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Review: Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine

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Abstract

Background – Antimicrobial resistance has become a major challenge in veterinary medicine, particularly in the context of bacterial pathogens that play a role in humans and animals.

Objectives – This review serves as an update on acquired resistance mechanisms in bacterial pathogens of human and animal origin, including examples of transfer of resistant pathogens between hosts and of resistance genes between bacteria.

Results – Acquired resistance is based on resistance-mediating mutations or on mobile resistance genes. While mutations are transferred vertically, mobile resistance genes are transferred also horizontally (by transformation, transduction or conjugation/mobilization), contributing to the dissemination of resistance. Mobile genes specifying any of the three major resistance mechanisms – enzymatic inactivation, reduced intracellular accumulation or modification of the cellular target sites – have been found in a variety of bacteria from animals. Such resistance genes are associated with plasmids, transposons, gene cassettes, integrative and conjugative elements or other mobile elements. Bacteria, including zoonotic pathogens, can be exchanged between animals and humans mainly via direct contact, but also via dust and aerosols or via the food chain. Proof of the direction of transfer of resistant bacteria can be difficult and depends on the location of resistance genes or mutation in the chromosomal DNA or on a mobile element.

Conclusion – The wide variety in resistance and resistance transfer mechanisms will continue to ensure the success of bacterial pathogens in the future. Our strategies to counteract resistance and preserve efficacy of antimicrobial agents needs to be equally diverse and resourceful.
Introduction

Antimicrobial agents are used extensively in aquaculture, horticulture, and to treat bacterial infections in humans and animals. Due to this extensive use, antimicrobial resistance has become a significant problem in both human and veterinary medicine, mediated by a multitude of mechanisms.\textsuperscript{1, 2} Although the presence of resistance genes in bacteria is not a new phenomenon – as recently highlighted in a study describing resistance genes in bacterial DNA from permafrost soil samples\textsuperscript{3} – what is new is the selective pressure exerted on bacterial pathogens through antibacterial use. Since the 1950s, the selective pressure imposed on bacteria by the use of antimicrobial agents for various clinical and nonclinical purposes has increased dramatically. As a consequence, bacteria have developed and refined various ways and means to resist or escape the inhibitory effects of the antimicrobial agents.\textsuperscript{1, 2} In addition, certain bacterial pathogens have managed to accumulate or develop resistances to multiple classes of antimicrobial agents at the same time. Such multidrug-resistant, extensively resistant or even pan-drug resistant pathogens\textsuperscript{4} typically succeed in human and veterinary healthcare establishments or in patients repeatedly requiring antibacterial therapy. Risk groups include dogs with recurrent pyoderma. Such patterns of resistance may seriously compromise the prognosis of infected patients. As a result, for the first time in decades, the prognosis for patients with infections caused by multidrug-resistant bacteria has been seriously compromised by the lack of effective antimicrobial agents. This development has threatened the advancement of modern medicine.\textsuperscript{5}

Antimicrobial resistance

A bacterium is defined as being clinically resistant to an antimicrobial agent when the drug – after recommended dosing – does not reach a concentration at the site of infection that is able to effectively inhibit the growth of the bacterium or to kill it.\textsuperscript{6} This definition takes into account the pharmacological parameters relevant for systemic therapy of the antimicrobial agent in the patient species concerned. It also considers the minimum inhibitory concentration (MIC) of the causative bacteria to the antimicrobial agent applied. These factors, along with the results of clinical efficacy studies, play key roles in the definition of clinical breakpoints.\textsuperscript{6} Such clinical breakpoints are available for humans and various animal species as recommended by the Clinical and Laboratory Standards Institute (CLSI) and usually are applicable for a specific combination of host species/target bacterium/antimicrobial agent/disease condition, such as dog/Staphylococcus spp./tetracycline/skin and soft tissue infections.\textsuperscript{7, 8} In general, these breakpoints were derived from microbiological, pharmacokinetic (using accepted clinical doses) and pharmacodynamic data. In the veterinary field, clinical breakpoints applicable for bacteria involved in skin and soft tissue infections are available for the canine, feline and equine bacteria shown in Table 1.

In general, antimicrobial resistance in bacteria can be either intrinsic or acquired. Intrinsic resistance is a bacterial genus- or species-specific characteristic and is often based on either the absence or inaccessibility of the target structures in the respective bacteria,\textsuperscript{1} for example, resistance to β-lactam antibiotics and glycopeptides in cell wall-free bacteria such as Mycoplasma spp. or vancomycin resistance in Gram-negative bacteria due to the inability of vancomycin to penetrate the outer membrane. It can also be due to the presence of export systems or the
production of species-specific inactivating enzymes in certain bacteria, such as the AcrAB-TolC system and the production of AmpC β-lactamase in Escherichia coli. In addition, some bacteria, such as enterococci, are not dependent on a functional folate synthesis pathway, but instead can use exogenous folates. As a consequence, they are intrinsically resistant to folate pathway inhibitors, such as trimethoprim and sulfonamides. In contrast, acquired resistance is a strain-specific property which can be based on a wide variety of resistance mechanisms present in the different bacteria. Such acquired resistance mechanisms can be due to mutations of cellular genes or to the acquisition of novel/foreign genes, commonly referred to as resistance genes. The following basic considerations are important in the context of acquired resistance genes:

1. Acquired resistance genes can confer resistance to an entire class of antimicrobial agents or can be specific for only a single member of an antimicrobial class.
2. Certain acquired resistance genes can confer resistance to members of different classes of antimicrobial agents.
3. Acquired resistance to a specific class of antimicrobial agents can be due to several different resistance mechanisms.
4. The same acquired resistance mechanism can be encoded by different genes.
5. Different acquired resistance mechanisms and resistance genes can be present at the same time.
6. Definitions of multidrug-resistance vary but a bacterium is typically referred to as multidrug-resistant if it shows acquired resistance to members of at least three classes of antimicrobial agents.

Resistanc mechanisms and associated resistance genes

Acquired resistance mechanisms can be divided into one of the three major categories: (i) enzymatic modification or inactivation of antimicrobial agents, (ii) reduced intracellular accumulation of antimicrobial agents or (iii) alterations at the target sites of the antimicrobial agents.

Enzymatic inactivation of antimicrobial agents is widespread among Gram-positive and Gram-negative bacteria (Table 2). In the case of enzymatic modification, bacteria produce enzymes that chemically modify the drug molecule by the attachment of acetyl, adenyl or phosphate groups to specific sites of the antimicrobial molecule. Such modified antimicrobial molecules can no longer bind to their target site and consequently cannot maintain antimicrobial activity. This mechanism is commonly used for the enzymatic inactivation of nonfluorinated phenicols, such as chloramphenicol, by acetylation, or of aminoglycosides by acetylation, adenylation or phosphorylation. Other enzymatic inactivation processes include the phosphorylation of macrolides, nucleotidylation of lincosamides, and acetylation of streptogramin A antibiotics.

