

Responsible use of antimicrobials



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Disclaimer: this booklet is intended as a reference for veterinary professionals and aims to support them in making antimicrobial therapy choices. The veterinarian is responsible for the prescription of veterinary medicines to animals. Dopharma is not responsible for the veterinarian's therapy choice nor other decisions arising from the use of this booklet.

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Preface

Since establishment in 1969, the veterinary pharmaceutical company Dopharma focuses on providing distinct veterinary pharmaceuticals for livestock.

In order to ensure efficacy and safety for animals, people and the environment, investing in pre-marketing research & development, pharmacovigilance and promotion of responsible use of our products, is our top priority.

This booklet is an outcome of Dopharma's Technical Support team's commitment to provide reliable and valuable support to clients. For questions you can contact the veterinarians of the Technical Support team.



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1 Introduction



There is an increasing interest in the prudent use of antimicrobials in veterinary medicine. The responsible use of these medicines is essential, not only for the efficacy in the target species, but also to minimize the induction of resistance and to ensure food safety.

Responsible use of antimicrobials does not only mean reducing their use, but also choosing the right antimicrobial and administering it in an appropriate manner. Performing sensitivity tests is a very important tool in determining the preferred antimicrobial substance. When it is known to which antimicrobials the bacterium concerned is sensitive, there is often still a choice available between several active ingredients. Knowledge of the pharmacodynamic and pharmacokinetic properties of the available options is then necessary to make the right decisions. Also when veterinary medicines have to be used off label this knowledge is essential to choose the right therapy.

This booklet can be used as a reference document for information about the pharmacodynamics and pharmacokinetics of the most frequently used veterinary antimicrobial products. At the end of this booklet you can find a table in which an overview is given of the active ingredients most often used in veterinary medicine and their most important properties.

1.1 PHARMACODYNAMICS AND PHARMACOKINETICS

Pharmacodynamics describe how the active ingredient of a medicine achieves its effect once inside the body. The antimicrobials in the table in the appendix are categorized based on their mode of action, which is the most important pharmacodynamic parameter. This will be described in more detail in the chapters about the active ingredients. The spectrum of antimicrobials is also important, but will not be discussed in this booklet, unless relevant.

Pharmacokinetics describe how the body deals with the active ingredient. The most important pharmacokinetic components are: Absorption, Distribution, Metabolism and Elimination (ADME). The most important parameters are: C_{max} (maximum concentration), T_{max} (time after which the maximum concentration is reached), C_L (clearance), $T_{1/2EL}$ (elimination half-life) and V_d (volume

of distribution). These parameters depend on the characteristics of the active ingredient, but also on the formulation, the route of administration and the dosage regime.

1.2 BACTERICIDAL OR BACTERIOSTATIC EFFECT

The difference between bactericidal and bacteriostatic antimicrobials is generally known; bactericidal antimicrobials kill the bacteria, while bacteriostatic antimicrobials inhibit their growth. In vitro it is difficult to make a distinction: not all bactericidal antimicrobials will kill all bacteria within the set time, while some bacteriostatic antimicrobials will kill certain bacteria when the concentrations are high enough.

In practice it is often assumed that bactericidal antimicrobials are needed in acute infections or when the immune system is not functioning properly. However, in some situations bacteriostatic antimicrobials are preferred. Bactericidal antimicrobials could cause rapid death of the bacteria involved and when these bacteria produce endotoxins, this may result in an endotoxin surge which can enhance the clinical signs of disease.

1.3 LIPOPHILICITY & DISSOCIATION CONSTANT

To be effective, the antimicrobial substance drug must, of course, reach the bacteria in various tissues and it is therefore often necessary that the drug can pass cell membranes. In addition to active transport and diffusion through pores, passive diffusion of antimicrobials is also important for the passage over membranes. Passive diffusion mainly depends on the lipophilicity and the dissociation constant of the antimicrobial drug used. In the table at the end of this booklet both parameters are given for each group of antimicrobials. Highly lipophilic substances can pass the phospholipid structure of membranes more easily than substances with a low lipophilicity. As a result, highly lipophilic substances often reach effective concentrations in the synovial fluids, the eye and the cerebrospinal fluid. Diffusion of moderately lipophilic substances to these tissues depends on the plasma protein binding and the volume of distribution. These parameters are also shown in the appendix.



The dissociation constant (pK_a) of a drug indicates to which extent drugs are ionized or non-ionized at a particular pH. Passage of membranes by passive diffusion is only possible if the substance concerned is in non-ionized state. When there is a pH difference between two different tissues (e.g. plasma and lung tissue), the antimicrobial substance will accumulate in one of these tissues. Where depends on the pK_a of the substance. In general, weak bases will accumulate in the tissue with the lower pH and weak acids will accumulate in the tissue with the higher pH. This phenomenon is called ion trapping. In the body the pH difference between plasma and different tissues and the difference between the intracellular and extracellular pH are mainly of interest. Examples of tissues with a pH lower than the pH of the plasma are the lungs, the prostate, milk and the urine of carnivores. The urine of herbivores has a pH higher than the pH of plasma. To illustrate: in order to obtain high concentrations of an antimicrobial drug in the lungs, it is best to choose a lipophilic antimicrobial substance with a high pK_a .

1.4 CONCENTRATION OR TIME DEPENDENT EFFECT

For concentration dependent antimicrobials, an increase in concentration will result in a faster and better effect on the bacteria. The most important parameter for these antimicrobials is C_{max}/MIC . This is the ratio between the maximum plasma concentration (C_{max}) and the minimal inhibitory concentration (MIC). This is illustrated in Figure 1. To achieve maximum results, the C_{max} should be ten times as high as the MIC. In order to obtain a concentration high enough, it is possible to administer the drugs by means of pulse dosing. The duration that the concentration of the antimicrobial drug remains high is less important for these antimicrobials, because these antimicrobials usually have a significant post-antibiotic effect (PAE).

For the time dependent antimicrobials the efficacy depends on the period during which the bacteria are exposed to the antimicrobial drug. The most important parameter is the time period in which the concentration is higher than the MIC ($T > MIC$), as illustrated in Figure 2. To achieve maximum effect, the period that the concentration is higher than the MIC should at least be as long as half the dosing interval. When pharmacokinetic studies for example show that the concentration of a particular antimicrobial drug remains above the MIC for a period of six hours, a dosing interval of up to twelve hours can be used. In general it can be assumed that these

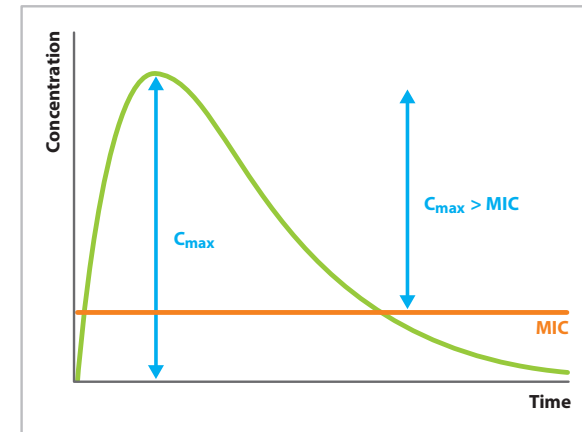


Figure 1 Pharmacokinetic parameters of concentration dependent antimicrobials.

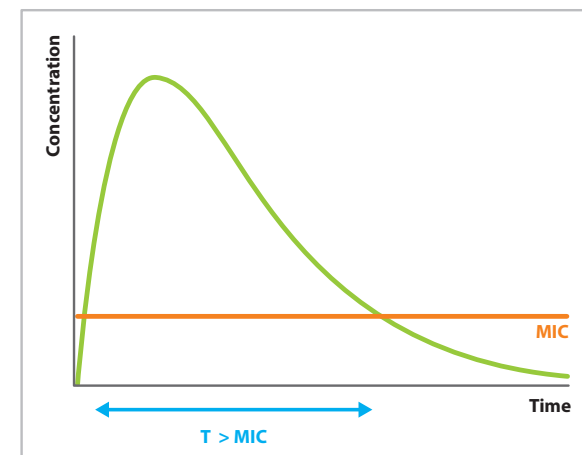


Figure 2 Pharmacokinetic parameters of time dependent antimicrobials.

antimicrobials should be provided as often as possible, preferably continuously in the drinking water.

