

# Responsible use of antibiotics in aquaculture



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# Responsible use of antibiotics in aquaculture

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by

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# Preparation of this document

“Antibiotic resistance as a phenomenon is, in itself, not surprising. Nor is it new. It is however, newly worrying because it is accumulating and accelerating, while the world’s tools for combating it decrease in power and number.”

(IOM, 1998)

With this statement in mind, and considering that prompt action is needed to reduce the overall misuse of antibiotics in all areas – human medicine, veterinary medicine, animal production and plant protection – the Fish Utilization and Marketing Service, Fisheries Division, FAO, took the initiative to develop this review, with the aim of raising awareness of the antibiotic resistance problem in fish farming and related sectors, promoting prudent use of these drugs according to the FAO Code of Conduct for Responsible Fisheries.

This work focuses on antibiotics misuse and the concomitant threat of resistance development, considering this topic to be a public health concern that affects the population worldwide. Aspects such as the toxicity and allergic effects of antibiotic residues, the mechanism of transmission of antimicrobial resistance and environmental impact were also taken into account.

As the terms “antibiotic” and “antimicrobial” are often used indiscriminately, it should be noted that, for the purposes of this document, discussion is limited to just those antibiotics as defined in the glossary, although many aspects of the topic may be common to other antimicrobials used in animal husbandry or aquaculture.

## Important notice

Information regarding antibiotics in use, authorized or banned should be read in relation to the data and other information of the reference. Since the status of veterinary regulations varies very often in many countries, the interested reader should reconfirm/update the specific information. Information given in this review is mainly for didactic purposes and in support of responsible use of antibiotics in aquaculture.

# Abstract

Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants. They have long been present in the environment and have played a crucial role in the battle between man and microbe.

Many bacterial species multiply rapidly enough to double their numbers every 20-30 minutes, so their ability to adapt to changes in the environment and survive unfavourable conditions often results in the development of mutations that enable the species to survive changing external conditions.

Another factor contributing to their adaptability is that individual cells do not rely on their own genetic resources. Many, if not all, have access to a large pool of itinerant genes that move from one bacteria cell to another and spread through bacterial populations through a variety of mobile genetic elements, of which plasmids and transposable elements are two examples. The capacity of bacteria to adapt to changes in their environment and thus survive is called resistance.

Drug choices for the treatment of common infectious diseases are becoming increasingly limited and expensive and, in some cases, unavailable due to the emergence of drug resistance in bacteria and fungi – resistance that is threatening to reverse much medical progress of the past 50 years.

Dissemination of resistant micro-organisms may occur in both hospitals and communities. It is recognized that a major route of transmission of resistant micro-organisms from animals to humans is through the food chain.

In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. The contribution to antimicrobial resistance of antibiotics used in aquaculture is reviewed here, using a risk analysis framework. Some recommendations on responsible conduct in this context are proposed, aimed at diminishing the threat of build up of antimicrobial resistance.

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# Acronyms

ADI	Acceptable daily intake
AFA	Antibacterial feed additives
ALARA	As low as reasonable achievable
AOAC	Association of Official Analytical Chemists (to 1991; now International Association of Analytical Communities)
APUA	Alliance for the Prudent Use of Antibiotics
AST	Antimicrobial susceptibility testing
CAP	Chloramphenicol
CCFH	Codex Committee on Food Hygiene
CDC	Centres for Disease Control and Prevention (United States of America)
CE	Competitive exclusion
CLA	Conjugated linoleic acid
COMISA	Confédération mondiale de l'industrie de la santé animale (World Federation of the Animal Pharmaceutical Industry)
CRL	Community Reference Laboratory (European Union)
CVM	Center for Veterinary Medicine (FDA, United States of America)
CVMP	Committee for Veterinary Medicinal Products (EMA)
DT	Definitive phage type
EC	European Community
EMA	European Medicines Agency (formerly known as the European Agency for the Evaluation of Medicinal Products)
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (United States of America)
GC	Gas chromatography
GESAMP	IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
HHS	Human Health Service (United States of America)
HLGR	High-level gentamicin resistance
HLGRE	High-level gentamicin-resistant Enterococci
HPLC	High-performance liquid chromatography
HUS	Haemolytic uraemic syndrome
ICES	International Council for the Exploration of the Sea
ILSI	International Life Sciences Institute
IPB	Inhibitor-producing bacteria

IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOQ	Limit of quantification
MEC	Minimum effect concentrations
MIC	[Bacterial] minimum inhibitory concentration
MR	Multidrug-resistant
MRL	Maximum residue limit
NACA	Network of Aquaculture Centres in Asia–Pacific
NARMS	US National Antimicrobial Resistance Monitoring System
NCCLS	US National Committee for Clinical Laboratory Standards
NOAEL	No observed adverse effect level
NOEL	No observable effect level
OIE	Office international des épizooties (now the World Organisation for Animal Health)
PFGE	Pulsed field gel electrophoresis
QD	Quinupristin-dalfopristin
SANCO	Commission européenne, Direction générale santé et protection des consommateurs
SOU	Statens Offentliga Utredningar (Sweden)
SVARM	Swedish Veterinary Antimicrobial Resistance Monitoring
USDA	US Department of Agriculture
USP	United States Pharmacopoeia
VICH	Veterinary International Committee for Harmonization of Technical Requirements for the Registration of Pharmaceuticals
VRE	Vancomycin-resistant <i>Enterococci</i>
WHO	World Health Organization

# 1. Introduction

With the development and widespread application of antibiotics and vaccines, and through improvements in urban sanitation and water quality, death from infectious diseases has reduced dramatically. Progress was so great that, three decades ago, some experts predicted the end of infectious diseases.

However, this optimism was premature. There is a global resurgence of infectious diseases, with both newly identified infectious agents and a re-emergence of older infectious diseases associated with the rapid spread of antimicrobial resistance. Antibiotic resistance is a serious clinical and public health problem on a global basis. Drug choices for the treatment of common infectious diseases are becoming increasingly limited, expensive and, in some cases, useless, due to the emergence of drug resistance in bacteria and fungi, and this loss of treatment options is threatening to reverse much of the medical progress of the past 50 years (HHS, 1999a).

Following the discovery of the growth promoting and disease fighting capabilities of antibiotics, fish farmers and livestock producers began using such drugs in animal feeds. Antibiotics routinely used for treatment of human infections are also used for animals, for either therapy, prophylactic reasons or growth promotion. For the last-named purpose, subtherapeutic doses of antibiotics usually have been used, and this has contributed to promoting resistance.

The accumulated scientific evidence is that certain uses of antibiotics in food-producing animals can lead to antibiotic resistance in intestinal bacteria, and this resistance can then be transmitted to the general population, causing treatment-resistant illness. These uses of antibiotics can also create antibiotic resistance in non-pathogenic bacteria, the resistance genes of which can be transferred to disease-causing bacteria, resulting in antibiotic-resistant infections for humans.

The report from the invitational European Union conference on *The Microbial Threat* (EU, 1998) recognized that the major route of transmission of resistant microorganisms from animals to humans is through the food chain. This trend is confirmed by other authors (Nawaz *et al.*, 2001).

There have been significant increases in developed countries in the occurrence of resistance in non-typhoidal *Salmonella enterica* and in *Campylobacter* spp., and to a lesser extent in Vero cytotoxin-producing *Escherichia coli* 0157 (VTEC 0157). There is also an increase in the occurrence of resistance in non-typhoidal *S. enterica* in developing countries, but, in contrast to the situation observed in developed countries, these increases have been almost entirely associated with the use of antimicrobials in human medicine (Threlfall *et al.*, 2000).

According to a study by the European Federation of Animal Health (FEDESA), farm animals in 1999 consumed 4 700 tonne (35 percent) of all the antibiotics administered in the European Union, while humans consumed 8 500 tonne (65 percent). Of the antibiotics that were given to animals, 3 900 tonne (29 percent of total usage) were administered to help sick animals recover from disease, while 786 tonne (6 percent of total usage) were fed to farm animals as growth promoters. The survey estimated that the amount of antibiotics used as growth promoters had fallen by half since 1997, when animals consumed around 1 600 tonne as feed additives (EU, 2002b).

At the same time, the drug resistance problem has an economic cost. A study conducted in New York estimated that resistant infections due to *Staphylococcus aureus* cost an additional US\$ 2 500 per infected patient, or US\$ 7 million annually, in New York City alone. Another report estimated that the emergence of antimicrobial resistance

among six common bacteria in hospitals added approximately US\$ 661 million per year in hospital charges. This estimate did not include indirect costs or costs of infections caused by other resistant pathogens. If the spread of resistance continues, this cost will almost certainly increase. The foregoing points to great potential for economic savings by preventing antibiotic-resistant infections in human beings (HHS, 1999a).

The indiscriminate use of antibiotics for veterinary purposes has increasingly become a matter of public concern, and legal requirements are being reinforced. Regulatory authorities license antibiotics for use if the agents meet scientific criteria for quality, efficacy and safety. The authorities have to consider safety in relation to the treated animal, to the consumer and to the individuals handling the product during treatment.

In the fish farming (aquaculture, mariculture, etc.) sector, the widespread use of antibiotics for treating bacterial diseases has been associated with development of antibiotic resistance in *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *E. ictturali*, *Vibrio anguillarum*, *V. salmonicida*, *Pasteurella piscida* and *Yersinia ruckeri* (De Paola, Peeler and Rodrick, 1995), and controlled studies are needed to determine the effect of antimicrobial therapy on the ecology of aquaculture ponds, particularly at the micro-organism level.

The contribution to antimicrobial resistance of antibiotics used in the aquaculture industry is reviewed in this work, using a risk analysis framework. Some recommendations on responsible conduct in this context are proposed, aimed at diminishing the antimicrobial resistance threat.

## 2. Antibiotics

### 2.1 DEFINITION OF ANTIBIOTICS

Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants. Such chemical agents have been present in the environment for a long time, and have played a role in the battle between man and microbes.

In the last century, the discovery of new antibiotics revolutionized the treatment of infectious diseases, leading to a dramatic reduction in morbidity and mortality, and contributing significantly to improvements in the health of the general population.

### 2.2 MECHANISM OF ACTION OF ANTIBIOTICS

#### 2.2.1 Anti-infectious agents

Table 2.1 illustrates the typical mechanisms of action of antibiotic.

#### 2.2.2 Growth promotion

The feeding of antibiotics is associated with decreases in animal gut mass, increased intestinal absorption of nutrients and energy sparing. This results in a reduction in the nutrient cost for maintenance, so that a larger portion of consumed nutrients can be used for growth and production, thereby improving the efficiency of nutrient use.

Antibiotics act by eliminating the subclinical population of pathogenic micro-organisms. Eradicating this metabolic drain allows more efficient use of nutrients for food production. Antibiotics alter the non-pathogenic intestinal flora, producing beneficial effects on digestive processes and more efficient utilization of nutrients in feeds. It has been estimated that around 6 percent of the energy in a pig's diet could be lost due to microbial fermentation occurring in the stomach and small intestine.

TABLE 2.1  
Typical modes of action of common antibiotics.

Mechanism	Comments	Examples
Damage cell membrane, allowing contents to leak out. Bactericidal.	High toxicity to animals and humans; topical use only.	polymixins.
Inhibitors of bacterial cell wall synthesis.	Animals and humans not affected because their cells do not have walls.	penicillins; aminopenicillins; cephalosporins (cephalexin); bacitracin (topical); vancomycin.
Inhibitors of folic acid synthesis. Folic acid is needed to make RNA and DNA for growth and multiplication, and bacteria must synthesize folic acid. Bacteriostatic.	Animals and humans obtain folic acid from their diets, so they are not affected.	sulphonamides; sulfasalazine; trimethoprim; co-trimoxazole.
Inhibitors of DNA function. DNA is needed for cell growth and division. Most are bactericidal.	Drugs used affect bacterial (or fungal) cells more than animal or human cells.	nalidixic acid; ofloxacin; metronidazole; rifampin; enrofloxacin; sarafloxacin
Inhibitors of protein synthesis. Proteins are synthesized on cell structures called ribosomes. Bactericidal or bacteriostatic.	High doses can affect animals and humans because some ribosomes are similar to those in bacteria.	tetracyclines; aminoglycosides; chloramphenicol; florfenicol; macrolides; spectinomycin; lincosamides.

Source: Pharmacology, 1997.

Antibiotics may prevent irritation of the intestinal lining and may enhance the uptake of nutrients from the intestine by thinning of the mucosal layer.

Intestinal bacteria inactivate pancreatic enzymes and metabolize dietary protein with the production of ammonia and biogenic amines. Antibiotics inhibit these activities and increase the digestibility of dietary protein.

Experimental results obtained with some antibiotics commonly used as growth promoters (chlortetracycline, penicillin and sulfamethazine) have shown that treated pigs have higher serum levels of an insulin-like growth factor. In this way, the effect may extend beyond digestion in the intestine and stimulate metabolic processes (Committee on Drug Use in Food Animals, 1999; Doyle, 2001).

## 2.3 CLASSIFICATION OF ANTIBIOTICS FOR VETERINARY USE

The classification is based on that of the United States' Pharmacopoeia (USP, 1999, 2000a–m).

### 2.3.1 Beta-lactams

#### Cephalosporins

Antibiotic	Spectrum	Some common brand names for veterinary use
First-generation cephalosporins		
Cefadroxil Cefazolin Cephalexin Cephalothin Cephapirin Cephradine	These cephalosporins have the highest activity against Gram-positive bacteria, including most <i>Corynebacteria</i> , <i>Streptococci</i> and <i>Staphylococci</i> , particularly <i>Staphylococcus aureus</i> .  Activity against Gram-negative bacteria: <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Haemophilus influenzae</i> , <i>Pasteurella</i> and <i>Salmonella</i> . Many anaerobic bacteria are susceptible to these antibacterials, with the exception of beta-lactamase-producing <i>Bacteroides</i> and <i>Clostridium difficile</i> .	Cefadroxil: Cefa-Drops Cefa-Tabs
Second-generation cephalosporins		
Cefaclor Cefamandole Cefmetazole Cefonicid Cefotetan Cefoxitin Cefprozil Cefuroxime	Have slightly less efficacy than first-generation cephalosporins against Gram-positive pathogens. Are more efficient than first-generation drugs in the treatment of infections caused by Gram-negative organisms.	Veterinary-labelled products are not commercially available
Third-generation cephalosporins		
Cefixime Cefoperazone <sup>(1)</sup> Cefotaxime Cefpodoxime, Ceftazidime <sup>(1)</sup> Ceftizoxime Ceftriaxone	The most effective of the cephalosporins against antibiotic-resistant Gram-negative bacteria.  Less effective than other cephalosporins against Gram-positive bacteria.	Veterinary-labelled products are not commercially available
New-generation cephalosporins		
Ceftiofur	Broader Gram-positive activity, including good activity against <i>Streptococci</i> but less activity against <i>Pseudomonas</i> than third-generation cephalosporins. Active against beta-lactamase-producing strains, as well as against anaerobes.	Ceftiofur: Excenel Excenel RTU Naxcel

Note: (1) Ceftazidime and Cefoperazone are active against *Pseudomonas*, but most of the third-generation cephalosporins commonly used in veterinary practice are not.

Source: USP, 1999.

(Cont.)



### Penicillin G

Antibiotic	Spectrum	Some common brand names for veterinary use
Penicillin G benzathine, G potassium, G procaine, G sodium	Active against aerobic and anaerobic Gram-positives. Susceptible aerobes include most beta-haemolytic <i>Streptococci</i> and beta-lactamase-negative <i>Staphylococci</i> . Most species of anaerobes are susceptible, including <i>Clostridium</i> species, but excluding beta-lactamase-producing <i>Bacteroides</i> species. Ineffective against beta-lactamase producers and those bacteria that are resistant to other mechanisms, such as those having a relatively impermeable cell wall. Has therefore little activity against many <i>Staphylococci</i> and most Gram-negative bacteria.	Agri-cillin; Ambi-pen; Aquacillin; Benza-pen; Benzapro; Combicillin; Crystiben; Crysticillin; Depocillin; Derapen; Dual-cillin; Duo-pen; Duplocillin; Durapen; Ethacilin; Hipencin 300; Long Acting Penicillin; Longisil; Microcillin; Pen-aqueous; Pen PB-48; Pendure Neat; Pen-G; Penject; Penject+B; Penlong XL; Penmed; Penpro; Pfi-pen G; Pot-Pen; R-Pen; Twin-pen.

Source: USP, 2000a.

### Aminopenicillins

Antibiotic	Spectrum	Some common brand names for veterinary use
Amoxicillin	Active against penicillin-sensitive Gram-positive bacteria and some Gram-negative bacteria.  Gram-positive spectrum includes alpha- and beta-haemolytic <b>Streptococci</b> , some <i>Staphylococci</i> species and <i>Clostridia</i> species.  Gram-negatives: <i>Escherichia coli</i> , many strains of <i>Salmonella</i> , and <i>Pasteurella multocida</i> . Is susceptible to destruction by beta-lactamases.)	Amoxicillin; Amoxi-Bol; Amoxi-Drop; Amoxi-Inject; Amoxil; Amoxi-Tabs; Biomox; Moxilean; Robamox-V.
Ampicillin	Active against alpha- and beta-haemolytic streptococci, including <i>Streptococcus equi</i> , non-penicillinase-producing <i>Staphylococcus</i> species, and most strains of <i>Clostridia</i> . Also effective against Gram-negative bacteria, such as <i>Escherichia coli</i> , <i>Salmonella</i> and <i>Pasteurella multocida</i>	Polyflex; Amp-equine.

Source: USP, 2000b.

### 2.3.2 Macrolides

Antibiotic	Spectrum	Some common brand names for veterinary use
Erythromycin: Base Estolate Ethylsuccinate Glucetate Lactobionate Phosphate Stearate Thiocyanate	Primarily against Gram-positives, such as <i>Staphylococcus</i> and <i>Streptococcus</i> species, including many that are resistant to penicillins by means of beta-lactamase production. Active against <i>Campylobacter</i> and <i>Pasteurella</i> species. Has activity against some anaerobes, but <i>Bacteroides fragilis</i> is usually resistant. Most <i>Pseudomonas</i> , <i>Escherichia coli</i> , and <i>Klebsiella</i> strains are resistant to erythromycin. Resistant strains of <i>Staphylococci</i> and <i>Streptococci</i> have been reported. Cross-resistance to other macrolides can also occur.	Base: Erythro-100; Erythro-200; Gallimycin-100; Gallimycin-200.  Phosphate: Gallimycin PFC Ery-Mycin.  Thiocyanate: Ery-Mycin Gallimycin Gallimycin 50.
Tilmicosin Phosphate	<i>In vitro</i> activity against Gram-positive micro-organisms and <i>Mycoplasma</i> . Active against certain Gram-negatives, such as <i>Haemophilus somnus</i> , <i>Pasteurella haemolytica</i> and <i>P. multocida</i> .  Gram-negatives such as <i>Enterobacter aerogenes</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> and <i>Serratia</i> species are very resistant to Tilmicosin	Micotil Pulmotil
Tylosin Base Tylosin Phosphate Tylosin Tartrate	Spectrum of activity similar to that of erythromycin but more active than erythromycin against certain <i>Mycoplasmas</i>	Base: Tylan 50; Tylan 200; Tylocine; Tylocine 200.  Phosphate: Tylosin 10; Tylosin 40; Tylan 10; Tylan 40; Tylan 100

Source: US Pharmacopoeia, 2000c

### 2.3.3 Spectinomycin (Aminocyclitol)

Antibiotic	Spectrum	Some common brand names for veterinary use
Spectinomycin	Active against: a variety of aerobic Gram-negative and Gram-positive micro-organisms. Anaerobics are generally resistant. Its use is limited by the ready development of bacterial resistance.	Adspec; Prospec; Spectam; Spectam Scourt-Halt; Spectam Water-Soluble Concentrate

Source: US Pharmacopoeia, 2000d.

### 2.3.4 Chloramphenicol

FDA regulations ban chloramphenicol from use in animals intended for food production. The Canadian Health Protection Branch, the European Union and Japan apply the same measure. However, chloramphenicol was used in shrimp culture in Latin America and Asia, where shrimp are grown primarily for export to European, Japanese and North American markets (GESAMP, 1997).

Antibiotic	Spectrum	Some common brand names for veterinary use
Chloramphenicol	Broad spectrum. Effective against: <i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Escherichia coli</i> , <i>Proteus vulgaris</i> , <i>Aerobacter aerogenes</i> , <i>Salmonella</i> species, <i>Pseudomonas</i> species, anaerobic bacteria.	Azramycine S125; Azramycine S250; Chloromycetin; Chlor Palm 125; Chlor Palm 250; Duricol; Karomycin Palmitate 125; Karomycin Palmitate 250; Novochlorocap.

Source: US Pharmacopoeia, 2000e.

### 2.3.5 Florfenicol (Fluorinated derivative of thiamphenicol)

Antibiotic	Spectrum	Some common brand names for veterinary use
Florfenicol	Broad spectrum, primarily bacteriostatic. Activity similar to chloramphenicol, including many Gram-positives and Gram-negatives and without the risk of inducing human aplastic anaemia associated with chloramphenicol	Aqua-flor; Nuflor

Source: US Pharmacopoeia, 2000f.

### 2.3.6 Tetracyclines

Antibiotic	Spectrum	Some common brand names for veterinary use
Tetracyclines: Chlortetracycline Doxycycline Oxytetracycline Tetracycline	Broad spectrum with activity against Gram-positives and Gram-negatives, including some anaerobics.  Active also against <i>Chlamydia</i> , <i>Mycoplasma</i> , Protozoa and several Rickettsiae, including <i>Ehrlichia</i> and <i>Haemobartonella</i> . Active against <i>Escherichia coli</i> , <i>Klebsiella</i> species, <i>Salmonella</i> species, <i>Staphylococcus</i> species and <i>Streptococcus</i> species.  However, resistance has been acquired by some coliforms, mycoplasmas, streptococci and staphylococci	Oxytetracyclines (in USA): OXTC 10; Terramycin 10; OXTC 30; OXTC 50; OXTC 100; OXTC 100 MR; Terramycin TM-50  Oxytetracyclines (in Canada): Oxy-110; Oxysol-110; Oxytetracycline 50; Terramycin-50; Oxi-220; Oxysol-220; Oxytetracycline 200; Terramycin-200; Terramycin-Aqua

Source: US Pharmacopoeia, 2000g.

### 2.3.7 Quinolones

Antibiotic	Spectrum	Some common brand names for veterinary use
Enrofloxacin <sup>(1)(2)</sup>	Broad spectrum. Bactericidal at relatively low concentrations, highly bio-available following either oral or parenteral administration in most species, and achieves good penetration of body tissues and fluids	Baytril; Baytril 100.
Sarafloxacin <sup>(3)</sup>	<i>Escherichia coli</i> infections	Saraflox Injection; Saraflox WSP.
Oxolinic acid	Against Gram-negative bacteria	
Flumequine	Against Gram-negative bacteria	

Notes: (1) Biotransformation: enrofloxacin is de-ethylated to form ciprofloxacin, an antimicrobically-active metabolite in many species.

(2) Not labelled for use in food-producing animals (United States of America and Canada).

(3) Not labelled for use in laying hens producing eggs for human consumption.

Sources: USP, 2000h, 2000m; GESAMP, 1997.

### 2.3.8 Sulphonamides

Antibiotic	Spectrum	Some common brand names for veterinary use
Sulphonamides: Sulfachlorpyridazine Sulfadimethoxine Sulfamethazine Sulfanilamide Sulfaquinoxaline Sulfathiazole	Antibacterial; antiprotozoal; broad spectrum; inhibiting Gram-positives and Gram-negatives and some Protozoa, such as <i>Coccidia</i> .  Ineffective against most obligate anaerobes and should not be used to treat serious anaerobic infections.  Resistance of animal pathogens to sulphonamides is widespread as a result of more than 50 years of therapeutic use. Nevertheless, still used in combination with other medications.	Sulfachlorpyridazine: Vetisulid; Vetisulid Boluses; Vetisulid Powder.  Sulfadimethoxine: Albon; Albon Injection; Albon Soluble Powder; Albon Tablets; Di-Methox, S-125, S-250.  Sulfamethazine: Hava-Span; Calfspan; Sulfa 25; Sulfa-Max III; Sulmet.  Sulfaquinoxaline: Sulquin 6-50 34 percent; Sul-Q-Nox.

Source: USP, 2000i.

### 2.3.9 Lincosamides

Antibiotic	Spectrum	Some common brand names for veterinary use
Lincosamides: Clindamycin Lincomycin Pirlimycin	Activity against many Gram-positive bacteria and many anaerobic bacteria, but not effective against most Gram-negatives.  Effective against <i>Staphylococcus</i> species, <i>Streptococcus</i> species (except <i>Streptococcus faecalis</i> ).	Clindamycin: Antirobe; Antirobe Aquadrops; Clindacure; Clindadrops; Clindrops.  Lincomycin: Lincocin; Lincocin Aquadrops; Lincomix; Lincomix 20; Lincomix 50; Lincomycin soluble; Lincosol Soluble Powder; Moorman's LN 10.  Pirlimycin: Pirsue Aqueous Gel.

Source: USP, 2000j.

### 2.3.10 Rifampin

Chilling	Freezing	Some common brand names for veterinary use
Rifampin <sup>(1)</sup>	Gram-negative aerobic bacteria as well as facultative anaerobic micro-organisms. Especially active in the treatment of staphylococcal infections and in the eradication of pathogens located in difficult-to-reach target areas, such as inside phagocytic cells.  Resistance to rifampin can develop quickly, so it is most often used in combination with other antimicrobials.	

Note: (1) Rifampin is not labelled in the United States of America or in Canada for use in animals, including food-producing animals.

Source: USP, 2000k.

### 2.3.11 Aminoglycosides

Antibiotic	Spectrum	Some common brand names for veterinary use
Amikacin Dihydrostreptomycin Gentamicin Kanamycin Neomycin Streptomycin Apramycin	Used for the treatment of infections caused by aerobic Gram-negatives. Effective against some Gram-positives like <i>Staphylococcus aureus</i> .  The use of aminoglycosides in the treatment of infection in animals has been tempered by toxicity considerations in the animal treated. Often, systemic use is limited to the treatment of serious Gram-negative infections resistant to less toxic medications.  Acidic or purulent conditions can hamper their effect and the presence of cations (calcium or magnesium ions) can decrease antibacterial effects	Amikacin: Amiglyde-V.  Dihydrostreptomycin: Ethamycin.  Gentamicin: Gentocin; Garacin.  Kanamycin: Kantrim.  Neomycin: Neomix.  Streptomycin: Strep-Sol.  Apramycin: Apralan; Apralan 75.

SOURCE: USP, 2000l.

## 2.4 ANTIBIOTICS BANNED FOR ANIMALS INTENDED FOR FOOD PRODUCTION

Chloramphenicol is a potent, broad-spectrum antibiotic used only at therapeutic doses for treatment of serious infections in humans. Due to the unpredictable effects of doses on different patient populations, it has not been possible to identify a safe level of human exposure to chloramphenicol. Therefore, United States of America Federal regulations prohibit its use in food-producing animals and animal-feed products (FDA, 2002b).

According to the European Medicines Agency (EMA, 2000b), a number of antibiotics are no longer available for use in veterinarian medicine (Table 2.3).

TABLE 2.2  
Antibiotics banned for animals intended for food production.

Antibiotic	Country	Reason	Reference
Spectinomycin	USA	Its use is limited by the ready development of bacterial resistance	USP, 2000d.
Enrofloxacin	USA	Its use is limited by the ready development of bacterial resistance (quinolone)	USP, 2000h.
Cloramphenicol	Argentina, Canada, EU, Japan, USA	Induces human aplastic anaemia	USP, 2000e; GESAMP, 1997; SANCO, 2001a.
Rifampin	Not labelled in USA or Canada for use in animals, including food-producing animals	Tumorigenicity and teratogenic effects on experimental animals	USP, 2000k.

TABLE 2.3  
Antibiotics no longer available for veterinary purposes

Antibiotic	Indication	Species	Examples of alternatives
Cefuroxime	Treatment of clinical mastitis in lactating cattle and treatment of subclinical infections and reduction of the risk of new infections in dairy cattle	Cattle	There are several drugs available against mastitis
Chloramphenicol	Treatment of bacterial infections (against broad spectrum of bacteria)	Cattle (calves), pigs, poultry	Thiamphenicol; Florfenicol; Amoxicillin
Polymixin B Sulfate	Treatment of clinical mastitis due to Gram-negative bacteria	Cattle	There are several drugs available against mastitis and infection by Gram-negative bacteria
Nystatin	Treatment of candidiasis	Poultry	Natamycin

## 2.5 ANTIBIOTICS AUTHORIZED FOR USE IN AQUACULTURE

TABLE 2.4  
Antibiotics authorized for use in aquaculture

Antibiotic	Treatment of	Reference
Oxytetracycline <sup>(1)</sup> (for medicated feed)	Furunculosis in salmonids (salmon or trout) caused by <i>Aeromonas salmonicida</i> Gafkemia in lobsters (caused by <i>Aerococcus viridans</i> ). Hemorrhagic septicaemia due to <i>Aeromonas hydrophila</i> , <i>A. sobria</i> and <i>Pseudomonas</i> . Cold water disease in salmonids, caused by <i>Cytophaga psychrophilia</i> . Columnaris disease in salmonids, caused by susceptible <i>Chondrococcus (Flexibacter) columnaris</i> . Enteric redmouth disease, caused by susceptible <i>Yersinia ruckeri</i> . Indicated for the control of <i>Pseudomonas</i> disease in catfish and salmonids. Indicated for the control of ulcer disease caused by susceptible <i>Haemophilus piscium</i> in salmonids (salmon, trout).	USP, 2000g
Florfenicol <sup>(2)</sup> Premix	Indicated in the treatment of furunculosis caused by susceptible strains of <i>Aeromonas salmonicida</i> .	USP, 2000f
Sarafloxacin	Indicated in the treatment of furunculosis, vibriosis and enteric redmouth in Salmonidae.	EMEA, 1997
Erythromycin	In the treatment of bacterial kidney disease ( <i>Renibacterium salmoninarum</i> ) and streptococcosis in yellowtail in Japan.	GESAMP, 1997
Sulphonamides potentiated with trimethoprim or ormethoprim	Against furunculosis, enteric redmouth disease and vibriosis.	GESAMP, 1997

Notes: (1) Incompatibilities: salmonid and lobster feeds having a high ash content (calcium, copper, iron or zinc) may bind oxytetracycline and prevent absorption. Oxytetracycline should not be administered with feeds containing bentonite. *Additional information:* For fish, this medication should not be used when the water temperature is below 16.7°C (62°F) for catfish, or below 9°C (48.2°F) for salmonids (USP, 2000g).  
(2) Is rapidly metabolized at water temperatures of 8.5° to 11.5°C and the major metabolite is florfenicol amine.  
*Caution:* Handlers must avoid inhalation of dust and contact with skin and eyes. Protective clothing should be worn when handling the medication and hands should be washed after administration (USP, 2000f).