In the case of enzymatic inactivation, bacteria produce enzymes that bind directly to the antimicrobial molecule and disintegrate it. This is commonly done by hydrolytic cleavage of specific bonds within the antimicrobial molecule. Such cleaved antimicrobial molecules also do not exhibit antimicrobial activity. Examples of this mode of enzymatic inactivation are the β-lactamases, which occur in Gram-positive and Gram-negative bacteria and, depending on the type of β-lactamase, may exhibit a more or less expanded substrate spectrum that can include penicillins,
cephalosporins, monobactams and/or even carbapenems.\textsuperscript{12, 13} Other examples are esterases which confer macrolide resistance or lactone hydrolases which inactivate streptogramin B compounds.\textsuperscript{14}

**Reduced intracellular accumulation** of antimicrobial agents can be achieved in two ways: reduced influx or enhanced efflux (Table 3). It is known that certain outer membrane proteins (OMPs), so-called porins, represent an entry point for antimicrobial agents to enter the bacterial cell. As such, OmpF is involved in the uptake of tetracyclines, \( \beta \)-lactams and chloramphenicol in E. coli, whereas OmpD is involved in the uptake of carbapenems in Pseudomonas aeruginosa.\textsuperscript{1} Reduced influx of antimicrobial agents is usually the consequence of downregulation, structural modification or even functional deletion of the genes coding for these porins. In such cases, the outer membrane of Gram-negative bacteria can represent a permeability barrier for antimicrobial agents.

By contrast, increased efflux describes a way by which incoming antimicrobial agents are actively pumped out of the bacterial cell. This can be achieved by multidrug transporters or specific transporters.\textsuperscript{1, 2} Multidrug transporters are present in virtually every bacterium and are mainly responsible for the transport of toxic substances from the cell metabolism. However, studies have shown that some multidrug transporters can also export antimicrobial agents. Most of them belong to the resistance-nodulation-cell division (RND) family. RND transporters mainly occur in Gram-negative bacteria and are composed of a cytoplasmatic and a periplasmatic component which can interact with different outer membrane components. Examples are AcrAB-ToIC transporter in E. coli and Salmonella enterica or the MexAB-OprM transporter in P. aeruginosa which can export chloramphenicol, fluoroquinolones, tetracyclines, \( \beta \)-lactams and macrolides among others.\textsuperscript{1, 15} It should be noted that multidrug-transporters increase the MICs for their substrates, but not necessarily to a level that correlates with clinical resistance.

Specific transporters involved in antimicrobial resistance commonly belong to the following families: (i) major facilitator superfamily (MFS), (ii) ATP-binding cassette (ABC) family or (iii) multidrug and toxic-compound extrusion (MATE) family.\textsuperscript{15, 16} MFS transporters often consist of 12–14 transmembrane segments, exchange a drug molecule against a proton and use the proton-motive force of the membrane as an energy source for the translocation. Examples of MFS transporters are the tetracycline transporters Tet(K) and Tet(L) in Gram-positive bacteria and Tet (A-E, G, H) in Gram-negative bacteria as well as the phenicol transporters FexA in Gram-positive bacteria and FloR, CmIA and CmIB in Gram-negative bacteria.\textsuperscript{17, 18} ABC transporters use the energy of ATP hydrolysis for the translocation of substrates across biological membranes. They represent a highly diverse class of transporters which are not only involved in antimicrobial resistance, but also in the uptake of nutrients and the secretion of proteins among other functions.\textsuperscript{19} ABC transporters involved in antimicrobial resistance are seen mainly in staphylococci and enterococci. Examples are the transporters Vga(A), Vga(C), Vga(E), Lsa(E) and Sal(A) conferring combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics or Msr(A) involved in resistance to macrolides and streptogramin B antibiotics.\textsuperscript{20, 21} MATE proteins are also located in the cytoplasmatic membrane and act in a similar way to MFS transporters. However, in contrast to MFS proteins, they are rarely involved in antimicrobial resistance. Examples of MATE proteins that export antimicrobial agents are NorM (hydrophilic fluoroquinolones) from Vibrio parahaemolyticus and MepA (glycycyclines) from Staphylococcus aureus.\textsuperscript{15, 16}
Alterations at the target sites of the antimicrobial agents represent the third and most variable group of resistance mechanisms (Table 4). These include mutational and chemical modifications, protection of the target sites, the replacement of sensitive targets by functionally analogous but insensitive ones, and overproduction of sensitive targets.22

Mutational alterations of the target sites are best known for (fluoro)quinolone resistance in various Gram-positive and Gram-negative bacteria. Within the genes for DNA gyrase (topoisomerase II and topoisomerase IV), a specific region known as the quinolone-resistance determining region (QRDR) has been defined where mutations accounting for (fluoro)quinolone resistance are located. Resistance to (fluoro)quinolones usually occurs in a step-wise manner by which the MIC is increased with each additional mutation.23, 24 Such a step-wise increase in resistance illustrates well the advantage of using mutant prevention concentrations (MPCs) as a measure for antimicrobial potency rather than MICs.25 Because two mutations are required for full (fluoro)quinolone resistance to occur, and with mutations occurring randomly, the likelihood that bacteria with double mutations will persist after treatment is low and measurable only in a large population of cells (i.e. in large numbers of colony forming units in the laboratory). To date, MPC measurement has not been applied routinely in clinical microbiology laboratories, possibly hampered by practical constraints.26

Mutations in the gene fusA, which encodes the elongation factor G (EF-G), have been found to account for resistance to fusidic acid in S. aureus as well as in meticillin-susceptible (MSSP) and meticillin-resistant Staphylococcus pseudintermedius (MRSP).27, 28 Mutations in 16S ribosomal RNA (rRNA) have been described to account for resistance to streptomycin in Mycobacterium tuberculosis, to tetracyclines in Propionibacterium acnes and to spectinomycin resistance in Pasteurella multocida.1, 29 Mutations in 23S rRNA are known to cause macrolide resistance in various bacteria including Mycobacterium spp., Brachyspira hyodysenteriae, Campylobacter coli, Campylobacter jejuni, Haemophilus influenzae and Streptococcus spp. among others.1 In addition, mutations in the genes for specific ribosomal proteins are associated with resistance to streptomycin and spectinomycin.1, 29 Mutations in the gene rpoB, which codes for the β-subunit of the enzyme RNA polymerase, have been described recently to cause high-level rifampicin resistance in Rhodococcus equi and in MRSP.30, 31

