ELSEVIER

International Journal of Food Microbiology 50 (1999) 33–44

International Journal of Food Microbiology

Resistance responses of microorganisms in food environments

C.K Bower, M.A. Daeschel*

Department of Food Science and Technology, 240 Wiegand Hall, Oregon State University, Corvallis, OR 97331, USA

Abstract

Food borne microorganisms display a broad spectrum of resistance responses to naturally occurring and intentionally added antimicrobial agents. Resistance may be conferred by innate structural features of the bacterial strain such as an impermeable outer membrane or a mechanism for antibiotic-inactivation. Bacteria previously susceptible to an antimicrobial compound can acquire resistance through mutation or through genetic transfer processes such as transformation, transduction, and conjugation. Resistance can also be conferred by biofilm formation on food processing surfaces as an adaptive response to protect colonies from cleaning and sanitation. Resistant pathogens are a global problem, facilitated by international trade of raw and processed foods. Cross resistance between clinical and nonclinical antimicrobials can exist and is of concern. The development of resistant foodborne pathogens has been attributed to increased antibiotic use in hospitals, outpatient facilities, and veterinary applications. Resistant microorganisms can also develop as a result of physical processes used in food preservation, such as acid treatments and irradiation processes. Strategies to effectively counter resistance development include: changing current practices of antibiotic usage, developing new antibiotics, applying hurdle preservation approaches, preventing bacterial adhesion, and utilizing competitive exclusion. This paper presents an overview of problems arising from the development of microbial resistance, and explores possible solutions for detecting and defeating the adaptive changes of microorganisms.

Keywords: Resistance; Microorganisms; Food environments

1. Introduction

Microorganisms are ubiquitous, coexisting and coevolving with all plant and animal life on Earth. Although most microorganisms are not harmful to humans, (and some are even beneficial), many bacterial strains exist that can cause disease. Effective clinical treatments exist for most human pathogens found today, however, when microorganisms) develop resistance to commonly used preservation methods, serious complications can result. This is especially relevant for the very young, the elderly, and the immunocompromised members of society.

The importance of studying bacterial growth, and the resistance responses that may develop, extends beyond the role of simply halting the spread of infectious disease. Microorganisms also play key roles in energy production, agricultural development, and the processing of food. For example, the petroleum industry is concerned with microbial growth since crude oil can be degraded by microorganisms, necessitating specialized conditions during drilling,

*Corresponding author. Tel.: + 1-541-737-6519; fax: + 1-541-737-6525.

E-mail address: daeschem@bcc.orst.edu (M.A. Daeschel)

0168-1605/99/$ – see front matter
PII: S0168-1605(99)00075-6

recovery, and storage. In agriculture, microbial infection of plants can affect all major food crops, resulting in a significant degree of food loss even before harvesting. Unwanted microbial growth in a food processing environment can also be disastrous. Large amounts of food (and money) are wasted each year as a direct result of food spoilage organisms (Wallerstein, 1980). The emergence of microorganisms resistant to the physical and chemical processes of traditional food preservation is posing a new hazard to the safety of our food supply.

2. Characteristics of antimicrobial agents

New techniques for inhibiting unwanted microbial growth must continually be developed as microorganisms adapt to survive in the presence of previously effective methods of control. Understanding *why* each preservation method is effective will be the first step toward predicting the emergence of resistance responses and controlling them as they develop.

2.1. Origin of antibiotic molecules

Antibiotics are produced by microorganisms as secondary metabolites. These compounds are apparently nonessential, leaving researchers to puzzle over the nature of an antibiotic's true function. Contrary to popular belief, it is unlikely that antibiotics are synthesized by one microorganism for use as a weapon against a competing microorganism. If antibiotics were produced to give one species of microorganism a competitive edge over another species during colonization of a new environment, then these antibacterial compounds would likely be small, simple molecules diverting little of the vital energy needed for survival and growth of the producing organism. However, this is generally not true, judging by the size of most antibiotics, and the complexity of their production pathways (Davies, 1990). Furthermore, these antibiotic metabolites are produced when the bacteria is in stationary phase, rather than in the competitive growth phase when their presence would make a greater contribution to colony survival. Although valuable resources must be expended by some bacteria to protect themselves from the action of their own antibiotics, the fact that these pathways are conserved suggests that they may confer some survival advantage upon the producing microorganism.

One theory concerning the origin of antibiotics is that these compounds at one time served as biological effectors, participating in cellular pathways such as peptide synthesis (Davies, 1990). This process may have involved specific binding to cellular structures such as ribosomes, but these pathways became less important as more efficient enzyme systems evolved. The modern antibiotic that binds to an enzyme and interferes with its cellular function may actually be the remnant of a molecule that once participated in an important biochemical process within the cell. There are still a variety of common antibiotics that can act as metabolic effectors for the producing organism, stimulating transcription, translation, and cell growth (Amábile-Cuevas, 1993).

2.2. Cellular targets of antimicrobial agents

The fundamental principle of antimicrobial action is that the agent must be selectively toxic, damaging only the unwanted microorganisms. Antimicrobial compounds exert their effect by interfering with the metabolic machinery of a microbial cell, thereby compromising its ability to survive and reproduce. Penicillin, the first antibiotic discovered, interferes with cell wall synthesis in an actively growing cell (Sternbach and Varon, 1992). Without the support and protection of a cell wall, the affected bacteria are vulnerable to osmotic changes as well as chemicals in their environment. Similarly, antimicrobial agents can damage microbial cells by inhibiting protein synthesis, targeting the cytoplasmic membrane, or interfering with an essential metabolic reaction used by the microorganism. Antibiotics that interfere with the synthesis or operation of the genetic machinery of cells (DNA or RNA), have also proven effective, however bacteria are capable of rapid mutation such that a single genetic change by a bacterium can quickly render these antibiotics useless.

3. Mechanisms of microbial resistance

Microorganisms owe their existence to their ancestors ability to adapt and change. An unfortunate consequence of this process, however, is the development of microbial resistance to clinical anti-

biotics, food preservation agents, and disinfectant processes. When a population of bacteria first encounters an antimicrobial compound, cells that are highly susceptible are rapidly killed. However, cells that already possess some degree of resistance, or acquire it later (through mutation or genetic exchange), may survive and proliferate. A variety of mechanisms for microbial resistance have been discovered.