## 3. Risk assessment

### 3.1 HAZARD IDENTIFICATION

#### 3.1.1 Antimicrobial resistance

Many bacterial species multiply rapidly enough to double their numbers every 20–30 minutes, and their ability to adapt to changes in the environment and survive unfavourable conditions often results in the development of mutations that protect them.

In addition, a factor contributing to their adaptability is that individual cells do not rely on their own genetic resources alone. Many, if not all, have access to a large pool of itinerant genes that move from one bacterial cell to another and can spread through bacterial populations on a variety of mobile genetic elements, of which plasmids and transposable elements are two examples.

Bacterial capacity to adapt to external changes using these mechanisms is called resistance development in the face of selection pressures, and the development of resistance allows the resistant organisms to proliferate in the prevailing conditions.

Resistance takes two forms.

- (i) Inherent or intrinsic resistance, i.e. the species is not normally susceptible to a particular drug. This may be due to the inability of the antibacterial agent to enter the bacteria cell and reach its target site, or lack of affinity between the antibacterial and its target (site of action), or absence of the target in the cell.
- (ii) Acquired resistance, where the species is normally susceptible to a particular drug but certain strains express drug resistance, which may be mediated through a number of mechanisms that will be discussed later in this document.

When resistance develops, the antibiotic is no longer capable of curing or treating the disease caused by the infective agent. A low level of resistance may be detected by a slight increase in the minimal inhibitory concentration (MIC) for the antibiotic from the usual value, which is not necessarily of clinical significance.

A higher degree of resistance is characterized by an MIC that exceeds, sometimes by several orders of magnitude, the concentrations of drug safely attainable in the patient's tissues.

#### 3.1.2 Epidemiology of antibiotic resistance

Emerging antimicrobial resistance, due to use of antimicrobials, is a public health concern in human and animal medicine worldwide.

According to the Centres for Disease Control and Prevention (CDC) (HHS, 1999a), resistant strains of three micro-organisms causing human illness – *Salmonella* sp., *Campylobacter* sp. and *Escherichia coli* – are linked to the use of antibiotics in animals. Young children, the elderly and immunocompromised are the population at risk. These bacteria infect humans through ingested contaminated foods, especially foods of animal origin. Animals serve as reservoirs for many food-borne pathogens, including *Salmonella* and *Campylobacter*. Antibiotic-resistant organisms may be present in or on animals as a result of drug use and these resistant food-borne pathogens can contaminate a carcass during slaughter or processing. When these resistant bacteria cause illness in a person, requiring medical treatment, medical therapy may be compromised if the pathogenic bacteria are resistant to the drug(s) available for treatment.

In England, in studies of 5 400 strains of *Campylobacter jejuni* and 376 of *Campylobacter coli* reported by Frost and Thwaites (1998) and by Threlfall *et al.* (2000), 11 percent were resistant to ciprofloxacin at concentration exceeding 8 mg/litre, with resistance being most pronounced in *C. coli*.

It must be assumed that a proportion of ciprofloxacin-resistant isolates originated in food producing animals. In the United States of America, it has been demonstrated that a considerable increase occurred in incidence of *Campylobacter*-resistant isolates in poultry, associated with the licensing in the United States of America of fluoroquinolone antibiotics for use in chickens. In the Netherlands, a direct association between the licensing of fluoroquinolones for water medication for poultry and resistance developing in animal isolates was demonstrated, while at the same time resistance in human isolates increased. A similar situation has been reported for Spain (Wegener *et al.*, 1999).

*Campylobacter*, the most common bacterial cause of food-borne illness, infects an estimated 2.4 million people annually in the United States of America. Fluoroquinolones (e.g. ciprofloxacin) are commonly used in adults to reduce the severity and duration of the symptoms.

Fluoroquinolones have also been used since late 1995 in chickens. Since chickens are the most common source of *Campylobacter jejuni* infections, a study done in cooperation with the Food Net Working Group (United States of America) tested for fluoroquinolone-resistant *Campylobacter* strains, isolated from ill persons and from chickens purchased from grocery stores. A high prevalence of fluoroquinolone resistance was detected among the *Campylobacter jejuni* isolates. Chickens represent a significant reservoir for fluoroquinolone-resistant *Campylobacter jejuni*, to which humans are routinely exposed.

The continued use of fluoroquinolones in chickens threatens the efficacy of fluoroquinolones for treatment of *Campylobacter* infections in humans, and so mitigating action is needed to preserve the efficacy of fluoroquinolones (Rossiter *et al.*, 2000).

The FDA Center for Veterinary Medicine (CVM) developed a risk assessment model that evaluates the risk of fluoroquinolone-resistant *Campylobacter* infections from consumption of chicken and attributable to use of fluoroquinolones in chickens. This risk assessment indicates that approximately 5 000 people who are ill with fluoroquinolone-resistant infections could be treated with a fluoroquinolone and that treatment would be potentially compromised due to resistance. Surveillance data can be used to update the model annually and will indicate changes in level of resistance and incidence of campylobacteriosis. These changes may reflect alterations in food animal production and processing or may indicate changes in bacterial virulence or a change in the susceptibility of the human population. Forecasting potential changes in level of resistance in chickens could provide a means to mitigate the human health impact (Hollinger *et al.*, 2000).

Each year, *Salmonella* bacteria infect an estimated 1.4 million persons in the United States of America; these infections result in several hundred deaths annually. One of the most common strains isolated from humans is multidrug-resistant (MR) *Salmonella enterica* serotype Typhimurium definitive type 104 (DT 104). This strain was first isolated from humans in 1984 in the United Kingdom, where it emerged as a major cause of human illness in the late 1980s, before its emergence in the United States of America and elsewhere in the mid-1990s.

Most of the infections are caused by *Salmonella* Typhimurium DT 104, which is usually resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline, and has acquired resistance against trimethoprim and fluoroquinolones, most probably because affected groups of animals could only be treated with these antibiotics (van den Bogaard and Stobberingh, 2000). This strain was first isolated in the



UK from exotic birds, and, with the exception of a human outbreak in Scotland in the mid-1980s, it was not isolated from human beings until 1989. During the next five years, the strain became epidemic in bovine animals, and common in poultry (particularly turkeys), pigs and sheep. It is often discussed whether resistant *Salmonella* develops primarily as a result of antibiotic use in agriculture or in human medicine. Although both uses always play a part, it is more probable that antibiotic resistance in *Salmonella* causing infection mainly reflects resistance developed in the animal reservoirs. This is supported by the facts that humans are not often carriers of *Salmonella* compared with food animals, that antibiotics are usually given to animals for long periods and often in subtherapeutic doses, and, finally, that resistance to antibiotics used for food animals (tetracycline, apramycin), but not for treatment of *Salmonella* in humans, has been observed in *Salmonella* (Wegener *et al.*, 1999).

Human infection has been associated with the consumption of chicken, beef, pork sausages and meat paste, and to lesser extent with direct contact with farm animals. In the 1990s, the infection was recognized in cattle and humans in the United States of America, and during recent years this MR strain has been responsible for infections in European countries, Israel and Canada (Threlfall *et al.*, 2000). It is important to note that all DT 104 isolates contained the same cassette gene, which codifies for resistance irrespective of source (food animal or human), or country of origin.

Since 1992, the DT 104 strain has acquired resistance against trimethoprim and ciprofloxacin, and, as consequence, since 1997, 15 percent of the isolates have been resistant to trimethoprim, and 13 percent have shown decreased sensitivity to ciprofloxacin. The appearance of resistance to trimethoprim has been attributed to the use of this drug to combat infections caused by DT 104.

The emergence of isolates of MR DT 104 with reduced sensitivity to ciprofloxacin has followed the licensing in the United Kingdom of a related fluoroquinolone drug, enrofloxacin, for veterinary use. This drug has been used with prophylactic and therapeutic purposes in poultry and cattle in the UK and as consequence, resistance against nalidixic acid has rapidly emerged in food producing animals in the United Kingdom, particularly turkeys, chickens and cattle.

In an outbreak of DT 104 in Denmark, attributed to the consumption of pork, lack of response to fluoroquinolone has been described (Threlfall *et al.*, 2000). The Danish researchers were unable to discover how the DT 104 strain entered the food chain. The pigs suspected of carrying this resistant strain had not been fed any fluoroquinolones, but the compounds may previously have been used at the farms. Wild animals or equipment may have spread the bacteria environmentally, and concomitantly with globalization of trade such outbreaks could become more common (Swint, 1999).

More recently, in the light of these findings, a series of proposals to ban the use of quinolones in food animals have been proposed (Sundlof, 2000; Tollefson, 2000; Environmental Defense, 2000).

CVM is withdrawing its approval of two fluoroquinolones for use in poultry (sarafloxacyne hydrochloride (= sarafloxacin) and enrofloxacyne (= enrofloxacin) (APUA, 2000). The two fluoroquinolones, enrofloxacin and sarafloxacin, had been approved in 1995 for use in poultry in the United States of America (CDC, 1996a) and have been used widely since the mid-1990s in chickens and turkeys to reduce mortality due to *Escherichia coli* and *Pasteurella multocida*.

A nationwide United States of America study, for which all state and territorial public health laboratories were asked to forward every tenth *Salmonella* isolate to CDC for antimicrobial susceptibility testing, showed that 275 (28 percent) of 975 *Salmonella* serotype Typhimurium isolates from humans in the United States of America in 1995 were resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline, with R-type ACSSuT resistance, the pattern commonly associated with MR DT 104 isolates (MR-DT 104).

In contrast, only 8 (7 percent) of 108 *S. Typhimurium* isolates from humans in sentinel counties in 1990 and 7 (5 percent) of 135 in 1985 were R-type ACSSuT. An isolate collected in 1985 probably represents the earliest isolate of MR-DT 104 in the United States of America. After emerging in the mid-1990s, MR-DT 104 has remained prevalent in the United States of America. In 1999, 114 (31 percent) of 362 human *S. Typhimurium* isolates received by the National Antimicrobial Resistance Monitoring System (NARMS) for Enteric Bacteria were R-type ACSSuT; most of these isolates had been collected from cattle and pigs.

The genetic determinants responsible for the R-type ACSSuT in MR-DT 104 are located in the chromosome. Molecular characterization of *S. Typhimurium* DT 104 R-type ACSSuT isolates from Europe and United States of America showed that resistance to ampicillin, streptomycin and sulphonamides is associated with the presence of two class-1 integrons first described by Stokes and Hall (1989). Integrons are a group of apparently mobile elements that can contain one or more antimicrobial resistance genes. Integrons represent an important and efficient mechanism by which many bacteria, including *S. Typhimurium* DT 104, can acquire resistance to antimicrobial agents. These genetic elements have been found in a wide variety of organisms and are thought to be largely responsible for the dramatic increase in MR bacteria. Integrons carrying antimicrobial resistance genes have been found in plasmids and transposons (and transposon-like elements) and in the chromosomal DNA of some bacteria. The fact that integrons are widespread among Gram-negative bacteria suggests that these genetic elements have evolved into a highly adaptable and very efficient mechanism by which cells can acquire and express antimicrobial resistance genes (Ribot *et al.*, 2002).

A study was proposed to compare the integron structure and gene-cassette content, plasmid profiles and pulsed-field gel electrophoresis (PFGE) patterns of recent *S. Typhimurium* DT 104 (R-type ACSSuT) isolates (1995) with the earliest identified isolate of MR-DT 104, collected in 1985, as well as isolates from 1990. The resulting data would suggest whether the emergence of MR-DT 104 in the mid-1990s in humans and animals resulted from the dissemination of a strain already present in the United States of America or from the introduction of a new strain (Ribot *et al.*, 2002).

The data suggest that the strain of MR-DT 104 that became prevalent during the mid-1990s had not caused frequent human illness in the United States of America in 1985 and 1990. Local health officials interviewed the four patients from whom these MR-DT 104 isolates were collected in 1985 and 1990; none of them reported travelling outside the United States of America for 30 days prior to onset of illness. None reported other underlying illnesses, and none reported taking any antibiotics before specimens were collected.

These data suggest that domestic transmission of MR-DT 104 to humans, perhaps through contaminated food, occurred in 1985 and 1990. Human infection with MR-DT 104 did not become prevalent, however, until the mid-1990s. The factors that led to the widespread dissemination of MR-DT 104 in humans in the United States of America in the mid-1990s are unknown. However, the limited animal data available indicate that MR-DT 104 became disseminated in food animals at approximately the same time. Factors that contributed to the dissemination of MR-DT 104 in animals are poorly understood. Since food animals are the reservoir for most domestically acquired human *Salmonella* infections, and transmission from animals to humans occurs through the food chain, the rapid dissemination of MR-DT 104 among humans in the mid-1990s was probably the consequence of dissemination of MR-DT 104 in food animals during the same period. If this hypothesis is correct, it would parallel the experience of the United Kingdom, where MR-DT 104 was identified as early as 1984, but did not become epidemic in humans until it was established in cattle in the late 1980s (Ribot *et al.*, 2002).

In 1998, 163 (29 percent) of 557 *S. Typhimurium* isolates from animals received in NARMS were R-type ACSSuT; most of these isolates had been collected from cattle and pigs (NARMS, 1998). Limited data are available, however, on the prevalence of MR-DT 104 in food-producing animals before 1996. A recent report indicates that MR-DT 104 became prevalent in cattle in the Pacific Northwest of the United States of America in the mid-1990s (Besser *et al.*, 2000).

Molecular evidence supports the suggestion that some of the antimicrobial resistance determinants found in *S. Typhimurium* DT 104 R-type ACSSuT may have emerged, perhaps in Asia, in the early 1980s in other bacteria and were transferred horizontally to DT 104 (Angulo *et al.*, 2000a). For example, chloramphenicol resistance in MR-DT 104 is encoded by a *flo*-like gene that confers resistance to both chloramphenicol and florfenicol (Bolton, *et al.*, 1999; Kim and Aoki, 1996). *flo* was first identified in *Pasteurella piscicida*, the causative agent of pseudotuberculosis, a common disease of marine fish in Asia (Kim and Aoki, 1993). Florfenicol was evaluated as a therapeutic agent in fish in Asia in the early 1980s (Kim and Aoki, 1996; Yasunaga and Tsukahara, 1988). Kim, Yoshida and Aoki (1993) reported the emergence of florfenicol-resistant strains of *P. piscicida* due to the acquisition of transferable resistance plasmid containing the *flo* gene, in addition to other antimicrobial resistance markers (ampicillin, kanamycin, sulphonamide and tetracycline). Furthermore, nucleotide sequence analysis of the DNA region containing the florfenicol resistance gene and the two tetracycline genetic determinants showed a 94 percent similarity to a sequence found in a plasmid from *P. piscicida* (Kim and Aoki, 1996).

Additional evidence supporting the idea of horizontal transfer of multiple antimicrobial resistance determinants comes from a recent study conducted on MR *Salmonella enterica* serotype Agona isolates containing a DT 104-like antimicrobial resistance gene cluster in their genome (Cloeckaert *et al.*, 2000). These data and the fact that food-producing animals are the reservoirs for most human *Salmonella* infections in the United States of America suggest that the emergence of MR-DT 104 during the mid-1990s probably resulted from the dissemination of MR-DT 104 in food-producing animal reservoirs (Ribot *et al.*, 2002).

MR *Salmonella enterica* serovar Typhimurium DT 104 is now acknowledged as an internationally distributed zoonotic pathogen. Since 1991, this phage type has been second only to *S. Enteritidis* phage type 4 as the principal agent of human salmonellosis in England and Wales. MR DT 104 is characterized by resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (R-type ACSSuT) (Lawson *et al.*, 2002). In MR DT 104, these antibiotic resistance genes have been accumulated in chromosomally encoded gene cassettes, a process mediated by the presence of class 1 integrons. Some isolates possess additional plasmid-mediated resistance to trimethoprim and low-level resistance to ciprofloxacin because of point mutations in the *gyrA* gene. The potential exists for the horizontal transfer of genetic elements such as antibiotic resistance gene cassettes between *Salmonella* serotypes and phage types. Evidence of this transfer was reported in 2000, when the presence of an MR DT 104-like antibiotic resistance gene cluster was reported in *S. Agona* (Lawson *et al.*, 2002).

Throughout the 1990s, the ACSSuT R-type was also identified in isolates of *S. Typhimurium* DT 12 and DT 120, although as yet numbers remain relatively small (27 of 84 and 22 of 109 of DT 12 and DT 120 isolates, respectively, were of R-type ACSSuT, out of a total of 2 651 *S. Typhimurium* isolates received at the Central Public Health Laboratory, London, in 2000).

ACSSuT-resistant MR DT 12 incidence has remained fairly constant since the mid-1990s, but as isolations of sensitive DT 12 have diminished, the relative proportion of MR DT 12 has increased (Lawson *et al.*, 2002).

In Japan, *Salmonella* serotype Typhimurium DT 12 isolated from a 35-day-old infant with diarrhoea was highly resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, gentamicin, sulfamethoxazole/trimethoprim, nalidixic acid and fluoroquinolones (Nakaya *et al.*, 2003).

MR *Salmonella enterica* serotype Typhimurium DT 12 and DT 120 are more closely related to DT 104 than to non-MR strains of their respective phage types. MR DT 12 and DT 120 appear to have arisen due to changes in phage susceptibility of DT 104 rather than horizontal transfer of resistance genes (Lawson *et al.*, 2002).

Subsequent to the introduction in 1988 of fluoroquinolones for food animal use in Germany, the emergence of fluoroquinolone-resistant variants of the MR *Salmonella* Typhimurium clone DT 204c was observed (WHO, 1998). The resistance observed reached a prevalence of 50 percent in isolates from calves in a defined area of the country. Subsequently the prevalence of these resistant strains has diminished, but data associating this change in prevalence with changes in fluoroquinolone usage in animals are unavailable.

There is uncertainty about the relative contribution to the emergence and dissemination of quinolone-resistant *Salmonella* of direct selective pressure versus the spread of resistant strains in the presence or absence of quinolone use. Variation is reported in the rate of emergence of animal strains with reduced susceptibility to fluoroquinolones in different countries after the introduction of fluoroquinolones for use in food animals. Lack of data on usage of quinolones in many countries makes interpretation of this variation difficult (WHO, 1998).

In contrast to patients with uncomplicated gastroenteritis, effective antimicrobial agents are essential for the treatment of patients with bacteraemia, meningitis or other extraintestinal *Salmonella* infections. In approximately 6 percent of the culture-confirmed cases reported to CDC, *Salmonellae* are isolated from specimens collected from extraintestinal sites, usually from blood (Hargrett-Bean, Pavia and Tauxe, 1988; CDC, 1996b).

Since approximately 40 000 culture-confirmed cases are reported to CDC each year, effective antimicrobial agents are critical and may be life-saving for at least 2 400 persons a year. The selection of antimicrobial agents for the treatment of invasive infections has become increasingly restricted due to increasing antimicrobial resistance among *Salmonella* isolates. In the past, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole have been the "treatment of choice" for *Salmonella* infections (McDonald *et al.*, 1987; Riley *et al.*, 1984; Lee *et al.*, 1994). Should *Salmonella* develop antimicrobial resistance to these antimicrobial agents, suitable alternative antimicrobial agents are not currently available and serious adverse human health consequences can be expected.

CDC in the United States of America conducted surveys in 1985 (McDonald *et al.*, 1987), 1990 (CDC, 1996b) and 1995 (Angulo *et al.*, 2000a). Ampicillin use declined from 60 percent in 1985 to 5 percent in 1995, whereas the proportion of isolates resistant to ampicillin steadily increased (Angulo *et al.*, 2000a). Trimethoprim-sulfamethoxazole use, in contrast, remained constant, whereas trimethoprim-sulfamethoxazole resistance increased slightly. Importantly, the proportion of patients with salmonellosis treated with ciprofloxacin or extended-spectrum cephalosporins markedly increased without an emergence of resistance to either of these antimicrobial agents among human *Salmonella* isolates.

The continued susceptibility of human *Salmonella* isolates to fluoroquinolones and extended-spectrum cephalosporins was confirmed in more than 4 000 isolates in 1995 and 1 200 isolates in 1996 (CDC, 1996c). These data suggest there is little correlation between the antimicrobial agents used in persons with *Salmonella* infections and development of antimicrobial resistance among human *Salmonella* isolates.

If human antimicrobial use is not associated with the increasing antimicrobial resistance seen among *Salmonella* isolates, what is causing the increasing prevalence of antimicrobial-resistance observed among *Salmonella* isolates? Possible sources for an increased number of an unusual strain of *Salmonella* among human isolates may be indicated by the emergence of the same unusual strain among isolates from animals, foods, and other sources. Such investigations often reveal that the source of the increase has been traced to foods of animal origin. For example, beginning in 1969, there was a marked increase in human isolates of *Salmonella* Agona detected in the United States of America and several other countries (Angulo *et al.*, 2000a). *Salmonella* Agona had not been isolated in the United States of America before 1969, but by 1972 it was the eighth most common serotype isolated from humans in the United States of America (Clark, Kaufmann and Gangrosa, 1973). Field investigations and surveillance data determined Peruvian fishmeal fed to chickens was the source of the infections. The identification of *Salmonella* Agona from Peruvian fishmeal in routine surveillance sampling of fishmeal in 1970 was critical in identifying this new vehicle.

The widespread geographical distribution of unusual strains also supports a limited role for person-to-person transmission of *Salmonella* in the developed world. For example, the widespread emergence in the United States of America and Europe of *Salmonella* Agona, and more recently an indistinguishable clone of MR *Salmonella* Typhimurium DT 104 R-type ACSSuT, suggests transmission via the contamination of a widely distributed vehicle such as food, rather than from infected persons (Angulo *et al.*, 2000a). In Europe, from August to October 2002, a total of 41 human cases of MR *Salmonella enterica* subsp. *enterica* serotype Typhimurium resistant to ampicillin, tetracycline, streptomycin and sulfamethoxazole (R-type ASSuT) were reported in Denmark. Cases were spread over most of the country. Data from routine *Salmonella* surveillance of domestic animals (primarily poultry, pigs and cattle) and foods revealed that 83 percent of all *S. Typhimurium* DT 120 isolates with the current outbreak resistance profile originated from turkey meat from a major Danish producer of turkey and turkey meat products. Most of the rest of the *S. Typhimurium* DT 129 isolates found were from pigs. PFGE profiles of the first 28 isolates from humans, turkey meat, and pork were compared. Twenty-two of 28 human isolates shared the same PFGE profile. This profile was also seen in several isolates from both turkey and pork (WHO, 2003).

Another example of a MR strain is *Salmonella* serotype Newport. Between 1996 and 2001, 8 387 non-typhoidal *Salmonella* isolates were tested; 522 (6 percent) were *S. Newport*. Of the 522 *S. Newport* isolates, 77 (15 percent) were resistant to ampicillin, amoxicillin/clavulanate, cefoxitin, ceftiofur, cephalothin, chloramphenicol, sulfamethoxazole, streptomycin and tetracycline (Newport9+). Among the 77 Newport9+ isolates, 71 (92 percent) had decreased susceptibility to ceftriaxone, 13 (17 percent) were resistant to kanamycin, 6 (8 percent) to gentamicin, and 9 (12 percent) to trimethoprim/sulfamethoxazole; none were resistant to ciprofloxacin or amikacin.

The prevalence of Newport9+ among *S. Newport* isolates was 1 percent (1 of 99) in 1996-1997, <1 percent (1 of 77) in 1998, 17 percent (17 of 98) in 1999, 22 percent (27 of 124) in 2000, and 25 percent (31 of 124) in 2001. Newport9+ was identified at 17 sites. The median age of Newport9+ patients was 27.5 (<1-81 years); 55 percent were female (Angulo *et al.*, 2002).

Recently, a prolonged nosocomial outbreak of infection with fluoroquinolone-resistant *S. enterica* serotype Schwarzengrund was described in an institutional setting in Oregon (United States of America), where there is heavy use of antimicrobial agents. The index patient had been hospitalized in the Philippines and had probably acquired the infection there (Olsen *et al.*, 2001).

Shiga toxin-producing *Escherichia coli* O157:H7 (O157) causes diarrhoea in >73 000 persons annually in the United States of America; 5 percent of these develop haemolytic uraemic syndrome (HUS).

Treatment with antimicrobial agents is controversial; *in vitro* exposure to sub-inhibitory concentrations of some agents increases Shiga toxin production, and some studies suggest that antimicrobial treatment increases the risk of HUS.

For vero-cytotoxin-producing *E. coli* O157 (VTEC O157), it was observed that of 23 percent of the 1 087 strains tested in England and Wales in 1997, only 2 percent were MR, with the most common resistance patterns being: streptomycin, sulphonamides and tetracyclines, although resistance patterns were increasing for sulphonamides and tetracyclines. For animal isolates, the overall incidence of resistance had increased since 1994 but multiple resistance remained rare (Threlfall *et al.*, 2000).

In the United States of America, a CDC study of isolates collected in the early 1980s reported that none were resistant to only one antimicrobial agent, but 1 percent were resistant to more than one agent, all of which were resistant to the triad of streptomycin, sulfisoxazole and tetracycline. A national study of isolates from 1990–1992 found that 2.5 percent were resistant to a single agent and 5.9 percent were multidrug-resistant. Again, the most common resistance pattern was to streptomycin, sulfisoxazole and tetracycline, present in 3.4 percent of isolates. This triad was also the most commonly reported resistance pattern among O157 strains isolated from cattle and ground beef collected between 1988 and 1993 (Johnson *et al.*, 2000). Resistance to streptomycin, sulfamethoxazole and tetracycline was similar to past surveys. This triad of drugs are rarely used to treat diarrhoea in humans, but sulfa-containing drugs and tetracycline are used in animals.

Continued surveillance for antimicrobial resistance in O157 strains from human and various animal sources may be useful in identifying reservoirs for this pathogen and practices that encourage development of resistance (Johnson *et al.*, 2000). *Enterococci* infection is commonly hospital acquired. When these bacteria are antibiotic resistant, the infection may be fatal. Subtherapeutic doses of tylosin have been commonly used in livestock in Denmark, since, there, 90 percent of *Enterococci* isolated in pigs are resistant to tylosin. In contrast, in Finland, where tylosin has rarely been used subtherapeutically, only 15 percent of the *Enterococci* are tylosin resistant. In the Netherlands, avoparcin was used subtherapeutically in pigs and 39 percent of the *Enterococci* isolated from pigs are avoparcin resistant, as they are to the similar one used in human medicine, vancomycin.

In countries that have banned certain subtherapeutic uses of antibiotics, decreases in resistance to those antibiotics have been reported, restoring the effectiveness of those antibiotics for treating disease. For example, in Denmark, after a 1995 ban on the use of avoparcin as a growth promoter, glycopeptide-resistant *Enterococci* in Danish broiler flocks declined from 82 to 12 percent. No reduction has been seen in swine, due probably to the facts that swine production is continuous (in contrast to cyclical broiler production, which allows complete cleaning between flocks) and that swine producers changed from avoparcin to tylosine, which also selects for glycopeptide-resistant antibiotic, whereas Danish broiler producers stopped using any kind of antimicrobial growth promoters.

Nevertheless, in Norway, vancomycin-resistant *Enterococci* (VRE) were still isolated from broilers after three years from its banning, and resistant genes were appearing in *Lactococcus lactis* and *Streptococcus bovis* (Borgen *et al.*, 2001).

Giving antimicrobial agents to chickens and other food animals selects for antimicrobial resistance among bacteria (e.g. *Enterococcus faecalis*, *E. faecium*), and that resistance may then be transmitted to humans through the food supply via *Enterococci* and other bacteria. Antimicrobial-resistant *Enterococci*, particularly high-level gentamicin-resistant *Enterococci* (HLGRE) and VRE are an increasing cause of

human illness and death in the United States of America. Gentamicin and vancomycin are important antimicrobial agents for the treatment of human enterococcal infections. Quinupristin-dalfopristin (QD), a mixture of two streptogramins, was recently approved for the treatment of vancomycin-resistant *E. faecium* infections. Although neither vancomycin nor other glycopeptides have been widely used in food animals in the United States of America, gentamicin and virginiamycin, an analogue of QD, are frequently used for disease prevention and growth promotion in chickens.