Chemical modification of the target site by methylation has proved to be an effective way to confer combined resistance to macrolides, lincosamides and streptogramin B antimicrobial agents. The corresponding Erm methylases, which target the adenine residue at position 2058 in 23S rRNA, are widely distributed among Gram-positive and Gram-negative bacteria.32 To date, 46 different Erm methylases have been differentiated.33 Methylation of the adenine residue at position 2503, which is located in the overlapping binding region of phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics, results in resistance to these five classes of antimicrobial agents.34 The corresponding methylase gene, cfr, has been detected in various Staphylococcus spp., Enterococcus spp., Bacillus spp., Macrococcus caseolyticus, Jeotgalicoccus pinnipedialis, Streptococcus suis, E. coli and Proteus vulgaris.20, 35 Recently, the gene cfrB, which confers the same resistance phenotype but is <80% identical to cfr, has been detected in Enterococcus spp. and Clostridium difficile isolates.17, 33

Protection of the ribosomal target site has been noted in tetracycline resistance. So far, 12 ribosome protective proteins are known which show similarities to elongation factor EF-G. These proteins bind to the ribosome, do not interfere with
proteins, but protect the ribosome from the inhibitory effects of tetracyclines.\textsuperscript{36, 37} The gene fusB also codes for an EF-G-binding protein that protects the staphylococcal ribosomes from inhibition by fusidic acid.\textsuperscript{27} The replacement of a sensitive target by an alternative drug-resistant target is well known in sulfonamide and trimethoprim resistance. The sulfonamide resistance genes sul1, sul2 or sul3, which code for sulfonamide-insensitive dihydropteroate synthases, are widespread in Gram-negative bacteria.\textsuperscript{1, 2} Gram-negative and Gram-positive bacteria have acquired various dfr genes which code for trimethoprim-insensitive dihydrofolate reductases.\textsuperscript{1, 2} In addition, the genes mecA and mecC, present in various Staphylococcus spp., code for alternative penicillin-binding proteins which exhibit a substantially reduced affinity to virtually all \(\beta\)-lactam antimicrobial agents. Moreover, the genes vanA–vanE code for alternative D-Ala–D-Lac or D-Ala–D-Ser peptidoglycan precursors that render the respective bacteria resistant to glycopeptides, which also act at the level of cell wall synthesis.\textsuperscript{1, 2, 38} Sulfonamide resistance via the hyper-production of \(p\)-aminobenzoic acid has been observed in isolates of the genera Staphylococcus and Neisseria. Likewise, promoter mutations resulting in the overproduction of a trimethoprim-susceptible dihydrofolate reductase have been described to account for trimethoprim resistance in E. coli and Haemophilus influenzae.\textsuperscript{22}

Additional discussions of MIC distributions, as well as resistance genes and the mechanisms specified by them in bacteria involved in skin and soft tissue infections of animals, including staphylococci, streptococci and Gram negative bacteria, are available in other articles and book chapters.\textsuperscript{39-54}

**Horizontal gene transfer and mobile genetic elements involved**

As resistance-mediating mutations usually are located in essential chromosomal genes or in the 16S and 23S rRNA, they can only be transferred vertically during cell division.\textsuperscript{1, 2} It is important that such mutations should not negatively affect the fitness of the bacteria. In contrast, mobile resistance genes are transferred vertically and horizontally, and thereby contribute to the dissemination of resistance properties.\textsuperscript{1, 2, 55} Horizontal gene transfer (HGT) from the donor cell occurs via transformation, transduction or conjugation/mobilization and may include recipient cells of the same species, the same genus but also of different species and genera.

Transformation describes the transfer of “naked” DNA. It is the usual way used to transfer DNA under in vitro conditions. Although it also occurs in nature, it is believed to play a minor role in the transfer of DNA under natural conditions.\textsuperscript{1, 2, 55}

Transduction describes the transfer of DNA via bacteriophages. Limitations to transduction are (i) the size of the head of the transducing phages into which plasmids or other DNA elements are packaged and (ii) the requirement for receptors on the recipient cell to which the transducing phage can attach. Thus, only a limited amount of DNA, approximately 45 kb for staphylococci, can be transduced and transduction occurs mainly between members of the same or closely related bacterial species.\textsuperscript{1, 2, 55}

Conjugation, however, can also occur between bacteria of different species and genera. It describes the self-transfer of a conjugative element from a donor to a recipient cell. Plasmids and transposons can be conjugative, whereas integrative and conjugative elements (ICEs) are by definition always conjugative. The conjugative
A conjugative element harbours a tra gene complex which specifies the transfer apparatus. If a conjugative element provides its transfer apparatus to nonconjugative elements, mainly plasmids that co-reside in the same donor cell, such nonconjugative plasmids can move over to the recipient cell. This process is referred to as mobilization. Conjugation and mobilization of various mobile genetic elements are believed to play key roles in the dissemination of antimicrobial resistance in bacteria.\textsuperscript{1, 2, 55}

Furthermore, dissemination is thought to be particularly efficient amongst bacteria of the same species or clonal lineage. Barriers to HGT gene transfer, which protect bacteria against "foreign" DNA from other bacterial species or lineages, have been identified and are now widely described in many bacterial species.\textsuperscript{56} Barrier systems described in staphylococci, including S. pseudintermedius, include restriction-modification systems, competence genes and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems, and these have been linked to the successful spread of certain lineages and their ability to protect themselves from foreign DNA.\textsuperscript{57} However, their role in preventing acquisition of resistance genes, at least in S. pseudintermedius, is questionable based on finding them distributed randomly amongst multidrug-resistant and -susceptible isolates.\textsuperscript{28}

There are several mobile genetic elements (MGEs) which can harbour antimicrobial resistance genes and which are essential to horizontal gene transfer. All of them are double-stranded DNA molecules. Plasmids are the most abundant MGEs. They can vary distinctly in their sizes between <2 kb and >200 kb. Plasmids replicate autonomously and independently from the chromosomal DNA. They can carry antimicrobial resistance genes, heavy metal resistance genes, virulence genes and genes for a number of other properties, including metabolic functions. Plasmids can harbour transposons and gene cassettes/integrons.

Transposons differ distinctly in size and structure. In contrast to plasmids, they are replication-deficient and as such must integrate for their replication either into plasmids or the chromosomal DNA. They move by transposition, either into specific sites or into various sites in plasmids or in the chromosomal DNA. The importance of large transposons in the emergence of the extremely drug resistant phenotypes was recently highlighted by the identification of a Tn5405-like element carrying up to five antimicrobial resistance genes in all of 11 fully sequenced multidrug-resistant MRSP isolates of four different lineages.\textsuperscript{28}

Gene cassettes are the smallest MGEs which commonly carry only one gene, mostly an antimicrobial resistance gene, and a recombination site, known as the 59-base element. They can neither replicate nor transpose. They move by site-specific recombination and are commonly found in integrons. The integrase of the integron catalyses the integration and excision of the gene cassette using the 59-base element. As gene cassettes usually do not have an own promoter, the cassette-borne gene is transcribed from a promoter in the 5'-conserved region of the integron. Gene cassettes are rarely found at secondary sites outside of an integron.\textsuperscript{1, 2, 55}