3.1. Inherent microbial resistance

Resistance to antimicrobial compounds can be conferred by innate structural features of a microorganism, such as an impermeable outer membrane that resists penetration of antibiotics. Gram-negative bacteria have a thick lipopolysaccharide layer that acts as a barrier to limit diffusion of antibiotic molecules into the cell, while gram-positive bacteria characteristically have lipophilic substances in their cell walls that retard penetration of hydrophilic, cationic, antimicrobial compounds (Volk et al., 1996). In addition to barriers, microorganisms can possess a variety of other resistance mechanisms. For example, the organism may lack a transport system necessary for antibiotic uptake or be missing the biochemical target required for attachment and proper functioning of the antimicrobial compound (Volk et al., 1996). Resistance can also be achieved through production of an inactivator by an otherwise susceptible microorganism. Strains of both gram-positive and gram-negative bacteria have been found to contain enzymes capable of deactivating penicillins, cephalosporins, and other β-lactam antibiotics (Brooks et al., 1998). Another inherent method of microbial resistance involves the active efflux of an antibiotic compound from the bacterial cytoplasm. In this process, resistant bacteria synthesize an integral membrane protein that pumps the antibiotic out of the cell before it has a chance to find its intracellular target. This mechanism has been observed in antibiotic-resistant *Staphylococcus aureus* strains (Russell and Day, 1996).

3.2. Transfer of resistance genes

Even when a species of microorganism is initially sensitive to an antimicrobial agent, it may acquire resistance through spontaneous mutation, or through acquisition of genetic material from a resistant bacterium by transformation, transduction, or conjugation. Bacterial resistance arising from spontaneous mutation occurs at a frequency of about 10^{-9} or less per bacterial generation (Davies, 1994), although this number may be higher in cases of hypermutation sometimes induced during bacterial starvation (Hall, 1991). Given the rapid adaptation of bacteria to new environments, this suggests that the majority of genetic changes occur through other processes. It was originally believed that genes for chromosomal resistance could only be transferred to another bacteria during the conjugative process, aided by special organelles known as sex pili. Eventually it was recognized that the presence of antibiotic resistant genes on transferable plasmid DNA (or R Factors) contributed to an even wider dissemination of resistance characteristics in bacterial populations. R Factors are capable of duplicating themselves within the bacterium. Further complicating the problem is the close physical association on DNA between different genes that confer antibiotic resistance. For example, the genes for chloramphenicol are located directly adjacent to the genes that cause resistance to streptomycin, spectinomycin, and ampicillin, thus a bacterium that carries the R Factor for chloramphenicol will likely be resistant to at least three other major antibiotics (Reece and Phillips, 1995). The seriousness of this situation has become more clear as R Factors that carry genes for resistance to human antibiotics are being discovered in livestock and poultry (Ridley and Threlfall, 1998).

Acquiring resistant genes from another microorganism enables a bacterium to code for useful proteins such as those that modify target receptors so that the antibiotic is no longer able to effectively bind. For example, penicillin resistance can occur among previously sensitive gram-positive bacteria when they acquire genes capable of modifying target receptors, thereby preventing the attachment of beta-lactam antibiotics. With penicillin no longer able to effectively bind to its cellular target, the now-resistant bacteria can survive and reproduce in what was once a lethal environment (Russell, 1991). A bacterium with the appropriate genes can cause transglycosylation or transpeptidation of its peptidoglycan layer, thereby defeating an antibiotic's mode of action (Volk et al., 1996). Resistance can also occur when a bacterium acquires a gene that allows inactivation of the antibiotic. For example, aminoglycosides such as gentamycin and kanamycin can

be disabled when a bacterium attaches a functional group (e.g. through phosphorylation or acetylation) to the antibiotic molecule (Davies, 1994). This process modifies the antibiotic to prevent it from entering the cell and interfering with protein synthesis.

3.3. Resistance conferred by biofilms

It has been well documented that microorganisms colonizing surfaces as part of a biofilm matrix display more resistance to toxic compounds than their single-celled counterparts in suspension (Holmes and Evans, 1989; Nichols, 1989). This has been observed on food contact surfaces (Frank and Koffi, 1990; Krysinski et al., 1992), medical implants (Gilbert et al., 1990), and even contact lenses (Miller and Ahearn, 1987), and is usually attributed to the slow diffusion of biocides through the biofilm matrix. Cells deeply embedded in a biofilm were found to receive less oxygen and fewer nutrients than cells in suspension (Brown et al., 1988). In response to this condition of starvation, some buried cells displayed an altered physiology, including a significant decrease in growth rate (Evans et al., 1991) making them more resistant to the uptake of toxic agents. Bacteria in this quasi-dormant state demonstrated the decreased sensitivity to antibiotics, surfactants and sanitizers that is characteristic of biofilms.

By understanding the nature of microbial cell components and their functions, it may be possible to speculate about the biocide resistance exhibited by microorganisms in biofilms. With most bacteria, the extracellular capsule is polysaccharide in nature, although some species such as *Bacillus* may form a polypeptide capsule. The presence of a capsule can enhance microbial attachment, prevent dessication, and act as a defense against phagocytosis. Capsular material may also facilitate the adsorption of toxic agents, thus preventing their penetration into the cytoplasm (Cross, 1990). Therefore, a biofilm matrix where capsular material is an integral component would be expected to provide cells with protection against sanitizers.

Attachment to surfaces can also have an impact on bacterial resistance to disinfection (Le Chevallier et al., 1988). Theoretically, the topography of a surface affects the ability of a disinfectant to approach the cell. A freely suspended (planktonic) organism is susceptible to a disinfectant from all sides and at all angles, while an organism attached to a surface is susceptible from only one side. Removing adherent cells of *Listeria monocytogenes* from a surface increased their susceptibility to sanitizers equivalent to that of planktonic cells (Frank and Koffi, 1990). The resistance against antibiotics and sanitizers exhibited by cells in a biofilm appears to be surface-dependent. Stainless steel was more easily cleaned and sanitized than polyester or polyester/polyurethane surfaces, despite the observation that no significant topographical differences were detectable (by scanning electron microscopy) among the surfaces to account for this variation in sanitizer resistance (Krysinski et al., 1992).

