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Spread of antibiotic resistance with food-borne pathogens

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Abstract. This short review summarizes data on antibiotic resistance profiles of common food-borne pathogens like *Salmonella* sp., *Escherichia coli*, *Campylobacter* sp., *Listeria monocytogenes*, *Clostridium perfringens*, *Staphylococcus aureus*, and coagulase-negative staphylococci. As a flashlight on the literature of the last few years, it provides ample evidence that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them. Molecular analysis of the resistance genes, where available, shows that the food microflora is not separated from its human counterpart and conjugative transfer of resistance genes has been demonstrated in vitro and in a few cases in vivo. For example, for *Salmonella typhimurium*, resistance towards tetracyclines has increased from zero in 1948 to a 98% level in certain epidemic populations of *S. typhimurium* DT104 in 1998. The high incidence of food-borne pathogens in raw meat and milk together with a high level of therapeutic, prophylactic and nutritional application of antibiotics in agriculture reveals an antibiotic resistance problem of global dimensions. The resistance problem in human medicine will not be solved if there is a constant influx of resistance genes into the human microflora via the food chain.

Key words. Food-borne pathogens; antibiotic resistance; enterobacteria; *Listeria*; *Clostridium*.

Introduction

The detection, invention, and global uses of antibiotics and antimicrobial agents in human and veterinary medicine, agriculture and aquaculture have initiated a 'Darwinian' experiment bringing about the survival of resistant microorganisms coupled with the elimination of susceptible ones in antibiotic-containing environments.

Fifty years of increasing applications of antimicrobial agents have created a situation leading to an ecological imbalance [1]: the enrichment of multiple-antibiotic-resistant bacteria, both pathogenic and commensal, in human and animal habitats.

The purpose of this review will be to examine recent data on antibiotic resistance spread in food-borne pathogens including *Salmonella*, *Escherichia*, *Campylobacter*, *Listeria*, *Staphylococcus*, and *Clostridium* species from farm animals and food.

Although there is evidence to suggest that the inadvertant development of an antibiotic-resistant human microflora in hospitals is mainly caused by the use of antimicrobial agents by the medical profession [2], the role of the application of about half of the world production of antibiotics in agriculture [3] in resistance development and its spread still remains to be determined. The term spread has multiple dimensions, also in the agricultural context: (i) translocation of a resistance gene from one place in the bacterial genome (plasmid or chromosome) to another; (ii) horizontal spread of resistance genes from one individual bacterium to another of the same species or over species and genus borders e.g. by conjugation; (iii) spread of resistant bacteria from animal to animal and from animal to the environment (e.g. plants and water via manure); (iv) spread from animal to human by direct contact or via food; (v) global spread by export/import of live animals and products; (vi) spread of antibiotic-resistant bacteria in healthcare settings versus community transmissions e.g., by food, domestic animals, or person-to-person contacts.

The dimensions of the farm animal/antibiotic system

The production of meat, milk, and eggs in modern agriculture has attained industrial dimensions, animals

being kept in large numbers for the different stages of production (breeding, raising, fattening, milk, and egg production) in specific 'farms'. In many countries, this system is still accompanied by small family farming. The numbers of slaughtered and animals in stock reaches a total of about 48 billion animals (cattle, pigs, sheep, goats, chicken, and turkeys) that live on farms and are potential consumers of drugs and antibiotics every year [4].

It is the meat, if raw, that is a direct link between the microflora of an animal and the human population, since it is not possible to slaughter an animal without contaminating the carcass with part of the intestinal and skin/hide microflora [5]. For raw milk samples, contamination with food-borne pathogens has been determined to be about 50% [6]. Eggs have also to be included, since at least on the shell they are inevitably contaminated with the fecal microflora of the laying birds [7]. Farming productivities have become dependent on the use of antibiotics for three different purposes [8]:

1) Therapeutic use to treat infected animals (diarrhea, pulmonary inflammation, skin and organ abscesses, bacteremia, mastitis of dairy cows). The broad- and narrow-spectrum antibiotics used for systemic administration are the same as those employed in human medicine, but may vary according to law in different countries (penicillins, cephalosporins, tetracyclines, chloramphenicol, aminoglycosides, spectinomycin, lincosamides, macrolides, nitrofurans, nitroimidazoles, sulfonamides, trimethoprim-sulfonamide combinations, polymyxin, and quinolones [8]).