In the United States of America, laboratories in Georgia, Maryland, Minnesota and Oregon participating in the Emerging Infections Program (EIP)'s *Enterococci* Study, coordinated through NARMS-Enteric Bacteria (NARMS-EB), cultured human stools and chickens purchased from grocery stores. Isolates of *Enterococci* were forwarded to CDC for species identification and antimicrobial susceptibility testing by broth microdilution. Although no VRE were isolated, HLGRE and QD-resistant *E. faecium* were isolated from human stools and from a high proportion of chickens from grocery stores. These data provide supporting evidence that use of gentamicin and virginiamycin in chickens promotes the transmission of HLGRE and QD-resistant *E. faecium* to humans (Angulo *et al.*, 2000b).

This highlights concern that the food supply can provide the "seeds" of antimicrobial-resistant *Enterococci*, which may be carried in the intestinal flora of the general population. Once introduced into a medical facility, they may blossom to clinical importance under the increased selective pressures of antimicrobial use in humans. In particular, the continued use of virginiamycin in chickens may threaten the long-term effectiveness of QD in humans (Angulo *et al.*, 2000b). *Enterococci* are an important cause of hospital infections. High-level gentamicin resistance (HLGR) can complicate treatment, especially among patients with heart valve infections. In the United States of America, gentamicin is used in certain food animals, particularly chickens, turkeys and pigs, but is not often used in cattle. Gentamicin resistance among *Enterococci* isolated from meat purchased from grocery stores and from outpatient human stools were studied in the United States of America during the period 1998–2001 (Kretsinger *et al.*, 2003). HLGR was most common among *Enterococci* isolated from chicken, less so from pork samples, and rare among isolates from beef. HLGRE were also present in human stools. Such resistance may complicate the treatment of serious enterococcal infection. Additional studies are needed to further evaluate the reasons for the very high prevalence of HLGR in *Enterococci* from chicken samples and the relationship between isolates from animals and those from humans (Kretsinger *et al.*, 2003).

Bacitracin is widely used as a topical or ophthalmic antibiotic ointment for wounds. It is used in some hospitals as the primary antibiotic barrier for patients with severe burns. It is also widely used in the United States of America to promote the growth of chickens and other livestock. *Enterococci*, including *E. faecium* and *E. faecalis*, were isolated from most human stools and chickens. Almost all isolates were resistant to bacitracin (Rossiter *et al.*, 2001). In 1999, the EU suspended use of bacitracin in livestock for growth promotion, because of public health concerns.

Until recently, *aac(6)-Ie-aph(2)-Ia* was the only reported gene associated with HLGR in *Enterococci*. This bifunctional gene confers resistance to essentially all clinically available aminoglycosides except streptomycin, thereby eliminating synergism between aminoglycosides and a cell-wall-active agent such as ampicillin or vancomycin. This gene has been detected in various species of *Enterococci* significant to human infection and among *Enterococci* isolated from food-producing animals. Three recently identified gentamicin-modifying genes are also associated with gentamicin resistance in *Enterococci* and the elimination of synergy between aminoglycosides and cell-wall-active agents. The *aph(2)-Ib* gene is associated with gentamicin and other types of aminoglycoside resistance in *E. faecium* and *Escherichia coli* and appears to be linked with the *aac(6)-Im* aminoglycoside resistance gene in both *Enterococci*

and Gram-negative *Bacilli* (Chow *et al.*, 2001; Kao *et al.*, 2000). This gene has been detected in vancomycin-resistant *E. faecium* isolates from hospitalized patients (Kao *et al.*, 2000). The *aph(2<sub>-</sub>)-Ic* gene is associated with gentamicin and the elimination of ampicillin/gentamicin synergism, and was first described in 1997 in a veterinary isolate of *Enterococcus gallinarum*, and has also been identified in human *E. faecium* and *E. faecalis* isolates (Chow *et al.*, 1997).

The *aph(2<sub>-</sub>)-Id* gene, first described in 1998 in a human *Enterococcus casseliflavus* isolate, confers high-level resistance to gentamicin but not to amikacin (Tsai *et al.*, 1998). This gene has been detected in several vancomycin-resistant *E. faecium* isolates from hospitalized patients (Tsai *et al.*, 1998).

To further understand the potential spread of gentamicin resistance genes among *Enterococci*, Donabedian *et al.* (2003) evaluated the gentamicin-resistant *Enterococci* isolated from humans, food and farm animals from six states in the United States of America for strain relatedness and molecular mechanisms of resistance, using PFGE analysis. They confirmed gentamicin resistance from human stool specimens and from chicken and pork purchased in grocery stores, finding evidence of dissemination across a broad geographical area of the country. The *aac(6<sub>-</sub>)-Ie-aph(2<sub>-</sub>)-Ia* gene was the most common gene among the gentamicin-resistant isolates evaluated in this study, and was detected in various enterococcal species, including the *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. casseliflavus* isolates collected from human stools, chickens and pork purchased in grocery stores, and from on-farm chickens, dairy cattle, swine and turkeys. Their observations provide evidence of a large reservoir for this resistance gene in humans, food and food-producing animals, indicating widespread dissemination of this resistance determinant. Because they have shown that *Enterococci* isolated from animals and humans possess the same aminoglycoside resistance gene content, it will be important to determine the transferability of these resistance genes, since the dissemination of genes can occur by horizontal transfer. Their additional findings of a high prevalence of gentamicin-resistant *Enterococci* in the faeces of food-producing animals on farms also suggest that the occurrence of gentamicin-resistant *Enterococci* in food can be attributed to the presence of the organism in food-producing animals. Furthermore, it is likely that the occurrence of gentamicin-resistant *Enterococci* in food-producing animals is a consequence of gentamicin use in these animals.

In Sweden, all antibiotics have been banned as growth promoters since 1986, including avoparcin. There, avoparcin-resistant *Enterococci* and VRE have not been isolated from pig faecal samples. In other northern European countries, where avoparcin has been used as a growth promoter, *Enterococci* resistant to this antibiotic and also to vancomycin are common in healthy people. In contrast, in United States of America, where agricultural uses of avoparcin and vancomycin are banned, this kind of resistance is not observed (HHS, 1999b).

Tetracyclines have been widely used as growth promoters and as therapeutic agents in animal husbandry. Despite several warnings about the increased resistance of micro-organisms to tetracyclines and their banning as growth promoters, actually more of the 65 percent of the antibiotics prescribed in the European Community for veterinary therapeutic use are tetracyclines (Kuhne *et al.*, 2000). These authors demonstrated the presence of tetracycline residues in bones of slaughtered animals and assessed the potential risk from mechanical deboning of meat, and the use of meat and bone meal in animal feeding, pointing out that the thermal treatment required by the European Community for hazardous micro-organisms and agents is a minimum of 133°C, but bones must be heated to at least 140°C to obtain a significant decrease in tetracycline residues. Another potential risk that has to be considered is the use of chitin-derivate products for animal feeding or as source of peptone to prepare culture media.

Another aspect of the resistance problem that has also to be considered is that recently some similarities between bacterial resistance patterns to antibiotics and to



biocides (antiseptics, disinfectants, preservatives) have been reported. Gram-negative bacteria that have developed resistance to cationic biocides (chlorhexidine salts and quaternary ammonium compounds) may also be resistant to some antibiotics (Russell, 2000).

There is clear evidence that, with an increase in the consumption of antimicrobial agents by humans or animals, there is a resultant increase in antimicrobial resistance (Donabedian *et al.*, 2003).

### 3.1.3 Antimicrobial resistance in aquaculture

The current definition of aquaculture, according to FAO/NACA/WHO (1997), is “the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants”. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators.

Aquaculture is becoming a more concentrated industry of fewer but much larger farms. Infective diseases are always a hazard, and may cause major stock losses and problems of animal welfare. To control infectious diseases in aquaculture, the same strategies used in other areas of animal production are employed. Whenever antibiotics are used, they should be strictly controlled under the same code applying to other veterinary medicines. As there are no antibiotics specifically designed for aquaculture, authorized products developed for other areas of veterinary medicine are used.

In the United States of America, the majority of fish farming enterprises where antibiotics might be used have pond-like or tank structures, rather than open-water habitats, like oceans or lakes. Generally, after harvest, large commercial ponds for fish are not drained, so high levels of drugs may still remain, affecting newly growing fish, which are then exposed to the antibiotic residues and actively-resistant bacteria (Committee on Drug Use in Food Animals, 1999).

It is estimated that nearly 150 pounds of antibiotics are applied per acre ( $\approx 170$  kg/ha) of salmon harvested in the United States of America, and since pens are placed in natural seawaters, antibiotics and the resultant resistant bacteria are in contact with the environment. Some countries, such as Norway, utilize natural structures like fjords for salmon farming and, for this reason, there are concerns about the wastes that collect in fjord bottoms (FAO/NACA/WHO, 1997).

Aquaculture promotes the production of various sizes and types of aquatic organisms, and the use of antibiotics and drugs in the fish industry is complicated because of the need to administer the compounds usually direct into the water where the organisms live. Several factors have to be considered: the safety of aquatic fish products, the integrity of the environment, the safety of target animals, and the safety of the persons administering the compounds.

Antibiotics effective in human medicine, including oxytetracycline, sulfamerazine and ormethoprim, are used for treatment of bacterial infections in salmon, catfish, trout and other commercially-raised fish. The most frequent fish infections treated with antibiotics are skin ulcers, diarrhoea and blood sepsis. The micro-organisms responsible for these infections belong to bacterial families that also produce infections in humans. Therefore, transference of antibiotic resistance is highly probable.

Even when treatment is suspended before the fish is sold for consumption, the resistance can still be transmitted. For this reason, the environmental and health impact of the use of antibiotics in aquaculture is recognized; in many countries the use of antibiotics in aquaculture is under veterinary medicine control. In Table 3.1, the antibiotics used for aquaculture purposes in some countries are listed.

A similar range of antibiotics is permitted in most European countries for aquaculture purposes (FAO/NACA/WHO, 1997). In Canada, the use of oxolinic acid, chloramphenicol and furazolidone are not allowed for use in food fish (ICES, 1996).

Wider ranges of antibiotics are approved in Asian countries. A survey, supported by the Asian Development Bank in 1995, which involved 16 countries in the region, showed that the use of antibiotics in inland carp farms was very low, less than 5 percent, with oxytetracycline and oxolinic acid the compounds most frequently used. In extensive and semi-extensive coastal shrimp farms, antibiotic use was also very low.

In some countries, there was a higher use of antibiotics for intensive shrimp farming, with oxytetracycline and oxolinic acid being the antibiotics mainly used. When no antibiotic regulatory regime existed, farmers used any antibiotic they might obtain (FAO/NACA/WHO, 1997).

Some examples of use of antibiotics in aquaculture in Asian countries are shown in Tables 3.2–3.5. In addition to the information tabulated below, it was reported that mainly sulphonamides (sulfonamide+trimethoprim, sulfamonomethoxine and sulfadimethoxine), tetracyclines, chloramphenicol, oxolinic acid and virginiamycin were in use in Malaysia and Singapore (Southeast Asian Fisheries Development Center, Aquaculture Department, 2000).

For finfish and for crustaceans, antibiotics are usually administered in the feed, either compounded during the process or as surface-coated feed pellets. Oil is frequently used as vehicle, either by the feed manufacturer or at the farm.

In the shrimp industry, antibiotics are mainly used as a bath medication in the hatchery. For finfish or for juvenile shrimp, the antibiotic is used as an oil-based coating. The use of antibiotics in older shrimps is increasingly restricted due to concerns about antibiotic residues.

TABLE 3.1  
Antibiotics used for aquaculture purposes in certain countries.

Country	Antibiotic	Purpose	Reference
United Kingdom	oxytetracycline, oxolinic acid, amoxicillin and co-trimazine (trimethoprim- sulfadiazine).	Not mentioned	FAO/NACA/WHO, 1997.
Norway	benzylpenicillin+ dihydrostrepto-mycin, florfenicol, flumequine, oxolinic acid, oxytetracycline, co-trimazine.	Not mentioned	FAO/NACA/WHO, 1997.
United States (FDA approved)	sulfadimethoxine and ormethoprim.	To control furunculosis ( <i>Aeromonas salmonicida</i> ) in salmonids. To control enteric septicaemia ( <i>Edwardsiella ictturali</i> ) in catfish.	Committee on Drug Use in Food Animals, 1999.
United States (FDA approved)	oxytetracycline.	To control ulcer disease, furunculosis, bacterial haemorrhagic septicaemia and <i>Pseudomonas</i> disease in salmonids. To control bacterial haemorrhagic septicaemia and <i>Pseudomonas</i> disease in catfish.	Committee on Drug Use in Food Animals, 1999.
Mexico	enrofloxacin; oxytetracycline.		Gomez-Gil <i>et al.</i> , 2001.

TABLE 3.2  
Antibiotic use in aquaculture in India.

Antibiotic	Purpose	Mode of application
oxytetracycline	<i>Myxobolus</i> spp.	Supplemented in the feed
sulfadiazine+trimethoprim	Ulcerative and systemic type ( <i>Aeromonas hydrophila</i> )	Water dispersible powder
chlor-tetracycline	Not defined	Supplemented in feed
oxytetracycline	Columnaris disease	Supplemented in feed

TABLE 3.3  
Antibiotic use in aquaculture in Indonesia.

Antibiotic	Purpose	Mode of application
oxytetracycline <sup>(1)</sup>	bacterial fish and shrimp disease	bath and oral
chloramphenicol <sup>(2)</sup>	bacterial disease in shrimp and ornamental fish	immersion
erythromycin	bacterial fish and shrimp disease	bath
streptomycin	bacterial disease in shrimp and ornamental fish	long bath
neomycin	bacterial disease in shrimp and ornamental fish	bath
enrofloxacin	High skills required	bath

NOTES: (1) Negative effects: antibiotic application has resulted in emergence of drug-resistant bacteria, and detection of antibiotic residues in exported fish products has resulted in rejection for the Japanese market.  
(2) Currently banned for use in aquaculture in Indonesia.

Source: Southeast Asian Fisheries Development Center, Aquaculture Department, 2000.

TABLE 3.4  
Antibiotic use in Japan in aquaculture of yellowtail, rainbow trout and kuruma prawn.

Antibiotic <sup>(1)</sup>	Target species	Administration mode	Withdrawal period (days)
amoxicillin	yellowtail	oral	5
ampicillin	yellowtail	oral	5
erythromycin	yellowtail	oral	30
oxytetracycline hydrochloride	yellowtail	oral	20
oxolinic acid	yellowtail	oral	16
spiramycin	yellowtail	oral	30
novobiocin	yellowtail	oral	15
flumequine	yellowtail	oral	-
lincomycin hydrochloride	yellowtail	oral	10
florfenicol	yellowtail	oral	5
thiamphenicol	yellowtail	oral	15
oxytetracycline hydrochloride	rainbow trout	oral	30
oxolinic acid	rainbow trout	oral	21
sulfadimethoxine	rainbow trout	oral	30
sulfamonomethoxine	rainbow trout	immersion	15
florfenicol	rainbow trout	oral	14
oxytetracycline hydrochloride	kuruma prawn	oral	25
oxolinic acid	kuruma prawn	oral	30

Note: (1) Items regulated by the Drug Laws.

Source: Southeast Asian Fisheries Development Center, Aquaculture Department, 2000.

TABLE 3.5  
Antibiotic use in aquaculture in the People's Republic of China.

Antibiotic	Pond disinfection	Fish or egg disinfection	Bath treatment	Injection	Oral administration
sulfonamide					+
nystatine		+			
terramycin		+	+		+
aureomycin		+			+
penicillin			+	+	
streptomycin		+	+	+	
doxycycline					+
erythromycin			+		+
chloramphenicol <sup>(1)</sup>			+	+	+
oxolinic acid					+

Note: (1) Currently banned for use in aquaculture in China.

Source: Southeast Asian Fisheries Development Center, Aquaculture Department, 2000.

Some studies have been carried out to try to improve the antibiotic delivery dosage. Gomez-Gil *et al.* 2001) studied the possibility of delivering these antibiotics to shrimp larvae by bio-encapsulating the antibiotic in *Artemia franciscana* (brine shrimp eggs) and are currently active in research to try and standardize the bio-encapsulation technique. For this approach, it is very important to calculate if the bio-encapsulated antibiotic is sufficient to achieve more than the recommended MIC in the target organism. The number of nauplii that the target organism can consume in a given period of time will have to be taken into consideration.

As the antibiotic is mostly given as medicated food pellets, the food surplus not eaten by the fish, together with the drug excreted, will eventually reach the bottom around the pens. Previous research indicates that 70-80 percent of the drug ends up in the environment. Drug concentrations with antibacterial activity were found in the sediment underneath fish farm (Samuelsen, Torsvik and Erik, 1992). Their results confirm that, based on the concentration gradient of the antibacterial agents in the sediment, leaching accounts for the depuration. Variations observed in antibiotic half-lives are most probably caused by differences in water solubility and chemical-sediment interactions.

In fish therapy, a strain may be resistant or sensitive depending on some additional factors: the way the antibiotic is administered, the antibiotic distribution compared with the pathogen location in the fish, and the physicochemical environment of the fish.

An example of environmental influence is the well-known fact that the presence of  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  in the marine environment reduces the biological activity of oxytetracycline, quinolones, flumequine and oxolinic acid. Oxytetracycline and quinolones are reported to degrade photochemically and form divalent cationic complexes with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  in seawater, which produces a loss of antibacterial activity in less than one month through cation complexation or binding to sediment and associated organic matter, or a combination.

While the risks associated with persistence of oxytetracycline in saltwater are mitigated by complexation, when the drugs are used in freshwater, a greater fraction of the compound may exist in an uncomplexed form, and persistent antimicrobial activity in the environment could be an ever greater concern.

The out-washing of oxytetracycline from the marine sediment into the water column will result in its moving from one environment where its activity is inhibited to another with an increase of biological activity (Smith and Samuelsen, 1996).

The persistence of several antibacterial agents (oxytetracycline chloride, oxolinic acid, flumequine, sarafloxacin, florfenicol-sulfadiazine and trimetoprim) were studied by Hektoen *et al.* (1995), who reported that the initial concentrations of these antibiotics were present in the deeper layers of the sediment after 180 days, whereas in the upper layers they were depurated more rapidly. Quinolones were adsorbed to the sediment.

The use of antibiotics in aquaculture may contribute to an increase in the frequency of resistance in the related microflora (Table 3.6).

In the case of *A. salmonicida*, laboratory studies showed that resistance to oxolinic acid can readily be selected for in the presence of the antibiotic, and a similar situation was reported for the fluorinated quinolone sarafloxacin.

Horizontal transfer of resistance genes on plasmids has been demonstrated between bacteria in the water of fishponds and in marine sediments. Plasmids carrying resistance determinants have also been transferred *in vitro* from fish pathogens to human pathogens, including *Vibrio cholerae* and *Vibrio parahaemolyticus* (Angulo, 1999).

Bacteria present in aquaculture settings may be transmitted to humans who come in contact with this ecosystem. For example, *Vibrio* spp. are part of the normal warm marine flora and cause wound infections in persons with open wounds or abrasions

TABLE 3.6  
Plasmid-mediated resistance to antibiotics identified in various fish pathogens.

Bacteria	Antibiotic	Country
<i>Aeromonas salmonicida</i>	not mentioned	not mentioned
<i>Vibrio anguillarum</i>	not mentioned	not mentioned
<i>Aeromonas hydrophila</i>	not mentioned	not mentioned
<i>Pseudomonas fluorescens</i>	not mentioned	not mentioned
<i>Edwardsiella tarda</i>	not mentioned	not mentioned
<i>Pasteurella piscida</i>	not mentioned	not mentioned
<i>Yersinia ruckeri</i>	not mentioned	not mentioned
<i>Aeromonas salmonicida</i>	chloramphenicol	Japan
<i>Aeromonas salmonicida</i>	sulphonamides	Japan
<i>Aeromonas salmonicida</i>	streptomycin	Japan
<i>Aeromonas salmonicida</i>	Combinations of sulphonamides, streptomycin, spectinomycin, trimethoprim and/or tetracycline	Ireland
<i>Aeromonas salmonicida</i>	oxytetracycline	Scotland, United Kingdom

Source: FAO/NACA/WHO, 1997.

exposed to seawater or marine life. In 1991, an epidemic of *Vibrio cholerae* O1 infections affected Latin America; the epidemic strain in Latin America was susceptible to the 12 antimicrobial agents tested, except in coastal Ecuador, where the epidemic strain had become multidrug-resistant. The cholera epidemic in Ecuador began among persons working on shrimp farms. MR was present in non-cholera *Vibrio* infections that were pathogenic to the shrimp. The resistance may have been transferred to *V. cholerae* O1 from other vibrios (Weber *et al.*, 1994).

The presence of  $\beta$ -lactamases has been reported for *Aeromonas* spp., including those strains that are fish pathogens. The presence of MR *A. salmonicida* strains represents serious problems in the salmon farming industry. The widespread use of antibiotics, especially in hatcheries, has also led to the development of multiple resistance, and causes losses in shrimp production (FAO/NACA/WHO, 1997).

Bacteria from the aquaculture ecosystem may also be transmitted directly to humans through handling of fish. Recently, the fish pathogen *Streptococcus iniae* has caused invasive infections in persons who handled store-bought aquacultured tilapia; *S. iniae* was isolated from the aquaculture ecosystem and on fish in grocery stores (Weinstein *et al.*, 1997). Similarly, a new biotype of *Vibrio vulnificus* caused hundreds of serious infections among persons handling live tilapia produced by aquaculture in Israel (Bisharat and Raz, 1996).

Bacteria on fish may also be transmitted to humans when the aquacultured products are eaten, or when other foods are eaten that have been cross-contaminated by bacteria from fish. For example, *Vibrio parahaemolyticus* is a common food-borne disease in Japan, where infections have been linked to the consumption of aquacultured finfish (Ministry of Health and Welfare, 1999).

Antibiotic-resistant bacteria have been isolated from the carcasses of catfish from the retail market (De Paola, Peeler and Rodrick, 1995). These bacteria can be transferred during food preparation at home or by handling in the market.

Guardabassi *et al.* (2000) have reported an increase in the prevalence of oxolinic acid resistant *Acinetobacter* spp. in a stream that received the effluent from a freshwater trout farm following treatment with oxolinic acid-medicated feed. They observed an increase, at least 10-fold, in the MIC values for ciprofloxacin for the oxolinic acid-resistant isolates.

Since plasmid-mediated resistance to quinolones has not been reported in fish pathogens, presumably because these compounds appear to effectively inhibit the

process of conjugate plasmid transfer, bacteria that are not able to survive under environmental conditions, like most of the human pathogens, should not be affected by the use of quinolones in aquaculture (FAO/NACA/WHO, 1997; Guardabassi *et al.*, 2000).

It has to be pointed out that most of the studies mentioned above were realized under experimental conditions, which may not exactly reflect the real characteristics of aquaculture environments and realistic selection pressures. The potential diffusion of resistance in the environment has to be established.

### 3.1.4 Mechanism of resistance transfer

Resistance, like any other phenotypic characteristic, is determined by the bacterial genome. This may change as the result of mutation or by acquisition of new genetic material. Because resistance to many antibiotics does not arise by mutation of the bacterial chromosome, but rather by the acquisition of new genes whose products affect resistance by a variety of mechanisms, the study of resistance, particularly for Gram-negative bacteria, has had substantial impact on the understanding of the bacterial genomes and the way they evolve.

Resistant bacteria can transfer the resistance to other bacteria (even to bacteria of different genera) that have never been exposed to the antibiotic, and this phenomenon is known as horizontal gene transfer. One classical horizontal gene transfer example was observed in Germany where, in 1983, some farmers started using nourseothricin for growth promotion in swine. This practice resulted in the appearance of resistance in *E. coli* strains isolated from swine and pork products. In 1990, this resistance was transferred to farm workers, their families and citizens of the community where this antibiotic had been used, and patients were suffering urinary tract infections. Later, the resistance to nourseothricin was also observed in *Shigella*, a bacterium found in primates but not in swine. No nourseothricin-resistant bacteria were isolated in other parts of the country since this antibiotic had not been used elsewhere. This fact indicated that resistance from bacteria exposed to antibiotics on the farm moved to human pathogens.

At the same time, the fact that one micro-organism acquires resistance against an antibiotic seems to help it in becoming resistant against others; this capacity is known as co-selection. *Neisseria gonorrhoea*, for example, first became resistant to penicillin, then to tetracycline, then to fluoroquinolones and at present treatment relies on cephalosporins (IOM, 1998).

In the first Swedish Veterinary Antimicrobial Resistance Monitoring Report (SVARM, 2001), the observance of co-selection was confirmed: the use of one antibiotic can lead to increased resistance not only to itself, but also to another, unrelated antibiotic. In this study, the presence of linked resistance genes was pointed out. The danger of this element is evident because it means that a single transfer event conveys resistance to several antimicrobials in the recipient bacteria. The resistance transfer may occur through the exchange of part of the bacterial DNA (plasmids) or portions of their chromosomes that may contain the genes that codify for the resistance. The new genetic material may be acquired by three different mechanisms:

- **conjugation**, in which a plasmid is passed from one organism to another through a pilus. This may occur between the members of same species and between bacteria from different genera or families. This mechanism of resistance transference is called “infectious drug resistance”. The spread of genes coding for antibiotic resistance is facilitated by mobile genetic elements called transposons, which can move from plasmids to the bacterial chromosome and the in reverse direction;

- **transformation** is the second mechanism for acquisition of new genetic material, whereby DNA is assimilated from the external environment; and
- **transduction** is the third mode by which genetic material can be acquired from an infecting bacteriophage.

Another novel system for the movement of antibiotic resistance genes has been studied; it comprises an already large family of discrete mobile genetic units called cassettes, which may contain only one antibiotic resistance gene, and a family of receptor elements, integrons, that provide both the site into which gene cassettes are integrated and the enzyme responsible for gene movement (integrase). This enzyme can move these resistance cassettes in and out of the integron, thereby substantially increasing the horizontal mobility of antibiotic resistance adapting to environmental changes (SANCO, 2002a).

The integron mechanism as described has only been seen in the context of moving resistance genes, but ancestors to it have recently been discovered in *Vibrio cholerae* and other bacteria (Rowe-Magnus, Guerot and Mazel, 1999). Movement of gene cassettes, i.e. their integration into and excision from integrons, is effected by site-specific recombination (Hall, 1997). Most gene cassettes encode for antibiotic resistant determinants, but antiseptic resistant genes have also been described.

Other types of integrons coding for other determinants in Gram-negatives have been studied, such as for biochemical functions and virulence factors, which suggests they may be implied in bacterial genome evolution (Ploy *et al.*, 2000).

These events may occur in the animal gastrointestinal tract, which serves not only as the main reservoir and major site of bacterial propagation, but also as a place for the exchange of genetic information (Wegener *et al.*, 1999). Antibiotic resistance spreads both as a result of resistance genes spreading (infectious drug resistance) and to a major extent as a result of resistant bacteria spreading.

There are two main factors that have to be considered in resistance transference: the antibiotic as a selective agent in a particular environment, and the resistant gene as the vehicle of resistance.

These two elements compose the resistance equation. Parameters affecting the equation equilibrium will determine the presence or absence of resistance. If both elements are kept under control, the fact that drug-resistant organisms exist does not mean that they necessarily constitute a public health problem. If the two elements are not controlled, a drug resistant trait will be selected and propagated and the environment will be populated with different kinds of resistant bacterial flora.

The variables affecting the antibiotic side of the equation are the amount of antibiotics, the number of individuals in which they are used and the geographical extent of influence. Altering the dynamics among them may disrupt natural microbial ecology by selective pressure against bacterial strains susceptible to a given antibiotic and for strains that are resistant to it. On the genetic side of the equation, it is the resistant gene and the factors that affect its transmission. As these elements are continuously interacting against a background of continual exchange of microbes among human, animal and agricultural hosts, resistance transmutes into a public health problem.

Several studies have shown other examples of how the resistance transference may occur. Shoemaker *et al.* (2001) reported the prevalence of evidence for extensive resistance gene transfer among *Bacteroides* spp. and among *Bacteroides* and other genera in the human colon. They observed that resistance transfer is occurring in the community and is not limited to clinical environments.

Penicillins and cephalosporins ( $\beta$ -lactam antibiotics) are currently prescribed for medical use in hospitals; these products are available in more than seventy formulations, being well tolerated by human beings, with limited side effects. Nevertheless, the outstanding number of bacteria producing  $\beta$ -lactamase represents a serious threat to the clinical utility of those antibiotics. After the discovery of  $\beta$ -lactamase inhibitors,

it was thought that the resistance problem was solved. Unfortunately, bacteria have evolved new mechanisms of resistance to overcome the effects of  $\beta$ -lactamase inhibitors (Therrien and Levesque, 2000).

The *Enterococci* vancomycin resistance mechanism has been carefully studied. There are two types of vancomycin resistance known. The first type is intrinsic resistance: isolates of *Enterococcus gallinarum* and *E. casseliflavus*/*E. flavescens* demonstrate an inherent, low-level resistance to vancomycin. The second type of vancomycin resistance in *Enterococci* is acquired, through genetic information from another micro-organisms. Commonly, this type of resistance is observed in *E. faecium* and *E. faecalis*, but has also been observed in *E. raffinosus*, *E. avium*, *E. durans* and several other enterococcal species. Several genes contribute to vancomycin resistance in *Enterococci*: *van A*, *van B*, *van C*, *van D*, and *van E*. The isolates that contain *van A* and *van B* genes produce a lower level of resistance to vancomycin (MICs 16 to 64  $\mu\text{g/ml}$ ) and are susceptible to teicoplanin.

