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Potential Impact of Increased Use of Biocides in Consumer Products on Prevalence of Antibiotic Resistance

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INTRODUCTION

Problems associated with the development and spread of antibiotic resistance in the clinic have been increasing since the early 1960s and are currently viewed as a major threat to clinical practice (130, 180, 314). It is generally accepted that the main cause of this problem has been and still is widespread inappropriate use and overprescribing of antibiotics in clinical medicine, animal husbandry, and veterinary practice (71, 86, 79, 170, 225, 274). Concern about bacterial resistance has led to calls for increased education of both the public and professionals on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection (68, 83, 111, 214, 304; G. Delage, final recommendations, Global Consensus Conference on Infection Control Issues Related to Antimicrobial Resistance, 1999, p. 503–513; J. C. Wright, Association Internationale de la Savonnerie press response to antibiotic resistance, www.pestlaw.net/x/press/2000/ncis-20000300A.html, 1999).

In recent years, a number of scientists have expressed concern that the use of antimicrobial chemicals (biocides, preservatives) in general practice and in domestic and industrial settings may be a contributory factor to the development and selection of antibiotic-resistant strains (151–152, 192, 193, 240–241, 250, 256–257, 281, 299). This has been particularly the case with regard to the recent trend towards inclusion of antibacterial agents within a multitude of otherwise traditional consumer products (R. Beumer, S. F. Bloomfield, M. Exner, G. M. Fara, K. J. Nath, and E. Scott, Microbial resistance and biocides: review by the International Scientific Forum on Home Hygiene, http://www.ifh-homehygiene.org/2public/2pub03, 2000; J. C. Wright, Association Internationale de la Savonnerie press response to antibiotic resistance, www.pestlaw.net/x/press/2000/ncis-20000300A.html, 1999) and apparent increases in the environmental impact of many active ingredients used in personal care and consumer products, together with pharmaceuticals (66). The general concerns are (i) that commonality of target site between biocide and antibiotic might lead to selection of mutants altered in such targets by either agent and the emergence of cross-resistance (89), (ii) that subtle differences in the biocide and antiseptic susceptibility of antibiotic-resistant strains might facilitate their selection and maintenance in the environment by low, subeffective concentrations of biocides and antiseptics as well as the primary antibiotic, and (iii) that indiscriminate biocide application might cause the evolution and selection of multidrug-resistant strains through polygamous mechanisms such as efflux pumps.

While a number of laboratory in vitro studies have demonstrated possible associations between the exposure of bacterial cultures to subeffective concentrations of biocidal molecules and changes in antibiotic susceptibility, there is currently little or no direct evidence that this is significant in the development of antibiotic resistance in clinical practice (R. Beumer, S. F. Bloomfield, M. Exner, G. M. Fara, K. J. Nath, and E. Scott, Microbial resistance and biocides: review by the International Scientific Forum on Home Hygiene, http://www.ifh

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-homehygiene.org/2public/2pub03, 2000). This is supported by indirect, epidemiological evidence that shows a lack of correlation between the patterns of biocide use in clinical settings and the emergence of antibiotic resistance. In this respect, biocide use in hospitals has generally declined over the last 30 years, whereas the incidence of antibiotic resistance has steadily increased. Similar data are not available for the domestic environment, where broad exposure to a range of biocidal molecules is a recent phenomenon.

The current indications are that if the concerns that the widespread deployment of biocidal molecules impacts antibiotic efficacy are genuine, then its contribution is likely to be relatively minor. Conversely, the tremendous contributions of disinfection and acceptance of hygienic measures towards advances in public health over the last century cannot be denied. Indeed, if reductions in the number of infections requiring antibiotic treatment can be achieved through effective hygiene, including the use of biocidal products, then this is likely to decrease rather than increase the incidence of antibiotic resistance. Accordingly, it is important to ensure that biocide use, as an integral part of good hygiene practice, is not discouraged when there is real benefit in terms of preventing infection transmission. This means that it is also necessary to assess the possibility that the indiscriminate use of biocides and antibacterial products might compromise the in-use effectiveness of such biocides in truly hygienic applications (29). Use of such products must be associated with appropriate analyses of added value to the consumer, particularly when there is no apparent gain in public health.

In this review of the literature, it is our intention to consider the mechanisms by which bacteria may become less sensitive to biocide action and then to look at the potential links between antibiotic and biocide resistance and their implications for the inclusion of antibacterial agents within consumer products. The relevance of laboratory monoculture experiments in particular, where competitive selection pressures are absent, will be viewed in the context of field studies and complex ecologies. First, however, it is necessary to consider the precise meaning of some of the terms used and misused by various opinion-forming groups.

LESSONS IN TERMINOLOGY

The unwanted effects of microbial growth have long been controlled through the deployment of chemical agents, whether these are associated with hygiene delivery, preservation, or chemotherapy. It has also long been recognized that the susceptibility to such agents varies markedly between different groups of organisms and indeed within individual species and genera. The scientific literature is also littered with documentary evidence that altered susceptibility to biocides and antibiotics can be a phenotypic response to the macroenvironment (43, 94, 179, 238, 249). In all instances, there has been a need to quantify such susceptibility as a guide to the prediction of successful treatment outcomes. When assessing such data, it is noteworthy that while there is a common vocabulary relating to biocide and antibiotic susceptibility and a tendency to extrapolate from one situation to the other, this is often done without considering the fundamental differences between the mechanisms of action of antibiotics and biocides and the methods used to evaluate the efficacy of each. Care must be taken in interpreting the literature, especially regarding its deployment of the following terms. A full glossary of terms is provided.

Resistance

Resistance is a description of the relative insusceptibility of a microorganism to a particular treatment under a particular set of conditions. For antibacterial agents, it is usually quantified as the minimum concentration required to exert a definable effect (i.e., growth inhibition) on a population of cells. Resistance measurements should be related to the treatment conditions that prevail in the field. In this context, clinicians, guided by their clinical experience, use the results of antibiotic susceptibility tests to predict the likelihood of therapeutic success. Where there is a change in susceptibility that renders an agent ineffective against an infection that had previously been treatable by that agent, then the organism is referred to as resistant.

Many organisms have always been insensitive to and are thereby intrinsically resistant to particular therapeutic regimens by nature of their physiology or biochemistry. By contrast, much of the work investigating "resistance" to biocides is often solely laboratory-based and not guided by the evidence of treatment outcomes. Sadly, there has been a tendency in the disinfection and hygiene field to describe organisms with altered resistance as being resistant even where such changes are insufficient to result in treatment failures (for example, see reference 108). The literature is therefore peppered with references to biocide-resistant bacteria that do not necessarily correlate with failure of the product or process, i.e., failure to achieve disinfection or antisepsis, yet such associations are often inferred (221, 246). The measure of susceptibility often used in such instances is the minimum growth-inhibitory concentration (MIC), the appropriateness of which to biocides is discussed below. As in the clinical setting, certain groups of microorganism are innately resistant to certain groups of biocide and can be described as tolerant. Preferably the term resistance should be avoided, and reference should only be made to the susceptibility of an organism other than when treatment outcomes are changed.

Bacteriostatic, Bactericidal, and Biocidal Activity

The activity of antimicrobial agents is often quantified as a minimum concentration that is required to inhibit the growth of the target organisms (MIC) or as a concentration that leaves no detectable survivors after a specified contact time (minimum bactericidal concentration [MBC], generally taken as >99.9% killing). Antibiotics are generally pharmacologically precise and exert their action at a single physiological target, often an enzyme (i.e., dihydrofolate reductase). The most common response of the cell is to cease growing (bacteriostasis), but for certain classes of agent (i.e., β-lactam antibiotics), continued growth is permitted, with inhibition of the target leading to an indirect structural lesion in the cell and subsequently cell death. Both growth inhibition and cell death therefore relate to common physiological targets, and the MIC, which can be related to blood and serum levels, provides an appropriate

indication of susceptibility and treatment outcome. In the treatment of infection, bacteriostasis is often effective because the killing and elimination of the pathogen are mediated through host immune defenses. Such augmentation is typically absent in disinfection and hygienic applications.

Biocide formulations, on the other hand, contain antibacterial chemicals that are at sufficient concentration so as to affect multiple rather than singular cell targets. The action of such bactericides is seldom mediated through a single lesion and, unlike that of antibiotics, is a direct consequence of exposure to or chemical attack by the chemical. The actions of biocides are rarely pharmacologically precise and do not usually permit their use as therapeutic agents. The causes and targets related to growth inhibition and cell death are often unrelated, yet in many cases isolates have been described as biocide resistant based on the MIC of the biocode. In such instances the MIC reflects the susceptibility of the most sensitive target site and has little relevance to prediction of biocidal efficacy.

BIOCIDE ACTION MECHANISM

Studies of the mode of action of antimicrobial agents suggest that unlike antibiotics, whose high level of target specificity facilitates selective action against specific cell targets, biocides may act at one or several generalized sites within the cell. This has led to broad classifications of biocides as oxidative, thiol interactive, membrane active, etc. Nonspecific attack against such broad targets reduces their selectivity and negates their enteral and parenteral use in therapy. Relative to the wealth of data concerning the structure-action mechanisms of antibiotics, scant attention has been paid to biocidal molecules. General accounts of the mode of action of biocides have been presented elsewhere (69, 234, 235, 239, 242, 244, 247, 248).