2) Prophylactic use to avoid infection of a herd if one animal starts to show symptons of infectious disease. In dairy cows, antibiotics are routinely administered directly into the udder to cure and avoid mastitis (e.g., in dry-cow therapy). The same therapeutic antibiotics are delivered directly into the fodder or drinking water.

3) Nutritive use at subtherapeutic levels to induce growth promotion. Antibiotic addition to fodder at subtherapeutic levels is an integral part of modern agriculture worldwide [8]. The application of sublethal levels of antibiotics as practised in their use as growth promotors is specifically prone to select and enrich resistant bacteria [9].

Whereas we have information on which antibiotics are used for which purposes and animals, there seems to be no control of the flow of a particular antibiotic into farm animal production. The available information is usually rather approximate, e.g., about 50% of world production is said to be applied in agriculture and aquaculture [3]. Concrete data have been published for the therapeutic use of antibiotics for farm animals in France in 1989 and include 50,000 kg β-lactam antibiotics, 57,100 kg aminoglycosides, 99,600 kg chloramphenicol, 116,800 kg tetracyclines, 37,000 kg macrolides, 138,600 kg sulfonamides, 77,200 kg nitrofuran, 3700 kg quinoxaline, and 45,300 kg nitroimidazole [10]. For The Netherlands, veterinary usage of 300,000 kg of antibiotics in the year 1990 was calculated to result in 125, 430, and 55 mg/kg per year for pigs, poultry, and cattle, respectively [11]. The amounts used in Sweden in 1980 (before the ban on antibiotics for growth promotion) were 70,700 kg active substance in human medicine, compared to 41,270 kg in veterinary medicine and agriculture [12]. On the basis of metabolic body weight, human beings received 4.2 times more antimicrobial compounds than farm animals. The use of antibacterials (not including coccidiostats and antiparasitic compounds) dropped after the implementation of the feeding ban to 20,307 kg in 1996 [12]. The amount of growth promotors (active substance) in animal nutrition within the European Union (EU) has been estimated to be 1,520,370 kg for 1982, which breaks down to 67, 187, 115, and 122 mg/kg per year for pigs, broilers, veal calves, and cattle, respectively [13]. Recently, detailed consumption data have been released in the framework of a surveillance program in Denmark [14]: unofficial figures indicate that the consumption of therapeutic antibiotics in agriculture was 22,000 kg active substances in the first half of 1996, aminoglycosides, macrolides, penicillins, and tetracyclines accounting for almost 80% of this amount. In contrast, 94,000 kg of active compounds (more than 50% as tylosin) were used for growth promotion. In the USA, the National Academy of Sciences of America [15] estimated that in 1978, 6.08 million kg of antibacterials were produced for therapy while 5.58 million kg were for addition to animal feed. For the EU and Switzerland, the corresponding data for the year 1997 were reported to be 5.46 and 5.04 million kg, resp. [16].

A sober analysis of this situation—based on worldwide experience as to how antibiotics are applied by farmers and in some instances the veterinary profession—leads to the conclusion that an assessment of the prudent use of antibiotics and proper controls is very difficult.

Analysis of the quantitative and qualitative resistance situation of food-borne pathogens from farm animals and food produced or processed from them

Two lines of scientific evaluation of the development of antibiotic resistance in bacteria from farm animals are available: first, analysis of the resistance of bacteria isolated in mandatory food hygiene and animal health

investigations and second, analysis of resistance development under experimental conditions controlling all factors involved like fodder, antibiotics, drugs applied, and kinds and numbers of animals including general hygienic husbandry conditions.

