Antibiotics and the intestinal microflora

J.F. GUILLOT *,**, and J.P. LAFONT **

Summary: Antibiotics are major drugs active against infections of bacterial origin in human beings and animals, and they have made a considerable contribution to the health of farm animals.

Although their efficacy remains unimpaired, other short-term and long-term effects of antibiotics, involving both pathogenic bacteria and saprophytic bacteria of the microflora, have become manifest over the years.

The main effect is the selection of bacteria resistant to antibiotics, particularly bacteria carrying plasmids for multiple resistance. These plasmids may be auto transferred by conjugation, both in vitro and in vivo, and this promotes the spread of resistance genes within microbial populations. In the long term, bacteria resistant to antibiotics become established in the microflora of animals and human beings, even when no antibiotic is being administered.

Other effects of antibiotics on the microflora include effects on resistance to colonisation, adherence of bacteria to epithelia, carriage of potentially pathogenic bacteria like salmonellas, and effects on the growth rate of animals. These effects show the bioregulatory capacity of antibiotics for bacterial populations, particularly those of the digestive tract. This bioregulation may have favourable or unfavourable consequences for the host. Since the use of antibiotics as growth promoters for animals is now prohibited in most cases, work is underway to develop bacteria or substances having a probiotic effect, in order to continue the favourable bioregulatory effect of antibiotics on the host.

KEYWORDS: Antibiotics - Antibiotic supplements - Farm animals - Intestinal flora - Microbial ecology - Plasmids - Resistance.

INTRODUCTION

Since their discovery, antibiotics have proved to be very valuable in controlling diseases of bacterial origin in human beings and animals.

In animal husbandry they have made a considerable contribution, together with vaccines and improved hygiene, to the development of intensive husbandry systems.

An additional property of antibiotics, that of growth promotion, was first discovered in poultry. However, the bacterial world has become adapted to antibiotics, and strains resistant to antibiotics have appeared, reducing their efficacy.

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At the same time, advances in knowledge of the microbial ecology of the digestive tract have thrown light on the changes in this microflora produced by antibiotics, which can have adverse consequences for the host under certain conditions.

Such evolution explains the need to intervene in the microflora and its bioregulation, in order to enhance its favourable effect on the health of the host, manifested in farm animals by improved productivity.

**USE OF ANTIBIOTICS IN ANIMAL HUSBANDRY**

Antibiotics belong to many different chemical families which share the common property of inhibiting the growth of bacteria or killing them when present in low concentrations. This antibacterial property has been applied in the treatment and prevention of infections.

In parallel to the use of antibiotics in human medicine, their veterinary use developed rapidly, and is far from being negligible, for about 40% of antibiotics produced are used in animals (17) (Table I).

**TABLE I**

*Geographical distribution of antibiotic use (in percentages) (17)*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Man</th>
<th>Animals</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feed additive</td>
<td></td>
</tr>
<tr>
<td>Great Britain</td>
<td></td>
<td>58%</td>
<td>21%</td>
</tr>
<tr>
<td>Swann report of 1969 for the year 1967</td>
<td></td>
<td>58%</td>
<td>21%</td>
</tr>
<tr>
<td>Canada</td>
<td></td>
<td>65%</td>
<td>24%</td>
</tr>
<tr>
<td>official statistics for 1974</td>
<td></td>
<td>65%</td>
<td>24%</td>
</tr>
<tr>
<td>France</td>
<td></td>
<td>54%</td>
<td>30%</td>
</tr>
<tr>
<td>Source: Rhône-Poulenc for 1978</td>
<td></td>
<td>54%</td>
<td>30%</td>
</tr>
<tr>
<td>Sweden</td>
<td></td>
<td>62%</td>
<td>25%</td>
</tr>
<tr>
<td>Wierup et al., 1987, for the year 1980</td>
<td></td>
<td>62%</td>
<td>25%</td>
</tr>
</tbody>
</table>

They are used for therapeutic purposes, and to an increasing extent for prophylactic purposes.

Relatively high doses are used for these purposes, whether by injection or by adding to drinking water or feed, usually 100 ppm (parts per million = g/tonne) or more.