4. Implications of resistance

The emergence of resistant foodborne pathogens has been traced to the increase in antibiotic use in hospitals, outpatient facilities, and on farms. Each of these three ecosystems involves different antimicrobial compounds, different reasons for their usage, and different microorganisms of concern.

4.1. Resistant bacteria in healthcare

Stories about Ebola and hantavirus may capture our attention in the headlines, but it is the less exotic infectious diseases that are truly newsworthy as resistant strains of organisms emerge, hindering treatment of tuberculosis, malaria, cholera, diarrhea, and pneumonia around the world. Despite our impressive arsenal of antibiotics, it is still possible to die from a resistant bacterial infection. This increase in bacterial resistance is a direct result of our increased use of antibiotics. In hospitals, the prophylactic use of clinically effective antibiotics before surgical procedures is a generally accepted practice when the risk of bacterial infection is high. Using the wrong drug, too low of a dosage to ensure pathogen eradication, or broad spectrum antibiotic agents may also contribute to increased instances of resistant bacteria. As resistant organisms develop, they are easily spread throughout the population, especially through day care centers, nursing homes, and correctional facilities.

Originally physicians worried that the antibiotics

used to treat their patients might cause hypersensitivity or other toxic side-effects. Now their concern includes the possibility of finding antibiotic-resistant bacteria, and of having these highly resistant microorganisms transfer their resistance to sensitive cells. Methicillin (a semi-synthetic penicillin) was first introduced in 1960, but within a few years, a number of outbreaks involving methicillin-resistant strains *of Staphylococcus aureus* (MRSA) had occurred (Cohen, 1992). Vancomycin is an antibiotic used to effectively treat patients with MRSA, however the increased use of this antibiotic has produced vancomycin resistance in other bacterial species such as enterococcus (Neu, 1992). When fluoroquinolones, (another potent broad-spectrum group of antibiotics), were introduced in the 1980s, they were effective against 95% of MRSA, however within one year, the majority of those strains had become resistant (Neu, 1992). This increase in antibiotic resistance among organisms found in clinical medicine is seriously decreasing the treatment options for patients with bacterial infections.

The cost of antibiotic resistance in the healthcare system is substantial, not only from the standpoint of increased usage of one or more antimicrobial agents, but in terms of extended hospital stays and patient mortality as well (Shlaes, 1993). Frequently prescribed antibiotics may lead to an increase in the number of organisms that are resistance to that antibiotic (Phelps, 1989). The ultimate consequence of widespread resistance in human microbial flora will be when clinical treatment options no longer exist for invading pathogens. If resistant populations of bacteria in food animals develop cross-resistance to similar clinical antibiotics, healthcare problems will become even more severe.

4.2. Resistant bacteria in food animals

Antibiotics have also proven valuable for treating infections in food animals, however there is adequate data to believe that the sudden emergence of antibiotic-resistant bacteria is spreading from farm animals through the food supply, not from the treatment of sick animals, but from the subtherapeutic doses of antibiotics routinely added to animal feeds (Cohen and Tauxe, 1986). Animals consuming small amounts of antibiotics were found to gain weight more efficiently and stay healthier, with fewer losses to disease. Until 1979, the prophylactic use of antibiotics in the USA was a common practice to prevent disease, and to economically speed the growth of food animals. The US Food and Drug Administration has approved a variety of antibiotics for use in animal feed including penicillin and nitrofurans in poultry and swine; tetracyclines in all food animals; and sulfa in swine. However, subtherapeutic doses of these antibiotics favored the selection of antibiotic-resistant microorganisms, with each treated animal serving as a reservoir (Cohen and Tauxe, 1986).

When establishing tolerance levels for antibiotics in food, the development of bacterial resistance to these antibiotics may not be considered. Antibiotic residues commonly present in milk and meat have been found to contribute to the development of resistant bacteria (Brady and Katz, 1992; Brady et al., 1993). The significance of producing a population of bacteria resistant to antibiotics is not confined to the health of the animal producing the food. The effect of antibiotic residues on the complex microbial ecosystem of the human intestine is of equal importance and has not been adequately studied. Exposure to one antibiotic, such as ampicillin, has also been found to increase the resistance of bacteria such as *S. aureus* to other antibiotics (Brady et al., 1993). This suggests that antibiotic levels previously considered safe may actually be selecting for resistant populations of bacteria in our food supply.

The finding that pathogens isolated from *both* calves and humans after a *Salmonella* epidemic displayed identical patterns of antibiotic resistance, prompted the recommendation (Swann committee, 1968, Great Britain) that any antibiotic commonly prescribed for human therapy be banned from use as a feed supplement for livestock (Howells and Joynson, 1975; Lappe, 1982). Legislation was enacted, and now Europe no longer allows subtherapeutic levels of antimicrobials in animal feeds (Levy, 1992). The veterinary drug, avoparcin, was withdrawn from sale in the European Union (EU) following the recommendation of the European Commission. It was believed that since avoparcin was closely related to vancomycin, its continued use might promote the spread of resistant bacteria from farmyards to hospitals (Billstein, 1994). The ban was enacted despite being rejected by the EU's Scientific Committee on Animal Nutrition, an organization that

strongly disagreed with popular interpretation of available scientific data. Meanwhile, data continues to accumulate. A fluoroquinolone antibiotic (enrofloxacin) used by veterinarians in Europe may be selecting for strains of *Campylobacter* in poultry that can enter the food chain (Endtz et al., 1991). These resistant bacteria have also shown decreased susceptibility to a medical fluoroquinolone (ciprofloxacin), causing renewed concern for the human health implications of antibiotic growth promoters in the food supply (Piddock et al., 1993).