Based on the mean global prevalences of the microbiological hazards arising from swine, cattle, and poultry [8], the first method allows collection of a great number of bacterial strains unbiased by the original farm which produced the animal and the producer processing food from it. Such investigations have resulted in significant data regarding the kind of bacteria and their resistances; however, the amounts of antibiotic applied are unknown due to lack of control at the individual farm. Therefore, the results reflect the overall effects of antibiotic uses for different purposes in a specific agricultural environment. The second method has been applied in surveillance experiments with control of antibiotics and animals in both prospective and retrospective studies [1, 17]. However, such experiments can only be done on a limited quantitative basis which may lead to the argument that they do not reflect the actual situation.

From the insights into resistance development [1] we may predict that bacteria living in a biotope under constant antibiotic pressure will show a high degree of resistance against the applied compounds. On the other hand, bacteria coming from low-level-antibiotic environments should be more or less susceptible to antibiotics. If a new therapeutic or feeding antibiotic is being introduced, a specific resistance should appear. Such effects have been observed in common food-borne pathogens.

Salmonella species

These bacteria provide evidence for the spread of antibiotic resistance over the entire time scale of antibiotic application in human and veterinary medicine since 1948.

It is an almost universally accepted dogma that human salmonellosis is a zoonosis [8, 15, 18]. The continuing pandemic of human infections with *Salmonella enteritidis* phage type 4 (Europe) and phage type 8 (North America) is associated with the consumption of raw or lightly cooked shell eggs and egg-containing products [18]. The second most common *Salmonella*—serovar *typhimurium*—persists in the porcine, ovine, and bovine meat industries. It originates from environmental sources, contaminated feeds, and in parental and animal to animal transmissions of infections [18]. A high percentage of *Salmonella*-positive samples of ground meat from swine (40.3%) and cattle (46%) and of processed poultry products (56.3%) implies that a consumer has a 50/50 chance of carrying home live *Salmonella* with these products from the supermarkets. The number of resulting certified cases of food-borne salmonellosis has been estimated in different countries for the years 1989/1990 to amount to 1–250 per 100,000 capita [18].

S. typhimurium is a food-borne pathogen for which data on its resistance properties are available from the pre-antibiotic area to the present. One hundred strains each from farm animals (fowl) and humans isolated between 1940 and 1948 in the USA were all sensitive to tetracycline [19], providing a base line for the subsequent development of resistance. After introduction of tetracyclines into human and veterinary medicine as well as agriculture for growth promotion, the precentage of tetracycline-resistant *S. typhimurium* has been steadily increasing to 90% and more of the isolated and investigated strains in certain countries. The usefulness of tetracyclines to fight infectious disease caused by *S. typhimurium* in farm animals (e.g., calves) and infected humans was entirely destroyed within 50 years of their first application. The situation has become more serious with the spread of an epidemiologically predominant strain of *S. typhimurium*, namely phage type DT104. First recognized in bovine and human cases in the UK in the late 1980s, DT104 strains have now been reported from the USA, Canada, Denmark, Germany, France, and Austria [20]. DT104 is characterized in almost all the investigated strains by a specific resistance profile comprising ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines. Recently, additional resistances have been added, including trimethoprim, spectinomycin, and ciprofloxacin. For ciprofloxacin, a clear correlation of the introduction of fluoroquinolones into agricultural use and resistance development has been demonstrated in the UK and Germany [21, 22]. DT104 has been isolated from cattle, pigs, sheep, goats, chickens, turkeys, cats, and dogs and, recently, a gray seal [23]. Since DT104 *Salmonella* may have an increased pathogenicity specifically for the very young and the very old, the lack of effective antibiotics may soon become critical. In September 1998, a previously [24] healthy woman died from an infection with a multidrug-resistant DT104 strain in Denmark which was traced back to contaminated pork.