Finally, there are some antimicrobials which are both concentration- and time-dependent. When using these antimicrobials, not only a high concentration should be achieved but this should also be maintained for a certain period of time. The most important parameter for these antimicrobials is the AUC (area under the curve), which is shown in Figure 3. In general, the ratio AUC/MIC should be bigger than 125.

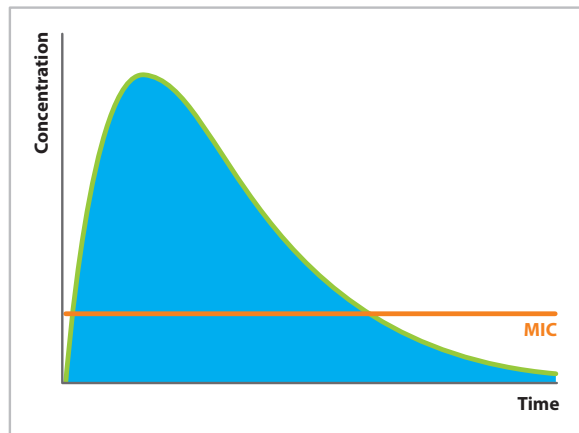


Figure 3 Pharmacokinetic parameters of antimicrobials which are both time and concentration dependent.



2 Penicillins & Cefalosporins



2.1 PHARMACODYNAMICS

Penicillins and cephalosporins are both β -lactam antibiotics. The bactericidal mechanism of action of β -lactam antibiotics is based upon the inhibition of the cell wall synthesis. The peptidoglycan of the cell wall is made up of different peptide chains and sugars, which are linked by penicillin binding proteins (PBPs) such as transpeptidase. β -lactam antibiotics bind irreversibly to these PBPs preventing peptidoglycan to be formed. β -lactam antibiotics only have an effect on replicating bacteria as the cell wall is only opened whilst bacteria replicate and peptidoglycan is then needed to close it.

Within the group of penicillins there is a clear distinction between penicillins that are only effective against Gram-positive bacteria and penicillins with a broad spectrum. This can partly be explained by the structure of the bacteria. In Gram-positive bacteria the peptidoglycan cell wall is situated on the outside of the cell making the PBPs readily available, as can be seen in Figure 4. In Gram-negative bacteria the peptidoglycan cell wall is surrounded by a lipid layer, making the PBPs more difficult to access. The narrow spectrum penicillins are not capable of passing the lipid outer layer of the cell wall of Gram-negative bacteria, while the broad spectrum penicillins generally are. Other factors that influence the susceptibility to penicillins are the structure of the PBPs, the amount of peptidoglycan (Gram-positive bacteria contain a larger amount of peptidoglycan) and resistance mechanisms. Different cephalosporins also show different spectra. The first and second generation cephalosporins (cephalexin, cefalonium, cefapirine) have a narrow spectrum and are only effective against Gram-positive bacteria. The newer cephalosporins (3rd generation: cefaperazon, ceftiofur; 4th generation: cefquinome) are increasingly effective against Gram-negative bacteria.

Because penicillins and cephalosporins disturb the cell wall synthesis, they are ineffective against bacteria that lack a cell wall such as *Mycoplasma* spp. Animals also lack PBPs, which explains the wide safety margin of penicillins.

2.2 LIPOPHILICITY & DISSOCIATION CONSTANT

Penicillins and cephalosporins have a low lipophilicity. The biological availability of penicillins and cephalosporins is in general not very good. Exceptions are amoxicillin and penicillin V (phenoxymethylpenicillin). These antibiotics have a better oral availability than other penicillins. After parenteral administration the biological availability is better and absorption from the injection site occurs quickly.

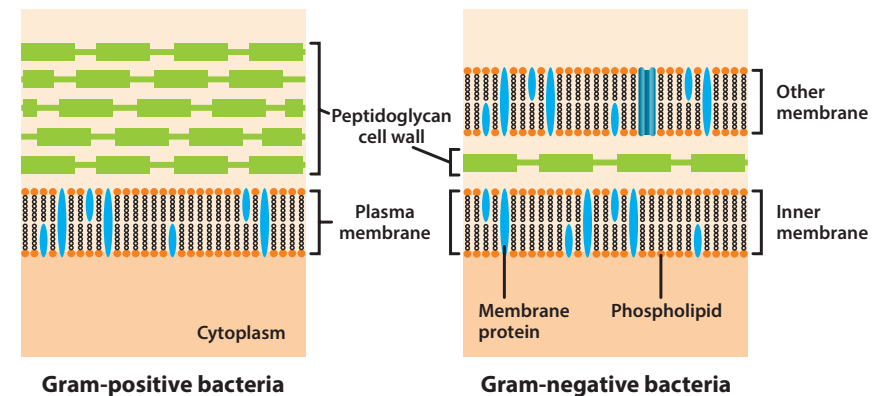


Figure 4 Cell wall of Gram-positive and Gram-negative bacteria (Openstax CNX)



The low pK_a of penicillins and cephalosporins is responsible for the small volume of distribution; in plasma a large part of these antibiotics is ionized. The ionized form is less capable of passing biological membranes than the non-ionized part. Consequently, the concentration in the udder for example is only approximately one fifth of the plasma concentration. This is the reason why cephalosporins are often administered locally (intramammary).

In the presence of an inflammatory process the passage over biological membranes increases. The (extracellular) pH of inflamed tissues is lower than the (extracellular) pH of healthy tissues. With the decline in pH, a larger concentration of penicillins in the tissue becomes non-ionized, which results in a better ability to pass membranes. This explains why penicillins do reach effective drug concentrations in inflamed tissues which they hardly penetrate in normal situations.

2.3 CONCENTRATION OR TIME DEPENDENT EFFECT

β -lactam antibiotics are time dependent antibiotics. This means they are particularly effective when the bacteria are exposed to this antibiotic for a sufficient period of time. Continuous administration is therefore preferred over pulse dosage. To achieve an optimal effect the dosing interval should be no longer than twice the period during which the tissue concentration is higher than the MIC. This period has to be determined in species specific pharmacokinetic trials. Injections with penicillins are often administered multiple times daily or contain penicillins bound to procaine, resulting in a slow release formulation.

In penicillins there is also another phenomenon called 'Eagle effect'. In certain bacteria (e.g. *Enterococcus* spp.) a higher than usual concentration appears to be less effective than the usual concentration. This effect is caused by the inhibition of growth, which is, as explained above, essential for the efficacy of penicillins. This highlights the importance of a correct dose regimen in which levels are sustained above the MIC for a sufficient period of time, but without high peak concentration.

2.4 COMBINING ANTIMICROBIALS

As described above, β -lactam antibiotics are only effective when used against replicating bacteria. This means they should never be combined with bacteriostatic antibiotics such as most protein synthesis inhibitors. Aminoglycosides are the exception to this rule as these protein synthesis inhibitors are bactericidal. The combination of β -lactam antibiotics with aminoglycosides has a synergistic effect. β -lactam antibiotics increase the permeability of the cell wall and the aminoglycosides benefit from this change.