Recently, isolates of *Salmonella typhimurium* with resistance to more than one antibiotic have emerged as an important cause of salmonellosis in the United Kingdom and the USA. Salmonellae are transmitted primarily by foods of animal origin and contaminated water. Some strains of Salmonella *(Salmonella enterica* subsp enterica ser Typhimurium DT104, where DT = Definitive Type) have been shown to display resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. This multi-drug-resistant strain can thrive in an animal being fed subtherapeutic doses of antibiotic when competing bacteria from the normal flora are suppressed. The resulting reservoir of infectious agent can persist and transmit itself to other animals, and eventually to the food supply (Poppe et al., 1998). Farm families working with infected animals or drinking unpasteurized milk are particularly at risk for acquiring a Salmonella infection. A study of *S. typhimurium* DT104 infections in humans and farm animals determined that 9 out of 23 human cases were associated with animals, suggesting that occupationally-acquired *Salmonella* in farmers may be significantly contributing to the increasing number of infections reported (Fone and Barker, 1994).

There is further concern that resistant pathogens such as DT104 could spread to the food supply from infected cattle during slaughter. Isolates of a chloramphenicol-resistant species of *Salmonella* have been traced from infected cows on the farm, through a slaughterhouse to a meat packaging company, and into hamburger consumed by a group of people who had become ill (Cohen and Tauxe, 1986). Multi-resistant *E. coli* isolates appeared in chickens that had been given spectinomycin in their drinking water (Gins et al., 1996), and young turkeys given subtherapeutic levels of virginiamycin developed bacteria resistant to quinupristin and dalfopristin (Welton et al., 1998).

4.3. Resistant bacteria in food processing

It is of critical importance to prevent the spread of resistant organisms through the food supply. In 1985, an epidemic of *S. typhimurium* was traced to pasteurized milk from a contaminated dairy in the USA. The *Salmonella* strain was resistant to five different antibiotics, and resulted in about 180 000 people being infected (Ryan et al., 1987). Bacteria that develop resistance to food preservatives and processes represent a serious concern in the food industry. For example, antibiotic-resistant strains of *S. aureus* and *S. epidermidis* have demonstrated plasmid-mediated resistance to organic cationic agents such as chlorhexidine and quaternary ammonium compounds (Russell, 1997), and strains of *E. coli* have been found that demonstrate resistance to common household disinfectants such as pine oil (Moken et al., 1997).

Many microorganisms exhibit resistance to chlorine treatments (Dychdala, 1991), leaving the food industry to seek alternative agents for disinfection. Unlike antibiotics, which generally have a specific target site, biocides usually exert their cytotoxic effects through multiple non-specific targets. While a single mutation or chemical transformation of a cellular target can confer antibiotic-resistance upon a bacterium, biocidal action is rarely affected by such an event. Resistance to biocides can occur when a cell develops reduced permeability (such as a mucopolysaccharide outer layer within a biofilm). There is some evidence that the acquisition of a plasmid could be responsible for changes in the cell envelope which subsequently render the bacterium less susceptible to biocides (Russell, 1991). This suggests that cross-resistance may exist between antibiotics and biocides (Russell and Day, 1996). Changes in the membrane lipid composition of *L. monocytogenes* were observed to confer resistance to previously effective biocides (Juneja and Davidson, 1993). A presumed altered lipid structure of differing nutrient fatty acid ratios was suggested as the cause of observed increases in resistance to paraben-type preservatives.

Nisin is an antimicrobial peptide with GRAS status that is finding increased use as a food preservative. It exerts its bactericidal effect by forming voltage-dependent pores in the cytoplasm of susceptible microorganisms. However, pathogenic bacteria have already begun to emerge that demonstrate

resistance to the antimicrobial action of nisin. For example, a strain of nisin-resistant *L. monocytogenes* was found to have a lower ratio of C15 to C17 fatty acids than the wild-type strain as well as an altered requirement for divalent cations (Mazzotta and Montville, 1997; Crandall and Montville, 1998). These genetic changes confer resistance by somehow disrupting the normal pore-forming mechanism of nisin. Cross-resistance to the class IIa bacteriocin pediocin PA-1 and the class IV leuconocin S have also been displayed by these nisin-resistant strains of Listeria. Additionally, cells and spores of *Clostridium botulinum* have demonstrated resistance to nisin, presenting a possible safety hazard in nisin-preserved, minimally processed foods (Mazzotta et al., 1997).

In addition to developing resistance to antimicrobial agents and biocides used in a food processing environment, microorganisms can also develop resistance to food processing treatments. Accurately measuring bacterial resistance when evaluating processing conditions may depend on growing the organism in its most resistant form, since prior growth conditions may influence the bacteria's ability to survive processing treatments such as hydrostatic pressure, irradiation, thermal processes, and acidic conditions. Bacteria that display resistance to acid have been found in grain-fed animals (Diez-Gonzalez et al., 1998). Cattle are deficient in the starch-degrading enzyme amylase, thus starchy grains in the diet will be fermented by bacteria in the colon, producing acid. There is concern that acid resistant *E. coli* (O157: H7) in grain-fed cattle may be capable of surviving in acidic foods and in the human stomach despite gastric secretions of pH 2.0.

Colbalt 60 gamma irradiation was found to be effective against *S. typhimurium* on chicken carcasses and eggs, although high doses (>4.0 Kgy) were required to inactivate 1 million cells per carcass (Kohler et al., 1989). 'Some bacteria such as *Lactobacillus sake* may exhibit resistance to gamma irradiation' (Hastings et al., 1986) with the effect being enhanced under nitrogen packaging (ave D_{10} kGy of 1.95) as compared to air (ave. D_{10} kGy of 1.47). (Hastings et al., 1986).

Exposure to ultraviolet radiation (254 nm) can generate UV resistant strains of *E. coli* (Rame et al., 1997). As a method of food preservation, UV radiation was recommended as best used in combination with other preservation techniques since cumulative damage to microbial DNA caused by irradiation, while sufficient to decrease the overall number of bacterial cells, did not result in complete sterilization (Rame et al., 1997).

Ultra-high pressure can inactivate microorganisms in certain foods without compromising the quality of the product. However, sporeforming bacteria demonstrate increased resistance to high pressure treatments, creating problems for food processors (Cheftel and Dumay, 1995). Effective sterilization treatments are found when high pressure is combined with another method of food preservation such as acidic conditions, or high temperatures, or addition of bactericidal compounds (e.g., nisin) (Roberts and Hoover, 1996). Heat-resistant organisms are also of concern. Isolates of *S. typhimurium* DT104 demonstrated increased heat tolerance when attached to pork muscle tissue (Humphrey et al., 1997). This suggests that *Salmonella*, if attached to muscle tissue as might normally occur during processing, could survive the cooking process and cause an outbreak of *Salmonellosis*.