Salmonella strains from food animals are passed to the human population via insufficiently cooked meat, eggs, and milk [18]. Since enteric infections with *Salmonella* in humans result in multiplication and excretion of the infectious agents in and from the human intestine, the animal-food-human spread must be regarded as an important contribution to the release of antibiotic-resistant bacteria from farm animals [24, 25]. Tourism enhances the transfer of *Salmonella* and other enteropathogens from one country to another [26]. Free-moving wild animals, like seals, gulls, and sparrows, add to the spread of antibiotic resistance [27].

In different epidemics of *S. typhimurium* phage types, a conjugative resistance transfer via R-plamids has been demonstrated [e.g., 22]. The current DT104 epidemic, however, is characterized by chromosomal-encoded multiple resistance in connection with recently identified integrons [28, 29]. Two different integrons have been characterized, each carrying a single resistance cassette in addition to the *sul*1 and *qac*ED1 genes characteristic for integrons. One encodes the *ant* (3″)-Ia gene that specifies spectinomycin and streptomycin resistance, the other the *pse*-1 β-lactamase gene [28, 29]. The recently developed resistance of DT104 to fluoroquinolones (ciprofloxacin and nalidixic acid) is a mutation of the *gyr*A gene consisting of two discrete base substitutions at Asp87 leading to substitution of Asp87 by Asn, a mechanism previously identified in other enteropathogenic bacteria like *Campylobacter* [30, 31].

Escherichia coli

E. coli is used as an indicator species to monitor fecal contaminations of e.g., drinking water and food. It has also been introduced as an indicator species in recent surveillance programs to analyze the antibiotic resistance status of the enteric microflora of both farm animals and humans.

The advent of *E. coli* strains pathogenic for animals and humans has initiated studies of their antibiotic resistances which might be a threat to a necessary antibiotic treatment [33].

E. coli has been a model to study the resistance levels of bacteria from persons involved in animal handling such as pig farmers, abattoir workers, and veterinarians [34, 35]. Compared to the overall community, persons with a high level of contact with farm animals have a significant percentage of antibiotic-resistant *E. coli* in their intestinal microflora. This phenomenon was seen in prospective studies on chicken farms years ago [3], and still holds true in retrospective studies under field conditions. Thirty percent of antibiotic-resistant *E. coli* and 33% of resistant *Salmonella* from chicken carcasses transferred their resistance to *E. coli* K12 in laboratory experiments [36]. Of interest is the resistance profile of *E. coli* O157:H7 and other verotoxin-producing serotypes, although antibiotic treatment of infected persons has been regarded as a risk factor and antibiotic treatment of such infections is presently not advised [33]. Since the normal host of these pathogens seems to be cattle, use of antibiotics for these animals (specifically calves) should be reflected in their resistance profiles. Among 20 human and cattle isolates (of serotypes verotoxin 1, 2, or both) of *E. coli* 0157:H7, O11:H −, and O26:H11, 80% were resistant to at least one antibiotic. Seventy percent were resistant to streptomycin, 65% to sulfonamides, 50% to tetracycline, 25% to ampicillin, 20% to sulfonamide/trimethoprim, 10% to chloramphenicol, 8% to cefalotin, and 5% to gentamicin [37]. Most of these markers occurred in combinations of at least two (e.g., SSu-R type) to up to six (e.g., CGSSuTTm-R type or AGSSuTTm-R type). Conjugal transfer of some resistance determinants from non-O157 strains to antibiotic-sensitive *E. coli* K12 has been demonstrated [37]. *E. coli* O157:H7 strains isolated between 1984 and 1987 in a Washington State (USA) investigation were sensitive to all tested antibiotics, but 7.4% of strains from the 1989–1991 period were resistant to streptomycin, sulfisoxazole, and tetracycline [38]. *E. coli* O157:H7 highly resistant to ampicillin and tetracycline was reported for the period beginning July 1996 [39].

