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

Stefan Schwarz*, Anette Loeffler† and Kristina Kadlec*

* Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany
† Clinical Sciences and Services, Royal Veterinary College, Hawkshead Campus, University of London, London, UK

Correspondence: Stefan Schwarz, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Höltystr. 10, 31535 Neustadt-Mariensee, Germany. E-mail: stefan.schwarz@fli.bund.de

Source of funding: This study was self-funded.

Conflict of Interest: Within the last 5 years, Stefan Schwarz has received consultancy payments and/or research funding from Bayer HealthCare, Pfizer Animal Health and Zoetis.
The other authors declare no conflicts of interest

Running title: Mechanisms and transfer of resistance

Keywords: Mutation, resistance gene, mobile genetic element, susceptibility, transfer

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.[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[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.[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[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.[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.[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.[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.[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,[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,[1] 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.[9] 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.[1] 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.

Resistance 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.[1, 2]

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,[10] or of aminoglycosides by acetylation, adenylation or phosphorylation.[11] 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.[12, 13] Other examples are esterases which confer macrolide resistance or lactone hydrolases which inactivate streptogramin B compounds.[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, β-lactams and chloramphenicol in E. coli, whereas OmpD is involved in the uptake of carbapenems in Pseudomonas aeruginosa.[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.[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-TolC transporter in E. coli and Salmonella enterica or the MexAB-OprM transporter in P. aeruginosa which can export chloramphenicol, fluoroquinolones, tetracyclines, β-lactams and macrolides among others.[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.[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, CmlA and CmlB in Gram-negative bacteria.[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.[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.[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 (glycylcyclines) from Staphylococcus aureus.[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 pinnepedialis, 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

protein synthesis, but protect the ribosome from the inhibitory effects of tetracyclines.[36, 37] The gene fusB also codes for an EF-G-binding protein that protects the staphylococcal ribosomes from inhibition by fusidic acid.[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.[1, 2] Gram-negative and Gram-positive bacteria have acquired various dfr genes which code for trimethoprim-insensitive dihydrofolate reductases.[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 β-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.[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.[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.[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.[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.[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.[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.[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

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.[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.[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.[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.[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.[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.[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.[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.[59, 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,

respectively.[65] 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.[66] 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, 69-72] 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),[76] 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.[79] 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.[69, 70, 80, 81]

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.[82-84] 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.[68]

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.[85] A study from Australia revealed that veterinarians often carry multidrug-resistant MRSA isolates.[86] 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.[87] 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.[87] 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.[88]

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.[89] 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.[90] 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.[91] 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.[92, 93] Another study showed that food animal transport in open crates resulted in the dissemination of bacteria, including resistant enterococci, into the environment.[94] In addition, indirect transmission via insects or rats can occur on farms.[95, 96]

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.[97] 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.[98] 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.[98] 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.[99] 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.[100] 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.[100] 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.[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.[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).[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.[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).[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.[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.[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 optrA 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.[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.[110] 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.[111, 112] 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.[10] 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.[10, 17]

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.[113] 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:[114]

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,[115, 116] the “Get smart” program of US Centers for Disease Control and Prevention,[117] and antibiotic awareness days promoted in Europe and Canada.[118, 119]

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.[120]

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.[121] These include the development of vaccines (especially for animal diseases), phage therapy[122, 123] and phage lysin therapy,[124-126] adjuvants, antivirulence therapies (including synthetic polypeptides that neutralize bacterial pathogenicity factors),[127] pre- and probiotics, immunostimulants, antimicrobial peptides (such as cathelicidins, defensins and dermicins),[128, 129] anti-biofilm therapies[130-132] and reprogrammed nucleases that target antimicrobial resistance genes.[133]