It is tempting to suggest that many of the biocides deployed as hygiene agents interact covalently with generalized chemical targets within and on microbial cells and that for this reason resistant cell lines are unlikely to evolve. Chemically reactive biocides, such as chlorine and oxygen-releasing agents, exert their bactericidal action most probably through oxidation of thiol and other chemical groups represented within a whole range of membrane-bound and intracellular enzymes. Agents such as alcohol and chlorhexidine at bactericidal concentrations produce a denaturation of cytoplasmic proteins and coagulation of cell contents. For other groups of agents, such as polymeric biguanides and quaternary ammonium compounds, action is centered upon physical disruption and partial solubilization of the cell wall and membrane. Thus, the bactericidal action of biocidal agents that exhibit surface-active properties, such as the quaternary ammonium compounds, phenols, and substituted phenols, results from a generalized disruption of the cell membrane. Polymeric biguanides, on the other hand, destabilize divalent cations associated with the cell envelope, disrupting the lipopolysaccharide, and self-promote their own entry into the cell (310). This is an action that parallels the mechanisms of entry of aminoglycoside antibiotics. Interestingly, it is thought that lack of penetration through the cell envelope is the primary reason for the resistance of pseudomonads (59, 109, 110) and *Providencia* spp. (128) to these agents.

Studies with many of these compounds suggest that the high concentrations used in practice to achieve rapid biocidal action produce generalized effects such as disruption of the cell membrane or inactivation of a broad range of enzymes. At lower growth-inhibitory concentrations, they may, however, act in much the same way as antibiotics, specifically affecting one or two cellular targets. Thus, phenoxyethanol (and its analogue 2,4-dichlorphenoxyethanol) affect a multitude of intracellular targets, dependent on concentration (90–92). At sublethal biocide levels, a variety of concentration-dependent inhibitory processes take place. These range from actions as potassium: proton antiporters and respiration uncouplers to competitive inhibition of NADH binding by malate dehydrogenase. Additionally, DNA biosynthesis is slowed relative to general anabolism in the cell at sub-MICs (93). Membrane-active phenolic molecules, such as tetrachlorsalicylanilide and fentichlor, also act as uncouplers of oxidative phosphorylation at concentrations that inhibit cell growth (28, 106).

Isothiazolone biocides, often described as thiol-interactive preservatives, possess subtle secondary targets. While their primary action on the cell is mediated through a chemical interaction with thiols (57), specificity is seen towards a number of respiratory enzymes (56), and at sub-MIC levels, they have been shown to induce filamentation both in bacteria and in the fission yeast *Schizosaccharomyces pombe* (55). While lethal doses affect thiol groups on proteins, sublethal doses more specifically affect DNA (57, 93). Phenylethanol is also known to inhibit the initiation of DNA replication and to cause a filamentation similar to that caused by the isothiazolones (267). More recent studies have shown that in *Escherichia coli* and *Mycobacterium smegmatis*, the widely used antibacterial agent triclosan has a specific action on the enzyme enoyl reductase, which is essential for fatty acid synthesis at concentrations which are growth inhibitory (181, 183, 276, 306).

FACTORS THAT AFFECT BIOCIDE SUSCEPTIBILITY

A number of mechanisms account for the wide range of sensitivity noted for the antibacterial action of antibiotics and biocides (116). Some organisms and genera, by virtue of the absence of critical target sites or an inability of the agents to accumulate at those targets, are intrinsically resistant to particular groups of agents under all growth conditions (110). Other groups of organism may undergo changes in susceptibility that reflect either the conditions under which they were cultivated or exposed (phenotypic change), the temporary expression of efflux pumps or synthesis and export of protective enzymes (inductive change), or mutations in the genes encoding or regulating a sensitive target site (chromosomal change). The acquisition or generation of extrinsic genetic elements that encode such resistance (plasmid-mediated change) can be responsible for the rapid development and horizontal spread of resistance within population groups, particularly where this entails the expression of efflux pumps and drug-inactivating enzymes. Phenotypic, genotypic-chromosomal mutation, and inductive mechanisms of resistance enhancement such as these are thought to have more practical relevance to biocide susceptibility than plasmid-mediated change (179, 238).

Phenotypic Changes in Biocide Susceptibility

The intrinsic susceptibility of an organism to a biocide, documented from an MIC or MBC determination by standard methods, is not a fixed value. Rather, the susceptibility pheno-

type expressed by the cell can vary significantly according to the prevailing physicochemical environment (62, 156). Notable in this respect are the effects, upon susceptibility, of nutrient depletion, physiological growth rate, and growth as a biofilm on a solid surface (39, 42–43, 94–95, 116).

Changes in susceptibility result, at least in part, from changes in the outer cell layers that increase barrier properties and prevent access of biocides to their site of action (243). Paradoxically, the documented MICs bear little or no relation to the in situ MIC (39), where in the domestic as in all other environments microbes typically exist in nutrient-depleted, slow-growing or nongrowing states as biomasses or adherent biofilms (309). In clinical situations, cells may grow as biofilms, again under nutrient-depleted conditions, on epithelial surfaces (13), or intracellularly in macrophages and amoebae (18–19, 301) and upon gut epithelia. In a recent study by Foley et al. (81), it is suggested that a general stress response to nutrient depletion and onset of stationary phase initiates the adoption of resting or dormant phenotypes, analogous to endospores, which are resistant to numerous physical and chemical agents (31, 72). Such events might be critical in chronic infections and result in at least a subpopulation of cells surviving therapy. They presented evidence for the expression of *rpoS*, the major general stress response regulator, in the sputum of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. In such situations, the activity of antiseptics and antibiotics may be substantially affected.

Growth as a biofilm further conspires to reduce the apparent susceptibility profile and is probably caused by a variety of factors, including nutrient depletion within the biofilm and hence expression of the general stress response, reduced access of the biocide to cells in the biofilm, chemical interaction between the biocide and the biofilm, and the production of degradative enzymes and neutralizing chemicals (40, 94–95, 179).

Typically, not only are those phenotypes expressed by growth under these conditions significantly more resistant to biocides and antibiotics, but the clinical or therapeutic impact of this reduced susceptibility may be as great as if not greater than that associated with other mechanisms of resistance. Indeed, it is often these factors that render infections caused by apparently sensitive organisms to be recalcitrant to therapy (i.e., infections associated with indwelling medical devices). In this respect, laboratory investigations have shown that the resistance of bacteria in the stationary phase of growth or in biofilms to many antibiotics and biocides is orders of magnitude greater than that of logarithmic-phase cells typically used in susceptibility testing (43, 76, 94, 314). Simulations of in-use conditions with *P. aeruginosa* and *Staphylococcus aureus* showed that, whereas the bactericidal concentration of benzalkonium chloride, producing a 5-log reduction in 5 min against laboratory-grown suspensions was 10 to 20 mg/liter, the concentration required to produce a similar effect against simple biofilms grown for 24 h on stainless steel disks was as much as 2,000 mg/liter (217, 268; S. F. Bloomfield and C. H. Sims, Abstr. 96th Annu. Meet. Am. Soc. Microbiol., 1996, p. 302). 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 a 1,000-fold increases in resistance to antibiotics and biocides compared with cells grown in conventional media (18, 19). Marrie and Costerton (175) showed that cells of *Burkholderia cepacia* and *Serratia marcescens* adsorbed onto glass surfaces were able to survive in the presence of in-use concentrations of chlorhexidine (0.5% up to 2%), isothiazolones, and quaternary ammonium compounds. Likewise, Stickler et al. (277, 278, 282) showed that mixed biofilms of *Citrobacter diversus*, *P. aeruginosa*, and *Enterococcus faecalis* and monoculture biofilms of *Escherichia coli* grown on silicone disks showed no significant loss of viability when treated with 0.2% chlorhexidine and povidone iodine (1% [wt/vol] available iodine) over 120 min. This should be compared to the response of planktonic cells, grown in samples of urine, which showed 2 to 3 logs of reduction. Equally, Berkelman et al. (23) showed that contamination of povidone iodine formulations (1% [wt/vol] available iodine) implicated in hospital-acquired bacteremia had resulted from the formation of biofilms on the surfaces of pipes of the manufacturing equipment by otherwise sensitive strains of *P. aeruginosa*.

Planktonic strains are unable to alter their susceptibility to povidone iodine after multiple passage (125), and, in a 6-month comparison of daily povidone use in continuous ambulatory peritoneal dialysis patients with hypochlorite, no changes in susceptibility were detected in coagulase-negative staphylococci (139). Similarly, major factors affecting the efficacy of chlorine-based biocides are aggregation, attachment of the target bacteria to surfaces, and starvation (149, 162).

In conclusion, while phenotypic resistance to biocides and antibiotics is frequently overlooked as a cause of therapeutic failure, its contribution to the in situ susceptibility of microorganisms is massive. Indeed, other than the ability to export drug-degrading enzymes, phenotypic resistance has probably the greatest influence of all. Such resistance is not amenable to quantification with MIC or MBC determinations by conventional culture methods. Since phenotypic change can cause susceptibility to alter by several orders of magnitude, it renders recorded changes in the MIC of 5- to 10-fold irrelevant. This is generally the range of susceptibility demonstrated within related species and clones. Large reductions in the susceptibility of natural communities of bacteria over those of laboratory monocultures does however increase the likelihood of situations in which the concentration of biocide will be subinhibitory for some cells and thereby impose a selection pressure towards the less susceptible clones. Such selection pressures will not occur when the mechanisms associated with decreased susceptibility relate to the adoption of a quiescent state. Rather, repeated treatments will cause a posttreatment clonal expansion of these lines.

Reduced Susceptibility to Biocides Associated with Inductive Change

Bacterial vomit response. Recent studies have shown that efflux pumps, sometimes with unusually broad specificity (towards mostly lipophilic or amphipathic molecules), also contribute to the intrinsic resistance of gram-negative bacteria to a variety of agents, including dyes, detergents, and antibiotics (154, 202, 203, 204). These pumps are chromosomally encoded in gram-negative bacteria, and their expression is induced

through sublethal exposure to a plethora of agents (88, 153; P. J. Marshall, P. Rumma, and E. Reiss-Levy, Presentation at the 11th National Conference of the Australian Infection Control Association, 7-9 May 1997, Melbourne, Australia; F. W. Whyte, D. G. Allison, M. V. Jones, and P. Gilbert, Abstr. 101st Annu. Meet. Am. Soc. Microbiol., 2001, abstr. A99). These include not only small hydrophilic antibiotics but also other xenobiotics such as quaternary ammonium compounds, pine oil, and salicylate (188, 190). Sublethal exposure of bacterial populations to some biocides might therefore induce multidrug resistance for as long as the pump is actively expressed.