2.5 INDUCTION OF RESISTANCE

Resistance against β -lactam antibiotics is usually based on β -lactamases. These enzymes cut open the β -lactam ring of β -lactam antibiotics so they can no longer bind to PBPs and are therefore ineffective. This mechanism of resistance is mainly transmitted via plasmids. Transmission via plasmids is particularly effective in Gram-negative bacteria. As a result, there is greater diversity of β -lactamases in Gram-negative bacteria than in Gram-positive bacteria. The degree of induction of resistance in narrow spectrum penicillins which are only effective against Gram-positive bacteria, is therefore very low. Broad spectrum penicillins on the other hand can induce resistance reasonably quickly. This was also seen in the Dutch monitoring program; resistance against penicillins is particularly prevalent in Gram-negative bacteria.

There are several penicillins that are not susceptible to β -lactamases. One example of a penicillin that is used in the veterinary field is cloxacillin. The newer generation cephalosporins are also less susceptible to β -lactamases than the penicillins and older cephalosporins. The difference in susceptibility is caused by an alteration of the structure. As a result, the β -lactam ring is situated at another position within the molecule. In addition, penicillins can be combined with β -lactamase inhibitors such as clavulanic acid. These inhibitors will neutralize the effect of β -lactamase enzymes.



Other mechanisms of resistance that may be of interest in the context of β -lactam antibiotics are adjustments of the PBPs and a decrease in the intracellular concentration of the antibiotic caused by efflux pumps or a decreased permeability. The decreased production of pores which are usually used to pass the outer membrane is only applicable to Gram-negative bacteria such as *E.coli*. This mechanism of resistance is often linked to a resistance mechanism that results in higher efflux of β -lactam antibiotics over the outer membrane. This is also only applicable to Gram-negative bacteria.

Specific groups of bacteria that may show resistance against penicillins are MRSA, and ESBL- and AmpC- producing bacteria. Methicillin resistant *Staphylococcus aureus* (MRSA) is resistant to almost all β -lactam antibiotics. Resistance in these bacteria is caused by a chromosomal mutation on the *mecA* gene. This gene encodes a PBP with a different structure, making it virtually impossible for β -lactam antibiotics to bind to it. Extended spectrum β -lactamase (ESBL) producing bacteria are insensitive to penicillins, cephalosporins and monobactams. These bacteria are capable of breaking down the β -lactam ring. These bacteria are however sensitive to carbapenems and β -lactamase inhibitors.

AmpC-producing bacteria are a subgroup of ESBL-producing bacteria. Bacteria that produce this enzyme are in contrast to the previous group also insensitive to β -lactamase inhibitors. Carbapenems are the only treatment option left in those cases, but there are no registered carbapenems in the veterinary field.



3

Polymyxines



3.1 PHARMACODYNAMICS

Like β -lactam antibiotics, polymyxins have the cell wall as their target and are bactericidal. Their mode of action is different however. Polymyxins disturb the rigidity of the cell wall of Gram-negative bacteria by binding to the lipopolysaccharides (LPS) that are present inside the cell wall of these bacteria (Figure 5). This results in a decreased integrity of the cell wall and eventually death of the bacteria. LPS is also an endotoxin and binding of polymyxins to LPS will neutralise their toxic effects. Calcium and magnesium also bind to LPS and the presence of these ions in the gastro-intestinal tract will result in competitive binding and a decreased efficacy of orally administered polymyxins.

3.2 LIPOPHILICITY & DISSOCIATION CONSTANT

Polymyxins have a low lipophilicity which results in poor absorption rates from the gastro-intestinal tract. After parenteral administration the biological availability is good and absorption from the injection site will occur quickly. The passage of membranes will however still be limited and polymyxins have a small volume of distribution. Due to the pharmacokinetics of polymyxins,

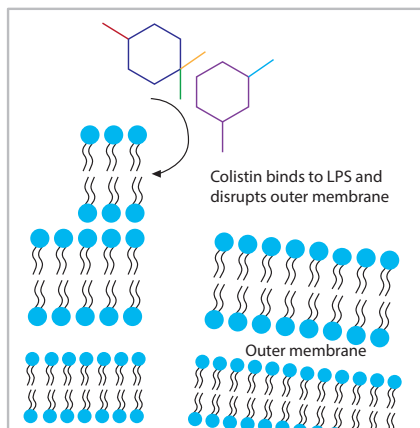


Figure 5 Mode of action of polymyxins (Expert Rev. Anti Infect. Therap© Future science group (2012))

colistin is usually administered parentally, or when administered orally, used for treatment of infections of the gastro-intestinal tract. Polymyxin B is mainly used for the topical treatment of infections. Polymyxins have a high pK_a which means that they will accumulate in tissues with a pH that is lower than the pH of plasma.

3.3 CONCENTRATION OR TIME DEPENDENT EFFECT

The effect of polymyxins is concentration dependent. The duration of exposure is therefore less important than the concentration that is achieved at the site of the infection. The peak tissue concentration is preferably ten times as high as the MIC of the bacteria that are to be treated.

3.4 COMBINING ANTIMICROBIALS

Polymyxins can be combined with sulphonamides and trimethoprim to achieve a synergistic effect against different *Enterobacteriaceae*, including *P. aeruginosa*. In the field polymyxins are often combined with amoxicillin. This combination is synergistic in the treatment of infections that are caused by a combination of bacteria. The combination of colistin with amoxicillin is however also used to inhibit the induction of resistance to amoxicillin. In humans polymyxins in combination with amoxicillin is used for the treatment of multi-resistant bacteria such as specific strains of *P. aeruginosa*, *E. coli* and *Enterobacter* spp.

3.5 INDUCTION OF RESISTANCE

Resistance against polymyxins is very rare, but does sometimes occur in *Salmonella* spp. When resistance occurs it is usually based upon a decreased amount of LPS molecules in the cell wall or a change in the prevalent LPS molecules based on changes in the polarity of these molecules. Due to a change in polarity, the distance between the LPS molecules is decreased, making it more difficult for polymyxins to bind to the LPS molecules. Recent research showed plasmid mediated resistance in *E.coli* strains isolated from animals. Resistance to one of the polymyxins always results in resistance to all other polymyxins. We can therefore say there is complete cross resistance.



4

Diaminopyrimidines & Sulphonamides



4.1 PHARMACODYNAMICS

Diaminopyrimidines and sulphonamides inhibit the DNA synthesis of bacteria by inhibiting the folic acid synthesis. Sulphonamides interfere with the first step of the folic acid synthesis; the step where para-aminobenzoic acid (PABA) is added to dihydropteroate diphosphate. Sulphonamides are structural analogues to PABA and compete for the enzyme dihydropteroate synthesis.

Diaminopyrimidines prevent the conversion of dihydrofolate into its active metabolite tetrahydrofolate by competitive binding of the enzyme dihydrofolate reductase. The bacterial folic acid synthesis and the mode of action of both diaminopyrimidines (trimethoprim) and sulphonamides is illustrated in Figure 6. Sulphonamides are bacteriostatic, while diaminopyrimidines are dose dependent bactericidal. The combination of both is bactericidal.

As explained, the mode of action of sulphonamides is based upon competitive binding with PABA. In an environment with a high concentration of PABA, sulphonamides will not be able to compete effectively, and as a result, have a reduced efficacy. High concentrations of PABA can be found in inflammatory processes with much pus, necrosis and/or debris. This explains why sulphonamides in some cases do not work in vivo despite a good in vitro antimicrobial sensitivity.

Diaminopyrimidines and sulphonamides have a broad spectrum. This can be explained simply by the fact that folic acid is essential for the production of DNA and RNA in almost all bacteria. Only some *Enterobacteriaceae* are capable of absorbing folic acid from their environment, which leaves them less susceptible to sulphonamides.

Folic acid synthesis is not only important to bacteria, but also to mammals, especially during pregnancy. Despite the fact that bacterial dihydrofolate reductase is much more sensitive to trimethoprim than the same enzyme in mammals, these antimicrobials should be used with caution in pregnant animals.