References

1. Schwarz S, Cloeckaert A, Roberts MC. Mechanisms and spread of bacterial resistance to antimicrobial agents. In: Aarestrup FM, ed. *Antimicrobial resistance in bacteria of animal origin.* Washington DC: ASM Press, 2006; 73–98.
2. Schwarz S, Noble WC. Aspects of bacterial resistance to antimicrobial agents used in veterinary dermatological practice. *Vet Dermatol* 1999; 10: 163–176.
3. D'Costa VM, King CE, Kalan L *et al*. Antibiotic resistance is ancient. *Nature* 2011; 477: 457–461.
4. Magiorakos AP, Srinivasan A, Carey RB *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18: 268-281.
5. World Health Organization website. Antimicrobial Resistance. http://www.who.int/mediacentre/factsheets/fs194/en/. Accessed Feb 14, 2016.
6. Generation, Presentation, and Application of Antimicrobial Susceptibility Test Data for Bacteria of Animal Origin; A Report. *CLSI document VET05-R*. Wayne, PA: CLSI 2011.
7. Performance Standards for Antimicrobial Susceptibility Testing – 26th edition. *CLSI document M100-S*. Wayne, PA: CLSI 2016.
8. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals – 3rd edition. *CLSI document Vet01-S*. Wayne, PA: CLSI 2015.
9. Zervos MJ, Schaberg DR. Reversal of the in vitro susceptibility of enterococci to trimethoprim-sulfamethoxazole by folinic acid. *Antimicrob Agents Chemother* 1985; 28: 446–448.
10. Schwarz S, Kehrenberg C, Doublet B *et al*. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* 2004; 28: 519–542.
11. Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. *Drug Resist Update* 2010; 13: 151–171.
12. Bush K, Jacoby GA. Updated functional classification of β-lactamases. *Antimicrob Agents Chemother* 2010; 54: 969–76
13. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14: 933–951.
14. Roberts MC, Sutcliffe J, Courvalin P *et al*. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; 43: 2823–2830.
15. Poole K. Efflux-mediated antimicrobial resistance. *J Antimicrob Chemother* 2005; 56: 20–51.
16. Putman M, van Veen HW, Konings WN. Molecular properties of bacterial multidrug transporters. *Microbiol Mol Biol Rev* 2000; 64: 672–693.
17. Roberts MC, Schwarz S, Tetracycline and phenicol resistance genes and mechanisms: Importance for agriculture, the environment, and humans. *J Environ Qual* 2016. Epub ahead doi:10.2134/jeq2015.04.0207
18. Kadlec K, Kehrenberg C, Schwarz S. Efflux-mediated resistance to florfenicol and/or chloramphenicol in *Bordetella bronchiseptica*: identification of a novel chloramphenicol exporter. *J Antimicrob Chemother* 2007; 59: 191–196.

19. Schneider E, Hunke S. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol Rev* 1998; 22: 1–20.
20. Wendlandt S, Shen J, Kadlec K *et al*. Multidrug resistance genes in staphylococci from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. *Trends Microbiol* 2015; 23: 44–54.
21. Wendlandt S, Kadlec K, Feßler AT *et al*. Identification of ABC transporter genes conferring combined pleuromutilin-lincosamide-streptogramin A resistance in bovine methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *Vet Microbiol* 2015; 177: 353–358.
22. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J Antimicrob Chemother* 2003; 51: 1109–1117.
23. Mascaretti OA. *Bacteria versus Antibacterial Agents – An Integrated Approach*. Washington, DC: ASM Press, 2003; 1–393.
24. Redgrave LS, Sutton SB, Webber MA *et al*. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol* 2014; 22: 438–445.
25. Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clin Infect Dis* 2001; 33 Suppl 3: S147–156.
26. Blondeau JM. New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach. *Vet Dermatol* 2009; 20: 383–396.
27. O'Neill AJ, McLaws F, Kahlmeter G *et al*. Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrob Agents Chemother* 2007; 51: 1737–1740.
28. McCarthy AJ, Harrison EM, Stanczak-Mrozek K *et al*. Genomic insights into the rapid emergence and evolution of MDR in *Staphylococcus pseudintermedius*. *J Antimicrob Chemother* 2015; 70: 997–1007.
29. Kehrenberg C, Schwarz S. Mutations in 16S rRNA and ribosomal protein S5 associated with high-level spectinomycin resistance in *Pasteurella multocida*. *Antimicrob Agents Chemother* 2007; 51: 2244–2246.
30. Riesenberg A, Feßler AT, Erol E *et al*. MICs of 32 antimicrobial agents for *Rhodococcus equi* isolates of animal origin. *J Antimicrob Chemother* 2014; 69: 1045–1049.
31. Kadlec K, van Duijkeren E, Wagenaar JA *et al*. Molecular basis of rifampicin resistance in methicillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. *J Antimicrob Chemother* 2011; 66: 1236–1242.
32. Roberts MC. Update on macrolide-lincosamide-streptogramin, ketolide, and oxazolidinone resistance genes. *FEMS Microbiol Lett* 2008; 282: 147–159.
33. Nomenclature Center for MLS Genes website. rRNA Methylase Genes Table. Available at: http://faculty.washington.edu/marilynr/ermweb4.pdf. Accessed Feb 14, 2016.
34. Long KS, Poehlsgaard J, Kehrenberg C *et al*. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* 2006; 50: 2500–2505.
35. Shen J, Wang Y, Schwarz S. Presence and dissemination of the multiresistance gene *cfr* in Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother* 2013; 68: 1697–1706.