The *mar* locus of *E. coli* regulates the expression of the *acrAB* efflux pump (88, 167–169) and was the first chromosomally encoded operon found to be involved in the intrinsic resistance of gram-negative bacteria to multiple drugs. Efflux pumps are operational in a wide variety of gram-negative organisms, are highly conserved, and may also be carried on plasmids (203). There are four major superfamilies of efflux pumps. Each superfamily contains pumps that are specific for single agents together with pumps that are responsible for multidrug efflux. The latter have broader specificity and are capable of removing a wide range of structurally unrelated antibiotics and disinfectants from the cell. Any of these types of efflux pump might be involved primarily with the extrusion of endogenous metabolites, or they may be involved primarily with the efflux of chemotherapeutic agents. Indeed, it is probable that these exporters evolved originally to extrude endogenous metabolites but that a coincident ability to exclude harmful substances has proven to be a desirable survival strategy that has been selected for, and incorporated, by almost every known genus and species of bacterium.

The *acrAB* efflux system in *E. coli* acts as a transporter for tetracycline, ciprofloxacin, fluoroquinolone, β-lactams, and novobiocin as well as ethidium bromide, acriflavine, phenylethylalcohol, sodium dodecyl sulfate, and deoxycholate (167–169, 197, 211; J. M. Buysse, W. F. Demyan, D. S. Dunyak, D. Stapert, J. C. Hamel, and C. W. Ford, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., 1996, HP abstr. C42). *acr* systems are also found in other species of *Enterobacteriaceae* such as *Salmonella* spp. (203, 286–7). In *P. aeruginosa*, the *mexAB*, *mexCD,* and *mexEF* multidrug efflux systems act as transporters for a variety of agents, including tetracycline, ciprofloxacin, fluoroquinolone, beta-lactams, and fusidic acid. These efflux systems act as transporters for a whole range of biocides and antibiotics that, coupled with the narrow porin channels in the outer membrane of this organism, restrict diffusion of many antimicrobial agents into the cells. This is probably responsible for the very high intrinsic resistance of this species to antimicrobial agents compared with other gram-negative species (261).

Studies with planktonic cultures have shown that expression of *acrAB* is enhanced under conditions of general stress, such as exposure to ethanol or simply entry into stationary phase (168). Continuous culture experiments conducted in *E. coli* with a *lacZ* reporter gene fused to $marO_{II}$ showed that *mar* expression was inversely related to specific growth rate (172, 173). Hence, *mar* expression will be greatest within the depths of the biofilm, where growth rates are suppressed, and might account for the long-term survival of the community when exposed to inducer molecules.

Realistically, it may be that antibiotic efflux caused by exposure to biocides is of no particular significance in the real world, since they represent only one of a whole series of inimical agents that elicit this effect. If these efflux pumps indeed evolved as a defense against antimicrobial agents occurring in the environment (188), then "natural" antimicrobial agents would also contribute to this problem. While George and Levy (88) and Cohen et al. (54) have shown that low levels of antibiotics such as chloramphenicol and tetracycline and biocides such as pine oil act (190) as weak inducers of the *mar* operon, so also do a variety of food supplements such as chili, mustard, and some herbs (F. W. Whyte, D. G. Allison, M. V. Jones, and P. Gilbert, Abstr. 101st Annu. Meet. Am. Soc. Microbiol., 2001, abstr. A99) and weak acids like salicylate (53). Sundheim (290) also noted that a novel disinfectant based on extracts of grapefruit seeds also served to select for decreased susceptibility to itself and some quaternary ammonium-based agents. Sulavick et al. (287) noted that the *marR* represser of *marA* is inactivated by a variety of phenolic-based compounds, many of which are naturally occurring and of plant origin.

Efflux pumps as a resistance mechanism are not restricted to gram-negative bacteria. The multidrug efflux pumps *qacA* to *qacG* contribute to biocide tolerance in *Staphylococcus aureus* (230) and have been the subject of a number of excellent reviews (269–270). These genes can become located on integrons and transferred between gram-positive organisms such as staphylococci and enterococci and also to gram-negative bacteria such as *P. aeruginosa* and the vibrios (135–136).

Efflux pumps contribute in a major way to the resistance of microorganisms to antibiotics and biocides. In order to gain further insight into their significance, we must consider what the normal physiological function might be. Current knowledge suggests that efflux pumps are part of the natural defense mechanisms against natural environmental toxicants. Although gram-negative bacteria defend against large hydrophilic toxicant molecules by utilizing narrow porin channels in their outer membranes, the lipid component of the membrane allows slow diffusion of lipophilic agents. Thus, *acrAB* is essential for survival of *E. coli* in the presence of bile salts in the gut (298). The wide substrate specificity of these pumps and, for the *acrAB* pump, regulation by global stress signals rather than specific substrates make such systems well suited for a general defensive role. Their broad substrate specificity however, probably causes as well as solves problems, since they will "accidentally" pump out key metabolites such as pyruvate or lactate (141).

Efflux may therefore be likened to a bacterial vomit response that enables populations to respond not only to changes in their environment, but also to the threat posed from natural bacterial inhibitors and chemotherapeutic agents. Indeed, Greenaway et al. (98, 99) recently proposed that rapid increases in levels of ppGpp which result from the growth-inhibitory effects of antimicrobials control the expression of *rpoS* and thereby *rpoS*-mediated efflux resistance determinants directed towards acid, peroxide, and osmotic fluctuation. If the primary function were to facilitate a continuous modulation of efflux activity in response to toxicants in the environment, then it seems unlikely that efflux systems that are constitutively expressed are the preferred or stable state. Rather, it is imperative that efflux be repressed when it is unnecessary.

In such a fashion, efflux per se of biocides that are both inducers and substrates should be a temporary event. Such biocides will only impact the pattern of antibiotic resistance in the environment when their use leads to a selection of clones that hyperexpress when induced. In this respect, it is notable that the level of expression of constitutive *mar* mutants is affected by the interactive binding of several transcriptional activators such as *marA*, Rob, SoxS, and Fis (3, 4). With the appropriate selection pressures, it is therefore possible to select populations that inducibly hyperexpress efflux. Thus, it is significant that in *mexAB*-deleted mutants of *P. aeruginosa*, sublethal exposure to many antimicrobials can select for cells that hyperexpress an alternative efflux pump, MexCD (51) and/or MexJK (52). Significantly, MexJK, while also part of the RND family of efflux systems, does not appear to be associated with the outer membrane porin (OprM) in order to efflux triclosan (52). Exposure to inhibitory molecules, such as triclosan and fluoroquinolones, that are substrates for efflux but not inducers will therefore dictate towards constitutive efflux pump expression, so long as the selection pressure is maintained.

Destruction of biocide. Some microorganisms are able to demonstrate intrinsic resistance through inactivation of biocides. Biocides are less likely than antibiotics to be inactivated by bacteria, but examples include the inactivation of phenols and some aldehydes in species of *Pseudomonas* (273, 302) and of triclosan (112, 186). Enzymatic degradation of formaldehyde release agents by species such as *Pseudomonas putida* is, in some instances, sufficient to degrade the preservatives and enable spoilage by secondary colonizers (48). Inactivation of quaternary ammonium compounds, chlorhexidine, and phenylethanol has also been reported, but only at concentrations below those used in practice. It is therefore unlikely to be a mechanism of resistance to these compounds (249), but will assist in the removal of such agents from the environment. Recently, Nishihara et al. (205) described the inactivation of didecyldimethylammoniun chloride by a strain of *Pseudomonas fluorescens*, and Meade (M. Meade, Abstr. 100th Annu. Meet. Am. Soc. Microbiol., 2000, abstr. A73) reported the detoxification of triclosan. In all instances, it is probable that chronic exposure of populations to subinhibitory concentrations of such agents will lead to an induced expression of biocide-degrading enzymes and a selection pressure towards those clones that can hyperexpress them. It is unlikely that such enzymes would confer cross-resistance to third-party antibiotics, and their existence is indeed essential if the affected biocides are to be biodegraded rather than recalcitrant within the environment.

Reduced Susceptibility to Biocides Associated with Chromosomal Change

Target modification. Since antibiotics usually act at specific sites within the bacterial cell, chromosomal mutations that alter those targets are likely to alter not only the functionality of that target but also its susceptibility to the antibiotic. Often such mutations impose a fitness cost that, in the absence of antibiotic stress, will severely reduce the competitiveness of that clone. Chromosomal gene mutations that confer resistance to antibiotics have been relatively well studied. Mechanisms include bypass of a sensitive metabolic step, alterations in the normal target site of the antibiotic such as a change in the bacterial ribosome or enzyme, and overexpression of the target enzyme or an efflux pump (above). Some biocides that also have specific singular target sites at growth-inhibitory concentrations have been identified, but in contrast to the field of antibiotic research, few studies of the effects of mutations at those sites have been made. There is, however, no obvious reason why the lessons learnt for chromosomal mutation and resistance to antibiotics should not apply equally to the growth-inhibitory action of biocides when the biological effects are governed by action at a single target.