Recently, a few *van D*-containing isolates of *E. faecium* with a moderate level of resistance to vancomycin (MICs 64 to 128  $\mu\text{g/ml}$ ) and teicoplanin (MICs 4 to 8  $\mu\text{g/ml}$ ) have been reported, as has a novel *van E*, containing *E. faecalis*, *E. gallinarum* and *E. casseliflavus*/*E. flavescens* isolates that are intrinsically resistant to vancomycin. These isolates contain *van C* genes that typically produce vancomycin MICs of 2 to 16  $\mu\text{g/ml}$  (Therrien, and Levesque, 2000).

To know the type of resistance is critical for infection-control purposes. While *van A* and *van B* genes are transferable and can be spread from one organism to another, *van C* genes, in contrast, are not transferable, have been associated less commonly with serious infections, and have not been associated with outbreaks (CDC, 2001).

Vancomycin interferes with bacterial cell wall formation, which surrounds the cell and its membrane, imparting structure and support. The bacterial wall is mostly composed of peptidoglycans. As the cell assembles this material, sugar units are linked together by an enzyme, called transglycosidase, to form a core. Every sugar unit along this core has a short peptide chain attached to it, formed of five amino acids, the last three being one L-lysine and two D-alanines. The enzyme transpeptidase hooks this peptide chain together, removing the final D-alanine and attaching the penultimate D-alanine to an L-lysine from a different sugar chain. As a result, the sugar chain is crocheted together through the peptide chain. All this linking and cross-linking creates a tightly-woven material that protects cells from differences in osmotic pressures. Vancomycin interferes with the formation of this material. It binds to the peptide chains before they are interlinked by the transpeptidase. The antibiotic fastens onto the terminal D-alanines, preventing the enzyme from acting. Peptidoglycan becomes weak, and cell death occurs.

Unfortunately, the bacteria have overcome this mechanism. From the molecular point of view, the binding mechanism described above entails five hydrogen bonds. For the VRE, the peptide chain is slightly different. Its final D-alanine is altered by a substitution: oxygen replaces a pair of atoms consisting of nitrogen bonded to hydrogen. This means that vancomycin can bind to the peptide chain with only four hydrogen bonds. In this form, the enzyme can pry it off, and the peptide chains can link up; the peptidoglycan then become tightly woven once again. In this form, drug activity is reduced by a factor of  $10^3$ .

To overcome this resistance mechanism, researchers have studied other antibiotics from the glycopeptide family: those that have long hydrophobic chains attached to them and that prefer to be surrounded by other hydrophobic molecules, such as those that built the cell membrane, which is protected by the peptidoglycan shield. Pharmaceutical researchers have attached hydrophobic chains to vancomycin, creating a vancomycin analogue. This drug connects to the cell membrane giving it more power



against the peptidoglycan. This analogue is effective against VRE and is now under clinical trials (Nicolaou and Christopher, 2001).

Other glycopeptides have a different action mechanism, namely dimerization. Here, two molecules bind together to form a single complex, and vancomycin dimers have enhanced drug strength. One molecule binds to the peptidoglycan, bringing the other molecule into proximity, making it more powerful. A number of dimeric vancomycin molecules, with exceptional activity against VRE, have recently been developed.

Recently, another *Enterococci* mechanism to overcome vancomycin action has been discovered: instead of substituting an atom in the final D-alanine, the bacterium adds an amino acid that is much larger than D-alanine to the very end of the peptide chain. In this way the amino acid prevents vancomycin from reaching its site of action (Nicolaou and Christopher, 2001).

Micro-organisms have developed another strategy to protect themselves from antibiotics: the formation of biofilms; they exist in layers that adhere to surfaces and this protects them from antibiotics and immune cells. Some researchers had attributed this resistance to the incapacity of the drug to diffuse into the film layer. Zilver (2000) attributed the resistance to the physiological heterogeneity within a biofilm, which results in areas of organisms with antimicrobial-resistant phenotypes.

Nevertheless, Sauer, Stewart and Givskov (2001) discovered that bacteria used a different strategy: the cells in the centre of the biofilm were less susceptible than those that grow on the outside because they ran out of oxygen, which spurred them to downshift and stop growing, making them less vulnerable to antibiotics. It means that conventional antibiotics that kill cells in the outer layers of the biofilm may not work on the inner cells, even if they reach them.

Recently, it has been discovered that some compounds, such as furanose, are able to block biofilm formation in *P. aeruginosa*, a micro-organism responsible for life-threatening lung infections in cystic fibrosis patients. This means that a similar drug can potentially defeat hard-to-treat chronic infections caused by biofilms (Sauer, 2001).

Biofilms formed in aquaculture system components incorporate microflora present in the water. Pathogenic micro-organisms were found in these biofilms causing recurrent exposure to disease and the presence of asymptomatic carriers. In a study recently carried out in aquaculture environments, some pathogenic bacteria have been identified: *Aeromonas hydrophila*, *Vibrios*, *Yersinia* and *Bacillus cereus*. Some of the micro-organisms isolated are pathogens for both animals and humans and can be significant in further-processed foods. Whether these micro-organisms' presence in biofilms could lead to food-borne illness is unclear, but the potential exists (King, 2000).

In the SVARM (2001) report, the resistance among indicator bacteria was tested (*E. coli* and *Enterococcus* spp.). The prevalence of acquired resistance to antimicrobials among bacteria of the normal enteric flora can serve as indicator of the selective pressure exerted by the use of antibiotics. Although it is unlikely that these bacteria may cause diseases, they constitute a reservoir of transferable resistance determinants from which resistance genes may spread to human and animal pathogens. For this reason, surveillance of resistance patterns among indicator bacteria can be of great value in detecting trends in resistance and to guide action taking (SVARM, 2001). The presence of acquired resistance amongst the bacteria of the animal digestive tract, developed primarily as a result of exposure to antibiotics used as growth promoters, represents a large pool of resistance genes. Since ingestion of bacteria derived from animals is common, there is a consequent potential for resistance genes in these bacteria to be transferred to human bacteria, although the magnitude of this risk has yet to be established.

The FDA, through CVM, is conducting several projects focused on the genetic characterization of novel and emerging antimicrobial resistant determinants, such

as efflux pumps and integrons, among both zoonotic and animal health pathogens, including *Campylobacter*, *E. coli* 0157:H7, *Salmonella*, *Enterococcus*, and *Staphylococcus aureus* (FDA, 2002c).

## 3.2 HAZARD CHARACTERIZATION

### 3.2.1 Human health risks associated with the use of antibiotics in aquaculture

#### 3.2.1.1 Resistance transference

To assess the likelihood of the risk of resistance transfer, two risks have to be considered independently:

- the risk associated with the transmission of resistant bacteria from aquaculture environments to humans, i.e. the increase of resistance in human bacterial pathogens as a direct consequence of the use of antibiotics in aquaculture; and
- the risk associated with the introduction, in the human environment of non-pathogenic bacteria containing antimicrobial resistance genes and the subsequent transfer of such genes to human pathogens.

Opportunistic pathogens, such as *Aeromonas* sp., and *Plesiomonas shigelloides*, have been isolated in aquatic environments and fish products all over the world (Koburger, 1988; Kirov and Hayward, 1993; Nedoluha and Westhoff, 1995; Hernández and de García, 1997; Hernández *et al.*, 1999; Ramos and Lyon, 2000). The public health risk is related to the indirect exposure of these micro-organisms to antimicrobials (FAO/NACA/WHO, 1997).

In developed countries, the risk that resistant bacteria from aquaculture may reach the drinking water supply is remote, because of the high dilution factors to which such bacteria would be subject and the fact that most fish pathogens are incapable of infecting humans. In some developing countries, drinking water is not treated; in this case, pathogenic fish bacteria are acclimatized to temperatures much nearer to that of the human body and are able to survive in the human intestinal tract.

According to the Committee on Drug Use in Food Animals (1999), the major impediment to assess the effect of antibiotics in animal foods on human health risk is the complexity of the food-animal drug interaction and how it is affected by the handling and processing process.

The evidence currently available implicates most of the food-animal pathogen events to the presence of enteropathogenic bacteria contracted by the consumption of contaminated foods.

#### 3.2.1.2 Toxicity of antibiotic residues

As animals are fed antibiotics for long periods, knowledge is vital concerning residue bio-accumulation, chronic toxicity and associated problems. These substances may have toxic or allergenic properties.

Toxic effects could be observed in target animal species, or non-target as a result of incidental intake, or in humans. Some antibiotics are poorly absorbed in the mammalian or bird intestinal tract and, for this reason, their residues are less important; others, like avilamycin, are absorbed to some extent, and tylosin, spiramycin, olaquinox and carbadox are easily absorbed after oral administration. For this reason, the possibility of accumulation in the animal must be addressed (SOU, 1997).

Animals and humans may be exposed via residues in animal products. Humans are also exposed when handling products containing the residues. Both ways of exposure could be harmful if the product has organ toxicity, mutagenicity or allergenicity.

For target species, some examples can be described: bacitracin has nephrotoxic properties but is not absorbed from the intestinal tract, and toxic effects are therefore

not expected despite alimentary ingestion. Some macrolides at therapeutic levels are responsible for gastrointestinal disorders, mainly diarrhoea. Carbadox and olaquinox are responsible for adrenal damage, since they affect the adrenal glomerular cells, producing several hormonal disturbances.

For non-target species, there are several possible routes to ingestion of antibiotic residues: contamination of feed at the feed mill, inadvertent feeding, or inclusion of poultry litter in animal feeds.

The susceptibility of intoxication will depend on the species. For example, ruminants and horses are very sensitive to disturbances in their intestinal microflora due to antimicrobial substances, which, in some cases, may cause fatalities; accidental feeding of low doses of tylosin to cows may cause loss of appetite, decreased milk production and hypersensitivity; contamination of dairy cows' feed may cause sudden drops in milk production.

As the accidental intake of antibiotic residues by non-target species may result in serious problems, adequate procedures for risk management, such as adherence to Good Manufacturing Procedures, have to be applied.

For substances with poor absorption by the intestinal tract, no residues in meat will be expected. Little is known about the possibility of residues due to faecal contamination. If the pharmacokinetics of these substances are similar in humans, ingested residues will not be absorbed to any large extent and no toxic actions will be expected.

For spiramycin and its active metabolite neospiramycin, liver accumulation was observed for pigs and poultry.

It was observed that tylosin is extensively metabolized in the animal and there is still some uncertainty as to the appropriate marker residue. Carbadox is rapidly decomposed to desoxycarbadox in kidney and liver samples, but it is stable in eggs and muscle. This drug has shown dose-related increase of benign and malignant liver tumours in long-term feeding studies in rats, giving positive results in 14 out of 15 mammalian and non-mammalian genotoxicity studies (FAO/WHO, 1990).

Experimental feeding of 60 ppm radiolabelled avilamycin to swine for 14 days resulted in residues in muscle, liver, kidney and fat at zero withdrawal time (SOU, 1997).

Most of the antibiotics administered in therapeutic and subtherapeutic form to domestic animals are also approved for human use. Distribution patterns of residues in food animal tissues vary according to the way the drug is administered.

The use of water and feed as the administration vehicle helps to obtain a uniform dose and avoid any potential for high localized concentration that might accumulate at the site of injection when intramuscular or subcutaneous routes of administration are used. Strict adherence to withdrawal times and suggested withdrawal intervals are critical, and sometimes the removal and discard of the tissue around the injection or treatment site is required (Committee on Drug Use in Food Animals, 1999).

The likelihood of direct toxicity from antibiotics or their metabolites in animals is extremely low (Corry, Sharma and Bates, 1983; Black, 1984). The exception could be chloramphenicol, which produces toxic aplastic anaemia not related to the dose used and which has been implicated in several cases of fatal aplastic anaemia (USP, 2000e). Residues of this antibiotic were found in 13 calves of 3 020 tested, confirming that the residues can be consumed with human food. That finding led to a ban on chloramphenicol use for food animals in United States of America, Canada and the EU (Committee on Drug Use in Food Animals, 1999).

Sulphonamides have been used widely at subtherapeutic and therapeutic concentrations in food animal production, but increasing concern about their carcinogenic and mutagenic potential and their thyroid toxicity has led to decreased use, longer withdrawal times and tighter residue monitoring. Those sulphonamides

approved for use in food animals are sulfamethazine, sulfadimethoxine, sulfaquinoxaline, sulfachlorpyridazine, sulfathiazole, sulfacetamide and sulfanilamide (Committee on Drug Use in Food Animals, 1999).

From the overall picture, the number of effects that are relevant for the assessment of low-level exposure to residues is limited. Potential effects that are not always related to dose, and which may be limited to predisposed humans, include allergic reactions.

Some studies on carcinogenicity properties of sulfamethazine have recently been conducted. The drug was evaluated by the 34th Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1989), which concluded that the thyroid tumours in rodents were most likely due to hormonal disturbance and that humans exposed to sulfamethazine below a threshold level would not be at carcinogenic risk (EMEA, 2001).

Drugs are administered in aquaculture not only as medicated feed but also through use of therapeutic baths. The use of medicated feed is the major source of aquaculture drugs, and antibiotics are the most widely used drugs. In terrestrial farming, antibiotics are accumulated in animal wastes that are released into the environment. Marine and brackish environments are exposed to these chemicals through aquaculture, through direct waste drainage and other ways.

The potential toxic effects of these drugs in the aquatic environments are not completely understood, although consequences such as chemical contamination, bioaccumulation and induction of microbial resistance have been described.

### 3.2.1.3 Allergy

Several antibiotics (notably macrolides, quinoxalines and bacitracin) are potent antigens and occupational exposure on a daily basis can lead to sensitization. Cases of occupational contact dermatitis and asthma have been reported in the literature. These reactions are probably due to airborne antigen or direct contact. Usage of special formulations can prevent this aerosol effect and reduce exposure.

Humans may be exposed to antibacterial feed additive (AFA) substances during their production and mixing in feed. Allergic reactions due to macrolides spiramycin and tylosin are reported to be frequent in farmers and people who handle these substances daily (Danese, Zanca and Bartazzoni, 1994). Allergic reactions to streptogramins (Pillette *et al.*, 1990) and bacitracin (Katz and Fisher, 1987) have been reported in association with clinical therapy.

Most of the reported allergic reactions are related to  $\beta$ -lactam antibiotic residues in milk or meat and the allergic reaction has been associated with exposure to antibiotic residues in foods. Many of the cases refer to people previously treated with antibiotics and hypersensitized to a degree that subsequent oral exposure evoked a response.

Some researchers explained the reasons for which antibiotics present in animal-derived foods are considered a relatively small risk as allergenic to humans: the antibiotics' molecular weight is too low to make them immunogenic by themselves, and when complexed to proteins of larger molecular weight that would transform them into immunogenics, the immunogenic number per protein molecule is extremely low, which minimizes the probability of a hypersensitivity reaction. At the same time, the heat applied during food preparation will degrade residue epitopes, reducing the potential for an allergic response.

Allergic reactions are mostly related to intramuscular drug administration rather than to oral administration and the epitope distribution of protein-bound antibiotic is so low that it is relatively insignificant and therefore not likely to be responsible for an allergic response when ingested (Committee on Drug Use in Food Animals, 1999).

Questions still exist regarding the ability of parenteral administration to call forth a stimulus, and regarding the consumption of penicilloyl residues as a trigger for hypersensitivity reaction (Committee on Drug Use in Food Animals, 1999).

### 3.2.1.4 Effects of the antibiotics on human intestinal flora

Bacteria are naturally part of the body's internal and external ecology and environment. Some bacteria are beneficial, most of them are benign, and their equilibrium is maintained by the organism's immune system. Microbial populations normally compete with foreign bacteria within a stable internal environment, and a stable internal environment is critical for maintaining health.

The colonic flora constitutes the most relevant flora to study regarding antimicrobial effects on human intestinal flora. The majority of the micro-organisms ingested with food are destroyed by gastric juice. The few survivors will have too low a permanence on duodenum and upper jejunum for any multiplication to have effect, even during a temporary colonization of the lumen.

The conditions for their growth become favourable as they reach the lower part of the small intestine. The caecum and colon allow the establishment of the relatively stable microbial complex that characterizes the flora of the large intestine and faeces, constituting a complex ecological system. This microflora interacts with its host (man), both locally, due to its intimate contact with the intestinal mucosa, and systematically, influencing diverse functions: physiological, anatomical, metabolic and toxicological.

The principal concern is to know how antimicrobial residues in food may affect human health either by: (i) exerting a selective pressure on the dominant intestinal flora; (ii) favouring the growth of micro-organisms with natural or acquired resistance; (iii) promoting, directly or indirectly, the development of acquired resistance in pathogenic enteric bacteria; (iv) impairing colonization resistance; or (v) altering metabolic enzyme activity of the intestinal microflora.

Based on present knowledge, therapeutic dosages of  $\beta$ -lactams antibiotics (ampicillin, amoxicillin, cephalosporins), tetracyclines (oxytetracycline) and macrolides (erythromycin) have demonstrated a distinct impact on the number of enterobacteria, *Enterococci*, anaerobic bacteria and the development of resistant strains in the human intestinal microflora. The lower-dose effects of some antibiotics have been investigated in a limited number of studies with human volunteers. The results showed that at a low level of exposure, effects on the human intestinal microflora might occur (EMEA, 2001).

### 3.2.2 Environmental risks

Several aspects have to be considered for the assessment of environmental effects of antimicrobial feed additives. The environmental burden, i.e. the amount of the substance that enters and is deposited in the environment, as well as the distribution and transport between different environmental compartments, defines the exposure.

Various effects have to be considered: effects on soil microbes, earthworms, algae, aquatic organisms, etc. Furthermore, safety for wildlife and other unintended recipients must also be considered. The presence of antibiotic residues in the environment is related to the number of animals fed these substances, duration of use, dose, metabolism, excretion pattern and waste disposal procedures. Since most of the antibiotics are not absorbed in the intestinal tract, the amount excreted in faeces is almost as large as the total amount fed to various animal species, and therefore the major impact in the environment is through excretion in faeces. Antibiotics may also be excreted in urine, and although the concentration in this substrate would be expected to be far lower than in faeces, evaporation and precipitation during storage could lead to higher concentrations, especially at the bottom of a storage tank or aquaculture pond.

The European Community requires that studies on excreted residues and an environmental assessment be performed on all feed additives (see the guidelines in EC Directive 94/40/EC; see EC, 1996). Antimicrobial substances that are released to the environment via faeces will disperse through a number of transport mechanisms.

A mathematical model has been proposed to estimate volatilization, degradation, diffusion, adsorption to sediment, losses to groundwater and streams (Addison, 1984).

Several studies indicate that most substances appear to have a half-life in soil of about 2–3 weeks at 20 °C, while lower temperatures generally cause a slower degradation. In sterile soils, no degradation was observed, indicating that microbes are essential for the process (SOU, 1997).

Antibacterial substances present in animal wastes in inhibitory concentrations will affect the environmental microflora. It was observed that tylosin at a concentration of 37 ppm or more is able to reduce soil nitrogen mineralization. A decrease in microbial respiration could be noted for 5 to 7 weeks after the addition of the antibiotic (SOU, 1997). Several studies suggested that antibiotic residues present in marine sediment might be removed through microbial degradation. Nevertheless, it has yet to be determined if aquatic environmental micro-organisms can degrade these compounds and whether an aerobic or anaerobic environment is required. It has also to be considered that compounds bound to a substrate cannot be degraded by micro-organisms (Bakal and Stoskopf, 2001).

These authors studied the sulfadimethoxine and ormetoprim fate in aquatic environments at different salinities, temperatures and pH, for a period of one year. Sulfadimethoxine was stable at 25 °C and 37 °C but showed a marked decrease in concentration at 4 °C.

Ormetoprim was stable at the different concentrations evaluated. Salinity and pH had no observed effect on the concentrations of both antibiotics after 365 days. Although this study was conducted under laboratory conditions, it shows that changes in environmental conditions may influence the free drug concentration in the water column, where it would be more available for biotic uptake by vertebrates and macro-invertebrates, potentially increasing the risk of antibiotic entering the human food supply, especially considering their prolonged half-lives.

Source-separated municipal solid waste and agricultural waste can be used for biogas production, but the presence of substances with effect against the anaerobic bacteria responsible for biogas (*gobar* gas) production will affect the process. Considerable effort is currently spent in optimizing biogas plants in order to meet requirements for more sustainable systems. In such highly efficient, modern, digestion plants, the process is strictly controlled and therefore the effects of antibiotic residues on these systems must be carefully evaluated.

Antimicrobial substances in the environment could affect treatment of wastewater, which generally involves a microbial process.

It also has to be considered that the presence of residues in manure or wastewater will impair microbial activity in the recipient habitats, lowering the turnover capacity of the microbiota.

### 3.3 EXPOSURE ASSESSMENT

At the thirty-second session of the Codex Committee on Food Hygiene (CCFH) (1999) the Delegation of Denmark presented a discussion paper considering all sources of antimicrobial resistance, recommending that this issue be dealt with at a multidisciplinary level. In Codex, the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and CCFH should have the mandate to deal with these problems according to their terms of reference.

The Codex Secretariat added that the twenty-third session of the commission had also established an Ad hoc Intergovernmental Codex Task Force on Animal Feeding to review antimicrobial resistance. The WHO representative, reporting on the work of WHO on this subject, in collaboration with Office international des épizooties (OIE)

and FAO, stressed the importance of establishing a risk profile, including a review of factors that might contribute to an increase in antimicrobial resistance (CAC, 2000b).

The EC suggested that the CCRVDF take the lead in antimicrobial resistance issues as this relates to the evaluation of residues of animal drugs in foods, in close cooperation with CCFH and the Codex Ad hoc Intergovernmental Codex Task Force on Animal Feeding.

According to the mandate of the CCRVDF, the antimicrobial resistance problem has two aspects:

- concerns that have been addressed in the past, namely evaluation of the risk related to the impact that certain residues have in the consumer intestinal flora, especially by residues that have antimicrobial activity; and
- concerns to be taken into account in the future in relation to antimicrobial resistance, including the effects related to use of antimicrobials as animal drugs in the development of antimicrobial resistance in certain bacterial populations that might represent an unacceptable problem for human health and in practices of animal breeding modification.

Because of serious concerns about the growing level of resistance to antibiotics in regular use in human medicine, the Scientific Steering Committee was asked by the EC to undertake a major review of the medical and non-medical use of antibiotics. Included in its Opinion of 28 May 1999 was the recommendation that the use of antibiotics as growth promoters in animal nutrition should be phased out (EC, 1999). This recommendation arose from the recognition that the presence of acquired resistance amongst the bacteria of the animal digestive tract, developed primarily as a result of exposure to antibiotics used as growth promoters, represents a large pool of resistance genes. Since ingestion of bacteria derived from animals is common, there is a consequent potential for resistance genes in these bacteria to be transferred to human bacteria, although the magnitude of this risk has yet to be established.

The Commission of the European Communities announced in a White Paper on Food Safety, 12 January 2000, its intention to pursue the prohibition or phasing-out of antibiotics used as growth promoters in the EU, depending on their potential use in human and veterinary medicine, as part of its broad strategy to control and contain antibiotic resistance (CEC, 2000). This proposal was adopted by the Commission on 5 February 2003. It will be made public as document COM(2003)52 Regulation of the European Parliament and of the Council on official feed and food controls (CEC, 2003a).

The removal from the market of a number of antibiotics used as growth promoters and the stated intention to phase out those remaining in use has changed emphasis in the safety assessment of micro-organisms intended for use as feed additives. The earlier requirement that microbial additives should be compatible with antibiotic growth promoters is now both superfluous and undesirable. Viable micro-organisms used as active agents in feed additives should not add to the pool of transferable antibiotic resistance genes already present in the gut bacterial population, or otherwise increase the likelihood of resistance transfer. This principle, if applied literally, would rule against most microbial products, since micro-organisms susceptible to all groups of clinically important antibiotics are rarely encountered. However, the basis of resistance varies greatly, as does the likelihood of intra- and inter-species resistance transfer.

In the European Union, any conclusion on the safety of microbial feed additives should be based on a current understanding of the mechanism of resistance and resistance transfer, and should be proportionate to the risk identified while remaining consistent with Commission Policy (SANCO, 2002b).

### 3.3.1 Guidelines on the establishment of MRLs

According to the Codex definition, a hazard is “a biological, chemical or physical agent with the potential to cause an adverse health effect”. Chemical substances may be associated with a number of different adverse health effects, not all of which would necessarily be expressed in a specific exposure scenario. Some experts dealing with chemical substances prefer to define the potential health effects as individual hazards, which need to be considered separately during the evaluation (Benford, 2000).

To evaluate the risk of use of antibiotics for animal food, two different aspects have to be considered: safety of the antibiotic residue, and resistance threat. Safety will be considered in the following paragraphs.

The likelihood or risk of antibiotic residues actually occurring in humans depends on the quantity of antibiotic encountered or taken into the body, i.e. the exposure. The hazard is an inherent property of the chemical substance but, if there is no exposure, there is no risk that anyone will suffer as a result of that hazard (Benford, 2000).

Risk assessment is the process of determining whether a particular hazard will be expressed at a given exposure level, duration and timing within the life cycle, and if so, the magnitude of any risk is estimated (Benford, 2000). The approach used by EMEA/CVMP for the evaluation of the safety of residues is based on the determination of the acceptable daily intake (ADI) on which MRLs are subsequently based (EMEA, 2000a).

The ADI is an estimate of the residue that can be ingested daily over a lifetime without a health risk to the consumer. The ADI may be set on the basis of toxicological, pharmacological or microbiological data, whichever is the lowest (Benford, 2000). Derivation of an ADI is a specific form of risk assessment since it will define exposure limits below which no harmful effects are expected to occur (Benford, 2000).

When using toxicological or pharmacological data, the lowest not-observable effect level (NOEL) with respect to the most sensitive parameter in the most sensitive tests species is identified from a battery of toxicological and pharmacological studies and, when such data is available, from observations in humans. Observed effects are referred to because assumptions cannot be made about effects not detectable by the methods used. Some effects observed in toxicity studies may represent adaptive responses with no implications for the health status of the animal and would generally not be used as a basis to establish ADI values.

Effects considered to result in harm to the animal are called “adverse”, and some experts are therefore considering the term “no observed adverse effect level” (NOAEL). It has to be stressed that the NOAEL is not an inherent property of a substance; it is an experimental observation influenced by the way in which a toxicity study is designed and it does not necessarily coincide with the threshold dose (Benford, 2000). Often a safety factor of 100, to correct for intraspecies variability and interspecies extrapolation, is used to extrapolate from NOAEL to ADI. Initially, the 100-safety factor was an arbitrary decision, but soon it became defined as comprising two equal components:

- a factor of 10 for interspecies differences, i.e. to allow for possible greater sensitivity of humans compared with the animal model, due to a slower elimination rate, greater balance of activation to detoxification reactions, or greater sensitivity to the toxic effect, or a combination, and
- a factor of 10 to allow for human inter-individual variation, i.e. the possibility that a proportion of the population may be at a greater risk because of differences in toxicokinetics or tissue sensitivity within that human population.

In aggregate, the equation becomes:  $ADI = NOAEL / (10 \times 10)$ .

The default  $10 \times 10$  factor is normally used, but according to the relevance and the quality of the toxicological/ pharmacological data, safety factors can range from 10 to 1 000 (Benford, 2000).



The ADIs for most of the antimicrobials are calculated on the basis of sensitivity testing of the relevant micro-organisms of the human intestinal flora, and the number of safety factors depend upon whether *in vivo* or *in vitro* models are used (EMEA, 2000a). The safety margin used to calculate the ADI is considered necessary to cover the substantial uncertainties in the models used and their relevance to a diverse population of consumers. The relevant residues and the marker residue are identified on the basis of pharmacokinetic and detoxification studies (EMEA, 2000a).

Also to be considered are the potential consumer intakes of residues in foods of animal origin on the basis of arbitrarily high fixed consumption values to ensure the protection of the majority of the consumers. For this calculation, a daily basket was developed by EMEA: 500 g of meat (it comprises, for ruminants, 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat; for pigs, fat and skin in natural proportions; and for poultry, 300 g of muscle, 100 g of liver and 10 g of kidney and 90 g of fat and skin in natural proportions); or 300 g of fish plus 1 500 g of milk, plus 100 g eggs and 20 g honey. Details of the development of the basket are given in EMEA (2000c), where it is noted that the basket was established on the basis of arbitrarily high fixed consumption values to ensure the protection of the majority of consumers.

This estimation also considers the residue concentration in the food commodities derived from the detoxification pattern of the substance in the target animal (EMEA, 2000a).

The determination of the MRLs is based on the ADI, the identified marker residue and total residues, the EU basket and the tissue distribution. MRLs are established in such a way that the maximum theoretical intake, as calculated from MRLs and the food basket, corrected for the ratio marker to total residues, does not exceed the ADI.

Once MRLs have been allocated, it is then necessary to determine a withdrawal period for such a veterinary product, to have some assurance that residues from the product concerned will not exceed the MRLs (EMEA, 2000a).

Withdrawal periods should be established for each veterinary medicinal product and, for this purpose, the following criteria have to be considered: the MRL established for the antimicrobial agent, the pharmaceutical form, the administration route, and the duration of the treatment (EMEA, 2000a).

During the 12th Session of CCRVDF, it was agreed that MRLs need to be set at the limit of quantification of the method to facilitate monitoring programmes and that, according to Good Practice in the Use of Veterinary Drugs, it is necessary to minimize exposure by establishing MRLs only as high as required associated with the use of antibiotics in conformity with Good Veterinary Practice as identified by countries (CAC, 2000a).

While establishing MRLs, consideration was also given to residues that occur in food of plant origin or the environment, or both.