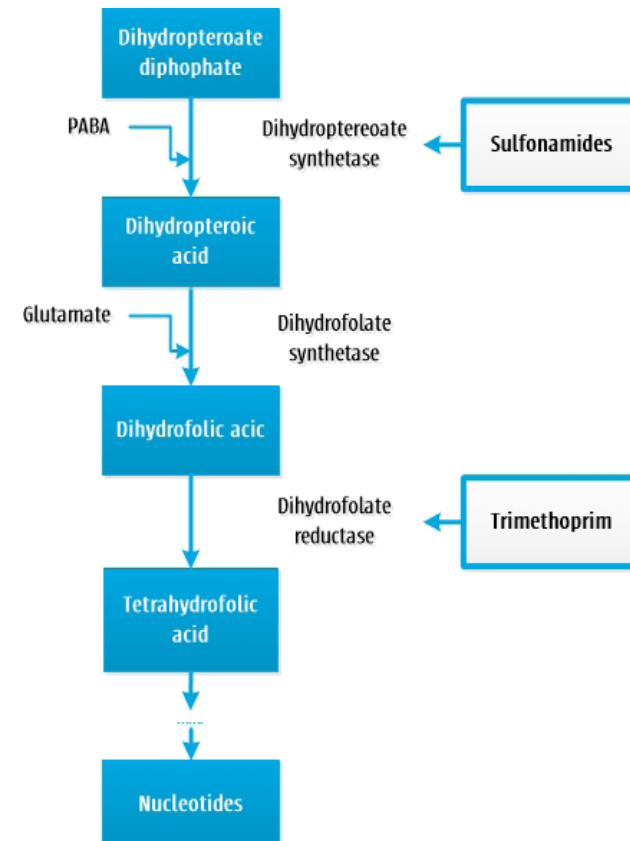


Figure 6 Bacterial folic acid synthesis and mode of action of trimethoprim and sulphonamides



4.2 LIPOPHILICITY & DISSOCIATION CONSTANT

Both sulphonamides and diaminopyrimidines have a moderate lipophilicity. The plasma protein binding of sulphonamides is usually high, but can vary greatly between different sulphonamides and between different animal species. Diaminopyrimidines have a moderate plasma protein binding. The volume of distribution of sulphonamides is small so they are not very effective intracellular. They are however usually well distributed over different tissues including synovial and cerebrospinal fluids. Diaminopyrimidines have a larger volume of distribution and also reach sufficient levels intracellular.

The pKa of the two antimicrobial classes differs: sulphonamides have a lower pKa than diaminopyrimidines. This results in accumulation of sulphonamides in tissues with a pH higher than the pH of the plasma, while diaminopyrimidines accumulate in tissues with a pH lower than the plasma pH.

Elimination of both sulphonamides and diaminopyrimidines partly takes place by biotransformation in the liver, and subsequent excretion via the kidneys, gall, milk and faeces. In addition, some of these antimicrobials are excreted unchanged via the kidneys. The amount of unchanged antimicrobials excreted is dependent on the pH of the urine; at an alkaline pH a higher proportion of the sulphonamides will be excreted unchanged while a larger part of diaminopyrimidines will be excreted unchanged when the urine is acidic. The differences in biotransformation rate are responsible for the variations in elimination half-life in different animal species.

The alkaline nature of sulphonamides makes them irritating when injected. Sulphonamides and combinations with trimethoprim should therefore be administered slowly.

4.3 RATIO EN ELIMINATION HALF LIVES

When using combined products of trimethoprim with one of the sulphonamides the desired in vivo ratio between these antimicrobials is 1 : 20 because trimethoprim is

approximately twenty times more potent than the sulphonamides. Human research has shown that this ratio can best be achieved by the administration of drugs that contain these antimicrobials in a ratio of 1 : 5.

The elimination half-life of sulphonamides varies per substance and between species. This means that the elimination half-life of the sulphonamide used does not always correspond to the elimination half-life of trimethoprim. As a result, the ratio between trimethoprim and the sulphonamide will correspond to 1 : 20 for only a short period of time. The elimination half-lives of trimethoprim and the most important sulphonamides are shown in Table 1 (page 30).

Despite this difference in elimination half-life, the clinical efficacy of this combination of antimicrobials is usually very good. There are several factors that might play a role in this phenomenon. Firstly, the large volume of distribution of trimethoprim ensures that concentrations in the tissues (where the inflammation usually occurs) are usually higher than the plasma concentrations and are usually maintained for a longer period. Additionally, in vitro research has shown that synergism between these antibiotics does not only occur at a range of 1 : 20, but at a much broader range (namely 1 : 1 to 1 : 1000). Finally, the pharmacokinetics in ill animals or at the site of inflammation can differ from pharmacokinetics in healthy animals.

4.4 CONCENTRATION OR TIME DEPENDENT EFFECT

Sulphonamides and diaminopyrimidines both have a time dependent mode of action. This means that the most important pharmacokinetic parameter is the time during which the concentration of the antibiotic is higher than the MIC of the bacterium targeted.

4.5 COMBINING ANTIMICROBIALS

Diaminopyrimidines and sulphonamides are often combined to achieve a bactericidal effect. This combination can however also be combined again with other antibiotics to broaden to spectrum to both aerobic and anaerobic bacteria and to make the combination effective in situations where



Table 1 Elimination half-lives of trimethoprim and different sulphonamides in cattle, calves, pigs and horses after intravenous administration (per hour)

	Cattle	Calf	Pig	Horse
Trimethoprim	1.0 – 2.0	1.9 - 2.1	2.7 - 2.8 ^{2*}	2.0 – 3.0*
Sulfadiazine	2.5	4.4 ¹	2.8	4.6
Sulfamethoxazole	2.3	12.8*	12.9 en 13.4 ²	3.5
Sulfachlorpyridazine	1.2	13.1*	3.0 ³	3.8
Sulfadoxine	10.8 – 13.0	12.9	8.2 en 8.4 ⁴	14.2

1 Elimination half-lives in calves decreases from 5.7 to 3.6 between the age of 1 and 42 days.

2 Elimination half-life in healthy animals and animals with pneumonia, respectively.

3 Elimination half-lives determined after oral administration of sulfachlorpyridazine in pigs.

4 Elimination half-lives after administration of sulfadoxine and sulfadoxine with trimethoprim, respectively.

* Method of administration is not mentioned in the study used.

the bacteria might be surrounded by pus and/or debris. This can for example be achieved by combining them with a penicillin.

Combining the combination of trimethoprim and sulphonamides with other veterinary medicines in drinking water is not recommended. The combination of trimethoprim and sulphonamides is poorly soluble because trimethoprim dissolves in an acidic environment, while sulphonamides dissolve best in an alkaline environment. Excipients are often added to these products to increase solubility, but the effect of these excipients can be neutralised when other veterinary medicines or their excipients are added.

At last, sulphonamides are better not combined with procaine or procaine penicillin, because procaine is a structural analogue of PABA and sulphonamides will compete with procaine for the enzyme dihydropteroate synthesis.

4.6 RESISTANCE

Resistance against sulphonamides is regularly demonstrated in bacteria isolated from cattle, pigs and poultry, especially in *E.coli* and certain *Salmonella* spp. Resistance against sulphonamides can be either chromosomal or plasmid mediated. It can be caused by three mechanisms of action:

1. Change of the enzyme dihydropteroate synthetase which causes inability of sulphonamides to bind to this enzyme;
2. Reduction of the intracellular concentration of sulphonamides by a decrease in the permeability of the outer membrane for these antimicrobials;
3. Production of PABA which can compete with sulphonamides.

Bacteria that are resistant to one sulphonamide are also resistant to other sulphonamides (complete cross-resistance). Multi-drug resistance is also known in which case bacteria might also be resistant to other antimicrobials such as trimethoprim or streptomycin. This is often seen when resistance is plasmid-mediated.

Resistance against diaminopyrimidines is often based on a change of the enzyme dihydrofolate reductase which leaves trimethoprim unable to bind to this enzyme. However, a decrease in permeability also occurs. For diaminopyrimidines, resistance can also be chromosomal or plasmid mediated.