Integrative and conjugative elements (ICEs) are large elements of >20 kb which integrate site-specifically into the chromosomal DNA. They can excise from the chromosomal DNA, form a circular intermediate and transfer themselves via a replicative cycle into new host cells where they integrate again into the chromosomal DNA. In terms of antimicrobial multidrug-resistance, the SXT element of Vibrio cholerae and the ICEPmu1 from P. multocida are well-studied ICEs.\textsuperscript{58-60} The latter...
has been shown to carry and transfer a total of 12 different antimicrobial resistance genes conferring resistance to eight classes of antimicrobial agents.\(^{58, 60}\) Other elements that integrate site-specifically into the chromosomal DNA of the respective bacteria include the various different types of the SCCmec elements in staphylococci, as well as the numerous variants of the integrative and mobilizable Salmonella genomic islands SGI1, SGI2 and PGI1 in S. enterica and Proteus mirabilis.\(^{1, 2, 38, 61-63}\)

Why the composition and predominant types of MGEs vary between species (e.g. plasmids predominate in S. aureus whereas transposons are more frequently described in S. pseudintermedius), remains to be answered.\(^{28, 40, 57}\)

**Consequences of the use of antimicrobial agents**

Whenever antimicrobial agents are applied to either humans or animals, a selective pressure is set under which susceptible bacteria are inhibited in their growth or killed, whereas resistant bacteria can propagate at the expense of the susceptible bacteria.\(^{64, 65}\) Antimicrobial agents do not differentiate between beneficial and pathogenic bacteria. They inhibit or kill all those bacteria for which MICs are at or below the antimicrobial concentration in the respective body compartment. As a consequence, the proportion of resistant bacteria increases during antimicrobial therapy and the composition of the microbiota is altered. This is true for virtually every antimicrobial agent and every human or animal host. Under the selective pressure imposed by the use of antimicrobial agents, antimicrobial resistance genes can also be disseminated between different bacteria within the same host.\(^{1, 64, 65}\)

However, when resistant bacteria are transferred between humans or between animals, they can also exchange their resistance genes with bacteria already resident in or on the new host.\(^{64, 65}\)

There are three basic requirements that favour the exchange of resistance genes: (i) close spatial contact between the exchange partners (which is present in the polymicrobial environments of the respiratory and intestinal tracts and also on the skin); (ii) location of the resistance genes on MGEs (which is given by the fact that most resistance genes are located on plasmids, transposons, gene cassettes and ICEs) and (iii) a selective pressure (which is provided by the application of antimicrobial agents).\(^{55}\) Exchange via horizontal gene transfer may involve obligatory and facultatively pathogenic bacteria as well as the commensal microbiota. If a multidrug-resistance MGE is transferred to new bacterial host and this host cell gains all the resistance genes associated with the MGE, the selective pressure imposed by the use of a single antimicrobial agent will ensure that the new host cell does not lose the multidrug-resistance MGE.\(^{64, 65}\) This means that the co-location of resistance genes furthers their co-selection and persistence even if no direct selective pressure is present. Thus, measures such as the voluntary withdrawal or even the ban of the use of an antimicrobial agent will not necessarily lead to a decrease in resistance. To better understand processes such as co-selection and persistence, and to judge the efficacy of the aforementioned measures, in-depth knowledge of the genetics of antimicrobial resistance is indispensable.

**Exchange of resistant bacteria between animals and humans**

As shown in Figure 1, the application of antimicrobial agents in human medicine as well as in veterinary medicine and food animal production can lead to the evolution and dissemination of resistant bacteria among humans and animals,
Depending on the virulence of the resistant bacteria, they may cause clinical diseases with limited treatment options. Transfer of bacteria — including resistant strains — can be exchanged between humans and animals in both directions by either contact, inhalation of dust and aerosols that contain bacteria, or via the food chain.65

Direct contact is likely the quickest and easiest way by which bacteria are transferred in either direction between humans and animals, particularly for those such as staphylococci which reside on body surfaces. Anyone who shares close contact with pets or companion animals may be affected. In this regard, it is important to consider the current role of dogs and cats as actual family members in many households in industrialized countries. A study published in 2014 revealed the presence of approximately 11.5 million cats, 6.9 million dogs, 6.1 million other pet animals (e.g. rabbits, guinea pigs, hamsters) and 3.4 million pet birds in German households.67 Pet owners often have extensive contact with their pets, especially to cats and dogs which may be allowed lick their owners’ faces and hands or to sleep in their owners’ beds.67, 68 Based on this close contact, a transfer of bacteria between pets and people is unavoidable and not surprising.66, 68-72 As “family members”, cats, dogs and other pet animals often enjoy not only an extensive support in terms of food supply and housing, but also broad medical care. In Germany, pet owners spent almost €4.8 billion for pet supplies in 2013, of which €3.75 billion accounted for pet food and €1.05 billion for equipment.67 For medical care of their pets, Germans spent approximately €2.1 billion in 2013.67 These data clearly show that pet owners have considerable interest in maintaining the health of their pets. As many infectious diseases in cats and dogs are caused by bacteria, particularly those infecting the skin of dogs,73 this also involves the application of antimicrobial agents. A wide range of antimicrobial agents has been licensed for use in cats and dogs. In addition, antimicrobial agents approved for use in human medicine may also be applied to nonfood-producing animals under the Animal Medicinal Drug Use Clarification Act (AMDUCA) in the USA or similar regulations in other countries.74 Although such applications should be kept to a minimum, it means that antimicrobial agents of last resort in human medicine, such as carbapenems, glycopeptides, oxazolidinones or lipopeptides, may be used in small animal medicine. However, no data are available to allow quantification of the use of these last resort agents for cats and dogs.

Animal transmission to companion animal owners

There have been numerous examples of the transfer of resistant bacteria, especially staphylococci and E. coli, between pets and people, beginning with the landmark report of the possible zoonotic spread of MRSA by a cat to hospitalized people.75 Reports of interspecies transmission of MRSA include: livestock-associated (LA-) MRSA ST398-t034 transferred from a colonized veterinarian to his dog,70 healthcare-associated MRSA ST225-t014 transferred from a family member (who suffered from an infected decubitus ulcer) to the family dog,70 MRSA ST80-t131 isolated from a woman who suffered from multiple recurrent skin abscesses and her husband, children and a cat living in the same household (where the patient’s disease resolved completely after topical decolonization of all family members including the MRSA-positive cat),78 and the likely horse-to-human transmission of a LA-MRSA ST398-t011.77 MRSA colonization of persons in contact with infected or colonized horses has been reported from the investigation of several outbreaks.78 Aside from MRSA, indistinguishable isolates of S. pseudintermedius ST33 have been reported from a dog and its owner.69
Typically, such reports are based on evidence from genetic typing studies which identify indistinguishable isolates from animals and in-contact humans. However, the direction of inter-host transmission can rarely be proven definitively, but rather, is often deduced from epidemiological characteristics. Even an MRSA outbreak investigation in a small animal hospital using whole genome sequencing of multiple isolates from each sample had to conclude that directions of transmission could only be suspected. For MRSA isolated from dogs and cats, for example, a predominantly human-to-animal direction of transmission is assumed because most isolates belong to MRSA clonal lineages that are also prevalent in human healthcare facilities and thus likely represent a “spill-over” to pets.