Such a view has been substantiated by the identification of enoyl reductases as growth-inhibitory targets for triclosan in *E. coli* and *Mycobacterium smegmatis* (113, 115, 181, 183, 276). Exposure of *E. coli* to sublethal concentrations of triclosan has been shown to select, at relatively high frequency, clonal mutations that are either modified in the *fabI* gene, encoding the enzyme, or where the gene has been repressed or deleted. In either instance, the susceptibility is reduced, giving rise to a series of mutants with increasing levels of resistance. Such mutants have been characterized and found to relate to a single-amino-acid changes in *fabI* at that codon for glycine 93 in *E. coli* (115) and at the codon for glycine 95 in *P. aeruginosa* (123). Levy's group postulated that, rather like an antibiotic, triclosan has a specific mechanism of action and blocks lipid synthesis for which enoyl reductase is an essential enzyme. *S. aureus* mutants that possess decreased susceptibility to triclosan (MIC > 1 μg/ml) have been isolated in a similar fashion (256–257, 289). The *S. aureus* mutants, like the *E. coli* mutants, show hyperexpression of a modified *fabI* gene product (77). In all cases, the mutant cells were no less sensitive to the bactericidal effects of triclosan than were the parent wild-type strains. This supports the view that the bactericidal effects of triclosan reflect a multiplicity of targets that include the enoyl reductase as the most sensitive and responsible for growth inhibition (178).

The identification of specific, highly sensitive targets responsible for the growth-inhibitory action of triclosan (180) offers the possibility that exposure of populations of bacteria to sublethal levels of the agent might coincidentally select mutant populations with reduced susceptibility to third-party therapeutic agents which possess this as their sole target.

The recent finding that an enoyl reductase enzyme in *M. smegmatis* (183) is the target not only for triclosan but also for the chemotherapeutic agent isoniazid is of concern. Deletion of the *inhI* gene, the *fabI* homologue, led to 1.2- to 8.5-fold increases in the MIC of isoniazid and 4- to 6.3-fold increases in the MIC of triclosan. Fortunately, further studies have shown that isoniazid-resistant enoyl reductase in *Mycobacterium tuberculosis* retains its susceptibility to triclosan, indicating that while the two agents have a common target enzyme, their interactions with that target are distinct. Thus, rather than threaten chemotherapy, molecules based on triclosan action offer the potential to develop not only novel antitubercular molecules (114), but also antimalarial drugs (220).

Earlier studies, reported by Cookson et al. (61), had suggested that low low-level triclosan resistance in methicillin-resistant *S. aureus* could be cotransferred with mupirocin resistance to sensitive *S. aureus* recipients. This implied the existence of a common resistance mechanism that could be

conveyed on a plasmid. Recent studies with *S. aureus* (288–289) have shown, however, that the acquisition of mupirocin resistance through a plasmid is unassociated with any change in triclosan susceptibility. Conversely, while mutants of *S. aureus* that stably inherit a decreased susceptibility to triclosan (>1 μg/ml) (274) have been isolated, these are no less sensitive than the parent strain (MIC of triclosan, 0.025 μg/ml) to a range of antibiotics or to the lethal effects of triclosan at 7.5 μg/ml. Similarly, whereas the MICs of *E. coli fabI* mutants were increased to 25 to 50 μg/ml, compared with 0.1 μg/ml for the wild type, tests with triclosan-containing products showed no differences in the rate of kill of the mutants (178).

As a biocide, triclosan is probably not unique in having a singular critical process associated with its activity that is shared with therapeutic agents. Such processes might relate to uptake as well as the interactive target. Therapeutic agents such as the aminoglycosides, for example, are known to gain access to gram-negative cells through a self-promoted mechanism (109, 292). In self-promoted uptake, the agent destabilizes cell envelope-associated cations to cause a reorganization of the lipopolysaccharide and thereby facilitate antibiotic entry. It is notable that many biocides, particularly polymeric biguanides (310), share this mechanism of cellular uptake in order to access their target sites at the cytoplasmic membrane.

Changes in the cell envelope, notably those that decrease the extent of cation binding to the outer membrane, coincidentally affect aminoglycoside and polymyxin susceptibility. *P. aeruginosa* cultures trained by passage to resistance against increasing concentrations of polymyxin (44) show decreases in their susceptibility to quaternary ammonium compounds (41). This is contrary to the result reported previously by Adair et al. (2), who had noted that the susceptibility to polymyxin B and polymyxin E was markedly enhanced in *P. aeruginosa* trained to benzalkonium chloride (1). The latter study also noted that the less-susceptible clones were enhanced in their susceptibility to the ion chelator EDTA, indicating changes in the association of cations with the outer membrane, this being the primary target both for EDTA, polymyxins, and quaternary ammonium compounds.

Guerin-Mechin et al. (101, 102) and Mechin (187) noted changes in inner and outer membrane fatty acid composition in quaternary ammonium compound-trained cells that would be consistent with changes in lipopolysaccharide or in the hydrophobicity of the membrane cores. In a similar vein, Ismaeel et al. (128) observed decreases in susceptibility of *Providencia stuartii* to chlorhexidine that could also be attributed to changes in the outer membrane. Nicoletti et al. (201) noted that changes in susceptibility induced by 12 weeks of passage of *Serratia marcescens* and *P. aeruginosa* gave concurrent alterations in susceptibility to quaternary ammonium-based formulations. Changes in inner and outer membrane composition may coincidentally affect susceptibility to chemically unrelated antibiotics in a nonspecific fashion (105). Prince et al. (224) reported similar changes after chlorhexidine passage but noted that only those within the *Serratia* spp. were stable after removal of the selection agent.

In the yeast *Saccharomyces cerevisiae*, reduced susceptibility to benzalkonium chloride has been associated with loss of a cytoplasmic membrane, active-transport leucine pump (209–210). The possibility arises that the quaternary ammonium biocides might also utilize such transport processes in order to gain access to bacterial cells and that deletion/repression might be associated with resistance. Mutants of *S. aureus* with concurrent reductions in susceptibility to a pine oil cleaner formulation and the antibiotics vancomycin and oxacillin have recently been reported (223). While the changes were insufficient to confer resistance to these agents, the trait appeared to be regulated by an alternative sigma factor, SigB. Significantly, *S. aureus* strains with intermediate resistance to glycopeptide were also less susceptible to this cleaner formulation. The concentrations of pine oil and the nature of other excipients within the cleaner were unknown.

Phenylethanol (267) is known to inhibit the initiation of DNA replication and to cause a filamentation similar to that observed after subinhibitory concentrations of the isothiazolone biocides. Filamentation is generally associated with an inhibition of DNA gyrase associated with chromosome replication or inhibition of peptidyl transferase. While there is no evidence to suggest that this is the case, it is not difficult to imagine how sublethal effects directed towards these targets might affect the action of quinolone and β-lactam antibiotics, respectively.

Chromosomal efflux mutants. The potential of various antimicrobial treatments to either directly or indirectly, through pppGppp and *rpoS*, to induce the expression of efflux has been discussed earlier. In such situations, it is clearly advantageous for cells to maintain efflux as an inducible function, with chronic treatments with antibacterial agent favoring those clones that can inducibly hyperexpress this phenotype. There are a number of agents, such as the antibiotic ciprofloxacin (140) and the antibacterial agent triclosan (262), that are substrates for efflux pumps but not inducers of their expression. Prolonged exposure to sublethal concentrations of such agents would select mutant cell lines that express the efflux pumps constitutively (bulimic bacteria). Further studies (51) have shown that exposure to triclosan selected a multidrug-resistant strain that hyperexpressed the *mexCD* efflux system genes from a susceptible population of *P. aeruginosa* mutants in which *mexAB* was deleted. In addition to reduced susceptibility to triclosan, this strain showed a marked decrease in susceptibility, as assessed by MICs, to several antibiotics, including tetracycline, ciprofloxacin, and trimethoprim. Under normal circumstances, such mutant clones might be expected to be disadvantaged with respect to wild-type cells but might come into dominance when all other members of the community succumb to the treatment agent. Clearly, in such circumstances, the selected efflux mutant might be less competitive but possess multiple resistance to a number of agents.

Thus far, only pine oil disinfectants and triclosan have been evaluated, but it has been suggested that further studies are likely to show that selection of efflux mutants is facilitated not only by exposure to various types of antimicrobial substances, including so-called natural antimicrobials, but also many other chemical agents, including various types of household surfactants and cleaning chemicals and other compounds which form part of daily life. In this fashion, mutations in the repressor genes of *E. coli* causing overexpression of *marA* or *acrAB* (182, 190) and in *P. aeruginosa* of *mexAB* (51, 261) are associated with laboratory experiments that expose monocultures to a wide range of chemicals and antibiotics (157–160, 222), engen-

dering generalized reduction in susceptibility through hyperexpression of efflux pumps. When the selective pressure is removed, it is suggested that mutant populations will decline in favor of wild-type populations that retain their ability to repress efflux in the absence of inducers.

In assessing the importance of multidrug efflux pumps and of the potential risks associated with the selection of efflux mutants, their significance to clinical practice must be evaluated. Much of this evidence relates to the opportunistic pathogen *P. aeruginosa*, for which the effects of efflux are compounded by the narrowness of its outer membrane pores. Thus, Rella and Haas (226) mutated the repressor gene of the *mexAB* operon to give hyperexpression and showed that the MICs of ciprofloxacin and carbenicillin were increased 8- and 32-fold, respectively. Among carbenicillin-resistant clinical isolates of *P. aeruginosa* collected in a United Kingdom study in 1982, almost 80% did not produce carbenicillin-hydrolyzing J3-lactamase and appeared to belong to the elevated efflux type (311). Similarly, a study conducted in France showed that approximately one third of ticarcillin-resistant *P. aeruginosa* isolates had susceptibility patterns that were characteristic of multidrug efflux (24).