5

Fluoroquinolones



5.1 PHARMACODYNAMICS

Fluoroquinolones inhibit the bacterial synthesis of DNA by preventing transcription and translation of DNA. They achieve this by inhibiting the essential enzymes DNA gyrase and topoisomerase. During the production of DNA these enzymes are responsible for the supercoiling of DNA strands. To do this the DNA strands are bound to the enzymes, cut open, turned, and closed. This is illustrated in Figure 7. Fluoroquinolones inhibit these enzymes by forming complexes with both the DNA strand and the enzyme. Besides inhibiting the DNA synthesis and the repair of DNA this also results in the release of broken DNA strands inside bacterial cells. This will cause oxidative stress for the bacteria. The combination of these factors results in the bactericidal effect of fluoroquinolones.

The spectrum of fluoroquinolones differs per active ingredient. This is explained by a varying affinity for the bacterial enzymes. Flumequine for example is only effective against *Enterobacteriaceae* like *E. coli* and *Salmonella* spp. The addition of an additional fluoride molecule in enrofloxacin results in the fact that this antimicrobial can also bind to Gram-positive bacteria and anaerobes. This broadens the spectrum and includes also *Bordetella bronchiseptica*, *Manheima haemolytica*, *Pasteurella* spp., *Chlamydomphila* spp. and *Actinobacillus pleuropneumoniae*.

5.2 LIPOPHILICITY & DISSOCIATION CONSTANT

Flumequine and comparable molecules have a reasonable biological bioavailability after oral administration. The newer generation fluoroquinolones such as enrofloxacin have a very good oral bioavailability. Due to the high lipophilicity and the low plasma binding the newer fluoroquinolones have a large volume of distribution and reach high tissue concentration in, amongst others, the brain, intestines, liver and urinary tract. Fluoroquinolones accumulate in phagocytic cells in which they are also effective against intracellular bacteria.

Feed components form complexes with fluoroquinolones and thereby decrease the rate of absorption of fluoroquinolones. The AUC (area under the curve) and thus the biological bioavaila-

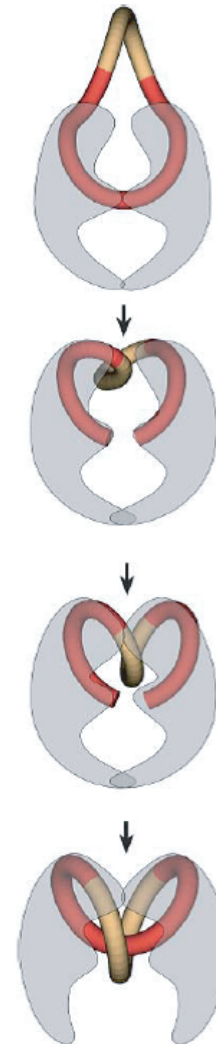


Figure 7 Mode of action of DNA gyrase (Vologodskii, 2004)



bility generally does not change. Only when the feed contains high concentrations of magnesium or aluminium, the biological bioavailability might be impaired significantly.

Fluoroquinolones are amphoteric molecules. This means that they have a double pK_a : one low and one high pK_a . The distribution is therefore good over tissues with a pH that is higher than the pH of the plasma, but also over tissues with a pH lower than the pH of plasma.

5.3 CONCENTRATION OR TIME DEPENDENT EFFECT

Fluoroquinolones have a concentration dependent effect. For these antibiotics the duration of exposure is less important than the peak concentration that is achieved at the site of the infection. To obtain an optimal treatment efficacy, the tissue peak concentration should be ten times as high as the MIC of the bacterium that is treated.

5.4 COMBINING ANTIMICROBIALS

Fluoroquinolones can be combined with β -lactam antibiotics or aminoglycosides to achieve a synergistic effect.

5.5 INDUCTION OF RESISTANCE

Fluoroquinolones inhibit the enzymes DNA gyrase and topoisomerase. One of the functions of these enzymes is the monitoring of the formed DNA strands and the repair of mutations that have occurred during DNA synthesis. Inhibition of these enzymes will result in an increased amount of mutations in the bacterial DNA. As a result, the number of mutations that will induce resistance will also increase.

Resistance against fluoroquinolones is induced by a change of the binding site of the enzymes, a reduction in the permeability of the efflux pumps and/or blocking of the binding site. A reduced permeability is caused by a decrease in the number of OMP (outer membrane protein) pores in the outer membrane of Gram-negative bacteria.

This mechanism of resistance will often result in resistance against other antimicrobials that use these pores. Examples of such antimicrobials are tetracyclines and β -lactam antibiotics.

Bacteria that develop resistance against one of the fluoroquinolones are often also resistant for other antimicrobials. This is especially true for the older molecules and for bacteria that have developed several mechanisms of resistance.

In the Netherlands the occurrence of resistance against fluoroquinolones is not uncommon. Resistance against enrofloxacin has been shown for *E. coli* (cattle) and *Enterococcus* spp. (poultry). Resistance against flumequine has been found in cattle (*E. coli*, *Salmonella* spp., *M. haemolytica* and *Pasteurella* spp.), chicken (*E. coli*) and pigs (*B. bronchiseptica*).



6

Tetracyclines & Aminoglycosides



6.1 PHARMACODYNAMICS

Tetracyclines and aminoglycosides bind reversibly to the 30S subunit of the bacterial ribosome. The 30S subunit is the small subunit, as can be seen in Figure 8. This subunit is responsible for decoding the RNA in order to incorporate the correct amino acids into the protein chain. Tetracyclines interfere with the first phase of this process; the binding of tRNA to the ribosome. This results in the inability to read tRNA and thus prevents formation of any peptide. When no peptides are formed, the bacteria cannot replicate and these antibiotics are therefore bacteriostatic.

Aminoglycosides bind to another receptor of the 30S subunit. They do not prevent tRNA from binding, but they cause misreading of the tRNA. The wrong amino acids are incorporated into the protein. When these default proteins are incorporated into the cell wall, this will result in disruptions of the permeability and finally lysis of the bacteria. This explains why aminoglycosides are the only bactericidal protein inhibitors.

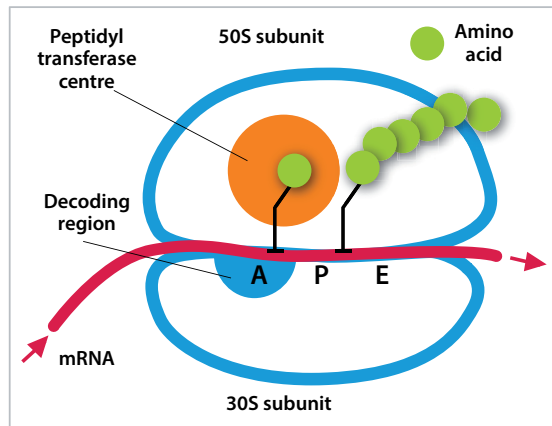


Figure 8 Bacterial ribosome (Williamson, 2000)

6.2 LIPOPHILICITY & DISSOCIATION CONSTANT

In the introduction it was described that the lipophilicity and the pK_a are important for the passage of membranes. In case of tetracyclines and aminoglycosides this is especially important because they will have to pass the cell membrane to reach the bacterial ribosome.

Tetracyclines have a moderate lipophilicity and a large volume of distribution. The absorption from the intestinal tract is reasonable, but tetracyclines are chelated agents which bind to food particles. Therefore, the presence of food in the gastrointestinal tract has a negative influence on the absorption of tetracyclines.

Due to the large volume of distribution of tetracyclines, the tissue concentrations are generally higher than the plasma concentrations. Tetracyclines reach the lungs and udder in high concentrations, but effective concentrations are rarely reached in the cerebrospinal fluid. This can be explained by the moderate lipophilicity. Doxycycline has a higher lipophilicity than the other tetracyclines, so the absorption from the gastro-intestinal tract is better and higher concentrations are reached in the tissues. Tetracyclines are amphoteric molecules. This means that they have a double pK_a : one low and one high pK_a . The distribution is therefore good over tissues with a pH that is higher than the pH of the plasma, but also over tissues with a pH lower than the pH of plasma.