Evidence for transmission of Gram-negative pathogens between animals and humans is only just beginning to emerge, but already includes some highly drug-resistant nosocomial pathogens, such as E. coli ST410 and other multidrug-resistant Extended Spectrum Beta Lactamase-producing (ESBL) E. coli. Escherichia coli isolates, which belonged to the same phylogenetic group (B2 or D) and exhibited the same Amplified Fragment Length Polymorphism patterns, were detected among family members and their dogs.

**People with occupational contact to animals**

In addition to pet and companion animal owners, people who have occupational contact with animals also are at risk for acquisition of bacteria from animals. Notably, these include veterinarians, but also veterinary students, farmers, abattoir workers and other animal caretakers. These people often work in an environment where they care for sick animals and in which antimicrobial agents are applied. Besides direct contact with animals, dust and aerosols, especially on farms and in abattoirs, may also play a role as vehicles that transport resistant bacteria and are inhaled by animals and humans.

There are a number of published reports which suggest occupational transmission in various settings. In a small animal clinic, multidrug-resistant Staphylococcus epidermidis ST5 was shown to be present at various locations in the stationary area and the quarantine ward, as well as in feline patients and in the nose of one veterinary nurse. A study from Australia revealed that veterinarians often carry multidrug-resistant MRSA isolates. A study conducted in Germany showed that 97 (85.8%) of 113 swine farmers but only five (4.3%) of their 116 family members were positive for LA-MRSA. Likewise, 22 (44.9%) of 49 swine veterinarians but only four (9.1%) of their 44 family members were positive for LA-MRSA in another report. These observations suggest that the human-to-human transfer of LA-MRSA occurs distinctly more rarely than the animal-to-human transfer. A study involving 26 dairy farms in the Netherlands revealed that the same LA-MRSA types, based on pulsed-field gel electrophoresis (PFGE) type, spa type and resistance patterns, were detected not only among dairy cattle and their contact personnel (e.g. milkers), but occasionally also among other animals living on the same farm.

LA-MRSA isolates with the molecular characteristics ST398-t011-dt11a and ST9-t1430-dt10a, both with very similar PFGE patterns and resistance phenotypes, were detected among poultry and workers in a Dutch poultry abattoir. The analysis of turkey flocks and their carers revealed that almost 60% of the farm personnel were colonized by LA-MRSA that exhibited the same spa type and SCCmec type as the
turkeys. A study on the transmission of LA-MRSA on broiler farms in the Netherlands revealed the presence of MRSA ST398-t034-dt10q with indistinguishable PFGE and resistance patterns among the broilers, dust samples from the broiler house and the farmer. The emission of bacteria from pig fattening and broiler chicken farms to the surrounding area was confirmed by the detection of ESBL-/AmpC-producing E. coli in air samples from inside as well as outside the farm buildings. Another study showed that food animal transport in open crates resulted in the dissemination of bacteria, including resistant enterococci, into the environment. In addition, indirect transmission via insects or rats can occur on farms.

**Transmission via the food chain**

Transfer of resistant bacteria via the food chain usually occurs by ingestion of raw or insufficiently heated, contaminated food. In this regard, it is worth noting that (i) the number of ingested bacteria must be sufficiently high to survive the passage through the acidic environment in the stomach, which varies according to the type of foodborne pathogen and (ii) the virulence of most food-borne pathogens is more relevant than their antimicrobial resistance due to the fact that antimicrobial agents are not recommended for use in uncomplicated self-limiting cases of intestinal infections. However, when resistant bacteria are ingested, they may transfer antimicrobial resistance genes to members of the intestinal microbiota of the host. Unfortunately, there are little if no data which provide reliable information about the extent at which bacteria transfer their resistance genes during transient colonization of a new host.

**Proof of transfer of resistant bacteria and resistance genes**

In view of the many opportunities for exchange of resistant bacteria and resistance genes amongst human and animal hosts and the respective selection pressures, a key question is: what proportion of resistance problems in human medicine is caused by bacteria of animal origin? One study has assessed the impact of antimicrobial resistance in different bacterial species and of the contribution of animal sources to resistance in human infections. Based on the results of a questionnaire sent to recognized experts in the UK and elsewhere, the authors concluded that bacteria from animal sources, mainly nontyphoid Salmonella enterica serovars, E. coli O157, Campylobacter spp. and vancomycin-resistant enterococci, might account for 3.88% of the human antibiotic resistance problem. It should be noted that this survey was conducted at a time when LA-MRSA and ESBL-producing E. coli were not yet recognized as emerging zoonotic problems. Nevertheless, this survey suggested strongly that most of the resistance problems encountered in human medicine as well as in veterinary medicine are self-made problems in either sector. Only a minority results from the transfer of zoonotic bacteria.

A study on zoonotic MRSA colonization and infection in Germany showed that zoonotic transmission of LA-MRSA CC398 from livestock to humans occurs predominantly in people with occupational livestock contact, whereas dissemination in the general population is limited so far. LA-MRSA CC398 currently causes about 2% of all human MRSA infections in Germany, but up to 10% in regions characterized by a high density of livestock farming. Likewise, a study investigating 629 ESBL-producing E. coli from people in the Netherlands, Germany and UK, which
were collected during the years 2005-2009 and examined by DNA microarray and multi-locus sequence typing (MLST), showed that the majority of the human isolates differed distinctly from isolates of animal origin due to diversity in virulence and antimicrobial resistance genes.\textsuperscript{101} It was concluded that attempts to minimize the human-to-human transfer of ESBL-producing E. coli are essential to limit the dissemination of these bacteria among humans. ESBL-producing E. coli from animals may play a role as a reservoir of virulence and antimicrobial resistance genes rather than directly causing infections in humans.\textsuperscript{101}

The methodological attempts to prove the transfer of resistant bacteria or resistance genes strongly depend on the location of the resistance gene. For bacteria such as MRSA, where the meticillin resistance genes mecA or mecC are located on a chromosomally integrated SCCmec cassette, molecular strain typing methods can be applied. These include pattern-based techniques, such as PFGE, or sequence-based methods such as MLST, single locus sequence typing via spa and dru typing, as well as multiple loci VNTR analysis (MLVA).\textsuperscript{102, 103} In addition, the presence of the relevant resistance genes can be detected by PCR. Whole-genome sequencing with subsequent SNP analysis can also be used as the ultimate proof.\textsuperscript{81, 104} The results of these methods can enable definite proof of clonality and transference of resistance genes.