Other gram-negative organisms, such as *E. coli* and *Salmonella* spp., have much wider porin channels, and the significance of multidrug resistance to clinical practice is far less apparent (169). In such organisms, the positive regulator *marA* (produced by the *mar* operon) controls the major efflux pump, *acrAB* and efflux mutants, related to *acrAB*, occur most readily through mutations in the *marR* repressor locus (3). Thus, of 28 quinolone-resistant isolates of *E. coli* examined by Maneewannakul and Levy (174), 3 possessed elevated *marA* transcription. It must be borne in mind, however, that overexpression of *marA* also reduces the levels of a porin, encoded by *ompF*, a secondary factor that will also reduce the general susceptibility of such strains. Notable in this respect is the observation that silver-resistant mutants of *E. coli* resist silver by an efflux mechanism that is augmented by deficiencies in *ompF* and *ompC* expression (160). For some staphylococci isolated in the clinic, significant resistance to fluoroquinolones has been partly associated with overexpression of the multidrug efflux gene *norA* (133, 200, 317).

It is still unclear, however, even in the case of *P. aeruginosa*, what fraction of the resistant isolates of clinical origin corresponds to efflux mutants. Opinions also differ as to their importance in clinical practice. Thus, in recent reviews, Nikaido (202–204) conceded that multidrug efflux is "probably not yet the most frequent mechanism of resistance among clinical isolates" but notes that recent reports suggest that efflux-based resistance is now occurring with increased frequency. If one were to accept that exposure of microbial populations to low-level antibiotics is a causative factor in the emergence of multidrug efflux mutations which confer clinically significant levels of antibiotic resistance, it is a natural follow-on that exposure to low levels of biocide might have the same effect. In this context, it is worth noting that recent studies by Levy and coworkers (190) with efflux mutants of *E. coli* selected with pine oil disinfectant showed that the levels of resistance generated to tetracycline, chloramphenicol, ampicillin, and nalidixic acid were relatively low and unlikely to compromise their effectiveness in clinical use. It is also noteworthy that the majority of clinical infections relate to monocultures, whereas the environments in which biocides are likely to accumulate at sublethal levels are colonized by a plethora of competing species. While in the former the relatively sickly efflux mutants may retain sufficient activity to infect, particularly in the immune-deficient patient, in the latter instance efflux mutants are unlikely to retain their competitive position within the community.

Of concern also with the observation of single-site mutations (target modification), leading to changes in the MIC, is the possibility that such resistance determinants might become fused into plasmids or other transposable genetic elements and transferred from environmental organisms to those of clinical significance. If such genetic elements already bore unrelated antibiotic resistance determinants, it becomes possible that the persistent low-level exposure of a population to a biocide could reinforce the selection of antibiotic-resistant populations in the absence of a therapeutic agent (249, 279). This is unlikely to occur with efflux mutants because the mutations that are known all occur in the repressors, which are recessive, and will not have an effect if transferred by plasmid.

Reduced Susceptibility to Biocides Associated with a Plasmid

Horizontal transmission of resistance determinants, manifested as infectious drug resistance, has been well known since the middle 1960s. While such genetic elements can fuse to produce large multiresistance plasmids, the fusions are inherently unstable. The persistence of large multiple resistance plasmids therefore reflects the combined selection pressures to which the cells containing them are exposed. In this fashion, the resistance plasmids associated with hospital and other clinical environments often mirror the antibiotic usage pattern within the institution.

The first evidence that plasmids might be capable of encoding reduced susceptibility to biocidal agents as well as to antibiotics specifically related to heavy metals (silver, mercury, organomercurials) (49, 236) and copper resistance (58). Aspects of plasmid-mediated transmission of biocide susceptibility determinants have been reviewed (233, 236–237). Silver salts are important topical antimicrobials, and resistance to these has been noted in the clinic (103–104, 137), but most metal compounds that illustrate plasmid-mediated resistance are not widely used as disinfectants.

Significant numbers of publications have, however, assessed the MICs of various biocides and attempted to relate these to the presence of antibiotic resistance plasmids (for a review, see reference 8). Thus, plasmid-carrying methicillin-resistant *S. aureus* strains have altered susceptibilities to a variety of biocides that includes chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, triclosan, parahydroxybenzoates, and betadine (6, 7, 9, 45, 60, 127, 193, 300, 316). Other studies, for example with coryneforms associated with infections of the immunocompromised host, have concluded that there is no link between plasmid carriage and antiseptic resistance (155). More recent studies have associated the presence of plasmids in bacteria with low-level resistance to chlorhexidine, quaternary ammonium compounds, triclosan, and hexachlorophene. In none of these instances has a causal link been demonstrated, although it is likely that in those studies involving quaternary ammonium-based compounds, efflux might be implicated.

The transfer of biocide susceptibility traits has been associated with plasmid acquisition by wild-type organisms (9, 70, 296), but the extent of the associated change has, in all instances, been insufficient to confer resistance to use concentrations of the agents. The susceptibility change most probably relates, therefore, to indirect effects of the plasmid, such as changes in the cell envelope. In some instances, direct, firm associations have been made between the presence of plasmids and biocide susceptibility, and these have generally been linked either to efflux mechanisms and associated mainly with gram-positive bacteria such as *S. aureus* or, as is the case with resistance to inorganic and organic mercury, to inactivation of the biocide.

Generalized reductions in susceptibility to ethidium bromide, acriflavine, cetrimide, benzalkonium chloride, and diamidines such as propamide isothionate are mediated by a group of structurally related plasmids encoding quaternary ammonium compound efflux pumps (21, 22, 49, 163–166, 189, 213, 227, 297). The *qacA* gene can be found on the pSK1 family of multiresistance plasmids and codes for a multidrug efflux pump, whereas *qacB* is found on many beta-lactamase and heavy metal resistance plasmids such as pSK23 (230, 238). Although coding for a similar protein, *qacB* is more specific and relates only to intercalating dyes and quaternary ammonium compounds (213–5). *qacC* and *qacD* encode resistance to ethidium bromide and some quaternary ammonium compounds and are typically found on the plasmids pSK89 and pSK41. Expression of *qacA* is governed by QacR, a repressor of *qacA* expression, which is bound to inducer molecules in an analogous fashion to the MarR of gram-negative organisms (100).

Many studies (21, 22, 117, 119–20, 206) show that these *qac* genes are widely distributed in clinical and food isolates of *S. aureus*. Plasmid-encoded *qacA* and *qacC* genes have also been reported in *Staphylococcus epidermidis* (150). Of concern is the finding that the *qacA/B* family of genes show significant homology to other energy-dependent transporters, such as the tetracycline transporters found in tetracycline-resistant strains, while *qacA/B* can be borne on penicillinase plasmids (230, 238). Sidhu et al. (266) showed that while resistance to β-lactam antibiotics and quaternary ammonium compounds was carried on separate plasmids, these could be coselected during antimicrobial therapy.

Bolhuis et al. (32, 33) reported two types of efflux resistance in the gram-positive *Lactococcus lactis*, each conferring ethidium bromide insensitivity but driven by either ATP hydrolysis or, as for *qacA* efflux, a proton pump (*lmrP*). Reassuringly, Kucken et al. (142) found no evidence that the presence of *qacE* (216) had any impact on biocide efficacy against a range of gram-negative isolates that included *Stenotrophomonas maltophilia*.

On the positive side, there is evidence that the transfer of recombinant plasmids between donor and recipient cells is significantly reduced in the presence of biocides such as cationic agents and organomercurials (9). Thus, Pearce et al. (219) reported that sub-MIC concentrations of chlorhexidine, povidone iodine, and cetrimide were able to reduce conjugative and transductive transfer of resistance determinants. Equally, Christensen et al. (50) found that conjugative transfer of the TOL plasmid pWWO was low in the presence of phenol within a three-species biofilm community, and Lakshmi et al. (143) demonstrated that some naturally occurring phenolics could cure *E. coli* of some plasmid-linked resistance markers. Viljanen and Boratynski (303) showed that conjugative transfer of resistance was also affected by the presence of antimicrobial agents.

Maillard et al. (171) demonstrated that phage-mediated transduction was inhibited by concentrations of five different biocides at concentrations that were sublethal to the phage.

Bordas et al. (35) presented data based on sequential passage of *S. aureus* in the presence of combinations of antibiotics and pesticides (carbaryl, malathione, and captan). They suggested that simultaneous exposure to antibiotics and chemical residues synergizes any decreases in susceptibility obtained. It is possible, however, that in this study the pesticides were selecting for efflux mutants. Equally, Sasatsu et al. (256, 258) characterized an *emr* gene found in all staphylococci with a natural function to pump out toxic substances. In a small proportion of staphylococci, the *emr* gene had become multicopy, and its hyperexpression appeared to account for decreased susceptibility to a number of antiseptic molecules, including triclosan.

Evidence of plasmid-borne resistance to biocide molecules in gram-negative species is relatively limited. Such plasmid-encoded changes appear to relate to changes in the relative abundance of particular outer membrane proteins and have been associated with decreased susceptibility to formaldehyde (134). Similarly, the presence of some plasmids (i.e., RP1) in *E. coli* has been shown to alter the composition of the outer membrane lipopolysaccharide and to reduce the expression level of porin proteins. Such changes could be associated with decreased susceptibility to cetrimide (229), chlorhexidine, and phenol (138).

Earnshaw and Lawrence (73) examined the susceptibility of 19 *Listeria monocytogenes* strains isolated from poultry products and bearing antibiotic resistance plasmids to commercial disinfectant formulations by both MIC determination and bactericidal efficacy tests. They found no significant differences in biocide susceptibility between these and plasmidless isolates and concluded that the persistence of such strains could not be attributed to the use of or resistance to the commercial disinfectants used.