5. Strategies for control

Mechanisms for combating the emergence of resistant microorganisms range from the obvious (e.g., improved hygienic practices in hospitals and on farms), to the fantastic (e.g., newly designed synthetic antibiotics whose mode of action cannot be defeated by a mutating bacterium). Meanwhile, new food processes such as organic food production limit the use of certain preservatives and sanitizers, putting a greater burden on the allowable ones which may not be as effective against emerging bacterial strains. Surveillance programs are underway to monitor the development of antimicrobial resistance, and regulatory guidelines for antibiotic use are being enacted in countries around the world as different strategies for control are carefully evaluated.

5.1. Change antibiotic usage practices

Perhaps the simplest strategy proposed for reducing the rise in antimicrobial-resistant bacteria is to change the current practices for antibiotic usage. If widespread use of antibiotics has given rise to an increased number of antibiotic-resistant organisms, then prudent use or even disuse should result in

decreased numbers of resistant microorganisms, especially through reduction or elimination of subtherapeutic usage of antibiotics in food animals. Although this strategy has merit, it will not solve the problem by itself. Temporarily ceasing to use an antibiotic does not cause the resistant microorganisms to disappear from the gene pool. There may be a significant decrease in the incidence of resistant bacteria, but the population seldom falls to zero (Nowak, 1994), with surviving members of the resistant strain ready to quickly rebound if antibiotic use is resumed. However, it is still reasonable to reduce or eliminate the practice of adding subtherapeutic levels of antibiotics to animal feeds. Sweden banned the use of antibiotic growth promoters in 1986, however, this issue is once again raising controversy in Europe. After joining the EU in 1995, Sweden was allowed to keep its ban only until the end of 1998 unless it could convince the rest of the EU states to adopt its ban on antibiotic growth promoters.

Several countries are now monitoring the occurrence of antimicrobial resistance in bacteria and establishing guidelines (either through voluntary or regulatory means), to control their increase. In Denmark, the DANMAP program (Danish Integrated Antimicrobial Resistance Monitoring and Research Programme) exists to monitor the spread of resistant bacteria, and to provide guidelines for medical and veterinary antimicrobial chemotherapy. In the USA, there is a grassroots American counterpart: Alliance for the Prudent Use of Antibiotics (APUA). The World Health Organization established the Division of Emerging and other Communicable Diseases Surveillance and Control (EMC) in 1995. The Antimicrobial Resistance Monitoring (ARM), coordinated by EMC, assists developing countries in establishing surveillance programs and prudently using antibiotic compounds.

5.2. Develop new antibiotics

One important method for discovering new antimicrobial agents involves the ‘random’ screening of natural products. A new antimicrobial protein, magainin, has been found in the skin of *Xeonpus laevis* (African clawed frog). Magainins can kill susceptible gram-negative bacteria by interacting directly with the lipopolysaccharide constituent of the cell walls, forming pores, and causing lysis of the cell (Rana and Blazyk, 1989). Other newly discovered antibiotics include everninomicins from soil organisms (Aarestrup, 1998), and squalamine from sharks (Moore et al., 1993).

However, the search for previously undiscovered antimicrobial compounds will only postpone the crisis of antibiotic resistance. Our ability to produce new antibiotics is being surpassed by the bacterial propensity to mutate and acquire resistant determinants. Synthetic antibiotics, based on new bacterial targets or on variations of previous antibiotics, are currently the focus of intense interest. This ‘structure-based’ strategy begins with the target receptor, and then designs an effective inhibitor that will possess significant structural and chemical complementarity (Service, 1995). Another group of compounds, oxazolidinones, are structurally distinct from current antibiotics. They exist as ‘bispecific antibodies’ capable of recognizing not only the microbial target on the bacterial cell, but the phagocytic cells of the human immune system as well (Service, 1995). By involving the body’s immune system in the process of locating and eradicating bacterial invaders, it may be possible to reduce the chances that resistant bacteria will develop.

In the ongoing struggle to control resistant microorganisms, the next generation of antibiotics may not involve conventional antimicrobial compounds at all. Vaccines may be used for controlling bacteria that have high rates of antibiotic resistance. A vaccine that prevents urinary tract infections caused by *E. coli* has already been tested. Unlike antibiotics, which usually are given after a bacterial infection has occurred, the new vaccine prevents the sticky pili of *E. coli* from attaching to a receptor on the bladder lining (Palaszynski et al., 1998). Similarly, a vaccine for *Haemophilus influenzae* type B introduced in 1988, appears to be protecting vaccinated children, who were reportedly less likely to get meningitis than unvaccinated children (Klein, 1994). Vaccines are also being developed for pneumonia and ear infections, making it likely that they can be adapted to protect against resistant food pathogens as well.

In addition to developing new antibiotics, it may be possible to reinforce the effect of currently used antibiotics. Genetic changes can occur rapidly among bacterial strains, quickly overcoming an antimicrobial agent’s destructive effect. In the case of β-

lactam antibiotics, previously susceptible bacteria suddenly began producing the enzyme β-lactamase to hydrolyze the β-lactam substrate. To overcome the bacterial advantage conferred by β-lactamase, inhibitors have been produced that are structural analogs of the B-lactam molecule. In successful tests, a β-lactam antibiotic (such as amoxicillin) was used in combination with an irreversible β-lactam inhibitor (such as clavulanic acid), to render the antibiotic therapy effective again (Davies, 1994).

5.3. Apply hurdle preservation approaches

Another strategy for reducing the possibility of bacterial resistance in a food processing environment relies on the prevention of all microbial growth and reproduction. To accomplish this, preservation methods are combined to create a series of 'hurdles' throughout the process, each representing a barrier that must be overcome by the bacteria to initiate food spoilage. Modified atmosphere packaging, high hydrostatic pressure, ultraviolet light, ethanol, and bacteriocins, are examples of secondary preservation methods. Additionally, bafflers to microbial growth might include a strategy of rotational antimicrobial and sanitizer use to maintain clean food-contact surfaces.