Finally, *E. coli* has been used as an indicator in a field study to show that the development of resistance to streptothricin (used for growth promotion of pigs in the former German Democratic Republic) occurred, shortly and consecutively after introduction of the drug, in the intestinal microflora of the pigs, the pig farmers, and members of the neighboring community [40]. The gene responsible for resistance was identified as a streptothricin acetyltransferase encoded on a novel type of transposon (Tn1825) linked to a gene for the streptomycin/spectinomycin adenyltransferase AAD-3″ [41]. The *sat* gene was later discovered in the same geographic area in a streptothricin-resistant human *Shigella* isolate [40]. Since this antibiotic has not been applied in human medicine, these observations prove that 'feed' antibiotics do induce resistances in the animal population which later move via resistant enterobacteria into the human population without a specific selection pressure from streptothricin but possibly from streptomycin in that new environment.

Campylobacter species

Campylobacter jejuni is a cause of human acute bacterial enteritis. It is isolated from the gastrointestinal tracts of most domestic animals (chickens, pigs, cattle, pets), but appears to be most highly adapted to the avian gut [42]. It may grow to levels of 10^{10} organisms per gram of cecal content in chickens without the animals having any symptoms of disease. If one animal in a flock acquires *Campylobacter*, the whole flock usually becomes colonized. The incidence of fresh meat (swine) or carcasses (cattle and poultry) found to be contaminated with *Campylobacter* species are 13.4, 27, and 66.2%, respectively [8]. Raw milk, untreated surface waters, and pets have to be regarded as three more important sources of campylobacteriosis [43].

Although *Campylobacter* infections are usually self-limiting, if diarrhea is frequent or bloody, or high fever is present, treatment with antibiotics is indicated. Erythromycin and fluoroquinolones are the antibiotics of choice. Both, however, are also applied in veterinary medicine. It is now very clear that introduction of fluoroquinolones has resulted in the enrichment of quinolone-resistant *Campylobacter* isolates from animals and human patients in many parts of the world (e.g., The Netherlands, UK, Finland, Spain, Germany, Canada, Sweden, France, Japan, and the USA) [for specific references see refs. 43–45].

Listeria monocytogenes

L. monocytogenes has emerged as a major concern in food microbiology during the last 10 years. *L. monocytogenes* causes severe diseases of non-enteric nature including meningitis, septicemia, and abortion coupled with a high case fatality rate of around 20 to 30% [46]. Ubiquitous in nature (plants, animals, humans, food, fodder, silage), the establishment in food-processing plants and food is aided by two properties: (i) it can survive for long periods of time in different environments containing organic material, and (ii) it is psychrotrophic, capable of growth down to about 1 °C. In addition, 2–6% of investigated healthy people had *L. monocytogenes* in fecal samples. The average contamination of meat is high, between about 30 and 60%. Two to 5% of tested bulk tank raw-milk samples contained *L. monocytogenes*, but fresh vegetables and processed products from meat may also contain it in low numbers. Raw and ready-to-eat seafood and specifically smoked fish contained up to 10^4 colony-forming units (CFU) per gram in 25% of investigated samples.

Under these circumstances, the availability of useful antibiotics is essential in the treatment of listeriosis. *L. monocytogenes* exhibits intrinsic resistance to third-generation cephalosporins, which are therefore incorporated into selective media for isolation. Until recently, the *Listeria* genus was thought to be uniformly susceptible to antibiotics active against Gram-positive bacteria including ampicillin or penicillin (combined with aminoglycosides), trimethoprim (alone or combined with sulfamethoxazole), tetracyclines, erythromycin, and gentamicin [46]. However, the first antibiotic-resistant *L. monocytogenes* was described in 1988, and many more resistant strains have been detected in food and sporadic cases of listeriosis since that time. The prevalence of antibiotic resistance of *Listeria* species pathogenic for humans and animals was studied in 1100 isolates (60 from cases of listeriosis, and 1040 from food and the environment). Sixty-one strains were resistant to tetracycline and minocycline due to *tetM* (57 strains) and *tetS* genes (4 strains) [47]. Of specific concern was the detection of trimethoprim-resistant strains from food and the environment. Antibiotic resistance in *Listeria* species is due to acquisition of movable genetic elements like self-transmissible plasmids and conjugative transposons [48, 49].