There have been suggestions in the literature of plasmid-mediated changes in the susceptibility of *Pseudomonas stutzeri* to chlorhexidine and cetylpyridinium chloride, which are mediated through a specific target, but such targets have not been characterized (293). Gentamicin-resistant, methicillin-resistant *Staphylococcus aureus* often carry a GNAB plasmid that encodes a nucleic acid binding site that is a common target for gentamicin and chlorhexidine, rendering the cells less susceptible to both agents (60, 75). Al-Masaudi et al. (10), however, found that while the presence of GNAB plasmids in methicillin-resistant *S. aureus* strains isolated in Great Britain and Saudi Arabia could be associated with changes in the MIC of quaternary ammonium compounds, these were accompanied by resistance to gentamicin only in the British isolates. Studies indicated that the British strains contained *qacA* genes, whereas the Saudi strains carried *qacB*, *qacB,* and *qacD*.

The evolutionary origins and environmental significance of the staphylococcal *qac* genes are unclear, but it has been sug-

gested (212) that they evolved long before the introduction and use of topical antimicrobials and disinfectants. This is because the chronological emergence, in clinical isolates, of plasmids containing these genes mirrors, rather than follows, the introduction and use of organic cationic compounds into clinical practice. Thus, although they appear to have been acquired by clinical pathogens for protection against hydrophobic antimicrobial agents and have now become widely disseminated due to the selection pressure imposed by the use of biocides such as acriflavine, benzalkonium chloride, chlorhexidine, and cetrimide in antiseptic and disinfectant formulations.

Evidence has been presented that illustrates the evolution of *qacA* from *qacB* (212). Paulsen et al. (214) supports the hypothesis that the emergence of the *qacA* in clinical isolates of *S. aureus* during the 1980s may have resulted from the extensive use of divalent cationic compounds such as chlorhexidine in hospital environments. Bacquero et al. (13), however, in a retrospective study of clinical isolates, found no evidence of association between prolonged chlorhexidine use and reduced susceptibility to chlorhexidine. Fang et al. (78), however, cloned a putative cation efflux pump (CepA) that was associated with 3- to 4-fold changes in chlorhexidine susceptibility. Such pumps are unlikely to efflux the agent because they act by tightly binding to the cytoplasmic membrane lipids, but might minimize the effects of osmoregulatory disruption brought about.

For gram-negative bacteria, it is generally assumed that changes in susceptibility to antibiotics and biocides mediated through the *acrAB* and *mexAB* multidrug efflux genes are unlikely to be transferable because they are already ubiquitous and chromosomally encoded. In their review of such efflux mechanisms, Saier et al. (254) concluded that the gene products for divalent cation efflux (CzC) are similar in construction to those of *acr* and *mex,* belong to the RND family of pumps, and can be found on plasmids.

It can be concluded that while plasmid-borne mercury resistance is significant in clinical isolates (137) and transferable by conjugation or transduction, this is not the case for the biocides commonly used as disinfectants. Indeed, the level and degree of the changes in biocide susceptibility associated with plasmid-mediated antibiotic resistance per se are sufficiently low that transfer of these plasmids is of little significance to biocide effectiveness. Also on a positive note is that the use of certain biocides in a clinical setting might contribute to reductions in the spread of antibiotic resistance by decreasing the success of conjugative and transductive transfer.

POSSIBLE ASSOCIATIONS BETWEEN BIOCIDE USE AND RESISTANCE—FIELD STUDIES

Association between chronic sublethal exposure of bacterial monocultures to biocides and changes in susceptibility to both the biocides themselves and third-party antibiotics has been demonstrated unequivocally in the laboratory. Such phenomena have not yet been demonstrated to have any relevance to the real world. In such situations, individual species of bacteria are in fierce competition with other forms of bacteria, and their competitive fitness determines their survival. Arguably, the clinic represents an environment where biocide use has been and still is extreme. If the increasing use of antibacterial agents within consumer products is likely to impact antibiotic resistance within the home, similar effects should already be apparent in clinical and hospital settings. Accordingly, a large number of studies have been carried out to evaluate whether clinical and environmental isolates taken from such settings show any evidence of significant reductions in their susceptibility to biocides and whether this might be linked with antibiotic resistance.

The results of such studies have been largely ambiguous. Thus, no differences were found in the MICs of hospital and laboratory gram-negative isolates for cationic antiseptics and two organomercurial compounds (107). Three separate studies by Stickler's group (280–281, 283) assessed the MICs of a range of antiseptics, disinfectants, and antibiotics for gram-negative bacteria isolated from a hospital environment and found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus,* and *Providencia* spp.) exhibited some level of reduced susceptibility to chlorhexidine and cetrimide and were also generally more resistant to multiple antibiotics. More recently, Block and Furman (27) isolated 251 strains of staphylococci, *Klebsiella, Pseudomonas, Acinetobacter,* and *Candida* spp. from a hospital environment and detected an inverse correlation between chlorhexidine use and susceptibility. It was noteworthy that when individual taxa were analyzed separately, no significant correlation was noted. This indicates a clonal expansion of existing less-susceptible strains rather than adaptation of individual species, as has been noted in other recent studies of hospital isolates (132, 144).

Similar results (196) showed that 12.8% of 148 clinical *E. coli* isolates selected for their elevated chlorhexidine MICs were no less susceptible to use concentrations. Such changes, in the case of the *Providencia* isolates, were thought to affect binding of the biguanides to the cell surface and therefore reflected envelope modification (74). Freney et al. (82) found no evidence of decreased susceptibility within 169 novel *Enterobacteriaceae* isolated from the general environment relative to clinical isolates. Arguably, such studies support the view that antiseptic use in hospitals does not contribute to the biocide susceptibilities of enterococcal isolates. Equally, Lear et al. (148) examined over 100 factory isolates and compared the MICs of triclosan and chloroxylenol for these to those of the equivalent culture collection strains. They concluded that there was no evidence suggesting that the residual levels of biocides in the factory environment had led to changes in susceptibility. Equally, Braid and Wale (38) showed that triclosan-impregnated storage boxes were effective at reducing the numbers of various challenge inocula and that the susceptibility of the strains was unaffected after repeated exposure on these treated items.

By way of contrast, Reverdy et al. (228) showed that antibiotic-sensitive *S. aureus,* and other staphylococci, for which the MICs of various antiseptics were elevated, were nevertheless less sensitive to a wide variety of antibiotics. Increased MICs for methicillin-resistant *S. aureus* strains have been reported for some biocides, including chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, triclosan, parahydroxybenzoates, and betadine (7, 10, 45, 60, 127, 189). Thus, while the MIC of chlorhexidine was higher against methicillin-resistant *S. aureus* clinical isolates (4 to 8 μg/ml) than for susecptible ones (0.37 to 21 μg/ml), there was no significant difference in

the efficacy of this agent when these strains were tested on the arms of volunteers with a bactericidal assay (60). No significant differences were noted in the chlorhexidine susceptibility of 33 clinical methicillin-resistant and -susceptible *S. aureus* isolates (97), and there was no loss of sensitivity to the bactericidal effects of triclosan when a clinical methicillin-resistant *S. aureus* isolate showing an elevated MIC (2 to 4 μg/ml) was challenged (288–289).

Bamber and Neal (17) found that of 16 methicillin-resistant *S. aureus* that exhibited low-level mupirocin resistance, none had increased MICs of triclosan, but Suller and Russell (288–9) found clinical methicillin-resistant *S. aureus* isolates to have slightly decreased susceptibility, relative to susceptible isolates, to a range of biocides that included chlorhexidine, cetylpyridinium chloride, benzalkonium chloride, and triclosan. Most of the strains described in the above studies remained equally susceptible to bactericidal concentrations of the biocidal agents, an observation that was repeated recently for vancomycin-resistant *Staphylococcus aureus* (L. M. Sehulster and R. L. Anderson, Abstr. 98th Annu. Meet. Am. Soc. Microbiol., 1998, abstr. Y3). Four antiseptic formulations (Savlon, Dettol, Dettol hospital concentrate, and Betadine) retained their bactericidal activity in a European suspension test (217) against a variety of antibiotic-resistant strains, including methicillin-resistant *S. aureus* and vancomycin-resistant enterococci. These data bear testimony to the multiplicity of target sites implicated in the bactericidal action of biocides.

Many other studies failed to observe any change whatsoever in MIC. Thus, Stecchini et al. (275) showed that, despite widespread antibiotic resistance in 100 strains of *Enterobacteriaceae* isolated from minced meat, these were not resistant to the bactericidal activity of an amphoteric Tego disinfectant. Similarly, among 330 psychrotrophic nonfermenting gram-negative strains isolated from vegetables, those antibiotic-resistant strains were demonstrated to be susceptible to the bactericidal action of quaternary ammonium compounds and hypochlorite disinfectants (80).

Baillie et al. (15) evaluated the chlorhexidine sensitivity of 33 clinical isolates of *Enterococcus faecium* that were sensitive to both vancomycin and gentamicin with vancomycin-resistant and gentamicin-resistant strains. The results showed no increase in resistance to chlorhexidine as indicated by MIC. lnterestingly, a study of 67 ciprofloxacin-resistant isolates of *P. aeruginosa* yielded four which were hypersensitive to chlorhexidine (MIC, 5 mg/liter), while none were found among 179 ciprofloxacin-sensitive isolates (16).

Marshall et al. (P. J. Marshall, P. Rumma, and E. Reiss-Levy, presentation at the 11th National Conference of the Australian Infection Control Association, 7-9 May 1997, Melbourne, Australia) reported that during an intensive policy of antiseptic handwashing involving a triclosan-based medicated soap, aimed at combating a methicillin-resistant *S. aureus* infection episode, not only did the incidence of methicillin-resistant *S. aureus* decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial. In a study of *Streptococcus mutans* isolated from the mouths of 114 schoolchildren and students from families in which about 70% used oral preparations containing chlorhexidine on a regular basis, there was no evidence of decreased susceptibility either to chlorhexidine or to a range of antibiotics, as tested with MICs (129).