During this meeting, the Committee agreed that the concept of ALARA (as low as reasonably achievable) had been applied in the case of benzylpenicillin because of the potential for allergic reactions associated with this antibiotic. The Committee concluded that, in its work, the following factors have to be considered: good practices in the use of veterinary drugs, good manufacturing practices, technical feasibility, substantive changes in food composition and quality characteristics, the need to minimize exposure, the ALARA concept, food consumption estimates, and residues from sources other than animal products.

Seven substances were toxicologically evaluated during the 52nd JECFA Meeting (February 1999), among them the antimicrobial agent thiamphenicol, and an ADI for this agent was approved and an MRL was recommended for thiamphenicol in fish muscle. Five substances were evaluated toxicologically at the 54th Meeting (February 2000), among them the antimicrobial lincomycin and an ADI was assigned for this substance, together with an MRL for flumequine in trout muscle tissue and

a temporary MRL for oxytetracycline in fish muscle (JECFA, 1999, reported in ALINORM, 2001).

According to USP (2000, various parts), withdrawal times have been established for antibiotics used in aquaculture (Table 3.7).

The Codex MRLs for veterinary drugs are consistent with the JECFA recommendations, prepared by a body of independent scientists who serve as experts and evaluate veterinary drugs to establish safe levels of intake and develop MRLs when veterinary drugs are used in accordance with good veterinary practices.

TABLE 3.7

**Withdrawal times established for certain antibiotics used in aquaculture.**

Antibiotic	Species	Withdrawal time for meat (days)
oxytetracycline for medicated feed	Catfish	21
	Lobsters	30
	Pacific salmon	7
	Salmonids	21
	Salmonids, 10°C or warmer	40
	Salmonids, below 10°C	21
florfenicol premix	Salmon	12

Source: USP, 2000, various parts.

### 3.3.2 Assessing the effects of antimicrobial residues in food on the human intestinal microflora

According to JECFA (1996), the possible adverse effects of residues of antimicrobial agents on gastrointestinal microflora should always be investigated. A decision regarding microbiological safety should not be limited to the performance of one simple test system or to the use of MIC data for deriving an ADI. To evaluate an antimicrobial agent, JECFA will always consider all available data on the agent's microbial activity, together with an evaluation report relating such data to possible effects on human intestinal microflora. These effects may be based on *in vitro* or *in vivo* model systems, or on other relevant data. The type of information useful for the Committee includes the drug's stability and bio-availability in the gastrointestinal tract, its spectrum of activity, its influence on the colonization barrier in *in vivo* and *in vitro* tests, and its potential to cause gastrointestinal disturbances in animal or man. The Committee has recognized the shortcomings of the current methods used to evaluate the effects on the human intestinal microflora. In addition, the microbiological test systems used to evaluate these effects have not been sufficiently validated. Therefore, to assess the safety of antimicrobial substances, the Committee recommended that sponsors submit data on the safety evaluation, providing:

- in the absence of data about effects on humans, the microbiological ADI could be based on *in vivo* model systems, such as studies on rodents, or *in vitro* models, such as MIC data or continuous culture systems; and
- if *in vitro* MIC data are to be used to estimate the risk of antimicrobial agents in the absence of *in vivo* data, the limitation of the assay has to be recognized. First of all, the micro-organisms chosen for evaluation have to be representative of the human intestinal flora and the techniques recommended must mimic conditions in the gastrointestinal tract used for making the MIC determination. According to the Committee, MIC<sub>50</sub> data should be used according to the following equation:

$$\text{ADI} = \text{MIC}_{50} (\mu\text{g/g}) \times \text{MMC} (\text{g}) / \text{FA} \times \text{SF} \times \text{BW} (\text{kg})$$

Where:

ADI = Upper limit of acceptable daily intake of an antimicrobial agent ( $\mu\text{g}$  per kg of body weight).

MIC<sub>50</sub> = The minimum concentration ( $\mu\text{g/g}$ ) of an antimicrobial agent giving complete inhibition of growth of 50 percent of the culture of a particular micro-organism, after a given period of incubation. For this evaluation, the MIC<sub>50</sub> value is the mean MIC<sub>50</sub> (for the strain(s) of the relevant species tested). Alternatively, the lowest MIC<sub>50</sub> for the most sensitive species can be used.

MCC = Mass of colonic content. A standard value of 220 g is used.

FA = Fraction of oral dose available to act upon micro-organisms in the colon.

SF = The safety factor used to account for uncertainty about the amount and relevance of MIC data available for review. Its value may range from 1 to 10. A value of 1 will be used when extensive relevant microbiological data are provided.

BW = Body weight (kg). A standard value of 60 kg is used.

The Committee promotes the development of better *in vitro* and *in vivo* methods that may be relevant for determining the effects of low concentrations of antibiotics on human colonic microflora. It is recognized that other *in vivo* and *in vitro* methods are under development and call for comments that allow a better assessment of the potential human health risk of the dietary intake of residues of antibiotics.



## 4. Risk management options

### 4.1 AT REGULATORY LEVEL

#### 4.1.1 Considerations from international forums

The emergence of antimicrobial resistance in human pathogenic bacteria raises concerns for public health. Considering the importance of this matter, OIE, during the 1997 World Wide Public Consultation on Antimicrobial Resistance (OIE, 1997), decided to launch an investigation that would include the role of international trade in animals, animal products and feed in the spread of antimicrobial resistance; and means to control the spread of resistance factors of infective agents. At the request of the OIE Regional Commission for Europe, such a study was undertaken and revealed that few countries had established official resistance monitoring programmes, that risk analysis was not commonly applied, and that there were very different approaches and methodologies in use within the European Region. As a consequence, during the 18th Conference of the OIE Regional Conference for Europe, 1998 (OIE, 1998), it was recommended that country members strengthen their activities in this field and that OIE establish an expert group to address all relevant aspects for antimicrobial resistance.

Acknowledging the importance of risk analysis and need for standardization and harmonization of laboratory methodologies, as well as strengthening programmes monitoring antimicrobial resistance, and recognizing the recommendations by the OIE European Scientific Conference (March 1999, with FAO participation; see OIE, 1999), the following mandate was assigned to the OIE Expert Group by the OIE International Committee: (i) to develop an appropriate risk analysis methodology for the evaluation of the impact of antimicrobial resistant bacteria of animal origin on public health; (ii) to develop technical guidelines on prudent use of antimicrobials; (iii) to develop technical guidelines on monitoring of the quantities of antibiotics used in animal husbandry; (iv) to harmonize national antimicrobial resistance monitoring programmes in animals and animal-derived foods; (v) to establish a priority list of relevant bacteria and antimicrobials to be included in resistance monitoring programmes; and (vi) to standardize and harmonize laboratory methodologies used for the detection and quantification of antimicrobial resistance. The outcome was a set of draft recommendations (OIE, 2000).

At the thirty-second session of CCFH (1999), the delegation of Denmark presented a discussion paper considering all sources of antimicrobial resistance, recommending that this issue be dealt with in a multidisciplinary manner. The Danish delegation noted the public health concerns related to the higher pathogenicity of *Salmonella*- and *Campylobacter*-resistant strains isolated from foods, and proposed that a risk profile and risk assessment policy should be defined.

CCRVDF and CCFH should have the mandate to deal with these problems according to their terms of reference. Mr Jorgen Schlunt, WHO representative, reported on work on this subject by WHO in collaboration with OIE and FAO, stressing the importance of establishing a risk profile, including consideration of factors that might contribute to an increase in antimicrobial resistance.

The European Community suggested that CCRVDF take the lead on the antimicrobial resistance issue, as it relates to the evaluation of residues of animal drugs in foods, in close cooperation with CCFH and the Codex Ad hoc Intergovernmental

Codex Task Force on Animal Feeding. The Task Force held its first session in June 2000 (CAC, 2000b).

According to the mandate of the CCRVDF, the problem of antimicrobial resistance has two aspects:

- concerns that have been addressed in the past, namely evaluation of the risk related to the impact that certain residues have in the consumer intestinal flora, especially by residues that have an antimicrobial activity; and
- concerns to be taken in account in the future in relation to antimicrobial resistance, including effects related to use of antimicrobials as animal drugs in the development of antimicrobial resistance in certain bacterial populations that might represent an unacceptable problem for human health, and in practices of animal breeding modification.

The CCRVDF has recommended MRLs for residues of veterinary drugs in foods and CCRVDF relies on its expert committee, JECFA, to propose MRLs. JECFA has used a risk management approach to establish MRLs, using different safety factors based on the amount and quality of data available to JECFA, and has formulated new guidelines when necessary to address new or emerging issues, such as the establishment of microbiological end points as safety criteria for antimicrobial drug residues. The EC proposed to CCRVDF a series of instrumental methods (high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectroscopy (MS)) to confirm the presence of single substances once the result of an investigation has produced an indication of the substance's presence (CEC, 2002).

In the summary reports of the fifty-second (February 1999) and fifty-fourth (February 2000) JECFA meetings, it was recognized that insufficient progress had been made in the selection of methods and that this problem had to be addressed as a matter of urgency. Nevertheless, the committee agreed that, since suitable validation existed, provisional status could be applied to the methods for some components, among them flumequin (quinolone) for fish muscle. In addition, the Committee agreed that the methods previously recommended with provisional status for tetracycline residues in edible tissues (AOAC 995.09) could be advanced to "Recommended" status.

During the fifty-second and fifty-fourth JECFA Meetings, it was agreed that the outcome of the Expert Consultation among the EU, International Association of Analytical Communities (AOAC) and International Union of Pure and Applied Chemistry (IUPAC) could provide the basis for the criteria to be developed by the Committee with respect to methods and validation of methods of analysis for veterinary drug residues. CCRVDF reviews the basis for JECFA recommendations prior to deciding whether to accept a proposed MRL; an MRL should not be adopted where adequate methods of analysis are not available for detecting residues in a specific animal-derived food, or because pertinent new information had been generated that was not available to JECFA when it undertook its evaluation. CCRVDF and CCFAC consider socio-economic and political issues in making their decisions.

In parallel, the United States of America Congress has instructed the USDA to commission a study by the National Academy of Sciences to summarize the use of drugs in food animal production, practices used to administer these drugs to animals, and the process for monitoring drug use and residues in the food chain.

In 1992, through the funding support of USDA, FDA/CVM, the Pew Charitable Trust, the American Veterinary Medical Association, the American Food Industry Association and the National Research Council (NRC), the Panel on Animal Health, Food Safety and Public Health was established, under the joint auspices of the Board in Agriculture and the IOM Food and Nutrition Board. The Panel convened the Committee on Drug Use in Food Animals, with the participation of private, public and institutional stakeholders and experts in agriculture, veterinary medicine, human medicine, epidemiology and economics. The Committee was given responsibility

for developing a strategy to identify the risks and benefits associated with the use of pharmaceutical products in food animals and for providing a report on the issues (Committee on Drug Use in Food Animals, 1999).

The WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO, 2000b) provide the framework for recommendations to reduce resistance problems deriving from the overuse or misuse of antimicrobials in food animals. They strengthen previous recommendations, such as the need to terminate the use of antimicrobials as growth-promoters pending comprehensive human health safety evaluations. Since antimicrobial resistance is a multifaceted problem that involves all stakeholders concerned with the use of antimicrobials in both food animals and humans, active participation was sought from many national and international entities, including FAO, OIE and the World Federation of the Animal Pharmaceutical Industry (COMISA).

The recommendations were designed for use by governments, veterinary and other professional societies, industry and academia. The main measures are:

- obligatory prescriptions for all antimicrobials used for disease control in food animals;
- termination or rapid phasing-out of the use of antimicrobials for growth promotion if they are also used in human medicine;
- creation of national systems to monitor antimicrobial usage in food animals;
- pre-licensing safety evaluation of antimicrobials, with consideration of potential resistance to human drugs;
- monitoring of resistance to identify emerging health problems and planning of timely corrective actions to protect human health; and
- guidelines for veterinarians to reduce overuse and misuse of antimicrobials in food animals.

With the same purpose in mind, the Committee on Drug Use in Food Animals (1999) identified four primary objectives to offer policy-makers, consumers, the communication industry, food producers, drug manufacturers, and other interested parties. Their recommendations for improvements needed were related to: (i) drug resistance monitoring; (ii) drug use and alternative strategies; (iii) the necessity of an integrated, continuous decision-making process with shared responsibilities among all stakeholders to enhance availability of needed drugs; and (iv) the necessity to move toward global harmonization of this process.

In relation with the topic, the American Public Health Association (1999) recommended:

- encouraging the education of health professionals in the judicious use of antibiotics, through clinical practice guidelines and other educational processes;
- encouraging the development of educational material for patients to increase their understanding of antibiotic usage;
- urging strengthening of State Public Health Departments' Surveillance Programs to determine patterns of resistance and to detect increases in resistance in a timely manner through surveillance efforts supported by CDC;
- actively disseminating relevant information to health care providers;
- urging FDA/CVM to work for regulation, eliminating the non-medical use of antibiotics and limiting the use of antibiotics in animal feed; and
- supporting the introduction of legislation for additional funding for population studies addressing antibiotic resistance and to improve the surveillance network.

The United States Department of Health and Human Services (HHS) released its Interagency Action Plan (HHS, 2001) for fighting antimicrobial resistance, setting priorities in the following areas:

- *Surveillance* CDC will be in charge of design and implementation of antimicrobial resistance surveillance, with a uniform methodology. FDA, USDA and CDC plan

to develop systems to monitor patterns of antibiotic use in human medicine, in agriculture and in consumer products.

- *Prevention and control* A national public education campaign will be launched to reduce the overuse and misuse of antibiotics and to improve their use in health care systems. Clinical guidelines for health professionals about the appropriate use of antibiotics will be designed as well. FDA has been responsible for assessing the human health impact of antibiotics used in animal food.
- *Research* Through the use of new technologies, including genetic blueprints for pathogens, it may be possible to identify targets for desperately-needed new diagnostics, treatments and vaccines that could assist in preventing the emergence and spread of resistant pathogens.
- *Product development* To identify and publicize priority health needs for new products that prevent resistance or treat resistant infections, HHS plans to create Interagency Antimicrobial Product Development.

According to WHO, few countries have had a surveillance programme for antimicrobial resistance in bacteria from food animals and food of animal origin (IOM, 1998). The existing programmes rarely involve all relevant zoonotic and commensal micro-organisms, and do not test for all the antimicrobials that may be relevant from a public health point of view. There is also a need to standardize the methods used to evaluate resistance, which should enable comparison of data obtained from disparate sources.

Other aspects to be considered are appropriate drug-use policies in human medicine, to be implemented through a public education campaign on appropriate antibiotics use, reducing inappropriate prescription through the development of clinical guidelines, considering regulatory changes, promoting behavioural change among clinicians, and informing the public.

Also to be considered is the necessity to improve diagnostic practices, encouraging the use of rapid diagnostic methods and trying to reduce the rates of infection transmission: this goal can be reached through public campaigns that promote vaccination and hygienic practices (EU, 1998).

To prevent and control resistance in agriculture and veterinary medicine requires:

- understanding of the risks and benefits of antibiotic use and of the ways to prevent the emergence and areas of resistance;
- development and implementation of principles for appropriate antibiotic use in the production of food animals;
- improvement in animal husbandry and production practices to reduce infection spread; and
- a regulatory framework to address the need for antimicrobial drug use in agriculture and veterinary medicine without compromising human health (United States of America Interagency Task Force on Antimicrobial Resistance, 2001).

The European Health Council adopted a recommendation on the prudent use of antibiotics and other antimicrobial agents in human medicine. The recommendation asks national governments to take measures to contain the spread of antimicrobial resistance by encouraging a more prudent use of antibiotics (EU, 2002b).

European member States are asked, *inter alia*, to raise awareness and provide information to the general public, to use a precautionary “by prescription only” approach, and to improve monitoring of consumption of these drugs.

The proposed recommendation represents the first attempt at European Community level to take action relative to human medicine, and complements the various actions under way with respect to veterinary and phytosanitary uses of anti-microbial drugs. The recommendation is one of the actions the Commission proposed in June 2001, when it adopted a Community Strategy to combat the threat to human, animal and plant health posed by antimicrobial resistance. The strategy gives a comprehensive



overview of the ongoing actions on surveillance, prevention, research and product development, as well as in international cooperation (EU, 2002b).

A conference in Brussels on 15–17 November 2001, hosted by the Belgian Council Presidency and endorsed by the European Commission, focused on current knowledge of antibiotic consumption in human medicine across Europe, the relation between antibiotics and resistance in the community and hospitals, and the determinants of antibiotic prescribing in primary care. The conference was also to initiate standardized and harmonized surveillance systems on antibiotic use in Europe (EU, 2001b).

The European Commission announced in its White Paper on Food Safety of 12 January 2000, its intention to pursue the prohibition or phasing-out of antibiotics used as growth promoters in the EU, depending on their potential use in human and veterinary medicine as part of its broad strategy to control and contain antibiotic resistance (SANCO, 2002b).

On 21 November 2002, David Byrne, the Commissioner responsible for Health and Consumer Protection, welcomed the European Parliament's adoption of the Keppelhoff-Wiechert Report on additives for use in animal nutrition. He hailed Parliament's vote as being an important step forward in the EU's drive to phase out antibiotics and other potentially harmful substances in animal feed. Strengthening rules on the safety of animal feed was one of the cornerstones of the EU's food safety strategy. Banning the use of antibiotics as growth promoters in feed was also vital to efforts to combat anti-microbial resistance (the phenomenon of antibiotic-resistant "superbugs"). The Council of Ministers was expected to respond rapidly to Parliament's First Reading and might agree a Common Position on the Regulation as early as the 16–19 December 2002 Agriculture Council (EU, 2002c).

The EU has already banned antibiotics used in human medicine from being added to animal feed. The new regulation would complete this ban on antibiotic growth promoters in feed by prohibiting the use of four drugs not used in human medicine that were still currently allowed in the EU. These antibiotics were monensin sodium, salinomycin, sodium avilamycin and flavophospholipol.

The EU Parliament voted on 21 November 2002 to back the Commission's proposal to phase out the last four antibiotics still used as growth promoters in animal feed, but was calling for the proposed deadline for withdrawing them to be brought forward by one year, to 1 January 2005, from the date of 1 January 2006 proposed by the Commission (EU, 2002c).

The Scientific Steering Committee of the EU recommended the progressive phasing out of the use of all antibiotics as growth promoters, while preserving animal health. This commitment is part of the Community Strategy to combat the threat to human, animal and plant health posed by anti-microbial resistance, which was adopted in June 2001 (EU, 2002b).

Under the regulation only additives that have been through an authorization procedure could be put on the market, used or processed. Authorizations would be for specific animal species and with a maximum dosage allowance. Authorizations for new feed additives would be for a ten-year period only. Companies marketing feed additives authorized under existing legislation would have to apply for re-evaluation and re-authorization within the next seven years. The new rules would require that companies demonstrate the additive's positive effect for the animal (efficacy) and the absence of risk for human health, animal health and the environment (safety). The European Food Safety Authority (EFSA) would be responsible for conducting these evaluations (EU, 2002b). The regulation covers all types of additives, not just antibiotic growth promoters. Companies marketing feed additives already authorized under existing legislation would need to notify EFSA accordingly (EU, 2002b).

After detecting chloramphenicol residues in fishery products from South-East Asia, a European Commission mission to China seemed to have identified a number

of serious dysfunctions as regards residue monitoring. Imports of products of animal origin from China were suspended in March 2002. At the request of several European Member States the question of the destination of positive-testing consignments were discussed in this respect, and the Commission and the Member States arrive at a common understanding, namely:

The Commission and the Member States agree that, following Article 5 of Council Regulation 2377/90/EC, the presence of chloramphenicol, at whatever limit, in foodstuffs of animal origin constitutes a hazard to the health of the consumer. Therefore all the consignments found positive to the presence of this antibiotic following a chemical test shall be destroyed in application of the Article 22.2 of the Council Directive 87/78/EC. (SANCO, 2001c).

As a consequence, an exchange of views and possible opinion of the Standing Committee on the Food Chain and Animal Health in Brussels, 19 March 2002, produced a Draft to deal with the problems of contamination for residues of veterinary medicines in certain products of animal origin imported from Myanmar and Viet Nam that might constitute a danger for animal or public health. The proposal concerns the presence of chloramphenicol and nitrofurans in shrimps imported from Myanmar. It was proposed that Member States carry out systematic control testing on all batches of these products to ensure their safety and notify the Commission of the results through the Rapid Alert System. The proposal received a unanimous vote (EU, 2002c).

In the United States of America, on 5–6 June 2002, a senior delegation of Chinese officials conferred with FDA to address the issue of chloramphenicol residues in shrimp and crayfish. The delegation informed FDA that on 5 March 2002, China had banned the use of chloramphenicol in animals and animal feeds. They also informed FDA that they were initiating testing of shrimp, crayfish and other animal-derived foods intended for export to ensure the absence of chloramphenicol and other drug residues. FDA and China exchanged information on testing methodologies. FDA informed the Chinese officials that the Agency would take enforcement action against products in violation of the requirements (FDA, 2002b). A range of measures aimed at correcting the identified weaknesses had in the meantime been adopted. These measures, together with guarantees provided by the Chinese authorities as regards the control and the production conditions of wild-caught fish, resulted in the gradual lifting of the import restrictions for certain fishery products. In view of subsequent information provided by the Chinese authorities, fishery products obtained by means other than aquaculture would be authorized. However, for eels and shrimps, the distinction between aquaculture and wild catches was not possible for the time being, except for catches of shrimps made in the Atlantic Ocean. Therefore the import of those products would remain prohibited.

Following the information provided by the Chinese authorities as regards the control and the production conditions of crayfish of the species *Procambarus clarkii* and surimi obtained from the fishery species already authorized for export, the European Commission proposed allowing the import of these products from China to resume. The import would be subject to intensified monitoring and 20 percent of consignments would be tested (EU, 2002a). In September 2002, after favourable results from the tests carried out on certain fishery products of some fish species imported from China, the European Commission proposed discontinuing the reinforced tests carried out on these species (SANCO, 2002c). On 20 September 2002, the Member States presented a Draft Decision repealing the protective measures adopted because of the presence of chloramphenicol and nitrofurans in fishery and aquaculture products imported from Viet Nam. The Commission explained that the Vietnamese competent authority has provided appropriate guarantees and that the results of the checks carried

out by Member States in shrimps imported from Viet Nam have been favourable (SANCO, 2002d).

For Thailand, the Commission provided detailed information about a joint EC/Thailand technical meeting held to discuss the issue of contamination with nitrofurans and chloramphenicol in shrimps and poultry products imported from Thailand. The objectives of the meeting were to review the current situation and move forward actions to control and prevent veterinary drug residues in the relevant products. The results of the meeting were intended to help restore normal trade relations between EU and Thailand as regards imports of poultry and shrimps. The minutes of the meeting were communicated to Member States' delegations. Following the meeting, the Commission received an action plan from the Thai authorities. The Commission indicated that this plan had to be assessed. The Commission also requested that the Member States inform the Office of Agriculture Affairs at the Thai Embassy, Brussels, of the confirmation of destroyed consignments (SANCO, 2002e, f).

After processing in HACCP-certified plants, the first batch of farmed shrimp carrying the new "Thai Quality Shrimp" label were reportedly soon to be shipped to France. Thailand hoped that if the shipment satisfied French health import requirements, exports to the EU could resume. Furthermore, the government wanted to see an end to the situation in EU ports where every shipment from Thailand was inspected for drug and chemical residues, preferring the previous system of random testing. During the first half of 2003, Thai shrimp exports to the EU were some 3 640 tonne, falls of 52 percent and 64 percent in terms of volume and value, respectively, compared with the same period of 2001 (Infofish, 2003b).

In Japan, with a view to improve consumer confidence in food safety, the Japanese Government proposed introduction of an overall policy on food safety. Under the proposed food safety law, a new food safety committee would be appointed to carry out scientific investigations on food additives and residual chemicals potentially harmful to human health, and to monitor government policies. The food safety committee would report its findings and advise the Ministry of Agriculture, Forestry and Fisheries, Japan (Infofish, 2003b). According to the bill, the government could take appropriate action without waiting for the results from the food safety committee's evaluation, which could be carried out in due course. The bill also indicated that the food processing industries, while entrusted with responsibility for guaranteeing food safety, are also required to provide accurate food safety information to consumers. The bill invited public comments to improve the government's understanding and handling of consumer concerns. The new bill represents a positive step towards building up a positive relationship between consumers and the food industry (Infofish, 2003b).

In the European Union, several Member States tabled a number of problems encountered with regard to the safeguard measures in relation to the findings of residues of nitrofurans and chloramphenicol in consignments of animal products from China and Brazil, particularly the problem of the absence of harmonization of detection and analysis methods for these residues in Member States and different approaches in Member States regarding the steps to be taken in the case of positive findings. Moreover, one delegation took the view that 100 percent checks of the consignments should be carried out by the third countries involved prior to export and that Member States should do only random checks. The Commission explained that the concept of official analytical methods had been superseded by the criteria approach, in which performance criteria and procedures for validation of methods are established (CEC, 2002). The Commission also made it clear that positive consignments have to be destroyed in accordance with Article 22 of Directive 97/78/EC (SANCO, 2002g).

This decision was modified on 13 March 2003 regarding the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin

(Decision 2003/181/EC of 13 March 2003 – see CEC, 2003b). According to this modification, it was necessary to provide for the progressive establishment of MRPLs of analytical methods for substances for which no permitted limit had been established, and in particular for those substances whose use was neither authorized nor specifically prohibited in the European Community, in order to ensure harmonized implementation of Directive 96/23/EC (CEC, 1996).

FDA prepared a draft guidance document entitled *Guidance for industry: evaluating the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern*. This draft guidance discussed a recommended approach for assessing the safety of antimicrobial new animal drugs, an approach that focused on the microbiological effects on bacteria of human health concern (FDA, 2002a). This draft guidance document discusses a recommended approach for assessing the antimicrobial resistance concerns as part of the overall pre-approval safety evaluation of new animal drugs. As draft guidance, the document represents the Agency's current thinking on a recommended approach for assessing the safety of antimicrobial new animal drugs with regard to their microbiological effects on bacteria of human health concern. An alternative approach may be used as long as it satisfies the requirements of applicable statutes and regulations. In particular, the draft guidance describes methodology that sponsors of new animal antimicrobial drug applications for food-producing animals may use to complete a qualitative antimicrobial resistance risk assessment. The draft guidance document outlines a process for integrating relevant information into an overall estimate of risk, and discusses possible risk management strategies. This guidance document was being distributed for comment purposes only (FDA, 2002a).

The thirteenth session of CCRVDF (CAC, 2003) agreed that a Drafting Group under the direction of the United States of America would further elaborate a proposed draft Code of Practice to Minimize and Contain Antimicrobial Resistance, for circulation, comment and further consideration at the fourteenth session of CCRVDF. The Committee also agreed that the JECFA Secretariat might be requested to provide specific input in this regard. The fiftieth session of the Executive Committee of the Codex Alimentarius Commission approved the elaboration of the Code of Practice as new work.

The delegation of the United States of America noted that the drafting group had identified the following issues that required the input of CCRVDF:

- definitions for “non-therapeutic” and “therapeutic”;
- establishing the criteria or definition, or both, of a “critical human disease” and “drugs of importance to human medical therapy”;
- environmental concerns; and
- determination of the concentration of active compound in the gut of the animal at the defined dosage level.

The EC supported the decision of the drafting group to refer to CCRVDF the matters of definitions of non-therapeutic and therapeutic use, as well as criteria to be applied for the definition of a critical human disease and drugs of importance to human medicinal therapy.

In addition, the EC recommended restricting the use of antimicrobials to therapeutic use, and therefore that they should in principle only be available on prescription (EC 2002, 2003).

#### **4.1.2 Laboratory methods for the detection and quantification of antimicrobial resistance**

Historically, veterinarians and medical practitioners have selected antimicrobials for therapeutic use based on their clinical experiences. The rapid increase and dissemination of antimicrobial resistance among many bacterial pathogens has reduced the success of

this practice. As a consequence, it is now a current practice to employ laboratory *in vitro* antimicrobial susceptibility testing (AST) of the relevant bacterial pathogens from properly collected specimens. AST was initiated globally after the introduction of antimicrobial therapy, driven by the need to identify the appropriate antimicrobials for clinical use. The need for laboratory reproducibility of AST methods appeared in order to ensure that data generated was accurate and consistent. This required that AST laboratories adopt quality control measures to assure the reporting of reliable and reproducible susceptibility data.

The *in vitro* AST test is performed to predict how a bacterial pathogen will respond to the antimicrobial agent *in vivo*. A “resistant culture” implies that the bacterium will not respond to a treatment with this particular agent. “Susceptible” means that the antimicrobial will be successful in treating the bacterial infection. “Intermediate” implies that the antimicrobial agent may be effective against this bacterium if high levels of this agent can be achieved at the infection site. It is essential that the bacteria subjected to AST have been isolated in pure culture from the submitted sample. The isolation and identification procedures have to be standardized to assure that the subject bacterium is consistently and correctly identified to the genus or species level. Once isolated in pure culture, it is necessary that the inoculum be standardized to obtain accurate susceptibility results. The selection of the appropriate AST methodology may be based on considerations that include ease of performance, flexibility, adaptability to automated or semi automated systems, costs, reproducibility, reliability and accuracy. Nevertheless, there are three primary methods that fulfil these requirements: broth dilution; agar dilution; and disk diffusion.