The 37-kb plasmid pIP811 from *L. monocytogenes* (isolated from a patient with meningoencephalitis) [48] encoded resistance to chloramphenicol, erythromycin, streptomycin, and tetracycline. This self-transmissible plasmid shared extensive sequence homology with plasmid pAMβ1, the prototype of broad-host-range plasmids in enterococci and streptococci. A similar plasmid has been reported in *L. monocytogenes* isolated in Switzerland encoding tetracycline (*tetM*), MLS (*ermB*), and chloramphenicol (*cat221*/*cat223*) resistance. Extensive homology to pIP501 from *Streptococcus agalactiae*, another broad-host-range plasmid of the pAMβ family was noted [50]. An identical *tetS* gene was recently identified on plasmid pK214 of a multiresistant *Lactococcus lactis* strain isolated from a French raw milk soft cheese [51, 52]. In other *Listeria* isolates from food, erythromycin resistance was attributed to an *ermC* gene. These strains also carry a complete *tetM* gene on a transposon [53]. The molecular characterization of such plasmids and transposons is of outmost importance to gain insights into the origin and spread of antibiotic resistance in microorganisms from food.

A report on an unusual incidence of multiple resistance to antibiotics in *Listeria* isolates (mainly *L. monocytogenes* and *Listeria innocua*) from Spanish meat products (pork sausages) warrants further research and monitoring. Twenty strains of *L. monocytogenes* out of 72 pork sausage samples were 100% resistant to chloramphenicol, 95% to amikacin and tetracycline, 90% to erythromycin, 80% to tobramycin, and 10% to ampicillin and penicillin G. A similar situation was recorded for 33 *L. innocua* strains from the same samples [54].

Human infections caused by antibiotic-resistant *L. monocytogenes* have been confidently predicted because the intestinal tract represents the port of entry for *Listeria* infections [46]. Whether the proposed antibiotic resistance exchange between *Listeria* and enterococci/streptococci took place in human or animal intestines, or in the food itself, remains to be established.

Staphylococci and *Clostridium perfringens*

The food-borne pathogens discussed above are infectious agents invading different parts of the human body. Other food-borne pathogens are those which produce enterotoxins and other toxic molecules by growth in contaminated food commodities e.g., *Staphylococcus aureus*, *Bacillus cereus* and *C. perfringens* [55].

Whereas the tolerance level for infectious bacteria in ready-to-eat food and drinks in national regulations and the FAO/WHO Codex Alimentarius is zero, the toxigenic bacteria may be present in numbers up to 10^4 CFU per gram before the contaminated food becomes unacceptable. In these cases, antibiotics are of no value in treating a patient suffering from food poisoning. A possible threat could be the uptake of antibiotic-resistant bacteria in high numbers into the human microflora and exchange of resistance genes with components of the indigenous flora.

S. aureus is the main enterotoxin-producing species. It is also the most common agent of bovine mastitis which may, together with the other mastitis agents [coagulase-negative staphylococci (CNS), streptococci like *S. agalactiae*, and *E. coli*] affect between 25 and 50% of dairy cattle in 1 year. The disease is of economic significance worldwide and the annual loss per cow has been calculated in the US to be USD 184.4 [8]. Antibiotics approved for lactating and dry cows as measures to avoid and treat mastitis include amoxicillin, cephapirin, cloxacillin, erythromycin, hetacillin, novobiocin, oxytetracyclin, pirlimycin, penicillin and its combinations with novobiocin or streptomycin. Due to the high prevalence of bovine mastitis and the associated antibiotic consumption, enrichment of antibiotic-resistant staphylococci is expected and has been observed. The phenomenon is dependent on the particular herd and the antibiotics being used for prophylaxis and treatment [56]. Resistance profiles of *S. aureus*, and more so of CNS, show around 50% resistance to penicillin and ampicillin in all tested isolates. Resistance levels to erythromycin, kanamycin, neomycin, oxacillin, cephalexin, sulfonamide/trimethoprim, enrofloxacin and tetracycline have been reported to be in the range of 2–20% [56–58]. The presence of antibiotic-resistant *S. aureus* and CNS has also been shown for sheep's milk [59]. In human infections, *S. aureus* and particularly CNS show an important rate of increased resistance to the standard antimicrobials used for therapy, the rate of emergence differing considerably between coagulase-positive and -negative staphylococci [60].