Carbon dioxide is a noncombustible, colorless gas that is sometimes favored as a preservative since it leaves behind no toxic residues in the food. It can also be used in modified atmosphere packaging. Solid CO_2 (dry ice) controls microbial growth by acting as a refrigerant during transport and storage. Then, as the dry ice sublimes, the CO_2 gas further inhibits bacterial growth by displacing the oxygen required by aerobic organisms, as well as by forming carbonic acid and thus possibly lowering the pH of the food to bacteristatic levels (Foegeding and Busta, 1991). Carbon dioxide does not seem to select for the growth of more adaptive microorganisms, and no $C0_2$-resistant bacteria have been observed (Clark and Takács, 1980), making it a useful secondary barrier against microbial growth.

Ultrasound waves are also capable of inactivating microorganisms by introducing alternating compression and expansion cycles in a liquid medium. The bactericidal effect of high intensity ultrasound waves is believed to be caused during the expansion phase of ultrasound, when small bubbles grow until they implode. The temperatures and pressures reached inside these bubbles can become extremely high (Suslick, 1990). Ultrasound has not been adapted for food preservation, probably because of its adverse effect on the quality of the treated food when applied at the intensity required to kill bacteria (Kyzlink, 1990). However, by using ultrasound in conjunction with pressure and heat, it may be possible to enhance the lethality of this technology for food applications (Raso et al., 1998).

5.4. Prevent bacterial adhesion

To prevent the development of resistant strains of bacteria forming on food-contact surfaces, it is essential to maintain cleanliness. Sometimes, the microtopography of a surface can complicate cleaning procedures when crevices and other surface imperfections shield attached cells from the rigors of cleaning. For this reason, proper equipment design is essential for avoiding cracks and dead areas where organic material could accumulate. Glass is sometimes used for food equipment because of its smooth and corrosion-resistant surface (Dunsmore et al., 1981). Stainless steel resists impact damage better than glass but is vulnerable to corrosion, while rubber surfaces are prone to deterioration and may develop surface cracks where bacteria can accumulate (LeClercq-Perlat and Lalande, 1994). Equally important in equipment design is the cleanability of the surface once bacteria have attached. Cleaning treatments that produce topographical defects in a surface will increase the number of attachment sites for microorganisms. Sanitizers, applied after cleaning to kill any remaining bacteria, can also lead to corrosion of surfaces. Thus many factors should be considered in designing food-contact equipment to resist microbial contamination.

If bacterial adhesion does occur, then a bacteria-filled biofilm may result. In the early stages of biofilm development, protein adsorbs to a surface as a conditioning layer. A useful strategy for inhibiting the initial adhesion of bacteria involves adsorption of a bioactive compound onto a clean food-contact surface. Surfaces with the adsorbed antimicrobial peptide, nisin, were found to decrease the incidence of surface contamination by *L. monocytogenes* on model food contact surfaces (Bower et al., 1995a,b). Alternately, chemical methods may be used to kill

attached bacteria (Russell and Russell, 1995), however, biologically inert biofilms still represent a hazard on food-contact surfaces since they can act as a substrate for new bacterial growth.

5.5. Utilize competitive exclusion

Another strategy for controlling the growth of resistant bacteria relies on the innate antagonism that exists between competing groups of microorganisms. When an antimicrobial is no longer present, susceptible organisms often have a slight survival advantage over resistant strains since they do not have to expend energy maintaining their resistance. Over time, they may outcompete the resistant group in their quest for habitat dominance. A common example of this competitive exclusion technique involves the consumption of yogurt containing live bacterial cultures. The benign *Lactobacillus* and *Streptococcus* cells are introduced into the intestinal tract, taking up available resources, and competing with any pathogens that may be present.

Competitive exclusion has been used successfully in chickens to reduce the amount of antibiotics needed to raise the birds to market size. Normal intestinal bacteria are isolated from healthy chickens and then grown in culture. These indigenous bacteria are then fed to day-old chicks to ensure that they develop healthy intestinal flora of their own. This treatment may reduce the presence of pathogens in the intestines of commercial birds (Bailey et al., 1998). Competitive exclusion may also have the potential to eliminate *E. coli* O157:H7 from cattle before slaughtering.

6. Conclusions

Although we can control and transform many processes of nature, it is unlikely that we will ever completely hold reign over the microbial world. As our understanding of microbial physiology increases, improved antimicrobial agents may be developed to take advantage of newly discovered bacterial targets. However, the problems associated with microbial resistance will likely still exist. Because this issue is of worldwide concern, we need to approach the task of controlling (and co-existing with) microorganisms as part of a world community, establishing guidelines, and agreeing to use antimicrobials according to recognized international policies.

References

Aarestrup, F.M., 1998. Association between decreased susceptibility to a new antibiotic for treatment of human diseases, everninomicin (SCH 27899), and resistance to an antibiotic used for growth promotion in animals, avilamycin. Microb. Drug Resist. 4, 137–141.

Amábile-Cuevas, C.F., 1993. Origin, Evolution and Spread of Antibiotic Resistance, Genes, R.G. Landes Company, Austin, TX.

Bailey, J.S., Cason, J.A., Cox, N.A., 1998. Effect of Salmonella in young chicks on competitive exclusion treatment. Poultry Sci. 77, 394–399.

Billstein, S.A., 1994. How the pharmaceutical industry brings an antibiotic drug to market in the United States. Antimicrob. Agents Chemother. 38, 2679–2682.

Bower, C.K., McGuire, J., Daeschel, M.A., 1995a. Suppression of Listeria monocytogenes colonization following adsorption of nisin onto silica surfaces. Appl. Environ. Microbiol. 61, 992–997.

Bower, C.K., McGuire, J., Daeschel, M.A., 1995b. Influences on the antimicrobial activity of surface-adsorbed nisin. J. Ind. Microbiol. 15, 227–233.

Brady, M.S., Katz, S.E., 1992. In vitro effect of multiple antibiotic/antimicrobial residues on the selection for resistance in bacteria. J. Assoc. Off. Anal. Chem. 75, 738–742.

Brady, M.S., White, N., Katzf, S.E., 1993. Resistance development potential of antibiotic/antimicrobial residue levels designated as ‘safe levels’. J. Food Prot. 56, 229–233.