- For the broth dilution method, a standardized suspension of bacteria is tested against varying concentrations of an antimicrobial agent in a standardized liquid medium. The aim of the method is to determine the lowest concentration of the assayed antimicrobial that inhibits the growth of the tested bacterium (MIC). Results are usually expressed in micrograms per millilitre ( $\mu\text{g}/\text{ml}$ ). This method can be performed either in tubes (macro-dilution) or in microtitration plates (micro-dilution). In the latter case, microtitration plates containing prediluted antibiotics are commercially available. This procedure has the additional advantage of eliminating potential errors that may arise due to the preparation and dilution of the antimicrobials in participating laboratories. Nevertheless, it can be costly and may not be the choice for laboratories with limited budgets.
- The agar dilution technique implies the incorporation of an antimicrobial into an agar medium in a concentration pathway with a geometrical progression of concentrations, followed by the application of a defined bacterial inoculum to the plate surface. This procedure can also be adapted to semi-automation. Agar dilution is usually referred to as the “defined standard” of AST. Nevertheless, it is expensive, labour intensive and requires extensive personnel training.
- The disk diffusion method involves the diffusion of an antimicrobial agent of known concentration from disks, tablets or strips into solid culture media inoculated with a standardized bacterial culture. The diffusion of antimicrobial agent into the culture media results in an antimicrobial gradient, which produces inhibition zones, having diameters that correlate with MICs for that particular combination of bacterium and antimicrobial agent. This method is easy to perform, reproducible and does not require expensive equipment. Given its many advantages, this could be the method of choice, but there are some aspects of this method that require standardization: the agar medium used, inoculum preparation (culture density), concentration of the antimicrobial tested in the carrier (disk, strip, tablet), growth and incubation conditions (pH,  $\text{O}_2$ ) and the interpretative criteria.

Traditionally, most laboratories have used disk diffusion methods. Reported results can be quantitative if zone diameters are recorded, resulting in bacterial MICs, but very often they are qualitatively reported as either susceptible, intermediate or resistant.

Regardless of the AST method used, appropriate quality control reference organisms need to be tested every time AST is performed to ensure accuracy of the data obtained (NCCLS, 1997, 1998, 1999).

Typically, laboratories have been restricted to reporting bacterial AST data as susceptible, intermediate or resistant. Such reports are primarily for the immediate needs of physicians and veterinarians as guidelines for appropriate antimicrobial therapies. Quantitatively reported data is required for epidemiological surveillance purposes, which allows the detection of shifts in antimicrobial susceptibility in bacterial strains, and for comparing this information with other surveillance programmes.

Some international networks have been created for the surveillance of antimicrobial resistance in veterinary and human medicine. To obtain comparable antimicrobial susceptibility data from different laboratories in the same country, or in different countries, laboratory methodologies need to be standardized and harmonized. This has resulted in the standardization and harmonization of AST methods among participating laboratories; they should adhere to strict standards of AST and quality control monitoring to ensure accuracy and comparability of the obtained data.

Unfortunately, currently there is no worldwide consensus on interpretative criteria for susceptibility testing. Additionally, a surveillance programme is required to monitor shifts in antimicrobial susceptibilities in target bacterial pathogens.

Given the lack of standardization and harmonization in the methodology used in different countries, it is difficult to compare susceptibility data among countries. To determine the comparability of results originating from different surveillance systems on a worldwide scale, AST tests results must be reported quantitatively, including information on the methods, quality control of micro-organisms and ranges of the antimicrobial tested. MIC values or diameter zones should be the desirable outcome of an AST test to enable determination of shifts in antimicrobial susceptibility among the target bacterial pathogens.

In the United States of America, all methods used to evaluate antimicrobial susceptibility should be of reference quality and should closely follow the documents published by NCCLS (1997, 1998, 1999).

#### **4.1.3 Methods of analysis and sampling for antibiotics residues**

The EC distributed a provisional list of National Reference Laboratories (NRLs) for the detection of residues, and encouraged States Members to comply with Directive 96/23/EC (SANCO, 2001b).

Recently, the laboratory of Fougères (France) has been named as the Community Reference Laboratory (CRL) for antimicrobial residues in food (EU, 2003b). The laboratory had been approved as reference laboratory for antibacterial substances, including sulphonamides and quinolones, carbadox and olaquinox, chloramphenicol, dapsone and nitrofurans (EU, 2002c). The European Union established work programmes for CRLs with the following objectives: to develop new analytical methods and validate them to be used as a reference, to assist NRLs by helping them to implement quality assurance systems; to provide technical advice, training courses; and comparative tests; to identify residues in case of disagreement between member States; and to provide the European Commission with technical and scientific advice (EU, 2002d). Directive 96/23/EC established the number of official samples to be taken in relation to the number of animals slaughtered of the species concerned, and details of the rules governing the collection of official samples to determine residues of veterinary drugs and contaminants such as antibacterial substances, including sulphonamides and quinolones (EC, 1996).

According to the Codex Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drug Residues in Foods (CCFH, 1993), two types of samples have to be considered:

- Samples that are taken at random from food considered safe. In this case, it is not necessary to retain these products while waiting for the results; the sampling plan has been predetermined according to a statistical procedure to ensure that samples are representative. The results obtained may be used to certify that exported food products are in compliance with Codex MRLs.
- When the food products are suspected of having residue concentrations that exceed the MRL, direct sampling is pursued. The product is detained while waiting for the results of laboratory testing, and is not released for human consumption unless the results are favourable.

The number of samples to be taken during the year for direct sampling may not, by definition, be predetermined. The results of direct sampling are not statistically representative. As an initial step, a screening method has to be considered. This kind of method should not require investment in complex laboratory instrumentation nor in costly reagents or operator training. These screening methods may be qualitative or semi-quantitative methods of analysis that detect the presence of a substance at a concentration that is equal to or higher than the MRL. For example, a series of tests have been proposed for screening purposes in bulk tank milk (Table 4.1).

TABLE 4.1

**Tests proposed for screening purposes in bovine milk in bulk tanks.**

Test kit name	Manufacturer	Analyte
SNAP Beta-lactam™ Test kit	IDEXX Laboratories Inc.	Ampicillin, cephalosporin, ceftiofur, penicillin
Delvotest™ P	DSM Foods	Amoxicillin, ampicillin, cephalosporin, ceftiofur, penicillin
Delvotest™ SP	DSM Foods	Amoxicillin, ampicillin, cephalosporin, ceftiofur, penicillin
Penzyme™ milk	Cultor Food Science	Amoxicillin, ampicillin, cephalosporin, penicillin

Source: AOAC International, 2000.

In the same way, a simple yoghurt culture test for antibiotic residues detection in farm milk is described by Yamani *et al.* (1999), and Delwiche *et al.* (2000) developed an enzyme immunoassay to detect penicillin residues in meat and dairy products.

Screening tests allow a high sample throughput and a high number of samples to be analysed in a relatively short time, and they are designed to minimize the number of false negatives. When a positive is found by a screening test, a confirmatory test is carried out, which normally involves a more sophisticated testing method that provides full or complementary information, enabling the substance to be identified precisely. These tests are intended to keep the number of false positives as low as possible.

In the case of inhibitors, the screening analysis is based on microbiological tests, whereby the sample is cultivated in different bacterial media. If, after the incubation period, the sample has inhibited the growth of the bacteria, it is considered to be positive, but the specific substance is not identified. Given that this is a qualitative analytical method, a misinterpretation of the results cannot be ruled out, and some false positives always occur. Chemical analysis provides information on the specific substance present in the sample.

In some cases, a positive result in a microbiological test is sufficient to reject the sample. This may mean that no confirmation by a physico-chemical method is carried out and there is thus no conclusive identification of the substance concerned. In other cases, a positive result in the screening test is confirmed by means of a physico-

chemical test, and it is then possible to identify the substance and establish whether its concentration is above the MRL. Another possibility is to analyse directly using a physico-chemical test (e.g. sulphonamides analysis) (SANCO, 2002b).

When necessary, a proper verification procedure through a HACCP Programme is required to assure the results are compatible with the MRL and, if necessary, to take appropriate corrective actions.

Early in 2002, sub-part-per-billion (ppb) amounts of chloramphenicol (CAP) were discovered by the EU in shrimp and honey imported from China. In the United States of America, with the purpose of providing Federal and State laboratories with validated screening methods for CAP, the FDA evaluated two commercial screening tests for CAP: the Charm II test and the RIDASCREEN test in shrimp and honey. Both tests were capable of detecting CAP at 0.3 ppb in shrimp; however, both tests exhibited a positive bias for negative samples. Nevertheless, for both kits, they were able to develop alternative protocols for data interpretation that would give a significantly lower percentage of false positive results while maintaining the ability to detect samples containing 0.3 ppb CAP in shrimp (Kijak *et al.*, 2003).

The Florida Department of Agriculture and Consumer Services and the Canadian Food Inspection Agency had recently developed appropriate liquid chromatography–mass spectrometry methods for shrimp and honey, respectively, which they shared with the FDA (Neuhaus, Hurlbut and Hammack, 2002). Recently, the FDA Pacific Regional North West Laboratory (PRL-NW) was charged with testing the performance of the abovementioned method developed at the Chemical Residue Lab of the Florida Department of Agriculture. This is a liquid chromatography–mass spectrometry method for qualitative and quantitative detection of CAP in shrimp at concentrations down to less than 1 ppb (the limit of detection (LOD) was estimated to be 0.08 ppb) (Neuhaus, Hurlbut and Hammack, 2002).

FDA validated Florida's method to confirm 0.1 ppb CAP in shrimp and crabmeat. FDA's existing shrimp method (LIB 4284) was adapted for analysis, lowering its limit of confirmation from 1 ppb to 0.1 ppb, and the modified method validated (Carson *et al.*, 2003).

According to the Codex Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drug Residues (CCFH, 1993), methods that are suitable for determining compliance with MRLs are those that have successfully completed an extensive, multi-laboratory study for specific tissue and species combination. In some cases, these methods may be considered reference methods, but reference methods frequently are not routine methods. Methods not validated by traditional interlaboratory study but providing results that may be compared and correlated to those obtained from validated methods or collaborative study, may serve regulatory purposes.

## 4.2 AT INDUSTRIAL LEVEL

### 4.2.1 Approaches to minimizing antibiotics use in food-animal production

#### 4.2.1.1 *As growth promoters (feed supplementation)*

There are some options that might be considered as alternatives to the use of antibiotics as growth promoter factors. The potential of some of these options represents a new area that requires further research.

#### *Enzymes and probiotics*

This approach aims to obtain a situation where a greater share of the nutrients supplied in the feed is made available for absorption by the animal. Improvement in feed efficiency has been associated with antibacterial feed additive (AFA) use (probiotics).



The amounts of nutrients excreted in faeces and urine are lowered in proportion to the decreased amount of feed consumed by the animals. Alternatively, some dietary manipulations can also reduce nitrogen excretion while maintaining maximum growth (Henry, 1996).

Appropriate feeding strategies will not only affect nitrogen excretion but also reduce pollution by other substances, such as phosphorus and trace elements. Animal feed with a protein content of 14 percent with added amino acids will result in similar or smaller nitrogen excretion per animal than feed with 18 percent protein content supplemented with AFA (Verbeke and Viaene, 1996).

Supplementation of feed with enzymes and probiotics may also aim at making more available the nutrients supplied in the diet.

As a consequence of the antibiotics ban in Sweden, the protein content in poultry and pig feed was reduced. This was possible since several crystalline amino acids are available at competitive prices. The alteration in feed composition primarily aimed at avoiding intestinal disturbances, but benefits in terms of nitrogen excretion were also observed (SOU, 1997). The Swedish experience from banning antibacterial growth promoters in 1986 was the emergence of clinical problems and health disturbances in the health status in piglets and broilers, which initially created a bigger demand for antibiotics at therapeutic dosages. This situation was corrected after a change in feed composition, hygiene improvement, and a change in rearing strategies (SOU, 1997).

### *Organic acids*

These products are widely distributed in plants and animals and are also fermentation products, and their salts are often used as food preservatives and to acidify feeds. At low pH (around 3.5), digestion of proteins and the population of beneficial bacteria (*Lactobacilli*) are maximized, and harmful bacteria are inhibited.

This is another approach that has been used to replace antibiotics as growth promoters. A greater weight gain was observed when fumaric acid was used to supplement piglet feed. The possible mechanism of growth promotion includes inhibition of undesirable microflora, increased digestibility of proteins and changes in the intestinal morphology.

Recent data indicate improved feed conversion ratios and growth-promoting effects of formates, citric acid and formic acid, showing that the effect was greater during growth of young pigs, especially in the finishing phase of growth. In addition, organic acids may improve the digestibility and absorption of proteins, minerals and other nutrients in the diet. Organic acids are commonly added to swine feed in many European countries, and their use has increased with the reduction in use of antibiotics (Doyle, 2001).

A Draft Regulation provisionally authorizing the new additive potassium diformate was unanimously approved as a growth promoter in Europe (SANCO, 2001b).

### *Minerals*

The use of zinc oxide in Denmark has led to decreased use of antibiotics in swine feed. Zinc improves pig performance and reduces incidence and severity of diarrhoea in piglets.

Rare earth elements have been used in China as feed additives. Recently, a rare earth mixture containing lanthanum, cerium and praseodymium was tested in swine, significantly improving weight gain and feed conversion (He and Rambeck, 2000).

### *Conjugated linoleic acid*

Conjugated linoleic acid (CLA) comprises a mixture of positional and geometric isomers of linoleic acid, with conjugated double bonds in the region of carbon atoms

8–13. With pigs fed up to 1 percent CLA in the diet, small improvements were seen in average daily weight gain and feed efficiency (Jahreis *et al.*, 2000).

### *Phospholipids*

It has recently been reported that lysoforte, a phospholipid, can aid in nutrient uptake from the digestive tract, significantly improving growth and feed conversion in piglets (Doyle, 2001).

### *Seaweed extracts*

Research work carried out by Texas Technical University, in conjunction with Mississippi State University, and Virginia Polytechnic Institute and State University, shows that cattle feed containing forage treated with, or directly fed, seaweed meal and extract showed marked positive effects on the animal's immune system, weight gain, carcass quality and even shelf life of finished meats. Researchers Allen, Thornton and Pond explained that applying a specific seaweed, *Ascophyllum nodosum*, as an extract to the pasture or to an animal's forage, or by directly feeding the extract or the seaweed meal to animals, enhanced their immune system and their ability to fight disease. The effects of the seaweed seen in the finished beef product include a more desirable colour, improved uniformity, decreased browning and less discoloration. They considered this research to be applicable to other species (Science Daily Magazine, 1999).

### *Immunologically-active compounds*

Some of the growth-promoting effects of the subtherapeutic use of antibiotics in feeds may result from their action against subclinical infections or competitive intestinal bacteria. Several immunologically-active compounds, such as antibodies, cytokines, spray-dried plasma and freeze-dried eggs, have been shown to affect the immune response and may enhance resistance to disease. For this reason, it has been suggested that the addition of these immunoactive compounds to feed may accomplish the same purpose.

#### *4.2.1.2 As therapeutic agents*

A series of studies have aimed to improve the therapeutic arsenal.

### *Application of genomics and bio-informatics to the development of new antimicrobials*

Recent advances in genomics have made an important contribution to drug design. The knowledge of genes and the synthesis of their proteins has allowed geneticists and chemists to use this information against bacterial resistance. Many of the known targets of antibiotics are essential genes. However, the proteins encoded by essential genes are not the only molecular-level targets that can lead antibiotic development.

Genes that encode for virulence factors are also important. Virulence factors are those responsible for overcoming the host's immune response, allowing bacteria to colonize. The host response used to make it difficult to identify these genes, especially because the events taking place during the immune response were difficult to reproduce in the laboratory. A new technique, known as *in vivo* expression technology (IVET), can insert a unique sequence of DNA – a form of tag that deactivates a gene – into each bacterial gene. Target bacteria are then used to infect an organism. The bacteria are later recovered and the tags identified. When a tag disappears, this means that the genes they were attached to were essential for the bacteria's survival, and the bacteria could not survive in the host without these genes.

Researchers expected that by identifying and inhibiting these virulence factors, they might aid the body's immune system in its fight against bacteria. In addition, this kind

of research is discovering which genes confer antibiotic resistance. This approach could rejuvenate previously ineffective antibiotics.

The standard process would be that whenever a medical need is identified, resistant bacteria DNA sequences are obtained, targets are selected, their essentiality determined and an assay developed. All the potential available targets can be evaluated almost simultaneously and target selection is determined by relationships among genomes. Some pharmaceutical companies are currently using this approach to develop new antibiotic targets.

Once the medical necessity is defined, such as the ability to deal with antibiotic-resistant Gram-positive bacteria, whole or partial DNA sequences are obtained from public and private sources and bio-informatics used to select potential targets. These can be of broad or narrow spectrum, or they may be organism-specific. In all cases, selection entails the application of a set of selection criteria and a process of comparison. *S. aureus* might be compared with mammalian, yeast or other bacterial databases to identify which genes are shared, leaving genes that are specific or unique to this micro-organism as a main focus of interest.

More specifically, *S. aureus* tRNA synthetases might be screened against different databases to identify synthetases that have low or no homology and are therefore more likely to be good targets. The next step is to determine whether the targets selected are essential for the micro-organism's growth under different conditions, e.g. minimal or rich media; different pH or temperature, *in vivo* conditions.

In essence, determinations may be made using gene knockouts, employing genomic footprint methods or preparing temperature-sensitive mutants, the two last methods being relatively rapid. The assays may be cell-free genetic assays based on phenotype; enzymatic assays; or binding assays. Since, even after the selection and essential determination, the selection of potential targets may be a big process, so pharmaceutical companies are interested in high-throughput methods that simultaneously permit assessing a number of targets.

The most important step in target evaluation is the screening for inhibitors of the gene targets. Finding such inhibitors requires screening from hundreds of thousand to millions of compounds, but fortunately the very large natural product, compound and combinatorial libraries required for this phase are available and continue to grow (IOM, 1998).

### **Immune modulators**

Recently, using a modern immunochemical technique, a new approach to fighting resistant bacteria has been proposed. Gupta (2001), at the Los Alamos National Laboratory, in New Mexico, United States of America, probed a new mechanism to distract the regular immune response against the "super antigens" developed by some bacteria such as *S. aureus*, responsible for food poisoning, toxic shock syndrome and many hospital infections.

Some strains of this bacterium are able to produce "super antigens" that can trigger an uncontrolled immune reaction, making the body attack itself. Gupta also developed a new strategy to try to stop the superantigen from binding to the cells responsible for the immune response. First, the parts of the gene for the super antigen that enable the toxin to bind to human cells were identified. Then those sequences were linked to make an artificial gene that was inserted in an *E. coli*. The *E. coli* then churned out the decoy protein. Some tests on human blood had shown that the decoy protein binds to the antigen-presenting cells and T-cells, like the super antigen, but, because the decoy complex has a different shape, it is not able to trigger a runaway immune reaction. Gupta thinks the decoy may also act against superantigens produced by other strains, such as methicillin-resistant *S. aureus*. This approach has the advantage that, as decoy

molecule targets human cells, the bacteria cannot develop any mechanism against it. Nevertheless, the problem might be getting the decoys to the right place in the body (Gupta, 2001).

### Genetic switch

Another advance was made by Levy at Tufts University in Boston (2001) and colleagues, working on a genetic switch in *E. coli*. The switch was originally named *mar* because it induced multiple antibiotic resistance, but this gene not only defeats antibiotics, it controls over 60 bacterial genes, including the ones coding for virulence. *mar* is found in many bacteria including *Salmonella* and *Shigella*. Levy and colleagues found small molecules that turn *mar* off. If these molecules are administered to a patient with an infection, they should make the bacteria harmless.

At the same time, Heithoff at the University of Santa Barbara, California (2001), discovered the enzyme called DNA adenine-methylase (Dam), which chemically alters genes or methylates genes and could control over 200 virulence genes in the food-poisoning *Salmonella*. As with *mar*, drugs that block Dam should disarm the bacteria. The researchers have created mutating bacteria in which Dam is either permanently inert or overproduced and they make very effective live vaccines in that way, having the advantage that the Dam vaccine protects against many strains since the mutant bacteria manufactures many different proteins that the immune system can target. Dam is found in many other bacteria, including *E. coli* 0157:H7 and the cholera bacterium, suggesting Dam vaccines should work for a wide range of bacteria.

Genetically engineered viruses may also be used against bacteria. Viruses that normally infect bacteria – bacteriophages – are harmless to humans. They only act against specific bacteria, into which they injected their genetic material, causing the cells to produce more viruses. The infected cell may burst releasing the new viruses. Phages had been used for many years to treat infectious diseases in humans, animals and plants. The technique has the disadvantage that natural viruses have evolved into replicating themselves, rather than killing cells. A new technique, used in genetic therapy, has been adapted to deliver a lethal message to disease-causing bacteria in such a way that the phage may inject altered DNA into the bacteria cell and this DNA codes for a protein that kills the bacteria (Schofield, 2001).

### New drugs development

WHO is actively promoting joint funding for research into new drugs, vaccines and diagnostic procedures, in addition to the development of ethical treatment guidelines, with input from both the private and public sectors (WHO, 2000b).

Recently, FDA approved a new antibiotic, for the first time in 35 years. The antibiotic is highly effective in the treatment of infections by Gram-positive bacteria in both adults and children. The new drug, Zyvox, an oxazolidinone of the class linezolid, blocks bacterial growth by disrupting the organism's protein synthesis, and is more effective than vancomycin, offering clinicians one more line of defence against such dangerous infections (APUA, 2001).

Nevertheless, the overuse or misuse of these drugs may lead to the development of resistance against them, making them worthless in the therapeutical arsenal.

#### 4.2.1.3 As prophylactic agents

In aquaculture, antibiotics have been misused as prophylactic agents. To prevent resistance problems, the aim must be to reduce antibiotic use to therapeutic purposes only. Whether a fish becomes diseased when a pathogen is present depends on factors that include fish health, water quality, and temperature, stocking density, pathogen load, vaccination status, handling practices, uniformity of grade, and proximity of neighbouring farms that may experience different disease threats.

Most of the infectious diseases reported in crustaceans refer to penaeid shrimp because they are subject to intensive aquaculture production. The main causative agents are viruses, fungi and bacteria. Viral diseases in crustaceans could be responsible for serious enzooties (or massive pandemics) in shrimp-farming countries. The principal viral agents known belong to the families of (i) *Parvo-like-viridae*, including Infectious Hypodermal and Haematopoietic Necrosis Virus; (ii) *Picornaviridae*, with the Taura Syndrome Virus, or the Yellow Head Virus group, which affects the Indo-Pacific shrimp industry; and (iii) *Baculoviridae*, the most important group of viruses affecting various decapod crustaceans and arthropods.

A number of preventive approaches are available to reduce the use of antibiotics for prophylactic purposes.

### *Hygienic procedures*

It is important to note that good aquaculture management practices are essential to maintain a healthy environment for farmed finfish and crustaceans. Priority should be given to good hygiene and other preventive efforts in containment of resistant infections (EU, 1998). As mentioned earlier, the most common diseases in aquaculture are infectious diseases, with various causative organisms. The use of antibiotics as prophylactic agents could be reduced, stressing hygiene measures, with proper handling practices. These practices will help prevent infectious diseases, including those of viral origin, which cannot be treated with antibiotics.

OIE (2001) established a series of hygiene measures available for aquaculture farmers. These measures are considered below.

### *Disinfecting of fish eggs with iodine*

This substance is generally effective for decontamination of egg surfaces, but the use of iodophor disinfectants cannot be relied upon to prevent vertical transmission of some bacteria, such as *Renibacterium salmoninarum*, and viral pathogens, such as infectious pancreatic necrosis virus, which may be present inside the egg. The pH of the iodophor solution must be between 6 and 8. At below pH 6, the toxicity for eggs increases, and at pH 8 or more, the antiseptic capacity decreases. Eggs must be rinsed in fresh water before and after disinfecting, or the iodine has to be neutralized with sodium thiosulphate. The disinfectant solution must be prepared using water free of organic matter. The solution has to be replaced when it becomes pale yellow, and before the colour disappears.

One litre of solution at a concentration of 100 mg per litre of disinfectant is indicated to disinfect 2 000 salmonid eggs. In the case of eggs that have to be transported, the packaging has to be disinfected as well, or destroyed in a manner that will not pose a contamination or health risk to water or other living organisms at the end destination. Disinfecting eggs with iodine can be carried out for various fish species, but it is most commonly used for Salmonidae. For other species, preliminary tests should be conducted to determine safer concentrations.

### *Efficiency limits using iodophors*

This procedure – using iodophors for disinfection – is ineffective when trying to avoid vertical transmission of infectious pancreatic necrosis, renibacteriosis and even infectious haematopoietic necrosis, for which this method was recommended initially. Several epidemiological surveys and laboratory tests have proven the ineffectiveness of iodine.

### *Neutralization of halogens (iodine and chlorine)*

As these agents are highly toxic for aquatic animals, it is necessary to prevent serious accidents that could result from a manipulation error; for this reason it is recommended

that this product be neutralized with sodium thiosulphate. The amounts to be used should be:

To neutralize chlorine:

Number of grams of thiosulphate to use =  $2.85 \times$  number of grams of chlorine.

To neutralize iodine:

Number of grams of thiosulphate =  $0.78 \times$  number of grams of iodine.

It is also possible to prepare a thiosulphate solution at 1 percent by weight, in which case the neutralizing volumes will be:

For chlorine: Volume of thiosulphate to use =  $28.5 \times$  [number of litres of the disinfecting solution  $\times$  concentration (mg/litre)] / 100.

For iodine: Volume of thiosulphate to use =  $7.8 \times$  [number of litres of the disinfecting solution  $\times$  concentration (mg/litre)] / 100.

### *Disinfecting fish farms*

The choice of substances and disinfecting procedures depends upon size, type and nature of the materials and sites to be disinfected. Tables 4.2 and 4.3 indicate some applicable methods based on various criteria. Chlorine and iodine have to be neutralized according to the procedures mentioned above.

### *Disinfecting mollusc farms*

This involves the application of chemical treatments in sufficient concentrations and for sufficient periods to kill pathogenic micro-organisms. Because of the inherent toxicity of disinfectants, disinfection can only reasonably be applied to hatcheries and tank holding facilities, as it is forbidden in open water or open-water systems and, as a rule, all disinfectants must be neutralized before release into the surrounding environments (OIE, 2001).

As mollusc farms are generally seawater-based, compounds produced during seawater disinfection (residual oxidants) must also be disposed of carefully.

### *Disinfecting eggs and larval stages*

This procedure is not considered practical for most molluscan systems. Besides, there is little information about specific disinfection procedures for pathogens of molluscs (i.e., *Marteilia* spp., *Haplosporidium* spp., *Bonamia* spp., *Perkinsus* spp., iridovirus and pathogen levels of marine microbes) or seawater. For this reason, disinfectants and concentrations are based on related pathogens or seawater sterilization.

*Marteilia refringens* and *Bonamia ostrea* are serious parasitic infections that have been studied by the Institut Français de Recherche pour l'exploitation de la mer (IFREMER, 2000) for several years. The pathogens have caused extensive economic losses in French production. *Marteilia refringens* is responsible for destroying the oysters' digestive system. *Bonamia ostrea* is an ulcerative disease with a high mortality rate. Due to high mortality rates for these diseases and the impossibility of applying a treatment, the most important measure is prevention, using good hygienic practices and a suitable culture density (Castillo, 1996).

According to OIE (2001), three stages of disinfection are possible in a hatchery:

- pre-treatment, using filters for the incoming water (1.0 and 0.22  $\mu$ m) or chemical disinfection;
- treatment within the facilities (especially recycling systems); and
- treatment of the effluent water in order to protect the environment.

Routine disinfection of pipelines and tanks is highly recommended; the frequency will vary according to stock turnover. High concentrations of molluscs should be rotated between disinfected tanks as often as practical or kept in seawater that has been disinfected with ozone or chlorine and subsequently neutralized, or a

combination. Each new batch of molluscs introduced into a facility should be placed in pre-disinfected tanks. Filtering all the incoming water is advised due to the presence of organic matter that could reduce disinfection capacity. All the surfaces must be thoroughly cleaned before disinfection. The detergent used must be compatible with the disinfectant used and both must be compatible with the surface on which they will be utilized. Regular air- or heat-drying of pipelines (daily), tanks and other equipment, in addition to disinfection of surfaces, is also recommended.

The following management practices (OIE, 2001) can be used to reduce opportunistic pathogen proliferation within a mollusc hatchery or holding facility:

- maintain pathogen-free algal stocks and cultures;
- use appropriate water filtration; regular disinfection of tanks, pipes, equipment and footbaths; and water changes;
- isolate infected stocks and associated equipment at the first sign of disease;
- discard infected stocks and sterilize equipment; and
- identify any source of infection within the holding facility to prevent further infection (algal stocks, seawater influent system, broodstock, larval stock).

### *Disinfection of crustaceans farms*

OIE (2001) proposes the following disinfection programmes.

- **Nauplii** Nauplii are much easier to collect than fertilized eggs in the hatcheries. Collect nauplii using a plankton net → running sea water for 1–2 minutes → formalin (400 ppm) for 30 seconds to 1 minute → iodophor (0.1 ppm iodine) for 1 minute → running seawater for 3–5 minutes → hatchery ponds.
- **Fertilized eggs** Fertilized eggs are more sensitive than *nauplii* to formalin. Collect fertilized eggs → running seawater for 1–2 minutes → formalin (100 ppm) for 1 minute → iodophor (0.1 ppm iodine) for 1 minute → running seawater for 3–5 minutes → hatchery ponds.

Prevention of infection by infectious hypodermal and haematopoietic necrosis virus may be achieved by using specific-pathogen-free crustacean populations. Although this approach has proven effective, it is still at an experimental stage.