S. aureus and CNS can contaminate milk and meat and survive in fermented foods like cheeses (about 10^2–10^3 CFU per gram) and sausages (up to 10^5 CFU per gram) if the raw material is not pasteurized during manufacture, or if recontamination occurs after heat treatment [61]. Penicillin-, neomycin-, gentamicin-, chloramphenicol-, tetracycline-, erythromycin-, and lincomycin-resistant *S. aureus* and CNS (*S. xylosus*, *S. lentus*, *S. caprae*, *S. epidermidis*, and *S. haemolyticus*) were noted in such products [61]. Chloramphenicol acetyltransferase genes were localized in several *S. xylosus* and *S. caprae* strains to plasmids ranging in size from 3.8 to 4.3 kb. Three *Staphylococcus* spp. encoded the information of an erythromycin efflux system (*msr*) on an 18-kb plasmid. The *lin*A gene was found in one *S. haemolyticus* on a 2.2-kb plasmid. All tetracycline-resistant *S. xylosus* had a *tet*K gene (tetracycline efflux) on a 4.4-kb plasmid [61].

Although animal and human staphylococci seem to be clonally independent and exchange genetic information only to a limited extent by processes like conjugation [62], we should be aware that certain food items do contain antibiotic-resistant microbes which likely originate from animals or other environmental sources including persons handling the food during production. The resistance situation in staphylococci of animal origin has been recently summarized [63].

The habitat of the anaerobic *C. perfringens* is the intestine of warm-blooded animals [64]. Multiply antibiotic resistant *C. perfringens* (tetracycline, erythromycin, lincomycin, and clidamycin of MLS-type resistance) were isolated from porcine feces as early as 1976 [65]. Tetracycline resistance is the most common resistance phenotype [66]. The conjugative plasmid pCW3 encodes two functional overlapping tetracycline resistance genes, *tetA(P)* and *tetB(P)*. The former encodes an efflux protein, the latter a tetM-like protein. All 81 tetracycline-resistant strains from the USA, Australia, France, Belgium, Japan, Canada, and Germany hybridized with a specific *tetA(P)* nucleotide probe. The hybridization level was 51% for a *tetB(P)*, and 40% for a *tetM* nucleotide probe, respectively. Chloramphenicol acetyltransferase genes and rRNA methylase genes (MLS resistance type) have been characterized at the molecular level. Conjugative plasmids and transposons exist in these isolates [66]. *C. perfringens* can accept transposon Tn*916* from *E. coli* and *E. faecalis* [67].

The response of *C. perfringens* to growth-promoting antibiotics was recorded for 95 field isolates obtained in 1991 and 1992 from poultry, pigs, and cattle [68]. All isolates were resistant to bambermycin and flavomycin, but susceptible to avoparcin, avilamycin, and salinomycin. Acquired resistance to tylosin, virginiamycin, and bacitracin were 5, 3, and 3%, respectively. *C. perfringens* clearly reveals an antibiotic resistance development similar to other bacteria found in antibiotic-abundant habitats.

Enterococci and lactic acid bacteria which are not regarded as food-borne pathogens, but may be opportunistic pathogens, are discussed in a recent review [69].