36. Connell SR, Tracz DM, Nierhaus KH *et al*. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother* 2003; 47: 3675–3681.
37. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* 2001; 65: 232–2360.
38. Becker K, Ballhausen B, Köck R *et al*. Methicillin resistance in *Staphylococcus* isolates: the "*mec* alphabet" with specific consideration of *mecC*, a *mec* homolog associated with zoonotic *S. aureus* lineages. *Int J Med Microbiol* 2014; 304: 794–804.
39. Wendlandt S, Feßler AT, Monecke S *et al*. The diversity of antimicrobial resistance genes among staphylococci of animal origin. *Int J Med Microbiol* 2013; 303: 338–349.
40. Kadlec K, Schwarz S. Antimicrobial resistance of *Staphylococcus pseudintermedius*. *Vet Dermatol* 2012; 23: 276–282.
41. Maddox TW, Clegg PD, Williams NJ *et al*. Antimicrobial resistance in bacteria from horses: Epidemiology of antimicrobial resistance. *Equine Vet J* 2015; 47: 756–765.
42. White DG. 2006. Antimicrobial resistance in pathogenic *Escherichia coli* from animals. In: Aarestrup FM, ed. *Antimicrobial resistance in bacteria of animal origin.* Washington DC: ASM Press, 2006; 145–166.
43. Ewers C, Bethe A, Semmler T *et al*. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clin Microbiol Infect* 2012; 18: 646–655.
44. Arais LR, Barbosa AV, Carvalho CA *et al*. Antimicrobial resistance, integron carriage, and *gyrA* and *gyrB* mutations in *Pseudomonas aeruginosa* isolated from dogs with otitis externa and pyoderma in Brazil. *Vet Dermatol* 2016. doi: 10.1111/vde.12290. [Epub ahead of print]
45. Harada K, Arima S, Niina A *et al*. Characterization of *Pseudomonas aeruginosa* isolates from dogs and cats in Japan: current status of antimicrobial resistance and prevailing resistance mechanisms. *Microbiol Immunol* 2012; 56: 123–127.
46. Aarestrup FM, Schwarz S. 2006. Antimicrobial resistance in staphylococci and streptococci of animal origin. In: Aarestrup FM, ed. *Antimicrobial resistance in bacteria of animal origin.* Washington DC: ASM Press, 2006; 187–212.
47. Moodley A, Damborg P, Nielsen SS. Antimicrobial resistance in methicillin susceptible and methicillin resistant Staphylococcus pseudintermedius of canine origin: literature review from 1980 to 2013. *Vet Microbiol* 2014; 171: 337–341.
48. Perreten V, Kadlec K, Schwarz S et al. Clonal spread of methicillin-resistant Staphylococcus pseudintermedius in Europe and North America: an international multicentre study. *J Antimicrob Chemother* 2010; 65: 1,145–1,154.
49. Schwarz S, Alesík E, Grobbel M et al. Antimicrobial susceptibility of streptococci from various indications of swine, horses, dogs and cats as determined in the BfT-GermVet monitoring program 2004-2006. *Berl Münch Tierärztl Wochenschr* 2007; 120: 380–390.
50. Schwarz S, Alesík E, Werckenthin C et al. Antimicrobial susceptibility of coagulase-positive and coagulase-variable staphylococci from various indications of swine, dogs and cats as determined in the BfT-GermVet monitoring program 2004-2006. *Berl Münch Tierärztl Wochenschr* 2007; 120: 372–379.