Aminoglycosides have a lower lipophilicity and a smaller volume of distribution. The oral biological availability is poor and orally administered aminoglycosides are particularly effective in the intestines. After parenteral administration, the distribution is mainly determined by the plasma protein binding; antibiotics that are bound to plasma proteins will remain in the plasma. Because aminoglycosides have low plasma protein binding, they will reach effective concentration in pericardial, synovial, pleural, perilymphatic and peritoneal fluids after parenteral administration. Diffusion to the joints increases when these are inflamed. The pH in inflamed joints decreases in comparison to healthy joints, and ion trapping will occur due to the high pK_a of aminoglycosides. Aminoglycosides, just like tetracyclines, reach the cerebrospinal fluids in insufficiently high



concentrations to be effective. Tetracyclines pass cell membranes by means of passive diffusion. In aminoglycosides the active transport in Gram-negative bacteria depends on an oxygen dependent process. This explains why these antibiotics are not effective under anaerobic conditions and in (optional) anaerobic bacteria. In addition, aminoglycosides have a reduced efficacy in an environment with purulent debris and low pH values, which may occur with tissue damage. The debris contains proteins which bind to the aminoglycosides so that they can no longer pass the cell membrane of the bacteria and when they cannot reach the ribosome, they are ineffective. Although drainage is often conducted because of other reasons, drainage of purulent debris may also contribute to an improvement of the effectiveness of these antibiotics.

6.3 CONCENTRATION OR TIME DEPENDENT EFFECT

Aminoglycosides have a concentration dependent efficacy and a long post antibiotic effect (PAE). Thus, it is important to reach a sufficiently high concentration at the site of the infection. Due to the PAE it is not necessary to continuously maintain concentrations higher than the MIC. In addition, repeated administration of aminoglycosides within one day is an important risk factor for the development of nephrotoxicity. Aminoglycosides are excreted renally and accumulate in tubulus cells where they cause toxicity. Accumulation, and hence toxicity, is particularly dependent on the duration of exposure and is minimal at a brief peak concentration. The combination of both of these factors results in the fact that aminoglycosides are often administered only once daily nowadays. Renal accumulation is also the cause of the long withdrawal period of most aminoglycosides. Tetracyclines do not have a specific time or concentration dependent effect. Both the concentration and the duration of exposure are important.

6.4 COMBINING ANTIMICROBIALS

In certain situations, it may be useful to combine antimicrobials, for example to broaden the spectrum. Protein synthesis inhibitors cannot be combined with β -lactam antibiotics like penicillins or cephalosporins. β -lactam antibiotics depend on the replication of bacteria

to be effective, and protein synthesis inhibitors will prevent this replication from occurring. Aminoglycosides are an exception to this rule. By using β -lactam antibiotics the cell wall will become more permeable for aminoglycosides. In addition, the aminoglycosides are the only protein inhibition synthesis inhibitors which are bactericidal. A combination of β -lactam antibiotics with aminoglycosides can have a synergistic effect against *Streptococcus* spp., *Enterococcus* spp. and *Pseudomonas* spp. and other Gram-negative bacteria.

6.5 INDUCTION OF RESISTANCE

Due to the widespread use of tetracyclines resistant bacteria can already be found in cattle, pigs and poultry. There are several mechanisms of resistance which bacteria can develop to become resistant to tetracyclines:

1. Changing the structure of the receptor on the ribosome or blocking of this receptor
2. Reduction of the intracellular concentration of the antibiotic
 - a. This can be achieved by efflux pumps; these energy dependent pumps exchange a proton against a complex of a tetracycline with a cation
 - b. The production of OMP pores can also be inhibited. These pores are normally used by tetracyclines to pass the outer membrane of the bacterium
3. Enzymatic inactivation of antibiotics (especially known for *Enterobacteriaceae* spp.)

Changing the structure of the receptor on the ribosome and the use of efflux pumps are the most important mechanism by which resistance is induced. Resistance to one tetracycline always results in resistance to all other tetracyclines. Induction of resistance is also known for aminoglycosides. For these antibiotics resistance is usually based on enzymatic inactivation of the antibiotic but all other mechanisms mentioned for tetracyclines may also occur for aminoglycosides. The occurrence of cross resistance is very unpredictable. In many cases, bacteria resistant to one aminoglycoside will still be sensitive to other aminoglycosides. This however should be tested in every individual case.



7

**Macrolides, Lincosamides,
Pleuromutilins and Fenicol**



7.1 PHARMACODYNAMICS

The antibiotics that belong to the groups of macrolides, lincosamides, pleuromutilins and fenicol all bind reversible to the 50S subunit of the bacterial ribosome. The 50S subunit is the large subunit which is responsible for the assembly of the various amino acids to one chain (peptide). An important enzyme in this process is peptidyl transferase.

Lincosamides, pleuromutilins and fenicol all inhibit the action of the above mentioned enzyme peptidyl transferase. As a result, the peptide chain cannot be assembled. Macrolides have a slightly different mode of action: they ensure that the peptide chain is released before it is completed. Thus, in the presence of macrolides only short and incomplete peptide chains are formed, while in the presence of an antibiotic from one of the other groups mentioned above, no peptide chains are formed at all.

These antibiotics are usually classified as being bacteriostatic. In some cases, however, the effect is bactericidal. This depends on the concentration of the antibiotic, the period during which the concentration is higher than the MIC, the bacterial strain that is being treated and the amount of bacteria. For florfenicol a bactericidal effect has been observed against *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*, when the concentration of florfenicol was higher than the MIC for 12 hours.

Besides the bacteriostatic effect, macrolides and lincosamides are also so-called immunomodulatory medicines. There are various ways in which these antibiotics can influence the course of an infection; they can both affect the bacterium and the interaction between the bacterium and the host, as well as the immune system of the host. However, these effects are only obtained when the molecules used possess a 14- or 15-membered lactone ring. Of these, only erythromycin is used in the veterinary field. To achieve the immunomodulatory effect, these antibiotics should be administered during a long period of time in a low concentration. This does not fit the framework of prudent use of antibiotics.

Research has shown that tilmicosin is effective not only in treatment of bacterial infections, but also in the treatment of PRRS infections. In vitro studies reveal a decrease in the virus titres in cell cultures, while in vivo trials show a decrease in mortality, the number of lung lesions and the virus titres in the lungs and serum. This effect can possibly be explained by the accumulation of tilmicosin in macrophages. Here, they prevent the replication of the virus.

7.2 LIPOPHILICITY & DISSOCIATION CONSTANT

The passage over membranes is very important for this group of antibiotics, since their target (the 50S subunit of the ribosome) is located inside the bacterium. As described previously, lipophilicity and dissociation constant are the main characteristics important for membrane passage. This group of antibiotics has a moderate to high lipophilicity and a high pKa. They are capable of passing biological membranes due to their good lipophilicity and their high pKa causes ion trapping of these antibiotics in tissues with a pH that is lower than the pH of the plasma, such as inflamed tissues.

Due to the large volume of distribution of these antibiotics, the tissue concentrations reached will often be higher than the concentration in the serum. The biological availability is good, both after oral and parenteral administration.

Tulathromycin is a semisynthetic macrolide with specific pharmacokinetic properties. Research has shown that when this antibiotic is administered intravenously or intramuscularly in pigs, high and homogenous concentrations are reached in the lungs which are maintained for a long period of time. For other animal species such as cattle and horses similar pharmacokinetic properties have also been found.