If a resistance gene is located on a MGE (e.g. plasmid-borne ESBL genes in E. coli) strain typing methods like PFGE, MLST or PCR-directed typing methods can still be applied. In addition, it is necessary also to characterize the resistance plasmid in question (e.g. by pMLST, replicon typing, restriction analysis or even whole plasmid sequencing).\textsuperscript{105} In the transfer of resistance plasmids, different scenarios are conceivable. Scenario 1 describes a situation where the transferred strain and its resistance plasmid multiply stably in the new host. In such a case, the aforementioned methods enable the verification of the transferred strain and the resistance plasmid.\textsuperscript{106} In scenario 2, the transferred strain cannot replicate in the new host, but transfers its resistance plasmid to bacteria of the new host. In this case, the transferred strain is not detectable any more, but the resistance plasmid may be detected in the new host bacteria. Scenario 3 describes a situation in which the transferred strain cannot replicate in the new host and the transferred plasmid cannot replicate in the new host bacteria but undergoes recombination with plasmids already residing in these new host bacteria. In this case, neither the original bacterial strain nor the original plasmid are detectable and the confirmation of transfer is not possible.

Another problem is the confirmation of the direction of transfer. In staphylococci, for instance, structurally closely related small mobilizable plasmids that carry the tetracycline resistance gene tet(K), the chloramphenicol resistance gene catpC221 or the MLSB resistance gene erm(C) are prevalent in various staphylococcal species from both humans and animals.\textsuperscript{107-109} Because tetracyclines, chloramphenicol and macrolides have been used in human and veterinary medicine for more than 60 years, it is impossible to determine in retrospect where and when these resistance genes first developed and which transfer events across species and host boundaries have taken place since then. In contrast, the recently identified phenicol and oxazolidinone resistance gene optR\textsuperscript{A} is likely to have developed in enterococci of animal origin in China under the selective pressure imposed by the use of florfenicol in livestock animals.\textsuperscript{110} Chloramphenicol was banned from use in food producing
animals in China in 2002, whereas florfenicol was licensed in 1999 for animals only and has been used widely since then. The first optrA-carrying E. faecium isolate of human origin orginated in 2005. This happened two years before linezolid, the sole commercially available oxazolidinone in China, was approved for use in human medicine in 2007.

The future of antibacterial therapy

For surface and superficial skin infections, and otitis involving multidrug-resistant bacteria, topical antimicrobial therapy is likely to remain effective in the future because very high concentrations of the drug, easily exceeding MICs, can be achieved at the site of infection. However, for deep infections or those requiring systemic therapy, new classes of antimicrobial agents are unlikely to be approved for veterinary medicine. All new classes of antimicrobial agents will first be tested for their suitability as therapeutics in human medicine. Only if a new class of antimicrobial agents is unsuitable for use in humans based on its pharmacological parameters, toxicity or adverse effects, may it be considered for veterinary applications. The antimicrobial agents approved for veterinary use during the last 15 years are all derivatives of already known substances. Thus, pradofloxacin is a fluoroquinolone with improved activity against canine and feline bacterial pathogens. Tulathromycin, tildipirosin and gamithromycin are macrolides for the control of bovine and porcine respiratory tract infections. Finally, florfenicol is a fluorinated phenicol with activity against chloramphenicol-resistant bacteria in which resistance is based on a chloramphenicol acetyltransferase. Florfenicol is an example where the detailed knowledge about the resistance mechanism has led to the development of a molecule which is resistant to enzymatic inactivation by acetylation. However, soon after the introduction of florfenicol into clinical veterinary use, genes specifying other phenicol resistance mechanisms, which also confer resistance to florfenicol, have emerged.

It is our responsibility to use the available antimicrobial agents wisely and try to preserve their activity for as long as possible. This needs to include following pharmacokinetic and pharmacodynamic data (and creating such data where they are not yet available) for agents that are not licensed for use in pets. One example is use of the published recommendations on minocycline. Most importantly, prudent use guidelines must be followed alongside the well-proven (but still too frequently neglected) concepts of rigorous hygiene measures. Moreover, improved microbiological diagnostics, which also include harmonized protocols for antimicrobial susceptibility testing of the various veterinary bacterial pathogens and additional veterinary-specific clinical breakpoints, especially for bacteria of poultry and fish origin, are urgently needed.

In summary, a multifaceted holistic approach which takes into account education as well as antimicrobial stewardship, is required.

Education of the public in addition to prescribers of antimicrobial drugs is needed. Understanding how antimicrobial agents work and under which conditions antimicrobial resistance develops and spreads promotes the awareness needed to implement measures that counteract resistance development. Examples of such educational measures are the pan-European e-Bug program, the “Get smart” program of US Centers for Disease Control and Prevention, and antibiotic awareness days promoted in Europe and Canada.
The search for new antimicrobial agents – natural and synthetic – should be stimulated by making the development of new agents more attractive to the pharmaceutical industry (e.g. by expanding the time of patent protection or lowering the administrative hurdles in the approval process). Public–private partnerships, which take the development of new antimicrobial agents forward, should be encouraged. As mentioned for florfenicol, more efforts also should be made to develop chemical modifications which provide antimicrobial derivatives that evade known resistance mechanisms.

Revival of “old” antimicrobial agents, including those discarded, not fully developed or even rejected, should be re-investigated. Combinations of antimicrobial agents with an inhibitor (e.g. an efflux inhibitor) should be explored for their ability to restore the activity of old antimicrobial agents.\(^1\)

Control of the use of antimicrobial agents: As the selective pressure imposed by the use of antimicrobial agents is a major driving force in the development of antimicrobial resistance, the nontherapeutic use of antimicrobial agents, for example, as growth promoters, must be discontinued worldwide. Antimicrobial agents in humans and animals should be made available by prescription only. Over-the-counter sales of antimicrobial agents should be forbidden worldwide. Monitoring of the consumption of antimicrobial agents in both human and veterinary medicine, including antimicrobial use in small animal practice, should be implemented.

Alternatives to antimicrobial agents: Novel nonantibiotic approaches for prevention of and protection against infectious diseases should be explored.\(^2\) These include the development of vaccines (especially for animal diseases), phage therapy\(^3\) and phage lysin therapy,\(^4\) adjuvants, antivirulence therapies (including synthetic polypeptides that neutralize bacterial pathogenicity factors),\(^5\) pre- and probiotics, immunostimulants, antimicrobial peptides (such as cathelicidins, defensins and dermicins),\(^6\) anti-biofilm therapies\(^7\) and reprogrammed nucleases that target antimicrobial resistance genes.\(^8\)
References


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Figure 1. Schematic presentation of the dissemination of resistant bacteria and resistance genes among different hosts with particular reference to the exchange between humans and animals. The thickness of the different arrows shall indicate the likelihood of the various transfer ways.