Anderson et al. (12) determined the susceptibilities of vancomycin-resistant and vancomycin-sensitive enterococci to various concentrations of commonly used hospital disinfectants, including quaternary ammonium compounds, phenolics, and a iodophore, at recommended use dilutions and extended dilutions with suspension tests. They concluded that there was no relationship between levels of vancomycin resistance and their susceptibility to disinfectants at the use dilution. Such findings have been confirmed by showing that a series of vancomycin-resistant and vancomycin-resistant enterococcal clinical isolates had no significant differences in their growth-inhibitory or bactericidal sensitivities to chlorhexidine, cetylpyridinium chloride, or triclosan (11, 288).

Published data for triclosan state that the expected MIC for staphylococci should be between 0.01 ppm and 0.1 ppm. Bamber and Neal (17) determined the MIC for 186 isolates of methicillin-resistant and methicillin-sensitive *S. aureus* and found 14 isolates (7.5%) with MICs greater than 1.0 ppm. These were, however, equally distributed between the methicillin-resistant and methicillin-sensitive *S. aureus* strains.

A series of antibiotic-resistant clinical and environmental isolates that included *P. aeruginosa*, *Klebsiella* species, *E. coli, S. aureus,* and *S. epidermidis* were found to be no less susceptible to the bactericidal activity of phenolic and quaternary ammonium disinfectants, chloroxylenol, cetrimide, and povidone iodine (217–218, 251–252). Similarly, some variation in the vancomycin susceptibility and biocide (chlorine, alcohol, aldehyde) susceptibility of enterococci has been noted, but the two did not correlate (37).

The food processing industry represents an environment other than the clinic where the use of biocidal products is high. In this respect, Heir et al. (117) reported that 13% of staphylococcal isolates from a food manufacturing environment had MICs of benzalkonium chloride that were between 4 and 11 mg/liter, compared with 70% of remaining isolates, which had MICs of less than 2 mg/liter. This resistance probably related to the presence of *qac* efflux mechanisms and encoded only small changes in susceptibility. Accordingly, suspension tests showed that recommended use concentrations of the agent produced the desired 5-log reduction in viable count in 5 min. In an examination of poultry carcasses, two strains of *Pseudomonas* were isolated that were deemed resistant to benzalkonium chloride by virtue of possessing a MIC greater than 200 μg/ml (146). Only one of these organisms failed the suspension test. A more recent study (118) showed that *S. aureus* cells that expressed *qacG* efflux suffered reduced killing in environments that contained low concentrations of benzalkonium chloride but 5-log reductions in viable counts at higher concentrations. The latter were nevertheless still well below the recommended use concentrations.

Latterly, Heir et al. (118) found a new member of the *qac* family of genes in *Staphylococcus saprophyticus* (*qacH*) isolated from a poultry processing plant. The same authors, however (120), conceded that quaternary ammonium compound use in the production facilities might have led to a selection for staphylococci bearing the *qacAB* genes. Bass et al. (20) demonstrated that approximately one third of diseased poultry carried plasmids that encoded multiple antibiotic resistance; 63%

of these contained markers for the class 1 integrons *intI* and *qacE* and were part of transposon Tn*21*. The selection pressure for Tn*21*, which also encodes mercury resistance, could not be determined.

The field studies discussed so far suggest strongly that the variable nature of the observable links between biocide and antibiotic susceptibility have no single underlying cause and that worries and concerns raised through laboratory monoculture experiments cannot be echoed in the environment. There are, however, a few published studies that indicate the contrary and show reductions in susceptibility to various oxidizing biocides that are sufficient to compromise their in-use effectiveness. In most instances, such studies make no distinction between phenotypic and hence reversible changes in susceptibility and that which may be acquired. In other instances, data were collected from large numbers of isolates taken from environments where biocide use is widespread but without reference to control habitats. The extent to which the data reflect adaptation to the biocides or the natural selection and clonal proliferation of existing strains is therefore often unknown. These studies are discussed below.

Several reports have described isolates, especially among gram-negative species, from various food processing environments that possess a reduced susceptibility to chlorine and quaternary biocides that relates to practical usage. Thus, an early report (122) noted that after changing the sterilization practices from steam to chlorine-based disinfectant compounds, there was a higher occurrence of dairy isolates that were resistant to hypochlorite. Similarly, Mead and Adams (184) and Bolton et al. (34) found that chlorine concentrations of 1 mg/liter produced a 4-log reduction in viability of *S. aureus* strains isolated from turkeys and turkey products, but only a 2-log reduction when tested against endemic strains that had colonized the processing equipment. All three reports could be related to growth of the resistant isolates as coaggregates within extracellular slime (34). This was also the explanation for the apparent resistance of lactobacillus strains isolated from packed meat that could survive exposure to 200 mg of benzalkonium chloride per liter (291). The resistance in all of these instances was therefore phenotypic in nature.

Pseudomonads are not generally noted for their susceptibility to quaternary ammonium compounds, a property that is generally attributed to the unique properties of the *Pseudomonas* cell envelope. Approximately 30% of *Pseudomonas* isolates taken from poultry carcasses were able to grow at concentrations of 200 μg/ml (146). While it was recognized that clonal selection of existing resistant strains, through a constant usage regimen involving benzalkonium chloride as the disinfectant, might have been the cause, these workers later reported (S. Langsrud and G. Sundheim, 1997, Pseudomonas ′97, p. 102) that the resistance was lost within 4 to 8 h of removal from the quaternary ammonium compound and was developed in batch culture only during the lag phase. These observations therefore more probably reflect a regulated process involving efflux genes, and the resistance shown for these cells could not be replicated in a bactericidal assay.

In a similar study, the susceptibility of 350 isolates collected from commercial chicken hatcheries to commercial preparations of quaternary ammonium compounds, phenolics, and glutaraldehyde was examined (312). Nineteen isolates (ca. 6%, including *Serratia marcescens*, *Bacillus* species, *Enterococcus* species, and *P. stutzeri*) from two of three hatcheries were resistant to disinfectant at and above the recommended use concentrations and exposure times. Some isolates were multiresistant, but only three showed resistance to quaternary ammonium compounds compared with seven to phenol and 15 to glutaraldehyde. The authors suggested that this might be correlated with the usage of glutaraldehyde in U.S. hatcheries over many years. No investigations were carried out to determine whether the resistance was reversible, although all isolates had been grown once through tryptone soy medium.

In a study of the effects of repeated antiseptic use on the bacterial flora of the urethral meatus in patients undergoing intermittent bladder catheterization (285), the bacterial flora was examined from the date of injury to the time at which urinary tract infection developed after daily washing with aqueous chlorhexidine (600 μg/ml). Prior to the regular application of chlorhexidine, the predominant flora comprised gram-positive, chlorhexidine-sensitive bacteria. These were superseded by a gram-negative flora that included some resistant strains (mainly *Proteus mirabilis*, *P. aeruginosa*, *Providencia stuartii*, and *Klebsiella* species) less sensitive to chlorhexidine, with MICs of 200 to 800 μg/ml. These were well above the levels of 10 to 50 μg/ml usually reported for gram-negative species.

In a subsequent study (281), the susceptibility to an array of antiseptics and disinfectants that included chlorhexidine, cetrimide, glutaraldehyde, and a phenolic formulation was assessed against a large collection of gram-negative isolates taken from a variety of clinical and hospital settings. The general conclusion drawn was that antiseptic and disinfectant resistance was not a widespread phenomenon in species responsible for urinary tract infections. They found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus,* and *Providencia*) exhibited some resistance to chlorhexidine, but these came from situations where there was extensive use of chlorhexidine.

It would appear therefore that in the earlier study (285), the routine application of chlorhexidine had eliminated the natural colonization resistance provided by the sensitive autochthonous flora and had enabled innately resistant environmental strains to infect. The innate recalcitrance of environmental gram-negative bacteria to antiseptics has been demonstrated by Nagai and Ogase (194–195). They isolated strains of *Achromobacter xylosoxidans* from a 0.4% chlorhexidine solution handwashing reservoir for which minimum bactericidal concentrations were more than 10-fold higher than the chlorhexidine solution in the reservoir. Two separate investigations with *Providencia stuartii,* (284) and an antibiotic-resistant clinical strain of *P. mirabilis that* was resistant to the growth-inhibitory action of chlorhexidine at 800 mg/liter (64) failed to show any evidence of a plasmid link. Both sets of authors concluded that the resistance was most likely an intrinsic property induced by persistent exposure to the biocide.

More recently, strains of *P. stutzeri* and *P. aeruginosa* have been shown to become much less susceptible to chlorhexidine and cetylpyridinium chloride when passaged through gradually increasing concentrations of each (293). Such decreased susceptibility was stable for *P. stutzeri* but not for *P. aeruginosa* and could not be transferred by conjugation. The authors concluded that resistance resulted from a nonspecific decreases in

cell permeability such as might arise from deletion or depression of a porin protein (294, 295). In this context, passage with increasing concentrations of isothiazolone biocides has been shown to repress the synthesis of an outer membrane porin protein (OmpT) that appears to facilitate the entry of this group of thiol-interactive biocides into the cell (313).

DISCUSSION

Various laboratory studies indicate that bacterial populations can simultaneously become less susceptible to both antibiotics and biocides. This establishes the possibility that continuous exposure to biocides could add to or enhance the selective pressure exerted by antibiotic use. The latter is currently assumed to be the predominant cause of antibiotic resistance in the clinic.

In assessing the implications of the currently available data, two criteria need to be assessed: the extent to which these mechanisms might occur in the environment or in clinical practice, and whether the level of antibiotic insusceptibility is sufficient to compromise clinical effectiveness or whether changes in biocide susceptibility influence their intended outcomes in domestic and clinical settings. Indications are that the latter is likely to differ according to the nature of the antibiotic, the target site, and the biocide involved.