7.3 CONCENTRATION OR TIME DEPENDENT EFFECT

Macrolides, lincosamides, pleuromutilins and fenicol are all time dependent antibiotics. This means that it is important that the concentration of these antibiotics at the site of the infection is



higher than the MIC during a sufficiently long period of time and a continuous administration is thus preferred over pulse-dosing.

7.4 COMBINING ANTIMICROBIALS

These antibiotics should not be combined with β -lactam antibiotics. The effect of the antibiotics that belong to the groups of macrolides, lincosamides, pleuromutilins and fenicolis are described as being bacteriostatic or bactericidal. However, during at least part of the time the concentration will be bacteriostatic. Bacterial growth is therefore inhibited. Since the efficacy of β -lactam antibiotics depends on bacteria replicating, this combination could result in a lack of efficacy of these antibiotics.

Caution should also be exercised when combining different antibiotics from within this group. Macrolides, lincosamides, pleuromutilins and fenicolis all have to bind to the 50S subunit to be effective. The binding sites of macrolides, lincosamides and fenicolis partially overlap. Combining antibiotics from these three categories is therefore not recommended. A combination of these antibiotics with tetracyclines or aminoglycosides is possible, because the tetracyclines and aminoglycosides bind to the other subunit of the bacterial ribosome.

Finally, when combining these antibiotics with other veterinary medicines the effect on the CYP-450 (cytochrome P450) system should be considered. These antibiotics inhibit the activity of the CYP-450 system in the liver. As a result, the metabolization of not only these antibiotics but also of other medicines that are metabolised by these enzymes is delayed. This could result in a relative overdose. Examples of other medicines that are metabolized by these enzymes are fluoroquinolones and ionophores.

7.5 INDUCTION OF RESISTANCE

The development of resistance against macrolides, pleuromutilins and lincosamides by Gram-negative bacteria is usually based upon a decreased permeability of the bacterial cell membrane.

In Gram-positive bacteria resistance is usually caused by an alteration of the binding site, active efflux of the antibiotic or enzymatic inactivation of the antibiotic. These mechanisms of resistance are also found in Gram-negative bacteria, but to a lesser extent. When resistance is caused by a modification of the binding site this often results in cross resistance between macrolides, lincosamides and streptogramin B. This can be explained the fact that the binding sites for these antibiotics overlap. This mechanism of resistance is also known as MLSB resistance.

Resistance against fenicolis is usually based on enzymatic inactivation of these antibiotics. Efflux systems are also known, but less often found. In Gram-negative bacteria resistance can also be caused by a decreased permeability of the cell wall or a transporter. In the last case, the transporter often also transports other antibiotics which results in multiresistant bacteria.



8

Literature



1. Altenburg, J., de Graaf, C.S., van der Werf, T.S., Boersma, W.G. (2011) Immunomodulatory effects of macrolide antibiotics – Part 1: Biological mechanisms. *Respiration*, 81: 67-74.
2. Baert, K., de Baere, S., Croubels, S., Gasthuys, F., de Backer, P. (2001) Pharmacokinetics and bioavailability of sulfadiazine and trimethoprim (trimazin 30%) after oral administration in non-fasted young pigs. *J Vet Pharmacol Therap* 24: 295-298.
3. Benchaoui, H.A., Nowakowski, M., Sherington, J, Rowan, T.G., Sunderland, S.J. (2004) Pharmacokinetics and lung tissue concentrations of tulathromycin in swine. *J.Vet. Pharmacol.Therap* 27: 203-210.
4. Brouwers, J.R.B.J. (1987) Pharmacokinetics of the newer fluoroquinolones. *Pharmaceutisch Weekblad Scientific Edition (Supplement)* 9: S16-S22.
5. Brown, M.P., Gronwall, R., Castro, L. (1988) Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares. *Am J Vet Res* 49: 918-922.
6. Centre for food security & public health – Iowa State University (2011) Methicillin resistant *Staphylococcus aureus*. <http://www.cfsph.iastate.edu/Factsheets/pdfs/mrsa.pdf>
7. Du, Y., Yoo, D., Paradis, M-A, Scherba, G. (2011) Antiviral activity of tilmicosine for type 1 and type 2 porcine reproductive and respiratory syndrome virus in cultured porcine alveolar macrophages. *Antivir Antiretrovir* 3: 28-33.
8. Formularium melkvee, werkgroep veterinaire antibioticumbeleid KNMvD. Versie 1.1 (2012), aangepast op 02-01-2014.
9. Giguere, S., Prescott, J.F., Baggot, J.D., Walker, R.D., Dowling, P.M. (2007) Antimicrobial therapy in veterinary medicine, fourth edition. Blackwell publishing, pp 121 – 159; pp 171 – 188, pp 191 – 205, pp 231-240; 207 – 229 and 241-285.
10. Guardabassi, L., Jensen, L.B., en Kruse, H. (2008) Guide to Antimicrobial Use in Animals. Blackwell Publishing.
11. Gustafsson, A., Baverud, V, Franklin, A., Gunnarsson, A., Ögren, G., Ingvast-Larsson, C. (1999) Repeated administration of trimethoprim/sulfadiazine in the horse-pharmacokinetics, plasma protein binding and influence on the intestinal microflora. *J Vet Pharmacol Therap* 22: 20-26.
12. Hamilton-Miller, J.M.T. en Shah, S. (1999) Effect of antibiotic concentration on the killing of *Staphylococcus aureus* and *Enterococcus faecalis*: Comparison of the novel penem, Men 10700 , with other β -lactam antibiotics. *J Antimicrob Chemother* 44: 418-420.
13. He, J., Tang, S., Li, L., Zhang, C., Li, X., Xia, X., Xiao, X. (2010) Pharmacokinetics of a novel amoxicillin/colistin suspension after intramuscular administration in pigs. *J Vet Pharmacol Therap* 34: 42-50.
14. Joint scientific report of ECDC, EFSA and EMEA on methicillin resistant *Staphylococcus aureus* (MRSA) in livestock, companion animals and food. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2009/10/WC500004306.pdf
15. Kaartinen, L., Gips, M., Laurila, T., Härtel, H., Soback, S., Pyörälä (2000) Pharmacokinetics of sulphadoxine and trimethoprim and tissue irritation caused by two sulphadoxine-trimethoprim containing products after subcutaneous administration in pre-ruminant calves. *Vet Res.* 31: 517-526.
16. Kaartinen, L., Löhönen, K., Wiese, B., Franklin, A., Pyörälä, S (1999) Pharmacokinetics of sulphadiazine-trimethoprim in lactating dairy cows (abstract). *Acta Vet Scand* 40: 271-278.
17. Kwiatkowska, B., Maslinska, M., Prygodzka, M., Dmowska-Chalaba, J., Dabrowska, J., Sikorska-Siudek, K. (2013) Immune system as a new therapeutic target for antibiotics. *Advances in Bioscience and Biotechnology*, 4: 91-101.
18. Leclercq, R., Courvalin, P. (2002) Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae*. *Antimicrobial agents and chemotherapy* 46: 2727-2734.
19. Melchior, M., Van Hout-van Dijk, J. (2011) Antibiotica, van werkingsmechanismen naar antibacteriële therapie. Deel III (van IV). *Tijdschrift voor Diergeneeskunde* 136(9): 646-652.
20. Mengelers, M.J.B., van Gogh, E.R., Kuiper, H.A., Pijpers, A., Verheijden, J.H.M., van Miert, A.S.J.P.A.M. (1995) Pharmacokinetics of sulfadimethoxine and sulfamethoxazole in combination with trimethoprim after intravenous administration to healthy and pneumonic pigs. *J Vet Pharmacol Therap* 19: 243-253.
21. Monitoring jaarverslag 2012, Gezondheidsdienst voor Dieren te Deventer.
22. Nielsen, P., Rasmussen, F. (1975) Trimethoprim and sulphadoxine in swine; Half-lives, volume of distribution and tissue concentration. *Zbl Vet Med A* 22: 564-571.