When antibiotics are used as feed additives to promote the growth of meat animals, the concentration ranges from 5 to 50 ppm. Such use is regulated in many countries (17). For more than ten years now, legislation has been introduced to progressively prohibit the use as feed additives for animals of antibiotics also used in human beings. There is a total prohibition in some countries, such as Sweden and Japan.
The selection pressure exerted by antibiotics on the microflora of farm animals now results essentially from their therapeutic and, above all, prophylactic use (8, 24, 36).

**EFFECTS OF ANTIBIOTICS ON THE MICROFLORA OF THE DIGESTIVE TRACT**

When used for therapeutic or prophylactic purposes, antibiotics have many effects on the microflora, depending on the particular antibiotic and the family to which it belongs.

**Selection of resistant bacteria**

Bacteria may be naturally resistant to an antibiotic, or they may acquire resistance.

Resistance results from the absence of a target or lack of penetration of the antibiotic into the bacterial cell. Thus *Pseudomonas* species are naturally resistant to chloramphenicol and other antibiotics, which partly explains their frequent selection as a result of antibiotic therapy.

Acquired resistance is the appearance of subpopulations of bacteria which are resistant to a given antibiotic, within a population of the same species which is usually sensitive to it. Two principle mechanisms responsible for this phenomenon are chromosomal mutation, and acquisition by a sensitive bacterium of plasmids carrying resistance genes.

The second of these mechanisms is the most important, since plasmids usually carry a number of genes for resistance to antibiotics of different families (multiple resistance), and many of them are autotransferable by conjugation between bacteria, even between those of different species, particularly Gram-negative bacteria.

Following therapeutic or prophylactic antibiotic intervention, the antibiotic administered selects those bacteria resistant to it, to the detriment of those which are sensitive. A similar mechanism applies to the emergence of fungi, such as *Candida*, which are insensitive to antibacterial antibiotics.

This selective effect is exerted in the short term and in the long term.

**Short-term effect**

During antibiotic therapy, many antibiotics produce profound changes in the microflora (2, 3, 27). This is the case with ampicillin, which diffuses readily and leads to the disappearance of sensitive enterobacteria, selecting resistant bacteria like pseudomonads.

In the short term, aminosides seem to have little effect on the microflora apart from selecting resistant enterobacteria.

Tetracyclines select resistant strains among the *Escherichia coli* population. There is no change in the total *E. coli* population, but strains resistant to tetracycline and one or two other antibiotics become dominant (Table II) (5, 12).

Colistin seems to have a variable effect on the microflora of man and animals.
Influence of tetracycline treatment on antibiotic resistance among *E. coli* of the digestive flora of chicks
(from ref. 5)

1. *E. coli* isolated on medium containing ampicillin

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>1st sampling</th>
<th>2nd sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>%</td>
</tr>
<tr>
<td>Ap</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ap Sm</td>
<td>198</td>
<td>68.7</td>
</tr>
<tr>
<td>Ap Tc Sm</td>
<td>85</td>
<td>29.5</td>
</tr>
<tr>
<td>Ap Cm Sm Tc</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Ap Km Sm Tc</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

2. *E. coli* isolated on medium containing tetracycline

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>1st sampling</th>
<th>2nd sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>%</td>
</tr>
<tr>
<td>Tc</td>
<td>136</td>
<td>94.4</td>
</tr>
<tr>
<td>Tc Sm</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>Tc Cm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tc Km</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tc Ap Sm</td>
<td>6</td>
<td>4.1</td>
</tr>
<tr>
<td>Tc Cm Sm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tc Cm Km</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tc Km Sm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tc Ap Cm Sm</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* before treatment ** after treatment

Resistance to:
Ap = ampicillin, Cm = chloramphenicol, Km = kanamycin, Sm = streptomycin, Tc = tetracycline

In the case of quinolones, the most recent derivatives are very active against Gram-negative bacteria, which are displaced from the digestive tract during antibiotic therapy. It remains to be seen if treatment, particularly when repeated or of long duration, will select resistant mutants.

In general, the microflora becomes re-established during the days following cessation of treatment, becoming similar to that existing before treatment (23, 27).

**Long-term effect**

Within a given bacterial species, this is frequently manifested by an increased frequency of resistance to various families of antibiotics among pathogenic strains. This phenomenon has been well studied in the case of *E. coli* strains enteropathogenic for calves, particularly those possessing K99 attachment antigen (Table III) (12, 25).

