resistant bacteria being brought into the home on humans and animals, and of their transfer within the home (Allen *et al.* 1997; Cefai *et al.* 1994). Wall *et al.* (1996) reported the health risk of multi-resistant *Salmonella typhimurium* DT104 in cats. It was found that 36% of all isolations of *Salmonella* from sick cats in the UK corresponded to this strain.

6. CONCLUDING REMARKS

As increases in antibiotic resistance continue to reduce our ability to treat infections, prevention of infection through hygiene in hospitals and in the community becomes even more important (Anon 1999a; 2000; Smith *et al.* 1999). When using biocides in the home environment it is vital to ensure that product usage does not contribute to the antibiotic resistance problem. It is also necessary to ensure that the biocides used retain their effectiveness in situations where they can have real health benefits in reducing infection transmission.

Overall, there is strong evidence that good standards of hygiene can have a significant impact in reducing the number of infections arising in the home. It is also concluded that within a risk approach to home hygiene in situations where failure to achieve hygiene carries a risk of serious consequences (e.g. food hygiene), or in the protection of vulnerable groups, we should not be afraid to intervene with a form of disinfection, either a heat process or an effective chemical disinfectant which will inactivate pathogens, including bacteria and preferably also viruses. In making recommendations on the use of disinfectants in the home and other environments, however, it is important to distinguish between 'general' use and 'targeted' use. Josephson *et al.* (1997) showed that introduction of a quat-based cleaner into 10 domestic homes over a 3 month period did not correlate with any significant reduction in microbial contamination levels unless participants were instructed in the targeted use of the products.

On the basis that concerns about antibiotic resistance remain unresolved, the consensus view of IFH is that there is need to ensure that biocides are used responsibly, i.e. in accordance with the recently published IFH 'Recommendations for the Selection of Suitable Hygiene Procedures for Use in the Domestic Environment' (Beumer *et al.* 2000, http://www.ifh-homehygiene.org/public/hypro00.htm). IFH also recommends that in order to avoid the possibility of any impact on antimicrobial resistance in the future, reactive biocides (e.g. peroxide and hypochlorite bleach), and those which evaporate (alcohols) and disappear rapidly, leaving bacteria with no residue to which to develop tolerance, should be preferred.

Targeted hygiene not only provides the most effective means of fighting infectious disease, it also offers a means of addressing concerns about 'too much hygiene' and 'too many antibacterials' amongst a public who have lost confidence regarding appropriate hygiene for their home environment. It is possible that the hygiene hypothesis may have benefited those concerned with infection control by promoting scientific interest in this neglected field of home hygiene and opening up the opportunity to develop an evidence-based approach whereby clear and effective guidance can be given.

7. ACKNOWLEDGEMENT

The author would like to thank Dr Peter Gilbert for valuable discussions and contributions to this paper.

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