TABLE 4.2  
Disinfection of fish farms – physical methods

Process	Indications	Method to use	Comments
Desiccation, light	Fish pathogens on earthen bottoms	Dry for three months at an average temperature of 18 °C or above	Drying period can be reduced by the use of a chemical disinfectant
Dry heat	Fish pathogens on concrete, stone, steel or ceramic surfaces	Flame-thrower; blowlamp	
Damp heat	Fish pathogens in transportation vehicle tanks	Steam at 100 °C or more for 5 minutes	
Ultra-violet rays	Viruses and bacteria	10 mJ/cm <sup>2</sup>	Minimum lethal dose
Ultra-violet rays	Infectious pancreatic necrosis (IPN) and nodavirus (VNN/VER) <sup>(1)</sup> in water	125–200 mJ/cm <sup>2</sup>	
Ultra-violet rays	<i>Myxosporidium</i> spores in water	35 mJ/cm <sup>2</sup>	

NOTES: (1) VNN/VER = Viral nervous necrosis/viral encephalopathy and retinopathy.  
Source: International Aquatic Animal Health Code. (Updated: 05/07/2001) (OIE, 2001).

TABLE 4.3  
Disinfection of fish farms – chemical methods

Agent	Indications	Concentration and method of use <sup>(1)</sup>	Comments
Quaternary ammonia	Virus, bacteria, hands	1 mg/litre for 1 minute	IPN virus is resistant
Quaternary ammonia	Gill bacteria, plastic surfaces	2 mg/litre for 15 minutes	
Calcium oxide <sup>(2)</sup>	Fish pathogens on dried earth base	500 g/m <sup>2</sup> for 4 weeks	Replace in water and empty disinfectant pools keeping the effluents at pH <8.5
Calcium hypochlorite <sup>(2)</sup>	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/litre left to inactivate for several days	Can be neutralized with sodium thiosulphate
Calcium cyanamide <sup>(2)</sup>	Spores on earthen bottoms	300 kg/ha on dry surfaces; leave in contact for 1 month	
Formalin	Fish pathogens in sealed premises	Released from formogenic substances. Comply with safety instructions	
Iodine (iodophors)	Bacteria, viruses		See special recommendations
Iodine (iodophors)	Hands, smooth surfaces	>200 mg iodine/litre for a few seconds	
Iodine (iodophors)	Eyed eggs	100 mg iodine/litre for 10 minutes	
Iodine (iodophors)	Gametes during fertilization	25 mg iodine/litre for several hours	
Iodine (iodophors)	Nets, boots and clothing	200 mg iodine/litre	
Ozone	Sterilization of water, fish pathogens	0.2-1 mg/litre for 3 minutes	Costly
Sodium hydroxide <sup>(2)</sup>	Fish pathogens on resistant surfaces with cracks	Mixture: Sodium hydroxide, 100 g Teepol, 10 g Calcium hydroxide, 500 g Water, 10 litre Spray: 1 litre/10 m <sup>2</sup> Leave for 48 hours	The most active disinfectant sodium (hydroxide) stains the surfaces treated; Teepol is a commercial tensioactive agent. Water to be turned on, pH to be checked.
Sodium hypochlorite <sup>(2)</sup>	Bacteria and viruses on all clean surfaces and in water	30 mg available chlorine/litre. Leave to inactivate for a few days or neutralize with sodium thiosulphate after 3 hours	
Sodium hypochlorite <sup>(2)</sup>	Nets, boots and clothing	200 mg available chlorine/litre for several minutes	
Sodium hypochlorite <sup>(2)</sup>	Hands	Rinse with clear water or neutralize with sodium thiosulphate	

NOTES: (1) The concentrations indicated are those for the active substance. The chemicals must be approved for the prescribed use and used according to the manufacturer's specifications.

(2) Dangerous. See precautions indicated in the OIE general recommendations.

Source: International Aquatic Animal Health Code (Updated: 05/07/2001) (OIE, 2001).

### Good practice in the use of veterinary drugs

In the United States of America, drugs used in aquaculture have to be approved by FDA and must be specifically approved for the selected use. Approved new animal drugs may be mixed in feeds only for uses and doses approved under FDA medicated-feed regulations. Besides fulfilling the abovementioned regulations, record-keeping is advised for any aquaculture activity and is a critical element in quality assurance programmes. This tool helps producers to keep track of the treatment employed, results obtained and the specific water and land involved. In this way, the treatment status of animals, ponds and other areas are known at all times. The records are needed to determine dosage rates and certify withdrawal times, and, at the same time, records



will help processors to demonstrate that all drugs and chemicals have been used properly (USDA, 1994).

Product withdrawal times must be observed to ensure that any product used for aquatic sites or for animals does not exceed legal tolerance levels in the animal tissue. In this way, products reaching consumers are safe and wholesome.

Antibiotics should be stored in their original container with the original label attached, at the temperature recommended on the label. These compounds should be stored away from bright light, because light may cause inactivation or deterioration of the product. Drugs should not be stored where flooding is possible or on sites where they might spill or leak into the environment or be exposed to high temperatures. Proper mixing, diluting and reconstituting are essential for their effectiveness and for safety reasons. Powders may be harmful or toxic if they are inhaled as dusts. Improper dilution may cause inappropriate concentration or dosage, with uneven effects ranging from ineffectiveness to overdose and toxicity. Careful reading of the manufacturer's instructions is strongly advised as they provide important information about mixing, diluting, storage and disposal.

Unused portions of a regulated product and empty containers must be properly disposed of. Product labels provide instructions for safe disposal. Improper disposal can result in toxicity, environmental contamination and reliability problems. The best approach is to purchase only the amount of material that will be used within a reasonable period of time and to use all of the product for its intended purpose (USDA, 1994).

The USDA gives very useful tips about the use of regulated products:

1. Obtain a diagnosis of the problems before applying any treatment.
2. Seek professional advice if ever in doubt as to how to use the regulated product.
3. Use the product only for those species and indications listed on the label, unless extra-label use is specifically prescribed by a veterinarian.
4. Read the product label carefully.
5. Use the proper dosage, amount or concentration for the species, area and specific condition.
6. Use the correct method and route of application or administration.
7. Calculate withdrawal times accurately.
8. Identify treated populations or stocks properly using clear markings.
9. Do not use medication for prophylactic purposes unless specifically approved for this purpose.
10. Do not substitute trade-name products that are labelled and approved for aquaculture or aquatic by unlabelled or generic products.
11. Keep accurate records.
12. Consider the environmental impact of discharging treated water, including possible effects on non-target organisms.
13. Adopt a quality assurance programme or a HACCP Programme that provides guidelines for preventing tissue residues violation and for producing high quality, wholesome products for consumers.
14. Be aware of personal safety measures and proper procedures for farm workers who handle or apply antibiotics.
15. Consider economic consequences, both short- and long-term effects.

In Norway, the Directorate of Fisheries has public responsibility for ensuring that cultivated fish does not contain medicine residues or other undesirable substances, and has therefore established good-practice procedures for the application of veterinary drugs, including antibiotics, in aquaculture. The Directorate of Fisheries receives copies of prescriptions issued by veterinary surgeons, chemists and feed-producing firms for the treatment of cultivated fish. The breeder is obliged to inform the Directorate of

Fisheries in good time before take-up and slaughter, and information regarding medical treatment during the previous 12 months is also required. This information is stored in a database at the Directorate of Fisheries (Directorate of Fisheries, 2001).

This system makes it possible to register the use of medicines at every single fish farm in Norway. Fish that have been treated with antibiotics or chemical therapeutics during the previous 12 months must be monitored prior to slaughter. This also applies to fish destined for slaughter in cases where neighbouring sea cages at the fish farm have been so treated.

Fish that have been treated with drugs shall not be slaughtered until drug residues are impossible to detect. Slaughtered fish are controlled anew with particular reference to residue of the drugs in question. Such controls are carried out by means of random selection of samples during unannounced controls or through the monitoring programme for undesirable substances and medicine residues. Control of medicine residues at the Directorate of Fisheries was established in 1988 (Directorate of Fisheries, 2001).

### Vaccines

Aquaculture, as a young industry in the 1970s, placed significant reliance on the use of antibiotics to combat a range of bacterial diseases, such as vibriosis and furunculosis. The industry's rapid expansion and the increasing market size brought about heavier investment in vaccine development, which allowed, by the beginning of the 1990s, for a range of effective vaccines to be available.

Vaccination offers aquaculture producers an effective way to lower both the risk of disease in their fish and their cost of production. Vaccines stimulate the immune response of fish to produce antibodies that help protect the fish from disease. Once exposed to the vaccine, the fish's immune system reacts, producing antibodies that bind with the disease-causing bacteria and destroy it. This learned response means that the immune system learns how to defend itself from disease by making bacteria-specific antibodies. When an outbreak occurs, these antibodies will help protect the fish from diseases. Vaccines are not impenetrable shields, and the resistance they impart can be destroyed if other risk factors are not considered.

The three common methods to administer a vaccine are: immersion, injection and oral. These methods vary in terms of fish species, ease of administration, cost, stress on the fish, survival rates, dosage control, amount of labour involved and duration of the protection. The decision about which method to use is based upon a combination of actual and perceived risk, age of the fish, farmer's own risk aversion and return on investment (Hugh, 1995). It is generally considered that injectable vaccines provide greater protection than immersion and oral vaccines, because they allow better dosage control, which results in greater efficacy and a longer duration of protection (Hugh, 1995). However, injectable vaccines are more expensive, more labour intensive, and can damage the fish if not administered with care. Injectable vaccines cause adhesions in the fish body; these adhesions are required for the immune response, but they should not affect the quality of the fish or interfere with the fish's ability to digest food. Careful attention when injecting will also reduce severe adhesions.

There is a tendency for the farmer to prefer a multivalent vaccine, but, for the same volume of vaccine, if there is no cross-protection between strains in the same vaccine, the antigenic capacity will be lower. There are times when protection from a multivalent vaccine may be preferable, such as when the manufacturer carefully develops the vaccine for an adequate antigenic mass (Hugh, 1995).

Some vaccines have been developed for use in aquaculture. Among them, there is one against *Streptococcus iniae*, an emerging pathogen in cultivated, hybrid striped bass, rainbow trout, yellowtail, eel and turbot (Weaver-Missick, 1999), and Table 4.4 list some authorized for use in United States of America.

TABLE 4.4  
Some vaccines licensed for by USDA for use in aquaculture in United States of America.

Vaccine based on	Licence or permit holder	Species for application	Active against
<i>Aeromonas salmonicida</i> Bacterin	BioMed, Inc	Salmonids	Furunculosis
<i>Aeromonas salmonicida</i> Vibrio	BioMed, Inc	Salmonids	Furunculosis, Vibriosis
Autogenous Bacterin	BioMed, Inc	Fish	Bacterial Disease
<i>Vibrio anguillarum</i> -Ordalii Bacterin	BioMed, Inc	Salmonids	Vibriosis
<i>Vibrio anguillarum</i> -Ordalii- <i>Yersinia ruckeri</i> Bacterin	BioMed, Inc	Salmonids	Vibriosis, yersiniosis (enteric redmouth disease)
<i>Yersinia ruckeri</i> Bacterin	BioMed, Inc	Salmonids	Yersiniosis (enteric redmouth disease)
<i>Vibrio salmonicida</i> Bacterin	BioMed, Inc	Salmonids	Vibriosis
<i>Vibrio salmonicida</i> Bacterin	BioMed, Inc	Salmonids	Vibriosis
<i>Vibrio anguillarum</i> <i>Salmonicida</i> Bacterin	BioMed, Inc	Salmonids	Vibriosis
<i>Aeromonas salmonicida</i> Bacterin	Jerry Zinn, Aqua Health, Ltd.	Salmonids	Furunculosis
Autogenous Bacterin	Jerry Zinn, Aqua Health, Ltd.	Fish	Bacterial diseases
<i>Edwardsiella ictaluri</i> Bacterin	Jerry Zinn, Aqua Health, Ltd.	Catfish	Enteric septicaemia
<i>Vibrio anguillarum</i> -Ordalii bacterin	Jerry Zinn, Aqua Health, Ltd.	Salmonids	Vibriosis
<i>Yersinia ruckeri</i> Bacterin	Jerry Zinn, Aqua Health, Ltd.	Salmonids	Yersiniosis (enteric redmouth disease)

Source: USDA, 1994.

## Other alternatives

### *Competitive exclusion (Nurmi effect)*

Competitive exclusion (CE) is a term that has been used to describe the protective effect of the natural or native bacterial flora of the intestine in limiting colonization by some bacterial pathogens. There is a lot of scientific literature on this subject, dating back over 25 years to the original studies by Nurmi. Only in the last few years have these concepts been developed to a point where commercial preparations of CE products are available for use in poultry. This represents another alternative to the use of antibiotics. Neonates or newly hatched chickens are fed CE products in one or a few doses to establish an intestinal microflora that will hinder colonization by pathogens. CE preparations are not always pure culture bacteria and their microbial composition may not be completely known. They have proven effective in protecting chickens from *Salmonella* infections (Jeffrey, 1999).

### *Antimicrobial peptides*

Although the use of these substances has recently been demonstrated in shrimp and crab, the production of antimicrobial peptides is a widespread mechanism of host defence in the living kingdom, from bacteria, protozoans, invertebrates to vertebrates (Bachère, Destoumieux and Bulet, 2000).

Three antimicrobial peptides have recently been characterized in *Penaeus vannamei*. These substances were purified from the plasma and haemocytes of shrimp collected from intensive shrimp farms. Their molecules were fully characterized, are highly homologous and were named penaeidins. The haemocytes were found to be a site of production and storage of these substances. Their antibacterial activity is predominantly against Gram-positive bacteria, with reported bactericidal activity against *Bacillus megaterium*, bacteriostatic effect against *Micrococcus luteus*, and a slow bactericidal effect on the crustacean-pathogenic *Aerococcus viridans* that causes

gafkemia. Under experimental conditions these molecules have no activity against Gram-negative bacteria such as Vibrionaceae, but are able to inhibit the growth of a large range of filamentous fungi, including *Fusarium oxysporum*, pathogenic for shrimp. The potential antimicrobial activity of these substances represents a new area that requires further research (Bachère, Destoumieux and Bulet, 2000).

#### *Plant compounds with antimicrobial activity*

Recently, several studies have been published on antimicrobial effects observed in some compounds. Thirty-eight plant-derived flavonoids were studied by Xu and Lee (2001) and their activity against antibiotic-resistant bacteria were tested. They used disc-diffusion assay and broth dilution assay. Among the flavonoids examined, four flavonols (myricetin, datiscetin, kaempferol and quercetin) and two flavones (flavone and luteolin) exhibited activity against methicillin-resistant *Staphylococcus aureus*. Myricetin was also found to inhibit the growth of VRE and other clinically important bacteria, including *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. Further research on these alternatives will be of utmost interest for the resistance problem.

Liu, Durham and Richards (2001) reversed vancomycin resistance of *Enterococci* by combining the flavonoid galangin with vancomycin, which could be of great help in establishing therapeutic regimes against resistant pathogens.

Riquelme *et al.* (2001) evaluated the feasibility of adding inhibitor-producing bacteria (IPB) to mass cultures of *Agropecten purpuratus* larvae in a commercial hatchery, with the aim of replacing the use of antibiotics to control the infections. They observed that for this Chilean high-commercial-value mollusc (Chilean scallop), the IPB obtained from several cultures (*Vibrio* sp., *Pseudomonas* sp. and *Bacillus* sp.) were able to control infections without affecting the scallop larvae.

#### *Removal of aquaculture therapeutants by carbon adsorption*

Activated carbon filtration has been used extensively for water treatment after ozone or chlorine treatment, and for organic colour removal. Organic chemical therapeutants, including antibiotics, can be effectively removed from the water column by adsorption onto activated carbon (Aitcheson *et al.*, 2000).

Because therapeutants could be in competition with other aquaculture components for the available adsorption sites on the carbon, some attempt have been made to design appropriate carbon filters. Aitcheson *et al.* (2001) proposed a series of alternatives for water treatment, reflecting the therapeutant's concentration in the effluent.

## 5. Risk communication

The Joint FAO/WHO Expert Consultation on the Application of Risk Analysis to Food Standards Issues (FAO/WHO, 1995) defined risk communication as an interactive process of exchange of information and opinion on risk among risk assessors, risk managers and other interested parties.

The practical application of risk communication involves all aspects of communication among risk assessors, risk managers and the public. This includes the mechanism of delivery; message content; timelines of communication; availability and use of supporting material and information; together with the purpose, credibility and meaningfulness of the communication.

According to the above, one approach to preventing the spread of antimicrobial resistance is through developing and disseminating practical public health messages to the medical community and the public regarding the scope of the problem and prudent use of antibiotics. Prudent use of these drugs is the key to decreasing, or even reversing, the spread of resistance. Physicians should prescribe antimicrobial drugs only when they will be beneficial and, when possible, prescribe drugs that are specific for the bacteria causing the infection. The public needs to be aware that antimicrobial drugs are not useful for colds, 'flu, most sore throats and other illnesses caused by viruses. Patients should avoid requesting antibiotics from their physicians unless clearly required. In human medicine, an important tool in decreasing antibiotic use is ensuring that those people at greatest risk of influenza and pneumonia, including the elderly and those with chronic illness, are vaccinated against those diseases. Unfortunately, many of these at-risk adults do not receive an annual influenza vaccine dose, and most adults at risk for pneumonia have not received pneumococcal vaccine. If they would, this could not only prevent serious illness and save lives, but also greatly reduce the use of antibiotics otherwise needed to treat pneumonia.

The public should be made aware of the appropriate use for antibiotics so that infections remain controlled. This objective can be achieved through public information campaigns. The pharmaceutical industry and pharmacists have an important role in educating the public, including transmitting messages about antibiotic resistance and discouraging the inappropriate use of antibiotics (HHS, 1999a).

### 5.1 PROMOTING THE PRUDENT USE OF ANTIBIOTICS IN HUMAN MEDICINE

Several organizations worldwide have established a series of programmes contributing to this phase of the risk assessment process. As for other public health issues, effective prevention programmes will require strong linkages between public health practitioners and those involved in clinical practice and their communities.

CDC considers (2001a) antimicrobial resistance a major focus area in its plans. CDC's programmes include activities that focus on working with other federal agencies, State and local health departments, academic centres, health care providers and networks, international organizations and other partners, with the aim of:

- Developing, implementing and evaluating educational and behavioural approaches for improving health provider adherence to practical recommendations and guidelines for the prudent use of antimicrobial drugs.
- Developing, implementing and evaluating educational and behavioural interventions that limit the emergence and spread of drug resistance by modifying the drug-prescribing practices of health care providers and educating patients on the appropriate use of drugs.

- Promoting professional education and training in the epidemiology, detection, prevention and control of diseases that are resistant to antimicrobial drugs, both in the United States of America and abroad. Such public health programmes should include:
  - infection control strategies in diverse settings;
  - behavioural and educational interventions for modifying drug-prescribing practices of health care providers;
  - behavioural and educational interventions for patients on the appropriate use of drugs and adherence to prescription instructions; and
  - health education programmes to promote the use of new vaccines for infectious diseases.
- Developing and disseminating practical recommendations and guidelines for the prudent use of antimicrobial drugs.

Therefore, to coordinate a timely international scientific and public health response to emerging antimicrobial resistance, the CDC designed an early warning system to monitor, analyse, control and prevent important events in the emergence of antimicrobial resistance at both the global and regional or local levels. Overall, this early warning system should trigger epidemiological and microbiological interventions to assess risk factors for emerging antimicrobial resistance, leading to more effective control (CDC, 2001).

#### **FDA activities**

FDA (2000a, b) is developing a series of activities related to antimicrobial resistance. Examples of recent or ongoing FDA activities include information on antibiotic resistance and prudent use of antibiotics in product labelling. The FDA is exploring other activities, through the United States of America FDA Task Force on Antimicrobial Resistance, for encouraging appropriate use of these drugs, such as working with industry to include messages about appropriate antibiotic use in their consumer-advertising campaigns. The United States of America FDA Task Force on Antimicrobial Resistance (FDA, 2000b) recommends developing a strategy to facilitate the safe and effective use of antimicrobials, embodying a number of points.

1. The Agency strongly supports the proposed antimicrobial-resistance labelling:
  - Product labelling offers FDA the opportunity to communicate important facts about drug safety and efficacy and provides key information that should be adequately presented in promotional activities. Antimicrobial resistance is an important potentially adverse effect of antimicrobial usage and may compromise efficacy. Adherence to basic principles of antibiotic use can reduce the likelihood of encouraging resistance.
  - Also, where possible, antimicrobial use is best guided by local epidemiology and resistance patterns. The U.S. Center for Drug Evaluation and Research (CDER) has been considering that labelling be required for all antibiotics and to include key information about resistance and to encourage judicious, safe and effective use. The Task Force strongly supports this effort (FDA, 2000b).
2. The FDA should work with the National Institute of Health, the CDC, the Agency for Healthcare Research and Quality (AHRQ) and others (e.g. health professionals, industry, health care organizations) to organize a conference or other process to develop and promulgate “Basic Principles for Antimicrobial Use”.
  - This action is closely linked to the set of issues embodied in the proposed antimicrobial labelling but extends the educational effort further and should include a full range of stakeholders. There is a need to develop a shared consensus on general principles for antimicrobial use that can be included in health professionals’ education, used by health systems and providers, and

by government agencies. Such a consensus process and the principles agreed upon would be very useful as benchmarks for quality care and in the future development of specific strategies for addressing emerging issues in anti-infective therapy.

3. The FDA should work towards ensuring that patient educational materials are provided with each antibiotic prescription and that they stress appropriate antimicrobial use. The FDA should use a variety of means (e.g. meetings, a new Website feature with outside links, publications) to better provide enhanced and consistent information to consumers and professionals regarding antimicrobial use and resistance, new antimicrobial approvals and related issues.
  - The effort to provide information about antimicrobial products and to address the resistance problem is complex and involves many partners.
4. Medication information provided by the pharmacist with each prescription represents an important educational opportunity. The FDA should work with health professionals, academia, pharmacist groups and others to design messages for these materials that stress appropriate antimicrobial use. It may be possible to accomplish this in conjunction with current private sector providers of pharmacy educational materials.
  - In addition, both traditional (e.g. meetings, liaisons with existing groups, medical journals) and less traditional or newer approaches (e.g. World Wide Web, women's and parents' magazines) should be used to make detailed information on appropriate use of approved antimicrobials widely available and well linked to other important sources. It may be particularly useful to health professionals for FDA to promptly post information on newly approved drugs and the clinical trials that led to their approval.
5. The Center for Drug Evaluation and Research (CDER) should develop a Guidance Document regarding both direct-to-consumer and professional promotion of antimicrobials that deals with key resistance issues and encourages appropriate promotion to preserve safety and efficacy of approved products.
  - Direct-to-consumer and professional promotion of antimicrobials are both significant sources of information to the public and the professionals, respectively. Issues of antibiotic resistance and principles of appropriate antibiotic use are only inconsistently addressed.

Some sponsors have included very helpful information to encourage appropriate use of their products and to minimize the development of resistance. The requirement for key information on antimicrobial use for inclusion in product labelling should be followed by the development of a more detailed guidance document that should serve to enhance the quantity, quality and consistency of information about resistance reaching the intended targets.

### **INSPEAR**

Because of the urgent need for infection control interventions to prevent further emergence of antimicrobial drug-resistant strains and for rapid distribution of information about emerging organisms, the International Network for the Study and Prevention of Emerging Antimicrobial Resistance (INSPEAR) was established, with the participation of CDC and numerous agencies and laboratories worldwide (Richet *et al.*, 2001).

The main objectives of INSPEAR are to:

- serve as an early warning system for emerging resistant pathogens;
- facilitate rapid distribution of information about emerging MR pathogens to hospitals and public health authorities worldwide; and

- serve as a model for the development and implementation of infection control interventions to prevent the emergence or transmission of antimicrobial drug-resistant pathogens in health-care facilities.

Another important function of INSPEAR is to assist microbiologists and infection control personnel in hospitals and countries that lack the expertise needed to conduct microbiological or epidemiological studies (Richet *et al.*, 2001).

INSPEAR was started as a collaborative effort between the Hospital Infections Program (CDC) and microbiologists and hospital epidemiologists in the United States of America and Europe. It is now a consortium of clinical microbiologists, hospital epidemiologists, infectious disease specialists, experts in the fields of antimicrobial resistance, epidemiology and computer sciences, with public health agencies and NRLs. One hundred and sixty health-care facilities in 40 countries have joined INSPEAR, with 50 percent of participants in Western Europe and 29 percent in Eastern Europe.

### Canadian activities

Canada, in its programme *Controlling Antimicrobial Resistance*, an integrated action plan for Canadians, recommends changing physicians' behaviour by: (i) establishing specific guidelines for diagnosis and management of common infections, e.g. pharyngitis, rhinitis/colds, sinusitis, bronchitis and acute otitis media in adults and children, and (ii) peer education, aimed at the practice level or at hospital rounds, whereby locally-respected professionals assume the role of educators.

In a similar way, the plan recommends changes in public attitude using educational materials such as videos, information sheets, posters and material specifically for day-care centres, walk-in clinics and emergency departments (Health Canada, 1997).

The Canada Health Protection Branch, Laboratory Centre for Infectious Disease Control, in collaboration with the Canadian Infectious Disease Society (CIDS), organized a multidisciplinary consensus conference on *Controlling Antimicrobial Resistance: an integrated action plan for Canadians* (Montreal, 1997). They recommended:

- improving the public's perception of the risk and benefits of micro-organisms and the risk and benefits of antimicrobial therapy;
- improving physicians' perception about the risk and benefits of micro-organisms and the risk and benefits of antimicrobial therapy; and
- incorporating consumers and local issues into the development of communications plans for each goal. These plans would include needs assessment and identified barriers. Consumers might help to identify positive factors.

For Communication Strategies, the meeting recommended:

- generating, interpreting and disseminating information that would support evidence-based approaches to dealing with antimicrobial resistance;
- communicating information that would improve understanding and actions concerning antimicrobial resistance;
- improving the public's perception about the risk and benefits of micro-organisms and the risk and benefits of antimicrobial therapy;
- communicating information that would improve understanding and actions concerning antimicrobial resistance;
- improving physicians' perception about the risk and benefits of micro-organisms and the risk and benefits of antimicrobial therapy; and
- incorporating consumer and local issues into the development of communications plans for each goal.

To develop a communication strategy for each goal, the meeting noted that it had to be established:

- Who would organize?
- Which are the targets?
- What resources were necessary (existing vs new)?



- What communications modality would be used?
- What were the time lines?
- How would the effect of the communication strategy be evaluated?

The communications package thus generated would promote behaviour change in usable, practical, attractive and achievable steps. Ongoing evaluation of the communications strategy would permit modification and improvement.

### European Community activities

In a similar way, a Council on the Prudent Use of Antimicrobial Agents in Human Medicine has been established in the European Community.

As a follow-up to the Council Resolution of June 1999 and the Council Conclusions of December 1999, a series of recommendations have been established, among which: the development of guidelines and principles on the prudent use of antimicrobial agents, including principles for evaluation of applications for marketing authorization, enhancing the knowledge on the problem by specialized education programmes for health professionals and raising of awareness on the problem of antimicrobial resistance by informing the general public (CEC, 2001a, b).

### WHO activities

WHO (WHO, 2000a, b, c) has established some recommendations:

- *Information, education and communication for consumers as well as providers.* Among the populations where communicable diseases are most prevalent, understanding of the potential dangers of misuse of antimicrobials is practically non-existent. Educating consumers is therefore necessary to ensure that policies and treatment guidelines are accepted and applied in practice. In many settings, the private sector is the most important source of patient care, including advice and dispensation of antimicrobials. Private providers may be uneducated drug-sellers operating in conditions where the application of any kind of regulation or norm is extremely difficult. Improving the practices of this informal private sector is one of the most daunting challenges to health systems; efforts need to be based on a solid understanding of economic and other factors governing the behaviour of providers and consumers. Social marketing is an approach that has already proven useful, for example, for sexually transmitted infections and malaria.
- *Intersectoral cooperation.* The emergence of resistance in microbes that transmit from animals to humans, such as *Salmonella*, indicates the need to ensure that activities to combat drug resistance should not be limited to the health sector.

In relation to resistance to antibiotics used for common bacterial diseases, WHO activities include the integrated management of childhood illness (IMCI). It works with national specialists to assess national data in order to formulate best practice guidelines for the use of antibiotics in treating the most common childhood diseases requiring antibiotic treatment.

WHO works with several countries to address the issue of antibiotics in hospital facilities through Hospital Drug Therapeutic Committees and development of innovative strategies.

In some countries, such as Cambodia and the Lao People's Democratic Republic, monitoring, training and planning (MTP) programmes with locally adapted indicators have been developed and field-tested in the public and private sectors. Indicators for the use of drugs – including antibiotics in health facilities supported by WHO – are used to monitor the pattern of drug use, to provide feedback to prescribers as a means of educational intervention, and to evaluate the impact of any intervention.

In the Philippines, public education materials for the rational use of antibiotics have been printed and distributed.

WHO created the Antimicrobial Resistance Information Bank, an interactive resource with open access and enabling all to contribute to a global understanding of antimicrobial resistance as a public health problem. This bank is hosted, developed and designed by the Anti-infective Drug Resistance and Containment Team in the Department of Communicable Disease Surveillance and Response, WHO, Geneva, in collaboration with the Institut National de la Santé et de la Recherche Médicale (INSERM), Paris (WHO, 1999).

The aim of WHO's Antimicrobial Resistance Information Bank is to gather and make accessible information on antimicrobial resistance surveillance networks and resistance data (in aggregate form) generated by these networks.