The same observations have been made on strains of human origin, as a result of the high and repeated selection pressure exerted by these strains following treatments.
TABLE III

Resistance of *E. coli* with and without K99 antigen to antimicrobial agents
(from ref. 25)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>% resistant <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K99 negative</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>64</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>81</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>61</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>70</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>80</td>
</tr>
<tr>
<td>Colistin</td>
<td>0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td>84</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>17</td>
</tr>
<tr>
<td>Furanes</td>
<td>15</td>
</tr>
</tbody>
</table>

Effect on resistance to colonisation

As described by Van der Waaij *et al.* (33), colonisation resistance is a property possessed by an established microflora of the digestive tract, against the implantation and durable persistence of bacteria ingested later. This property of a flora is due mainly to the presence and activity of anaerobic bacteria.

Antibiotics effective against strictly anaerobic bacteria – particularly the broad-spectrum antibiotics, ampicillin, streptomycin, kanamycin and metronidazole – diminish their populations, considerably impairing the effect of resistance to colonisation and favouring the implantation of other bacteria in the digestive tract (18, 35).

Similarly, modification of certain populations of bacteria by antibiotics like lincomycin and clindamycin indirectly augment the population of toxin-forming clostridia in the microflora. The amount of toxin produced may be sufficient to produce enterocolitis in horses, rabbits and human beings.

Effect of bacterial adherence

Adherence to the intestinal epithelium is an important characteristic of enteropathogenic bacteria. Some antibiotics, such as tetracycline, are capable in subinhibitory concentrations of reducing the adherence of virulent bacteria to the intestinal epithelium, urinary epithelium and dental plaques.

Studies of the attachment of *E. coli* K88⁺ to cells of the intestinal mucosa of pigs by Denke *et al.* (6) showed that oxytetracycline diminished adherence in concentrations as low as 0.001 µg/ml. By contrast, the antibiotic was less effective when the bacterial strain possessed a plasmid coding for tetracycline resistance.

This effect of antibiotics is important, because it reduces the severity of infections with enteropathogenic bacteria.
Decontamination by antibiotics

Despite certain undesirable effects, such as the appearance of resistant bacteria, antibiotics retain considerable efficacy when used wisely. They can be used to decontaminate the digestive tract if this becomes necessary, such as for surgical operations on the digestive tract, in immunodeficient children and for neoplasms.

The choice of a mixture of antibiotics, comprising about four non-absorbable compounds, is made according to their efficacy against the microflora present (7, 32, 34). Once bacteria have disappeared from the faeces, the patient is placed in an isolator prior to operation. On leaving the isolator, the microflora has to be re-established, and a selected flora may be administered by mouth for this purpose.

Colonisation by bacteria resistant to antibiotics

Sterile at birth, the young animal acquires colonising bacteria from its immediate environment. They come particularly from the parent, but also from feed, the environment and from human beings (animal attendants).

As a consequence of the long-term use of antibiotics, pathogenic bacteria capable of infecting farm animals, and also saprophytic bacteria which can colonise the animal from birth, are often resistant to antibiotics. This resistance is usually multiple and derived from plasmids.

Colonisation procedures

Taking the example of digestive tract colonisation in chicks, which has been studied thoroughly (13, 14, 15, 19, 20, 26), it may be concluded that:

- Colonisation by resistant enterobacteria, mainly \textit{E. coli}, takes place shortly after birth.
- Selection pressure exerted by antibiotics is not indispensable for implantation of bacteria in the digestive tract (Fig. 1).
- Contamination with few bacteria is sufficient to colonise the digestive tract, given that it is in a sterile state at birth.
- Resistant bacteria may be of maternal origin, or from contamination of the eggshell or from the incubator, or from the environment, particularly feed, which may be very slightly contaminated. In addition, the attendant may transfer small numbers of resistant bacteria on his or her hands from one batch of animals to another. The small number transported may be sufficient, in the case of \textit{E. coli}, to result in colonisation of the digestive tract of newly-hatched chicks, independent of any antibiotic intervention.
- Resistant bacteria implanted in the digestive tract persist throughout the economic life of the animal, and can be recovered from the body after slaughter.