Current laboratory-based evidence suggests that for antibiotic-resistant populations involving gram-negative efflux pumps, the levels of antibiotic resistance which are induced or acquired are relatively low and unlikely to compromise effectiveness. The significance of multiresistant plasmid transfer in gram-positive species, particularly in *Staphylococcus* species, requires further investigation, as does the possibility of mutation in shared target sites in both gram-negative and gram-positive species. If acquisition of multitarget plasmids that encode reduced biocide susceptibility alongside antibiotic resistance determinants is a real possibility, it is interesting to speculate whether this is likely to occur to any extent outside of the hospital environment. Logically, the sequential addition of antibiotic resistance determinants onto plasmids containing determinants conferring reduced susceptibility to biocides should only occur in environments where microbial populations are exposed not only to a persistent low levels of the biocide in question, but also to selective pressure from a series of different antibiotics (147). Such a situation is more likely to occur in the hospital than in the home, where antibiotic use (and perhaps, until recently, also biocide use) is much lower.

It must be borne in mind that, despite the fact that 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, there will be sublethal concentrations of biocide for any given cellular target at some point along this concentration gradient, providing a selection pressure for mutations in a multiplicity of cellular targets. Biofilm communities are ubiquitous in our homes and the environment and provide highly selective environments where sharp gradients of antimicrobial agents will prevail and selective pressures will be greatest (95).

Efflux or target site modification to resistance brings with it a fitness cost in terms of the ability of an organism to compete with other species. In infection, then the implicated microorganism often only has to compete with host defenses. In the general and domestic environment then bacteria compete with each other. Microbial communities develop which are highly competitive, in which compromised strains will be rapidly replaced by more competent immigrants (253). Thus, a number of environmental pseudomonads and *Serratia* spp. were found to be innately resistant to use levels of a novel disinfectant utilizing grapefruit seed extracts and would become enriched in environments utilizing this agent exclusively (290). Similarly, Scully (262) noted a proliferation of coliforms with a reduced susceptibility to chlorine (*Klebsiella* spp. etc.) in a U.S. wastewater plant, and chlorination of sewage-related bacteria has been noted to have a selective effect on the nature of the survivors (191).

While the regular application and use of antimicrobial handwashing products have been noted to bring about a change in skin flora, this has not been associated with fluctuations in resistance (131). Indeed, these authors concluded that the benefits of topical antibacterials in personal hygiene as well as in infection control far outweighs the risks of increased antibiotic or biocide resistance. Equally, other groups of authors (231, 248, 252) could find no evidence of any increased incidence of antibiotic resistance in home environments and demonstrated the continued effectiveness of traditional biocidal products (Lysol, quaternary ammonium based, and chlorine based).

While the majority of clinical isolates retain some degree of susceptibility to biocides and antibiotics, the converse is true for nonpathogenic environmental isolates. Thus, the majority of environmental pseudomonads are intrinsically resistant to all but the strongly oxidizing biocides. It is these innately resistant organisms that benefit from antimicrobial treatments by their clonal expansion into ecological niches previously occupied by susceptible clones and species. In this fashion, it is not unusual to find environmental isolates that resist killing by use levels of many nonoxidizing disinfectants (194, 198). In this context, it is reassuring that in most instances these organisms are relatively benign. Thus, pseudomonads actively contaminating a phenolic disinfectant over a period of 20 months within an intensive care unit could not be found associated with any of the severely compromised patients within it (199).

Field studies in environments where biocide use has been high therefore fail to demonstrate the evolution and selection of biocide and antibiotic resistant-clones; rather, they demonstrate a clonal expansion of existing, resistant but less competitive species. In some instances, these clonal expansions involve organisms that are not only intrinsically resistant but also are able to degrade the biocide, as has been shown recently for triclosan (112, 186). In this respect, it is surprising that while trace levels of triclosan have been detected in human milk and fish bile and in human plasma (126), there is no apparent residual from its daily use in dentifrices (14). It is possible that triclosan residues within breast milk are related to the topical application of medicated soaps before donation.

Since selection or transfer of determinants for reduced susceptibility will only apply to biocides which have selective target sites, it seems unlikely (although not impossible) that it could occur with chemically reactive agents such as chlorine or oxygen-releasing agents, or with solvent molecules such as alcohols (124). This 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.

Based on the current evidence presented in this paper, it seems that intrinsic and acquired antimicrobial resistance occurring in response to biocide exposure is not per se a significant problem. Various workers have raised concerns, however, that persistent exposure, particularly to low levels of biocides could cause the acquisition and/or expression of resistance determinants coding for reductions in biocide susceptibility that would ultimately enable such bacteria to persist for longer periods within environmental and clinical settings. The increased prevalence might thereby increase the chance of accumulating mutations or plasmids which produce high level stable antibiotic resistance (87, 202–203, 245). Currently there is no evidence to support this.

In response to recent concern about the growing impact of antibiotic resistance in clinical practice (79, 86, 225, 274), it is now widely accepted that more stringent measures, intended to reduce antibiotic misuse, are urgently required to deal with this problem (232, 307). Working parties across Europe and elsewhere are developing strategies aimed at reducing antibiotics in animal feeds and controlling antibiotic prescribing in humans more effectively (68, 96, 265). The need for improved hygiene is recognized as a vital component of these strategies (65, 84–86, 207, 208, 255, 260, 271–272). By reducing the incidence of infection through improved hygiene (263) the amount of antibiotic prescribing can be reduced, which in turn reduces the impact on antibiotic resistance. The benefits of this approach have been demonstrated in clinical settings where good hygiene has contributed to reduced antibiotic resistance through reduced prescribing (36, 259, 274; J. S. Garner and M. S. Favero, 1985, CDC guidelines for handwashing and hospital environmental control, www.cdc.gov/ncidod/hip/guide/handwash.html).

CONCLUSIONS

Overall, there is good evidence to suggest that good standards of hygiene in the domestic setting, which includes not only day-to-day cleaning of the home but food hygiene, hand hygiene, and hygiene related to the protection of vulnerable groups, can have a significant impact in reducing the number of infections arising in the home (25). Indeed, a number of recent studies have reported increased incidence of critical pathogens such as methicillin-resistant *S. aureus* into the home environment (5, 161, 177), often associated with household pets like dogs (47) and cats (305), and their transfer to humans (121). Such work highlights the need for targeted hygiene within the home (29). A variety of different procedures can be used to achieve hygiene in the home, and in some cases this may require the use of a disinfectant or antiseptic. This being the case, it can be seen that responsible use of biocides and antimicrobial cleaning products could contribute to reducing the impact of antibiotic resistance (29). Thus, if reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide use is not discouraged in situations where there is real benefit.

It is suggested that, although the practical implications of reduced susceptibility to antimicrobial agents associated with exposure to sublethal concentrations of biocides appears to be small, the impact must be balanced against the benefits of such use to public health and product durability. The risks must also be viewed in relation to the effects of natural agents (physical or chemical) that also have an inimical effect on the cells and to which microbial populations are continually exposed.

GLOSSARY

Antibacterial agents. Molecules, generally of synthetic or semisynthetic origin, that, above certain critical concentrations, have adverse effects on the growth or survival of bacteria.

Antibiotics. Molecules of biological origin used for the in vivo treatment of infection.

Antimicrobial agents. Molecules, generally of synthetic or semisynthetic origin, that, above certain critical concentrations, have adverse effects on the growth or survival of microorganisms (including bacteria, yeasts, molds, viruses, and protozoa).

Antiseptics. Formulations containing germicidal, microbicidal, or bactericidal agents that are safe for application to living things.

Bactericides. Antibacterial agents at concentrations that are capable of killing vegetative bacteria within a specified time.

Bacteriostats. Antibacterial agents at concentrations that will prevent the growth of specified groups of bacteria.

Biocides. Molecules, generally of synthetic or semisynthetic origin, that, above certain critical concentrations and under defined conditions, will kill living cells within specified times.

Disinfectants/sanitizers. Formulations containing germicidal, microbicidal, or bactericidal agents that are safe for application to inanimate surfaces and which kill specified groups of disease-producing microorganisms (not including bacterial or fungal spores) within specified times.

Genotypic resistance. A change in the genome, chromosomal or plasmid encoded, that causes the expression of a resistant phenotype.

Germicides. Antimicrobial agents at concentrations that are capable of killing defined groups of disease-producing microorganisms within specified times; may be applied to animate and inanimate objects and surfaces.

Microbicides. Antimicrobial agents at concentrations that are capable of killing microorganisms (including bacteria, yeasts, molds, viruses, and protozoa) within a specified time.

Microbistats. Antimicrobial agents at concentrations that will prevent the growth of specified groups of microorganisms (including but not limited to bacteria).

Phenotypic resistance. Expression of a phenotype mediated through the growth environment and reflecting normal gene expression that is resistant.

Preservative. A bacteriostat or microbistat that is included within a formulated product in order to kill and or prevent the growth of unwanted microorganisms. The preservative is intended to maintain these at levels that are safe for the designated shelf-life/use-life of the product.

Resistance (susceptibility). A descriptor, generally concentration, reflecting a standardized response (i.e., growth inhibition) by a microorganism to antibiotics, antibacterials, and antimicrobial agents.

Resistant. A state of a microorganism when resistance (susceptibility) is such that it will fail to respond to typical use concentrations of the specified agent.

Sporicides. Certain antimicrobial agents at concentrations that are capable of killing bacterial endospores and fungal exospores within a specified time.

Sterilants. Certain biocides at concentrations that are capable of sterilizng an object or surface within a specified time.

Susceptibility (resistance). A descriptor, generally concentration, reflecting a standardized response (i.e., growth inhibition) by microorganism to antibiotics, antibacterials, and antimicrobial agents.

Susceptible. A state of a microorganism when resistance (susceptibility) is such that it will respond appropriately to typical use concentrations of the specified agent.

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