## **5.2 PROMOTING THE PRUDENT USE OF ANTIBIOTICS IN VETERINARY MEDICINE AND ANIMAL PRODUCTION**

Due to the increasing evidence that certain uses of antibiotics in food-producing animals can lead to antibiotic resistance in intestinal bacteria that are then transmitted to human beings, causing treatment-resistance illness, FDA is proposing reactive responses. These actions include proposing a new framework for antibiotic use in food-producing animals in order to protect human health and prolong the period of usefulness of these drugs. FDA is implementing joint programmes with the American Veterinary Medicine Association, which include educational programmes for the judicious use of prescription of antibiotics (FDA, 2000b).

The judicious use of antibiotics in agriculture should reduce the emergence and spread of resistant bacteria in animals and on produce, and minimize public health risks. These drugs have been widely used in animals for therapy, prophylaxis and growth promotion and have also been used on some fruits and vegetables. The public health consequence of this exposure has been debated widely. There is agreement that antimicrobial resistance emerges in relation to this use (CDC, 1997).

In the United Kingdom, the Department of Health (2000) proposed a number of recommendations for the prudent antimicrobial use in animals.

- (i) to promote optimal prescribing in animals the veterinarian will:
  - encourage professional education; and
  - encourage the production and promulgation of guidelines and codes of practice for prescribing, including through the British Veterinarian Association, National Farmers Union and the National Office of Animal Health Ltd. (NOAH).
- (ii) to reduce unnecessary and inappropriate use of antimicrobials for non-therapeutic use in animals, the veterinarian will:
  - review appropriate usage, including as growth promoters, in light of advice from the Advisory Committee on the Microbiological Safety of Foods (ACMSF), the Veterinary Products Committee, and European Union decisions.
- (iii) to use the regulatory framework to improve optimal antimicrobial prescribing (in the UK and Europe), where appropriate, the veterinarian will:
  - critically assess existing products at the time of renewal of marketing authorizations and ensure that data sheets and product characteristic summaries are appropriate and consistent (with those for other products containing the antibacterial active ingredient); and
  - in the authorization process for new antimicrobials, require the development of optimized dosing rates and strategies based on recent advances in pharmacokinetic and pharmacodynamic data and, where necessary, require new doses rates and strategies for currently authorized antimicrobials.

The European Agency for the Evaluation of Medicinal Products (EMEA) is involved in activities that focus on requirements for market authorization and on the quality and content of the Summary of Product Characteristics (SPC), which, in particular, is the

basis for all promotional activities of an antimicrobial agent. Regulators in different European authorities have expressed concerns that different indications, doses, dose regimens (duration of treatment) and different pharmacodynamic information exist for the same and similar products already licensed in the EU. National competent authorities in consultation with EMEA are currently considering the issue of divergent product information. The authorization of veterinary medicinal products should ensure that recommended dosages and treatment regimes are optimum to minimize the development of resistance. Moreover, susceptibility patterns of populations of target bacteria may need to be monitored after the authorization.

EMEA/CVMP pointed out in its Report on Antimicrobial Resistance and Qualitative Risk Assessment that the vast majority of antibiotics used in veterinary medicine are related to, or identical to, human medicinal products and can select for cross-resistance or co-resistance. It also identified a lack of data and of harmonization that prevented a coherent and scientific approach at the European level. Furthermore, a Risk Management Strategic Plan has been developed, setting out proposals on resistance, and the following major areas of CVMP activity are current:

- Critical evaluation of the data related to MICs and of the current relevance of using MIC and kinetic data in the setting of dosage levels.
- Development of guidelines to satisfy the requirements for the resistance section in a regulatory dossier for antimicrobial agents, with particular emphasis on a description of the testing aimed at establishing the likelihood of resistance development to novel antimicrobial agents, i.e. pre-authorization sensitivity testing guidelines.
- Consolidation and standardization of phrases and formats used in the Summary of Product Characteristics to define clearly and consistently throughout the European Union, dosages and treatment regimes, target organisms and diseases, in accordance with prudent-use principles.
- Development of definitive guidelines for antimicrobial prophylaxis, combination therapies, in-feed and water mass medication, given that resistance is driven by the volume of active use and the route of administration (CEC, 2001a, b).

It is recognized that the emergence of antimicrobial resistance is a multifactorial problem, and thus requires a multifactorial solution. This involves all stakeholders concerned with the use of antimicrobials in both food animals and humans.

WHO has always sought the active participation of many national and international associations and federations associated with human and public health in the development of global principles. At present, WHO is strongly encouraging the surveillance of antimicrobial resistance and antimicrobial usage. The information obtained will be very useful for the development of national policies (intervention, education, legislation) for the containment of antibiotic resistance and for correlation with data from antimicrobial resistance monitoring in humans, animal and food and the implementation of the prudent use of antibiotics (WHO, 2001).



## 6. Recommendations

Some measures at the level of veterinary, phytosanitary and human medicine have to be taken to contain the growing public health problem of antibiotic resistance. International cooperation in efforts to combat anti-microbial resistance in international forums has to be reinforced.

Among doctors, veterinarians, farmers and patients, the “prescription only” use of antibiotics has to be supported in all sectors, including agriculture. Also, phasing out all uses other than direct use as therapeutic agents has to be pursued.

Collection and analysis of data of antibiotic-resistant micro-organisms available to prescribers, pharmacies, industry, health insurance providers, etc., is needed to detect potential links to intervention measures.

Principles on the prudent use of antibiotics should be developed and awareness of the problem of antimicrobial resistance should be raised through informing the public.

In a parallel way, there should be a tightening of controls covering the licensing of antibiotics so that the development of antimicrobial resistance in animals given antibiotics can be monitored, evidence of cross-resistance to other antibiotics detected and consideration given to appropriate action to minimize risks.

Establishing appropriate surveillance systems is a necessity and, for this purpose, standardized and harmonized procedures need to be established to evaluate micro-organisms’ resistance to a specific antibiotic.

In animal food husbandry, it is important to reduce the need for antibiotics – and the consequent transference of resistance – through applying good husbandry practices and reinforcing immunization programmes and hygienic conditions. In this way the use of antibiotics can be limited to therapeutic purposes alone.

In the light of a body of evidence, particularly the Scandinavian countries’ experience, reduced consumption of antibiotics in animal husbandry has contributed to slowing down the development of resistance. This very interesting approach deserves careful observation and should be considered a remarkable approach to combat the resistance threat.

In aquaculture, responsible conduct in the prudent use of antibiotics should be to reduce their use to therapeutic purposes alone; prophylactic use must be replaced by good husbandry practices, including adequate hygiene conditions and vaccination programmes. Among producers, the priority should be education programmes that emphasize proper drug use, e.g. which drugs are permitted and how to comply with HACCP protocols.

It should be noted that, for fish products, the use of dip solutions or ice with antibiotics for the purpose of extending product shelf life is forbidden.

Research projects should be encouraged that aim at better understanding of the mechanisms of emergence and spread of resistance within a species, and from animal to man and the environment.

Research projects should be promoted on pharmacology and pharmacokinetics of antibiotics in aquatic species in order to provide a more exact approach to establishing MRL values.



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# Glossary

- Absorption:** Movement of ions, molecules and water into an organism as a result of metabolic processes, frequently against an electrochemical potential gradient (active) or as a result of diffusion along an activity gradient (passive). In physiology, it refers to the movement of liquids and solutes into cells by way of diffusion or osmosis.
- Acceptable Daily Intake (ADI):** An estimate of the residue that can be ingested daily over a lifetime without a health risk to the consumer.
- Active site:** Region of an enzyme where substrates bind.
- Amino group:** A  $-NH_2$  group attached to a carbon skeleton, as in the amines and amino acids.
- Antibiotic:** A drug of natural or synthetic origin, with the capacity to inhibit the growth of or to kill micro-organisms. Antibiotics that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of man, animals and plants.
- Antibody:** Protein produced by animals in response to the presence of an antigen and which can combine specifically with that antigen.
- Antigen:** Substance that can incite the production of a specific antibody and that can combine with that antibody.
- Antimicrobial:** a drug that kills or inhibits the multiplication of micro-organisms.
- Antiseptic:** Agent that kills or inhibits microbial growth but is not harmful to human tissue.
- Bacillus:** Bacterium with an elongated, rod shape.
- Bacteria:** All prokaryotes that are not members of the domain *Archaea*.
- Bacterial infection:** The state when bacteria invade the body.
- Bacterin:** a vaccine made from killed bacteria. An **autogenous bacterin** is a bacterin made by a firm licensed to produce autogenous bacterins from organisms isolated from a particular farm, to be sold to and used on that farm only, and for a specified, limited period only.
- Bacteriocin:** Agent produced by certain bacteria that inhibit or kill closely related strains and species.
- Bacteriophage:** Virus that infects bacteria, often with destruction or lysis of the host cell.
- Bacteroid:** Altered form of cells of certain bacteria. Refers particularly to the swollen, irregular vacuolated cells of rhizobia in nodules of legumes.
- Biofilm:** Microbial cells embedded in an adhesive, usually a polysaccharide material, and attached to a surface.
- Bioremediation:** The process by which living organisms act to degrade or transform hazardous organic contaminants. Use of micro-organisms to remove or detoxify toxic or unwanted chemicals from an environment.
- Carbohydrate:** Any chemical compound that consists of only carbon (C), oxygen (O) and hydrogen (H) elements. For example, sugars, starches and cellulose are carbohydrates. The ratio of hydrogen to oxygen atoms in carbohydrates is usually 2:1.
- Catalyst:** Substance that promotes a chemical reaction by lowering the activation energy without itself being changed in the end. An enzyme is a type of catalyst.
- Cell:** Fundamental unit of living matter.

- Cell wall:** Layer or structure that lies outside the cytoplasmic membrane; it supports and protects the membrane and gives the cell shape.
- Chimeraplasts:** Chimeric RNA/DNA oligonucleotides. Chimeraplast-mediated gene correction is currently ideally suited for doing single-base exchanges in genomic DNA to correct a mutation in the genome. A chimeraplast has a sequence that is identical to the genomic sequence, except for a single mismatch at the mutation point. Evidence shows that when pairing occurs, cellular DNA mismatch repair mechanisms correct the mismatch, leading to restoration of a wild-type gene sequence. Advantages of this approach include that the target gene remains regulated by its own mechanisms and that exogenous DNA is not introduced.
- Chromosome:** the term was proposed by Waldeyer (1888) for the individual threads within a cell nucleus (from the Greek: *chroma* = colour, + *soma* = body). Genetic element carrying information essential to cellular metabolism. Prokaryotes have a single chromosome, consisting of a circular DNA molecule. Eukaryotes contain more than one chromosome, each containing a linear DNA molecule complexed with specific proteins.
- Clay:** Soil particle < 0.002 mm in diameter.
- Coccus:** Spherical bacterial cell.
- Coliform:** Gram-negative, non-spore-forming facultative rod bacterium that ferments lactose with gas formation with 48 hours at 35°C.
- Colonization:** Establishment of a community of micro-organisms at a specific site or in an ecosystem.
- Commensal relationship:** Generally established between members of two different species of organisms in which one individual is usually only slightly benefited, while the other member is not affected at all by the relationship.
- Competitive exclusion (CE):** The protective effect of the natural or native bacterial flora of the intestine in limiting colonization by some bacterial pathogens.
- Conjugation:** In prokaryotes, transfer of genetic information from a donor cell to a recipient cell by cell-to-cell contact.
- Cyst:** Resting stage formed by some bacteria, nematodes and protozoa in which the whole cell is surrounded by a protective layer; not the same as endospore.
- Cytoplasmic membrane:** Selectively permeable membrane surrounding the cell's cytoplasm.
- Deoxyribonucleic acid (DNA):** The molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases: adenine (A), guanine (G), cytosine (C) and thymine (T). In nature, base pairs form only between A and T and between G and C; thus the base sequence of each single strand can be deduced from that of its partner.
- Disinfectant:** Agent that kills micro-organisms.
- Drug:** Any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals; any substance (other than food) intended to affect the structure or any function of the body of man or other animals.
- Ecology:** Science of the study of the interrelations among organisms and between organisms and their environment.
- Ecosystem:** Community of organisms and the environment in which they live.
- Effluent:** Wastewater or other liquid – raw (untreated), partially or completely treated – flowing from a reservoir, basin, treatment process or treatment plant and passing to the environment.

- Enteric bacteria:** General term for a group of bacteria that inhabit the intestinal tract of humans and other animals. Among this group are pathogenic bacteria such as *Salmonella* and *Shigella*.
- Enzyme:** A protein that acts as a catalyst, speeding the rate at which a biochemical reaction proceeds but not altering the direction or nature of the reaction.
- Epitope:** The region of an antigen to which the variable region of an antibody binds.
- Eukaryote:** Cell or organism with membrane-bound, structurally discrete nucleus and other well-developed subcellular compartments. Eukaryotes include all organisms except viruses, bacteria and blue-green algae.
- Eutrophic:** Having high concentrations of nutrients, optimal, or nearly so, for plant or animal growth. Can be applied to nutrient or soil solutions and bodies of water.
- Extracellular:** Outside the cell.
- Extra-label use:** The actual or intended use of an approved new animal drug in a manner that is not in accordance with the approved label directions. Extra-label use is permitted only by or on the prescription of a licensed veterinarian when a valid veterinarian-client-patient relationship exists
- Exudate:** Low molecular weight metabolites that leak from plant roots into soil.
- Gene:** The term coined by Johannsen (1909) for the fundamental physical and functional unit of heredity. The word gene was derived from De Vries' term *pangen*, itself a derivative of the word *pangensis* which Darwin (1868) had coined. A gene is an ordered sequence of nucleotides located in a particular position (locus) on a particular chromosome that encodes a specific functional product (the gene product, i.e. a protein or RNA molecule). It includes regions involved in regulation of expression and regions that code for a specific functional product.
- Gene cassette:** A cassette is a mobile element, but it does not code for genes involved in its own mobility. Its movements depend upon the integrase, which interacts with the recombination sites, the *attL* site located in the 5'-conserved segment and the *attC* located at the 3'-end of each cassette to excise an integrate cassette. Cassette movement occurs through a conservative site-specific recombination mechanism, in which DNA-binding proteins play the primary role in bringing the partners together.
- Gene cloning:** Isolation of a desired gene from one organism and its incorporation into a suitable vector for the production of large amounts of the gene.
- Genetic code:** Information for the synthesis of proteins contained in the nucleotide sequence of a DNA molecule (or in certain viruses, of an RNA molecule).
- Gene pool:** the total of all genes in a population of a particular species
- Gene probe:** A strand of nucleic acid that can be labelled and hybridized to a complementary molecule from a mixture of other nucleic acids.
- Gene therapy:** Introduction of healthy genetic material to replace, augment or influence genes that do not function properly. In some cases the material can be injected with what is known as a genetic vaccination. In other cases, the material is introduced through bio-engineered viruses that carry the therapeutic gene to the cell. Globules known as liposomes can also be used to carry therapeutic genes to specific cells.
- Generation time:** Time needed for a population to double in number or biomass.
- Genetic engineering:** *In vitro* techniques for the isolation, manipulation, recombination, and expression of DNA.
- Genome:** All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

- Genotype:** Precise genetic constitution of an organism.
- Genus (plural: genera):** The taxon between family and species, and used to form the first element of the scientific name (binomial).
- Glycosidase:** Enzyme that hydrolyzes bonds in carbohydrates, glycoproteins and glycolipids.
- Gram stain:** Differential stain that divides bacteria into two groups, **Gram-positive** and **Gram-negative**, based on the ability to retain crystal violet when decolourized with an organic solvent such as ethanol. The cell wall of Gram-positive bacteria consists chiefly of peptidoglycan and lacks the outer membrane of Gram-negative cells.
- Growth factor:** Organic compound necessary for growth as it is an essential cell component or precursor of such components and cannot be synthesized by the organism itself. Usually required in trace amounts.
- Growth:** In microbiology, an increase in both cell number and cellular constituents.
- Growth promoters:** antimicrobials used in low concentrations to stimulate an animal's growth, resulting in increased daily live weight gain and feed conversion efficiency.
- Habitat:** Place where an organism lives.
- HACCP (Hazard Analysis Critical Control Points):** A system that identifies, evaluates and controls hazards that are significant for food safety.
- Half life:** when a property (e.g. radioactivity, enzyme activity, etc.) decreases at a rate proportional to its concentration, the time,  $t_{1/2}$ , taken for the property to decrease to one-half its initial value; related to the first-order rate constant,  $k$ , by  $t_{1/2} = \ln 2/k$ .
- Hapten:** A substance not inducing antibody formation but able to combine with a specific antibody.
- Hazard:** A biological, chemical or physical agent with the potential to cause an adverse health effect.
- Hazard analysis:** The process of collecting and evaluating information on hazards and conditions leading to their presence, especially to decide which are significant for food safety and therefore should be addressed in the HACCP plan.
- Haemocytes:** constitute part of the cellular defence system in some invertebrates. These cells are involved in the production of bactericidal peptides, in phagocytosis as well as in the encapsulation reaction. Haemocytes have the ability to internalize foreign material by a process called phagocytosis. They 'sense' the foreign material, migrate towards it (chemotaxis), attach to the material (adherence) and internalize it by extending pseudopods and pulling the material into the haemocyte where it will reside surrounded by host cell membrane forming a phagocytic vacuole.
- Humic acid:** Dark-coloured organic material extracted from soil by various reagents (e.g., dilute alkali) and precipitated by acid (pH 1 to 2).
- Humic substances:** Series of relatively high-molecular-weight, brown-to-black substances formed by secondary synthesis reactions.
- Hydrogen bond:** Chemical bond between a hydrogen atom of one molecule and two unshared electrons of another molecule.
- Immunity:** The ability of a human or animal body to resist infection by micro-organisms or their harmful products, such as toxins.
- Infectious disease:** Any of many diseases or illnesses (caused by micro-organisms) that can be transmitted from person to person or from organism to organism, produced when the micro-organisms multiply faster than the immune system can destroy them.



- Inhibition:** Prevention of growth or function.
- Integron:** Genetic unit that includes genes of a site-specific recombination system capable of capturing and mobilizing genes contained in mobile elements called gene cassettes.
- Intracellular:** Inside the cell.
- Ions:** Atoms, groups of atoms, or compounds that are electrically charged as a result of the loss of electrons (**cations**) or the gain of electrons (**anions**).
- In vitro:** Literally “in glass”; it describes whatever happens in a test tube or other receptacle, as opposed to *in vivo*. When a study is done outside a living organism.
- In vivo:** In the body, in a living organism, as opposed to *in vitro*; when a study or an experiment is done in the living organism, it is done *in vivo*.
- Leaching:** The removal of materials in solution from the soil.
- Lipopolysaccharide (LPS):** Complex lipid structure containing unusual sugars and fatty acids found in many Gram-negative bacteria.
- Liposome:** A tiny fat-encased pouch that can traverse cell membranes. It is also sometimes used to transport a gene into a body cell. Another approach employing liposomes, called chimeraplasty, involves the insertion of manufactured nucleic acid molecules (chimeraplasts) instead of entire genes to correct disease-causing gene mutations. Once inserted, the gene may produce an essential chemical that the patient’s body cannot, remove or render harmless, a substance or gene causing disease, or expose certain cells, especially cancerous cells, to attack by conventional drugs.
- Lysis:** Rupture of a cell, resulting in loss of cell contents.
- Metabolism:** All biochemical reactions in a cell, both anabolic and catabolic.
- Microbial biomass:** Total mass of micro-organisms alive in a given volume or mass of soil.
- Microbial population:** Total number of living micro-organisms in a given volume or mass of soil.
- Microenvironment:** Immediate physical and chemical surroundings of a micro-organism.
- Mineralization:** Conversion of an element from an organic form to an inorganic state as a result of microbial decomposition.
- Molecule:** Result of two or more atoms combining by chemical bonding.
- Morbidity:** A diseased condition or state; the incidence of a disease or of all diseases in a population.
- Mortality:** The mortality rate; the proportion of deaths to population or to a specific part of the population.
- Multivalent vaccine:** Protecting against several diseases.
- Mutagen:** Substance that causes the mutation of genes.
- Mutant:** Organism, population, gene or chromosome that differs from the corresponding wild type by one or more base pairs.
- Mutation:** The term which De Vries introduced into biological literature for an abrupt change of phenotype which is inherited. Any permanent and heritable change in DNA sequence. Types of mutations include point mutations, deletions, insertions and changes in number and structure of chromosomes.
- Mycobacterium:** A genus of aerobic bacteria found in soil and water, capable of biodegrading multi-ring compounds such as polyaromatic hydrocarbons (PAHs).
- Niche:** Functional role of a given organism within its habitat.
- Non target organisms:** Organisms at which treatment is not aimed but which contact the product and may be affected by it.

- Operon:** In genetics, site on a bacterial chromosome containing genes that control protein synthesis (structural genes) together with a gene that determines whether the structural genes are active or not (operator gene).
- Over the counter drugs (OTC):** Drugs that have adequate written directions for lay use and are authorized for sale without veterinarian or medical prescription.
- Pathogen:** Organism able to inflict damage on a host it infects.
- Pathogenicity:** Ability of a parasite to inflict damage on the host.
- Peptidoglycan:** Rigid layer of cell walls of bacteria, a thin sheet composed of N-acetylglucosamine, N-acetylmuramic acid, and a few amino acids.
- Phage:** A virus for which the natural host is a bacterial cell. Used as a vector for cloning segments of DNA.
- Phenotype:** Observable properties of an organism. The term coined by Johannsen (1909) for the appearance (Greek *phainein*, to appear) of an organism with respect to a particular character or group of characters (physical, biochemical and physiologic), as a result of the interaction of its genotype and its environment. Often used to define the consequences of a particular mutation.
- Pilus (plural: pili):** Fimbria-like structure present on fertile cells and involved in DNA transfer during conjugation. Sometimes called sex pilus.
- Plasmid:** Covalently closed, circular piece of DNA that, as an extrachromosomal genetic element, is not essential for growth. A **Conjugative plasmid** is a self-transmissible plasmid; a plasmid that encodes all the functions needed for its own intercellular transmission by conjugation.
- Polymer:** Large molecule formed by polymerization of monomeric units.
- Polysaccharide:** Long chain of monosaccharides (sugars) linked by glycosidic bonds.
- Probiotics:** Bacterial metabolic products with beneficial effects attributed to their capacity to:
- (1) adhere to the mucosal surface of the gastrointestinal tract (GI tract) to prevent being washed out by peristalsis;
  - 2.) survive the rigours of transit through the gastrointestinal tract (such as exposure to stomach and bile acids); and
  - (3) poses antagonism towards a particular, target pathogen.
- Prophylaxis:** The administration of antibiotics in advance of symptomatic disease.
- Protein:** A large molecule composed of one or more chains of amino acids in a specific order; the order is determined by the base sequence of nucleotides in the gene coding for the protein. Proteins are required for the structure, function and regulation of the body's cells, tissues and organs, and each protein has unique functions.
- Pseudomonad:** Member of the genus *Pseudomonas*, a large group of Gram-negative, obligately respiratory (never fermentative) bacteria.
- Recombination:** Process by which genetic elements in two separate genomes are brought together in one unit.
- Replication:** Conversion of one double-stranded DNA molecule into two identical double-stranded DNA molecules.
- Repression:** Process by which the synthesis of an enzyme is inhibited by the presence of an external substance (the repressor).
- Resistance threshold:** Level of resistance that is acceptable and is established for individual pathogens
- Restriction endonuclease (restriction enzyme):** Enzyme that recognizes and cleaves specific DNA sequence, generating either blunt or single-stranded (sticky) ends.

- Retrovirus:** Virus containing single-stranded RNA as its genetic material and producing a complementary DNA by action of the enzyme reverse transcriptase.
- Reverse transcription:** Process of copying into DNA information found in RNA.
- Ribonucleic acid (RNA):** Polymer of nucleotides connected via a phosphate-ribose backbone, and involved in protein synthesis; found in the nucleus and cytoplasm of cells. It plays an important role in protein synthesis and other chemical activities of the cell. The structure of RNA is similar to that of DNA. There are several classes of RNA molecules, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and other small RNAs, each serving a different purpose.
- Ribosomal RNA (rRNA):** Types of RNA found in the ribosome; some participate actively in the process of protein synthesis.
- 16S rRNA:** Large polynucleotide (about 1 500 bases) that functions as a part of the small subunit of the ribosome of prokaryotes and from whose sequence evolutionary information can be obtained; the eukaryotic counterpart is **18s rRNA**.
- Risk:** A function of the probability of an adverse health effect and the severity of the effect, consequential to a hazard.
- Risk analysis:** A process consisting of three interconnected components: risk assessment; risk management; and risk communication.
- Risk assessment:** A scientifically based process consisting of four steps: hazard identification; hazard characterization; exposure assessment; and risk characterization
- Risk communication:** The interactive exchange of information and opinions throughout the risk analysis process as regard hazards and risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, feed and food businesses, the academic community and other interested parties. It includes the explanation of risk assessment findings and the basis of risk management decisions.
- Risk management:** The process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if need be, selecting appropriate prevention and control options
- Site:**
- (i) In ecology, area described or defined by its biotic, climatic, and soil conditions as related to its capacity to produce vegetation.
  - (ii) Area sufficiently uniform in biotic, climatic, and soil conditions to produce a particular climax vegetation.
- Site-directed mutagenesis:** Insertion of a different nucleotide at a specific site in a molecule using recombinant DNA methodology.
- Species:** In microbiology, a collection of closely related strains sufficiently different from all other strains to be recognized as a distinct unit.
- Spectrum:** A measurable range of activity, such as the range of bacteria affected by an antibiotic.
- Strain:** Population of cells all descended from a single pure isolate.
- Substrate:**
- (i) Substance, base or nutrient on which an organism grows.
  - (ii) Compounds or substances that are acted upon by enzymes or catalysts and changed to other compounds in the chemical reaction.
- Succession:** Gradual process brought about by the change in the number of individuals of each species of a community and by the establishment of new species that gradually replace the original inhabitants.
- Symbiosis:** The living together in intimate association of two dissimilar organisms. The interactions between the organisms can be commensal or mutualistic.

- Synergism:** Association between organisms that is mutually beneficial. Both populations are capable of surviving in their natural environment on their own although, when formed, the association offers mutual advantages.
- Systemic:** Not localized in a particular place of the body; an infection disseminated widely through the body is said to be systemic.
- Target organism:** The plant, animal or micro-organism that is treated or at which treatment is aimed.
- Taxon (plural: taxa):** A group into which related organisms are classified.
- Taxonomy:** Study of scientific classification and nomenclature.
- Teratogenic effects:** The effects of exposure to medications or other drugs, chemicals or infections that may be harmful to an unborn child.
- Therapy:** The treatment of disorder or disease.
- Tissue residue:** The drug, pesticide, or toxic breakdown product remaining in the edible tissue after natural or technological processes of removal or degradation have occurred.
- Toxin:** Microbial substance able to induce host damage.
- Transcription:** Synthesis of an RNA molecule complementary to one of the two strands of a DNA double-stranded molecule.
- Transduction:** Transfer of host genetic information via a virus or bacteriophage particle.
- Transformation:** Transfer of genetic information into living cells as free DNA.
- Transgenic:** Describes genetically modified plants or animals containing foreign genes inserted by means of recombinant DNA techniques.
- Translation:** Synthesis of proteins using the genetic information in mRNA as a template.
- Transposable element:** Genetic element that can move (transpose) from one site of a chromosome to another.
- Transposition:** Movement of a piece of DNA around the chromosome, usually through the function of a transposable element.
- Transposon:** Transposable element that, in addition to genes involved in transposition, carries other genes; it often confers selectable phenotypes such as antibiotic resistance.
- Transposon mutagenesis:** Insertion of a transposon into a gene; this inactivates the host gene leading to a mutant phenotype and also confers the phenotype associated with the transposon gene.
- Vaccine:** A preparation of killed micro-organisms, living attenuated, fully virulent, or related non-virulent micro-organisms; or parts of micro-or macro-organisms that are administered to produce or increase immunity to a particular disease.
- Vector:** (i) Plasmid or virus used in genetic engineering to insert genes into a cell.  
(ii) Agent, usually an insect or other animal, able to carry pathogens from one host to another.  
(iii) Food that carries the hazard (pathogen, bacteria, toxin) and can affect human consumers.
- Vegetative cell:** Growing or feeding form of a microbial cell, as opposed to a resting form such as a spore.
- Viable:** Alive, able to reproduce.
- Vibrio:** (i) Curved, rod-shaped bacterial cell.  
(ii) Bacterium of the genus *Vibrio*.
- Virulence:** The degree or ability of a pathogenic organism to cause disease.
- Virulence factors:** Factors responsible for overcoming the host's immune response, allowing micro-organisms to colonize.

- Virus:** Any of a large group of submicroscopic infective agents that typically contain a protein coat surrounding a nucleic acid core, and are capable of growth only in a living cell.
- Withdrawal time:** The minimum waiting time required before treated animals can be slaughtered (processed) or released. Each withdrawal day is a full 24 hours, starting from the last time the animal received the drug, treatment or vaccine.
- Zoonosis:** Any disease and/or infection, which is naturally transmissible, directly or indirectly, from animals to humans.

Considering the overall misuse of antibiotics in all areas – human medicine, veterinary medicine, animal production and plant protection – this document aims to raise awareness of the antibiotic resistance problem in fish farming and related sectors, and promote the prudent use of these drugs according to the FAO Code of Conduct for Responsible Fisheries. This work focuses on antibiotics misuse and the concomitant threat of resistance development, considering this topic to be a public health concern that affects the population worldwide. Aspects such as the toxicity and allergic effects of antibiotic residues, the mechanism of transmission of antimicrobial resistance and environmental impact are also taken into account.

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