Brooks, G.F., Butel, J.S., Morse, S.A. (Eds.), 1998. Jawetz, Melnick and Adelberg’s Medical Microbiology, 21st ed., Appleton & Lange, San Francisco, CA, pp. 145–176.

Brown, M.R.W., Allison, D.G., Gilbert, P., 1988. Resistance of bacterial biofilms to antibiotics: a growth-rate related effect? J. Antimicrob. Chemother. 22, 777–780.

Cheftel, J.C., Dumay, E., 1995. Effects of high pressure on dairy proteins: a review. In: High Pressure Bioscience and Biotechnology: Proceedings of the International Conference on High Pressure Bioscience and Biotechnology, 5–9 November, Kyoto, Japan.

Clark, D.S., Takács, J., 1980. Gases as preservatives. In: Silliker, J.H., Elliot, R.P. (Eds.), Microbial Ecology of Foods, Factors Affecting Life and Death of Microorganisms, Vol. vol. I, Academic Press, New York, pp. 170–192.

Cohen, M.L., Tauxe, R.V., 1986. Drug-resistant Salmonella in the United States: an epidemiologic perspective. Science 234, 964–969.

Cohen, M.L., 1992. Epidemiology of drug resistance: Implications for a post-antimicrobial era. Science 257, 1050–1055.

Crandall, A.D., Montville, T.J., 1998. Nisin resistance in Listeria monocytogenes ATCC 700302 is a complex phenotype. Appl. Environ. Microbiol. 64, 231–237.

Cross, A.S., 1990. The biological significance of bacterial en-

capsulation. In: Jann, K., Kann, B. (Eds.), Current Topics in Microbiology and Immunology, Bacterial Capsules, Vol. vol. 150, Springer-Verlag, Berlin, pp. 86–95.
Davies, J., 1990. What are antibiotics? Archaic functions for modern activities. Mol Microbiol. 4, 1227–1232.
Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistant genes. Science 264, 375–382.
Diez-Gonzalez, F., Callaway, T.R., Kizoulis, M.G., Russell, J.B., 1998. Grain feeding and the dissemination of acid-resistant Escherichia coli from cattle. Science 281, 1666–1668.
Dunsmore, D.G., Twomey, A., Whittlestone, W.G., Morgan, H.W., 1981. Design and performance of systems for cleaning product-contact surfaces of food equipment: a review. J. Food Prot. 44, 220–240.
Dychdala, G.R., 1991. Chlorine and chlorine compounds. In: Block, S. (Ed.), Disinfection, Sterilization, and Preservation, 4th ed., Lea & Febiger, Malvern, PA, pp. 131–151.
Endtz, H.P., Ruijs, G.J., van Klingeren, B., Jansen, W.H., van der Reyden, T., Mouton, R.P., 1991. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrobiol. Chemother. 27, 199–208.
Evans, D.J., Allison, D.G., Brown, M.R., Gilbert, P., 1991. Susceptibility of Pseudomonas aeruginosa and Escherichia coli biofilms toward ciprofloxacin: effect of specific growth rate. J. Antimicrobiol. Chemother. 27, 177–184.
Foegeding, P.M., Busta, F.F., 1991. Chemical food preservatives. In: Block, S. (Ed.), Disinfection, Sterilization and Preservation, Lea and Febiger, Philadelphia, pp. 802–832.
Fone, D.L., Barker, R.M., 1994. Associations between human and farm animal infections with Salmonella typhimurium DT1O4 in Herefordshire. Commun. Dis. Rep. CDR Rev. 4, R136–R140.
Frank, J.F., Koffi, R.A., 1990. Surface-adherent growth of Listeria monocytogenes is associated with increased resistance to surfactant sanitizers and heat. J. Food Prot. 53, 550–554.
Gilbert, P., Collier, P.J., Brown, M.R.W., 1990. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrobiol. Agents Chemother. 34, 1865–1868.
Gins, C.A., Browning, G.F., Benham, M.L., Anderson, G.A., Whithear, K.G., 1996. Antimicrobial resistance in epidemiology of Escherichia coli in broiler breeder chickens. Avian Pathol. 25, 591–605.
Hall, B.G., 1991. Adaptive evolution that requires multiple spontaneous mutations: mutations involving base substitutions. Proc. Natl. Acad. Sci. USA 88, 5882–5886.
Hastings, J.W., Holzapfel, W.H., Niemand, J.G., 1986. Radiation resistance of lactobacilli isolated from radurized meat relative to growth and environment. Appl. Environ. Micro. 52, 898–901.
Holmes, C.J., Evans, R.C., 1989. Resistance of bacterial biofilms to antibiotics. J. Antimicrobiol. Chemother. 24, 84.
Howells, C.H., Joynson, D.H., 1975. Possible role of animal feeding-stuffs in spread of antibiotic resistant intestinal coliforms. Lancet 1 (7899), 156–157.
Humphrey, T.J., Wilde, S.J., Rowbury, R.J., 1997. Heat tolerance of Salmonella typhimurium DT1O4 isolates attached to muscle tissue. Lett. Appl. Microbiol. 25, 265–268.
Juneja, V.K., Davidson, P.M., 1993. Influence of altered fattyacid composition on resistance of Listeria monocytogenes to antimicrobials. J.Food Prot. 56, 302–305.
Klein, J., 1994. Antimicrobial treatment and prevention of meningitis. Pediatr. Ann. 23, 76–81.
Kohler, B., Hubner, H., Krautschick, M., 1989. The use of ionizing irradiation for decontamination of Salmonella containing slaughtered broiler chickens and powdered eggs. Z. Gesmate Hyg. 35, 665–668.
Krysinski, E.P., Brown, L.J., Marchisello, T.J., 1992. Effect of cleaners and sanitizers on Listeria monocytogenes attached to product contact surfaces. J. Food Prot. 55, 246–251.
Kyzlink, V. (Ed.), 1990. Principles of Food Preservation, Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 353–355.
Lappe, M., 1982. Germs That Won't Die: the New Threat of Antibiotic Resistant Bacteria, Anchor Press, Garden City, NY.
Le Chevallier, M.W., Cawthon, C.D., Lee, R.G., 1988. Inactivation of biofilm bacteria. Appi. Environ. Microbiol. 54, 2492–2499.
LeClercq-Perlat, M., Lalande, M., 1994. Cleanability in relation to surface chemical composition and surface finishing of some materials commonly used in food industries. J. Food Eng. 23, 501–517.
Levy, S.B., 1992. The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle, Plenum Press, NY, pp. 141–142, 241.
Mazzotta, A.S., Montville, T.J., 1997. J. Appl. Microbiol. 82, 32–38.
Mazzotta, A.S., Crandall, A.D., Montville, T.J., 1997. Appl. Environ. Microbiol. 63, 2654–2659.
Miller, M.J., Ahearn, D.G., 1987. The adherance of Pseudomonas aeruginosa to hydrophilic contact lenses and other substrata. J. Clin. Microbiol. 26, 513–517.
Moken, M.C., McMurry, L.M., Levy, S.B., 1997. Selection of multiple-antibiotic-resistant (mar) mutants of Escherichia coli by using the disinfectant pine oil: roles of the mar and acrAB loci. Antimicrob. Agents Chemother. 41, 2770–2772.
Moore, K.S., Wehrli, S., Roder, H. et al., 1993. Squalamine: an aminosterol antibiotic from the shark. Proc. Natl. Acad. Sci. USA 90, 1354–1358.
Neu, H.C., 1992. The crisis in antibiotic resistance. Science 257, 1064–1072.
Nichols, W.W., 1989. Susceptibility of biofilms to toxic compounds. In: Characklis, W.S., Wilderer, P.A. (Eds.), Structure and Function of Biofilms, John Wiley, New York, pp. 321–331.
Nowak, R., 1994. Flesh-eating bacteria: not new, but still worrisome. Science 264, 1665.
Palaszynski, S., Pinkner, J., Leath, S. et al., 1998. Systemic immunization with conserved pilus-associated adhesins protects against mucosal infections. Dev. Biol. Stand. 92, 117–122.
Phelps, C.E., 1989. Bug/drug resistance. Sometimes less is more. Med. Care 27, 194–203.
Piddock, L.J., Griggs, D.J., Hall, M.C., Jin, Y.F., 1993. Ciprofloxacin resistance in clinical isolates of Salmonella typhimurium obtained from two patients. Antimicrob. Agents Chemother. 37, 662–666.
Poppe, C., Smart, N., Khakhria, R., Johnson, W., Spika, J., Prescott, J., 1998. Salmonella typhimurium DT1O4: a virulent and drug-resistant pathogen. Can. Vet. J. 39, 559–565.