Plasmid transfer \textit{in vivo}

Certain plasmids, particularly those of Gram-negative plasmids, are autotransferable by conjugation between bacteria, even those of different species, independent of any selection pressure from antibiotics. This type of transfer can be reproduced in the digestive tract of animals (9, 10, 16, 29, 30).
Total enterobacteria • E. coli resistant to: chloramphenicol, tetracycline and sulphonamides.

E. coli resistant to: streptomycin, tetracycline and sulphonamides.

Enterobacteria resistant to kanamycin.

Enterobacteria resistant to ampicillin.

FIG. 1

Example of colonisation of the digestive tract of chicks by antibiotic resistant enterobacteria, in the absence of selection pressure from antibiotics (13)

Conditions favouring transfer are the variety of bacteria present, their population density and the extent of mixing.

Figures 2 and 3 demonstrate transfer between isogenic strains of *E. coli* in the digestive tract of the fowl, in the absence and presence of selection pressure (16). The rapid appearance of transconjugants and their prolonged persistence in the digestive tract are aspects worthy of note. When a microflora is present, the transfer is reduced considerably, but antibiotic therapy reveals and selects the transconjugants present. The ability of recipient bacteria to accept plasmids appears to be an important part of this process (29).

ANTIBIOTICS AND THE CARRIAGE OF PATHOGENIC BACTERIA BY HEALTHY ANIMALS

In addition to saprophytic bacteria, the digestive microflora often includes potentially pathogenic bacteria, such as *Salmonella* and *Campylobacter*.

Antibiotics may influence this carrier state, particularly when used as growth promoters. Among farm animals the carriage of salmonellae commences soon after birth. It is common and often persists throughout the economic life of the animal, as is the case in poultry.
Antibiotic supplementation influences the implantation, frequency of carriage and duration of excretion. The effect of penicillin in favouring carriage is clear-cut, but the results obtained with bacitracin, virginiamycin, nitrovin and avoparcin are divergent because of differing experimental conditions, particularly when the antibiotic treatment is not accompanied by controls (1, 11, 21, 28, 31).
Transfer of plasmids \textit{in vivo} between \textit{E. coli} strains (16)

\textit{In vivo} transfer of plasmid pGB99 CmTcSuTp in dixenic chicks in the presence of antibiotic

In the absence of experiments conducted under adequately controlled conditions, it is difficult to draw conclusions on this effect of antibiotics, which is important for public health. Further research on these mechanisms is required.
ANTIBIOTICS AND PROBIOTICS

Because of the consequences of injudicious use of antibiotics, particularly the emergence of resistance to antibiotics, legislation has become more and more restrictive for the use of these drugs as growth promoters.

Since antibiotics have beneficial effects and can to some extent improve nutrition as well, the search is on for micro-organisms or substances capable of replacing them, and this has led to the concept of probiotics.

Results obtained show that certain probiotics have a slight favourable effect on the productivity of farm animals, in most cases inferior to that obtained with antibiotics (4, 22).

Simultaneous administration of antibiotics and probiotics, however, does not seem to have a synergic effect (22). Moreover, frequent administration of antibiotics for therapeutic or prophylactic purposes to growing animals affects the digestive microflora, and may interfere with the effect of probiotics. This has not yet been examined in detail.

CONCLUSION

The growing use of antibiotics reflects the value of these substances for treating and preventing infections of bacterial origin. The frequency of resistant strains capable of colonising animals, even in the absence of selection pressure, is worrying, for any antibiotic therapy utilising drugs to which the strain is resistant will select this strain within the microflora. Moreover, the plasmid basis of much resistance also promotes the spread of antibiotic resistance.

Other effects of antibiotics on the microflora, including diminution of resistance to colonisation, effect on the adherence of bacteria to epithelia, effect on the carriage of pathogenic bacteria and the growth promoting effect, are evidence of the bioregulatory properties which antibiotics possess, even though not all of the mechanisms responsible have been elucidated.

These different actions on the bacterial flora make it necessary to select wisely the antibiotics used in therapy and prophylaxis in order to limit their undesirable effects. In the case of antibiotic supplementation, attention should be turned to other bioregulators, the probiotics, which should have the same effects on productivity without the inconvenient effects of antibiotics.

* * *

REFERENCES