Application of antimicrobial agents in veterinary medicine (pet, companion and food animals)

Evolution and dissemination of resistant bacteria among animals

animal-animal transfer of bacteria

Transfer of resistance genes between bacteria from animals

Clinical disease in animals with limited therapeutic treatment options

human-animal transfer of bacteria

Exchange of resistant bacteria between humans and animals

animal-human transfer of bacteria

Clinical disease in humans with limited therapeutic treatment options

human-human transfer of bacteria

Transfer of resistance genes between bacteria from humans

Evolution and dissemination of resistant bacteria among humans

Application of antimicrobial agents in human medicine
Table 1. CLSI-approved clinical breakpoints available for skin and soft tissue infections as well as wounds in animals\(^6\)

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Target bacteria</th>
<th>Antimicrobial agent</th>
<th>Clinical breakpoints (mg/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Dog</td>
<td><em>E. coli</em></td>
<td>Ampicillin</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td></td>
<td><em>S. pseudintermedius</em></td>
<td>Ampicillin</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus spp.</em>, <em>S. canis</em> (group G, β-hemolytic group)</td>
<td>Ampicillin</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>Amoxicillin-clavulanate</td>
<td>≤ 0.25/0.12</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em>, <em>S. aureus</em>, <em>S. pseudintermedius</em>, <em>Streptococcus spp.</em> (β-hemolytic group)</td>
<td>Cephalothin</td>
<td>≤ 2</td>
</tr>
<tr>
<td></td>
<td><em>E. coli, P. multocida</em>, <em>S. aureus</em>, <em>S. pseudintermedius</em>, <em>Streptococcus spp.</em> (β-hemolytic group)</td>
<td>Cefazolin</td>
<td>≤ 2</td>
</tr>
<tr>
<td></td>
<td><em>E. coli, P. mirabilis</em>, <em>P. multocida</em>, <em>S. aureus</em>, <em>S. pseudintermedius</em>, <em>S. canis</em> (group G, β-hemolytic group)</td>
<td>Cefpodoxime</td>
<td>≤ 2</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>Difloxacin</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>Enrofloxacin</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>Marbofloxacin</td>
<td>≤ 1</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>Orbifloxacin</td>
<td>≤ 1</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em>, <em>S. pseudintermedius</em></td>
<td>Pradofloxacin</td>
<td>≤ 0.25</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Antibiotic</td>
<td>Susceptibility</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp., <em>Streptococcus</em> spp. (β-hemolytic group)</td>
<td>Clindamycin</td>
<td>≤ 0.5</td>
<td></td>
</tr>
<tr>
<td><em>S. pseudintermedius</em></td>
<td>Doxycycline</td>
<td>≤ 0.12</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
<td>Tetracycline</td>
<td>≤ 0.25</td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td><em>E. coli</em>, <em>Staphylococcus</em> spp., <em>Streptococcus</em> spp.</td>
<td>Amoxicillin-clavulanate</td>
<td>≤ 0.25/0.12</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>, <em>P. aeruginosa</em>, <em>Staphylococcus</em> spp., <em>Streptococcus</em> spp.</td>
<td>Enrofloxacin</td>
<td>≤ 0.5</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus</em> spp., <em>Streptococcus</em> spp.</td>
<td>Marbofloxacin</td>
<td>≤ 1</td>
<td></td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em>, <em>Staphylococcus</em> spp., <em>Streptococcus</em> spp.</td>
<td>Orbifloxacin</td>
<td>≤ 1</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em>, <em>S. aureus</em>, <em>S. pseudintermedius</em>, <em>S. felis</em></td>
<td>Pradofloxacin</td>
<td>≤ 0.25</td>
<td></td>
</tr>
<tr>
<td><em>P. multocida</em>, <em>S. canis</em></td>
<td>Pradofloxacin</td>
<td>≤ 0.25</td>
<td></td>
</tr>
</tbody>
</table>

*S* (susceptible), *I* (intermediate), *R* (resistant)
Table 2. Examples of resistance to antimicrobials by enzymatic inactivation (modified from ref. 1)

<table>
<thead>
<tr>
<th>Resistance mechanism</th>
<th>Resistance gene(s)</th>
<th>Gene product</th>
<th>Resistance phenotype</th>
<th>Bacteria involved</th>
<th>Location of the resistance gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>chemical modification</td>
<td>aac, aad (ant), aph</td>
<td>acetyl-, adenyl-, phosphotransferases</td>
<td>aminoglycosides</td>
<td>various Gram+, Gram–, aerobic bacteria</td>
<td>T, GC, P, C</td>
</tr>
<tr>
<td></td>
<td>aad (ant)</td>
<td>adenyltransferases</td>
<td>aminocyclitols</td>
<td>various Gram+, Gram–, aerobic bacteria</td>
<td>T, GC, P, C</td>
</tr>
<tr>
<td></td>
<td>catA, catB</td>
<td>acetyltransferases</td>
<td>chloramphenicol</td>
<td>various Gram+, Gram–, aerobic, anaerobic bacteria</td>
<td>P, T, GC, C</td>
</tr>
<tr>
<td></td>
<td>vat(A-E)</td>
<td>acetyltransferases</td>
<td>streptogramin A</td>
<td>Staphylococcus, Enterococcus</td>
<td>P, C</td>
</tr>
<tr>
<td></td>
<td>mph(A-E)</td>
<td>phosphotransferases</td>
<td>macrolides</td>
<td>Escherichia, Shigella, Staphylococcus</td>
<td>P, T, C</td>
</tr>
<tr>
<td></td>
<td>lnu(A), lnu(B)</td>
<td>nucleotidyltransferases</td>
<td>lincosamides</td>
<td>Staphylococcus</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>tet(X), tet(37)</td>
<td>oxidoreductases</td>
<td>tetracyclines</td>
<td>Bacteroides</td>
<td>T, P</td>
</tr>
<tr>
<td>hydrolytic cleavage</td>
<td>blaZ, bla TEM, blaSHV, blaCTX-M, etc.</td>
<td>β-lactamases</td>
<td>β-lactam antibiotics</td>
<td>various Gram+, Gram–, aerobic, anaerobic bacteria</td>
<td>P, T, GC, C</td>
</tr>
<tr>
<td></td>
<td>ere(A), ere(B)</td>
<td>esterase</td>
<td>macrolides</td>
<td>E. coli, Staphylococcus</td>
<td>P, GC</td>
</tr>
<tr>
<td>vgb(A), vgb(B)</td>
<td>lactone hydrolases</td>
<td>streptogramin B</td>
<td>Staphylococcus</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
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<td>----------------</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

*a*  
P = plasmid; T = transposon; GC = gene cassette; C = chromosomal DNA
Table 3. Examples of resistance to antimicrobials by decreased intracellular drug accumulation (modified from ref. 1)