23. Nielsen, P., Rasmussen, F. (1977) Half-life, apparent volume of distribution and protein-binding for some sulphonamides in cows. *Research in Veterinary Science* 22: 205-208.
24. Nielsen, P., Romvary, A., Rasmussen, F. (1978) Sullphadoxine and trimethoprim in goats and cows: absorption fraction, half-lives and the degrading effect of the ruminal flora. *J Vet Pharmacol Therap* 1: 37-46.
25. Openstax CNX, Probiotic diversity <http://cnx.org/content/m45512/latest/?collection=col11487/latest>.
26. Pankey, G.A., Sabath, L.D. (2004) Clinical Relevance of Bacteriostatic versus Bactericidal Mechanism of Action in the Treatment of Gram-Positive Bacterial Infections. *Clinical Infectious Disease* 38; pp 864-870.
27. Plumb, D.C. (2011) *Plumb's Veterinary Drug Handbook*, 7th edition. Wiley-Blackwell.
28. Quintiliani, R. Pharmacodynamics of Antimicrobial Agents: Time-Dependent vs. Concentration-Dependent Killing. www.antimicrobe.org
29. Rasmussen, F., Gelsa, H., Nielsen, P. Pharmacokinetics of sulphadoxine and trimethoprim in horses. Half-life and volume of distribution of sulphadoxine and trimethoprim and cumulative excretion of [14 C]-trimethoprim. *J Vet Pharmacol Therap* 2: 245-255.
30. Rolinski, Z., Duda, M. (1984) Pharmacokinetic analysis of the level of sulfonamide-trimethoprim combination in calves (abstract). *Pol J Pharmacol Pham* 36: 35-40.
31. Romvary, A., Horvay, A. (1976) Data on the pharmacokinetics of sulfonamide-trimethoprim combinations in sucking pigs. *Zbl Vet Med* 23: 781-792.
32. Schwarz, S. Chaslus-Dancla, E. (2001) Use of antimicrobials in veterinary medicine and the mechanisms of resistance. *Vet Res* 32: 201-225.
33. Shoaf, S.E., Schwark, W.S., Guard, C.L. (1989) Pharmacokinetics of sulfadiazine/trimethoprim in neonatal male calves: effect of age and penetration into cerebrospinal fluid (abstract). *Am J Vet Res* 50: 396-403.
34. Shoaf, S.E., Schwark, W.S., Guard, C.L., Swartsman, R.V. (1986) Pharmacokinetics of trimethoprim/sulfadiazine in neonatal calves: influence of synovitis. *J Vet Pharmacol Therap* 9: 446-454.
35. Van Duijkeren, E., Vulto, A.G., Sloet van Oldruitenborgh, M.M., Kessels, B.G., van Miert, A.S., Breukink, H.J. (1995) Pharmacokinetics of trimethoprim/sulfachlorpyridazine in horses after oral, nasogastric and intravenous administration. *J Vet Pharmacol Ther* 18: 47-53.
36. Van Hout-van Dijk, J. en Melchior, M. (2011) Antibiotica; van werkingsmechanismen naar antibacteriële therapie. Deel I (van IV). *Tijdschrift voor Diergeneeskunde* 136: 494-499.
37. Van Hout- van Dijk, J. en Melchior, M. (2011) Antibiotica: van werkingsmechanismen naar antibacteriële therapie. Deel II (van IV). *Tijdschrift voor Diergeneeskunde* 136: 572-577.
38. Van Hout- van Dijk, J. en Melchior, M. (2011) Antibiotica: van werkingsmechanismen naar antibacteriële therapie. Deel III (van IV). *Tijdschrift voor Diergeneeskunde* 136: 646-652.
39. Van Hout-van Dijk, J. en Melchior, M. (2011) Antibiotica; van werkingsmechanismen naar antibacteriële therapie. Deel IV (van IV). *Tijdschrift voor Diergeneeskunde* 136(10): 730-733.
40. Villarino, N., Brown, S.A., Martin-Jiménez (2013) Understanding the pharmacokinetics of tulathromycin: a pulmonary perspective. *J. Vet. Pharmacol. Therap.* 37: 211-221.
41. Villarino, N., Lesman, S., Fielder, A., García-Tapia, D., Cox, S., Lucas, M., Robinson, J., Brown, S.A., Martin-Jiménez, T. (2012a) Pulmonary pharmacokinetics of tulathromycin in swine. Part I: Lung homogenate in healthy pigs and pigs challenged intratracheally with lipopolysaccharide of *Escherichia coli*. *J. Vet. Pharmacol. Therap* 36: 329-339.
42. Villarino, N., Lesman, S., Fielder, A., García-Tapia, D., Cox, S., Lucas, M., Robinson, J., Brown, S.A., Martin-Jiménez, T. (2012b) Pulmonary pharmacokinetics of tulathromycin in swine. Part 2: Intra-airways compartments. *J. Vet. Pharmacol. Therap* 36: 340-349
43. Vologodskii, A. (2004) Computational analysis of DNA gyrase action. *Biophysical journal* 87: 3066-3073.
44. Williamson, J.R. (2000) Small subunit, big science. *Molecular biology. Nature* 407: 306-307.



Appendix

Overview of antimicrobials based on mechanism of action

Mechanism of action		Group	Active ingredient
Cell wall	Inhibition of cell wall synthesis	Narrow spectrum penicillins	Penicillin-V Penicillin-G
		Broad spectrum penicillines	Amoxicillin Ampicillin
		Beta-lactam insensitive penicillines	Cloxacillin
		Cefalosporines	-
	Disruption of the cell wall	Polymyxines	Polymyxin-E (colistin)
DNA synthesis	Inhibition of folic acid synthesis	Sulfonamides	Sulfadiazin Sulfadoxin Sulfadimidin Sulfamethoxazol Sulfaquinoxalin
		Diaminopyrimidines	Trimethoprim
	Inhibition of transcription and translation of DNA	Fluoroquinolones	Flumequin Enrofloxacin
Protein synthesis	Inhibition of protein synthesis by binding the 50S subunit of the bacterial ribosome	Macrolides	Tylosin Tilmicosin Tulathromycin
		Lincosamides	Lincomycin
		Pleuromutilines	Tiamulin Valnemulin
		Fenicols	Florfenicol
	Inhibition of protein synthesis by binding the 30S subunit of the bacterial ribosome	Tetracyclines	Doxycycline Oxytetracycline Chlortetracycline
		Aminoglycosides	Neomycin Gentamicin Dihydrostreptomycin

Bactericidal / Bacteriostatic	Concentration / Time dependent	Lipophilicity	Volume of distribution	pK _a
Bactericidal	Time dependent	Low	Small	Low
Bactericidal	Time dependent	Low	Small	Low
Bactericidal	Time dependent	Low	Small	Low
Bactericidal	Time dependent	Low	Small	Low
Bactericidal	Concentration dependent	Low	Small	High
Bacteriostatic ¹	Time dependent	Moderate	Small	Low
Bacteriostatic ¹	Time dependent	Moderate	Large	High
Bactericidal	Concentration dependent	High	Large	Amphoteric ⁴
Bacteriostatic ²	Time dependent	Moderate	Large	High
Bacteriostatic ²	Time dependent	Moderate	Large	High
Bacteriostatic ²	Time dependent	Moderate	Large	High
Bacteriostatic ²	Time dependent	High	Large	High
Bacteriostatic	Co-dependent ³	Doxycycline: High Others: Moderate	Large	Amphoteric ⁴
Bactericidal	Concentration dependent	Low	Small	High

1 Combination of sulfonamides and trimethoprim is bactericidal.

2 These antibiotics are also defined as bactericidal depending on the concentration of the antibiotic, the time the concentration exceeds the MIC, the bacterial strain and the amount of bacteria.

3 The efficacy of tetracyclines is dependent on both the concentration of the antibiotic as well as the exposure time.

4 Amphoteric: these antimicrobials have both a low and a high pK_a.



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