Rame, J., Chaloupecky, V., Sojkova, N., Bencko, V., 1997. An attempt to demonstrate the increased resistance of selected bacterial strains during repeated exposure to UV radiation at 254 nm. Cent. Eur. J. Public Health 4, 30–31.

Rana, F., Blazyk, J., 1989. Outer membrane structure in smooth and rough strains of Salmonella typhimurium and their susceptibility to the antimicrobial peptides, magainins and defensins. Prog. Clin. Biol. Res. 292, 77–85.

Raso, J., Pagán, R., Condón, S., Sala, F., 1998. Influence of temperature and pressure on the lethality of ultrasound. Appl. Environ. Microbiol. 64, 465–471.

Reece, K.S., Phillips, G.J., 1995. New plasmids carrying antibiotic resistant cassettes. Gene 165, 141–142.

Ridley, A., Threlfall, E.J., 1998. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic Salmonella typhimurium DT 104. Microb. Drug Resist. 4, 113–118.

Roberts, C.M., Hoover, D.G., 1996. Sensitivity of Bacillus coagulans spores to combinations of high hydrostatic pressure, heat, acidity and nisin. J. Appl. Bacteriol. 81, 363–368.

Russell, A.D., 1991. Principles of antimicrobial activity. In: Block, S. (Ed.), Disinfection, Sterilization, and Preservation, 4th ed., Lea & Febiger, Malvern, PA, pp. 29–58.

Russell, A.D., Russell, N.J., 1995. Biocides: activity, action and resistance. In: Hunter, P.A., Darby, G.K., Russell, N.J. (Eds.), Fifty Years of Antimicrobials: Past Perspectives and Future Trends, Cambridge University Press, Cambridge, pp. 327–365.

Russell, A.D., Day, M.J., 1996. Antibiotic and biocide resistance in bacteria. Microbios 85, 45–65.

Russell, A.D., 1997. Plasmids and bacterial resistance to biocides. J. Appl. Microbiol. 83, 155–165.

Ryan, C.A., Nickels, M.K., Hargrett-Bean, N.T. et al., 1987. Massive outbreak of antimicrobial resistant Salmonellosis traced to pasteurized milk. J. Am. Med. Assoc. 258, 3269–3274.

Service, R.F., 1995. Antibiotics that resist resistance. Science 270, 724–727.

Shlaes, D.M., 1993. Clinical importance and public health concerns for the emergence of antibiotic resistant organisms in the human intestinal microflora. Vet. Human Toxicol. 35 (Suppl. 1), 24–27.

Sternbach, G., Varon, J., 1992. Alexander Fleming: the spectrum of penicillin. J. Emerg. Med. 10, 89–91.

Suslick, K.S., 1990. Sonochemistry. Science 247, 1439–1445.

Volk, W.A., Gebhardt, B.M., Hammarskjöld, M.L., Kadner, R.J., 1996. Antibiotic action and resistance: bacterial cell surfaces; and mechanisms of antibiotic action. In: Essentials of Medical Microbiology, 5th ed., Lippincott-Raven, Philadelphia, PA, pp. 253–265, pp. 266–284.

Wallerstein, M.B., 1980. The role of technology in food distribution systems in developing countries. In: Selvey, N., White, P.L., (Eds.), Nutrition in the 1980s: Constraints on our Knowledge: proceedings of the Western Hemisphere Nutrition Congress VI, in Los Angeles, California, August 1980.

Welton, L.A., Thal, L.A., Pen, M.B. et al., 1998. Antimicrobial resistance in enterococci isolated from turkey flocks fed virginiamycin. Antimicrob. Agents Chemother. 42, 705–708.