<table>
<thead>
<tr>
<th>Resistance mechanism</th>
<th>Resistance gene(s)</th>
<th>Gene product</th>
<th>Resistance phenotype</th>
<th>Bacteria involved</th>
<th>Location of the resistance gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>efflux via multidrug transporters</td>
<td>mexA-mexB-oprM, acrA-acrB-tolC</td>
<td>multidrug efflux in combination with specific OMP’s</td>
<td>chloramphenicol, β-lactams, macrolides, fluoroquinolones, tetracyclines, etc.</td>
<td><em>Pseudomonas, E. coli, Salmonella</em></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>emrE</td>
<td>4-TMS multidrug efflux protein</td>
<td>tetracyclines, nucleic acid binding compounds</td>
<td><em>E. coli</em></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>blt, norA</td>
<td>12-TMS multidrug efflux protein of the major facilitator superfamily</td>
<td>chloramphenicol, fluoroquinolones, nucleic acid binding compounds</td>
<td><em>Bacillus, Staphylococcus</em></td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>floR</td>
<td>12 TMS efflux system of the major facilitator superfamily</td>
<td>phenicols</td>
<td>various Gram– bacteria</td>
<td>T, P, C</td>
</tr>
<tr>
<td></td>
<td>cmlA, cmlB</td>
<td>12 TMS efflux system of the major facilitator superfamily</td>
<td>chloramphenicol</td>
<td>various Gram– bacteria</td>
<td>T, P, GC, C</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Antibiotics</td>
<td>Organism</td>
<td>Source</td>
<td></td>
</tr>
<tr>
<td>-------</td>
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<td>---------------------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>fexA</td>
<td>14 TMS efflux system of the major facilitator superfamily</td>
<td>phenicols</td>
<td><em>Staphylococcus</em></td>
<td>T, P, C</td>
<td></td>
</tr>
<tr>
<td>mef(A)</td>
<td>Efflux system of the major facilitator superfamily</td>
<td>14-, 15-membered macrolides</td>
<td><em>Streptococcus</em>, other Gram+ bacteria</td>
<td>T, P, C</td>
<td></td>
</tr>
<tr>
<td>msr(A)</td>
<td>Efflux system of the ABC transporter family</td>
<td>macrolides and streptogramin B</td>
<td><em>Staphylococcus</em></td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>vga(A), vga(C), vga(E), lsa(E), sal(A)</td>
<td>Efflux system of the ABC transporter family</td>
<td>streptogramin A, lin</td>
<td><em>Staphylococcus</em>, <em>Enterococcus</em></td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>optrA</td>
<td>Efflux system of the ABC transporter family</td>
<td>phenicols, linezolid, tedizolid</td>
<td><em>Enterococcus</em>, <em>Staphylococcus</em></td>
<td>P, C</td>
<td></td>
</tr>
</tbody>
</table>

a. P = plasmid; T = transposon; GC = gene cassette; C = chromosomal DNA
b. TMS = transmembrane segments
Table 4. Examples of resistance to antimicrobials by target site alteration (modified from ref. 1)

<table>
<thead>
<tr>
<th>Resistance mechanism</th>
<th>Resistance gene(s)</th>
<th>Gene product</th>
<th>Resistance phenotype</th>
<th>Bacteria involved</th>
<th>Location of the resistance gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>methylation of the target site</td>
<td><em>erm</em>(A-46)</td>
<td>rRNA methylase</td>
<td>macrolides, lincosamides, streptogramin B</td>
<td>various Gram+ and Gram– bacteria</td>
<td>P, T, C</td>
</tr>
<tr>
<td>methylation of the target site</td>
<td><em>cfr, cfrB</em></td>
<td>rRNA methylase</td>
<td>phenicols, lincosamides, linezolid, pleuromutilins, streptogramin A</td>
<td>various Gram+ and Gram– bacteria</td>
<td>P, C</td>
</tr>
<tr>
<td>protection of the target site</td>
<td><em>tet</em>(M, O, P, Q, S, T)</td>
<td>ribosome protective proteins</td>
<td>tetracyclines</td>
<td>various Gram+ and Gram– bacteria</td>
<td>T, P, C</td>
</tr>
<tr>
<td>protection of the target site</td>
<td><em>fusB</em></td>
<td>ribosome protective protein</td>
<td>fusidic acid</td>
<td><em>Staphylococcus</em></td>
<td>P</td>
</tr>
<tr>
<td>replacement of a sensitive target by an alternative drug-resistant target</td>
<td><em>mecA, mecC</em></td>
<td>penicillin-binding proteins with altered substrate specificity</td>
<td>penicillins, cephalosporins, carbapenems, monobactams</td>
<td><em>Staphylococcus</em></td>
<td>C</td>
</tr>
<tr>
<td>replacement of a sensitive target by an alternative drug-resistant target</td>
<td><em>sul1, sul2, sul3</em></td>
<td>sulfonamide-insensitive dihydropteroate synthase</td>
<td>sulfonamides</td>
<td>various Gram– bacteria</td>
<td>P, I</td>
</tr>
<tr>
<td>replacement of a sensitive target by an alternative drug-resistant target</td>
<td><em>dfrA, dfrB</em></td>
<td>trimethoprim-insensitive dihydrofolate reductase</td>
<td>trimethoprim</td>
<td>various Gram+ and Gram– bacteria</td>
<td>P, GC, T, C</td>
</tr>
<tr>
<td></td>
<td>Mutational Modification</td>
<td>Target Site</td>
<td>Antibiotics</td>
<td>Bacteria</td>
<td>Gene(s)</td>
</tr>
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<td>mupA, ileS2</td>
<td>mupirocin-insensitive isoleucyl-tRNA synthase</td>
<td>mupirocin</td>
<td>Staphylococcus</td>
<td>P</td>
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<td>vanA-E</td>
<td>alternative peptide-glycan precursors</td>
<td>glycopeptides</td>
<td>Enterococcus, Staphylococcus</td>
<td>T, P, C</td>
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<td>—</td>
<td>mutations in the genes for topoisomerase II and IV</td>
<td>fluoroquinolones</td>
<td>various Gram+ and Gram− bacteria</td>
<td>C</td>
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<td>—</td>
<td>mutation in the gene coding for ribosomal protein S12</td>
<td>streptomycin</td>
<td>several Gram+ and Gram− bacteria</td>
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<td>mutation in the gene for the ribosomal protein L3</td>
<td>tiamulin</td>
<td>E. coli</td>
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<td>tetracyclines</td>
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<td>fusidic acid</td>
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<td>C</td>
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<td>mutations in the marRAB soxR or acrR genes</td>
<td>fluoroquinolones</td>
<td>E. coli</td>
<td>C</td>
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</table>

*P = plasmid; T = transposon; GC = gene cassette; C = chromosomal DNA, I = integron*
Zusammenfassung

Hintergrund – Antimikrobielle Resistenz hat sich zu einer zunehmenden Herausforderung in der Veterinärmedizin entwickelt, insbesondere im Zusammenhang mit bakteriellen Infektionserregern, die bei Menschen und Tieren eine Rolle spielen.