Discussion

Antibiotic resistance in food-borne pathogens is reality, though substantial qualitative and quantitative differences exist. Milk and meat obtained from animals are inadvertantly contaminated with these bacteria. Antibiotic resistance in some bacterial species has approached

a 100% level, e.g., for tetracycline, chloramphenicol, streptomycin, and sulfonamides in epidemic isolates of *S. typhimurium* DT104, 80% for the same antibiotics in some *E. coli* strains, and 50% for penicillin and ampicillin in *S. aureus* from mastitis infections in cows. Opportunistic pathogens like enterococci, and harmless, commensal lactic acid bacteria like *Lactobacillus reuteri*, *Lactobacillus plantarum* or *Lactococcus lactis* have picked up tetracycline, erythromycin, and other antibiotic resistances. Resistance genes and molecular transfer mechanisms have been shown to be the same in bacteria from food and from pathogenic (animal and human) samples [51, 69].

This situation has led to scientific and political efforts to handle the problems of antibiotic resistance in agriculture. Ever since the Swann report [70] 30 years ago proposed restrictions on the use of antimicrobials important for human medicine in animal feeding, the scientific discussion has continued unabated. The impact of the arguments opposing antibiotic use in agriculture and aquaculture has been limited. The critical levels of antibiotic resistance in human pathogens as addressed by Harold Neu in 1992 [71] and others started a phase of new and in-depth scientific scrutiny of antibiotic usage, culminating in 1995 in an influential report by a task force of the American Society for Microbiology [72]. With regard to antibiotics in agriculture and food, a clear statement was given: 'Due to increased drug resistance in animal pathogens and changes in food production practices, there is a growing threat to food, the food industry, and hence the US economy. Due to increased foreign trade, travel and immigration, the threat of global spread of antibiotic resistance is greater than ever.'

In 1997, the World Health Organization for the first time ever published a report on the medical impact of the use of antimicrobials in food animals [73]. The main threats were formulated as: (i) an increase in the prevalence of resistant bacteria in animals; the transfer of resistant pathogens to humans via direct contact with animals, or through the consumption of contaminated food or water; (ii) the transfer of resistance to human bacteria; (iii) an increase in the incidence of human infections caused by resistant pathogens; (iv) potential therapeutic failures in animals and humans; and (v) frightening resistance situations in farm animals regarding *Salmonella*, *Campylobacter*, *Enterococcus* species and *E. coli*.

A recent report of the National Research Council and Institute of Medicine of the USA on the benefits and risks of antibiotic use in animals concludes that 'the use of drugs in the farm-animal production industry is not without some problems and concerns, but that it does not appear to constitute an immediate public health concern; additional data might alter this conclusion' [8]. This short-sighted conclusion is being challenged by daily reports of 'additional data' specifically from systematic surveillance programs like those in Denmark [14] and France [32].

Another thorough review of the effects of antimicrobial resistance in the food chain was released by the Ministry of Agriculture, Fisheries and Food (UK) in August 1998 [74]. It concludes that the reduction in antimicrobial resistance in the food chain is but one way of reducing the effect in humans; medical and veterinary sectors must be included.

In Europe, avoparcin which induces cross-resistance to vancomycin was banned as an antimicrobial feed additive in 1996, mainly on the basis of a high level of vancomycin-resistant enterococci in the intestines and meat of farm animals [40]. It would appear that the only country to have implemented a strict ban on animal feeding with antibiotics is Sweden. After initiation of the ban in 1986, Swedish agriculture adjusted to the necessary changes in feed composition, hygiene and farm management. The resulting improvements in the resistance situation have been amply documented in an elaborate report [12]. As of 1 July 1999, the EU has banned, in addition, the feed use of spiramycin, virginiamycin, tylosin, and bacitracin due to cross-resistance problems with antibiotics used in human medicine. Scientific reports on the antibiotic resistance of lactococci and enterococci in raw-milk cheeses [51, 52] sparked the 1998 decision of the Swiss parliament to ban all antimicrobial feed additives as of 1 January 1999. In addition, Swiss farmers will have to keep a protocol of the use of therapeutic and prophylactic antibiotics for their animals.

Surveillance programs of antibiotic resistance in farm animals and food are destined to provide new data necessary to support the prudent use of antibiotics in the agricultural sector. Thus it can be concluded that the overwhelming support for the control of antimicrobials in agriculture and related industries will gain impetus, with resulting benefits to human health.

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