51. Grobbel M, Lübke-Becker A, Alesík E et al. Antimicrobial susceptibility of Escherichia coli from swine, horses, dogs and cats as determined in the BfT-GermVet monitoring program 2004-2006. *Berl Münch Tierärztl Wochenschr* 2007; 120: 391–401.
52. Grobbel M, Lübke-Becker A, Alesík E et al. Antimicrobial susceptibility of Klebsiella spp. and Proteus spp. from various organ systems of horses, dogs and cats as determined in the BfT-GermVet monitoring program 2004-2006. *Berl Munch Tierarztl Wochenschr* 2007; 120: 402–411.
53. Werckenthin C, Alesík E, Grobbel M et al. Antimicrobial susceptibility of Pseudomonas aeruginosa from dogs and cats as well as Arcanobacterium pyogenes from cattle and swine as determined in the BfT-GermVet monitoring program 2004-2006. *Berl Münch Tierärztl Wochenschr* 2007; 120: 412–422.
54. Couto N, Monchique C, Belas A et al. Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. *J Antimicrob Chemother* 2016; 71: 1,479–1,487.
55. Schwarz S, Kadlec K, Silley P. *Antimicrobial Resistance in Bacteria of Animal Origin.* Steinen, Germany: ZETT Verlag, 2013; 1–128.
56. Thomas CM, Nielsen KM. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Review. *Nat Rev Microbiol* 2005; 3: 711–721.
57. Lindsay JA. *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. *Int J Med Microbiol* 2014; 304:103–109.
58. Burrus V, Marrero J, Waldor MK. The current ICE age: biology and evolution of SXT-related integrating conjugative elements. *Plasmid* 2006; 55: 173–183.
59. Michael GB, Kadlec K, Sweeney MT *et al*. ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: analysis of the regions that comprise 12 antimicrobial resistance genes. *J Antimicrob Chemother* 2012; 67: 84–90.
60. Michael GB, Kadlec K, Sweeney MT *et al*. ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: structure and transfer. *J Antimicrob Chemother* 2012; 67: 91–100.
61. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009; 53: 4961–4967.
62. Hall RM. *Salmonella* genomic islands and antibiotic resistance in *Salmonella enterica*. *Future Microbiol* 2010; 5: 1525–1538.
63. Schultz E, Haenni M, Mereghetti L *et al*. Survey of multidrug resistance integrative mobilizable elements SGI1 and PGI1 in *Proteus mirabilis* in humans and dogs in France, 2010-13. *J Antimicrob Chemother* 2015; 70: 2543–2546.
64. Schwarz S, Chaslus-Dancla E. Use of antimicrobials in veterinary medicine and mechanisms of resistance. *Vet Res* 2001; 32: 201–225.
65. Schwarz S, Kehrenberg C, Walsh TR. Use of antimicrobial agents in veterinary medicine and food animal production. *Int J Antimicrob Agents* 2001; 17: 431–437.
66. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother* 2004; 54: 321–332.
67. Ohr R. Heimtierstudie "Wirtschaftsfaktor Heimtierhaltung": Zur wirtschaftlichen Bedeutung der Heimtierhaltung in Deutschland, Göttingen 2014.
68. Damborg P, Nielsen SS, Guardabassi L. *Escherichia coli* shedding patterns in humans and dogs: insights into within-household transmission of phylotypes associated with urinary tract infections. *Epidemiol Infect* 2009; 137: 1457–1464.

69. Walther B, Hermes J, Cuny C *et al*. Sharing more than friendship--nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. *PLoS One* 2012; 7: e35197.
70. Nienhoff U, Kadlec K, Chaberny IF *et al*. Transmission of methicillin-resistant *Staphylococcus aureus* strains between humans and dogs: two case reports. *J Antimicrob Chemother* 2009; 64: 660–662.
71. Westgarth C, Pinchbeck GL, Bradshaw JW *et al*. Dog-human and dog-dog interactions of 260 dog-owning households in a community in Cheshire. *Vet Rec* 2008; 162: 436–442.
72. Westgarth C, Pinchbeck GL, Bradshaw JW *et al*. Factors associated with dog ownership and contact with dogs in a UK community. *BMC Vet Res*. 2007; 3: 5.
73. Hill PB, Lo A, Eden CA et al. Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. *Vet Rec* 2006; 158: 533–539.
74. U.S. Food and Drug Administration website. Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Available at: http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/ActsRulesRegulations/ucm085377.htm. Accessed Feb 13, 2016.
75. Scott GM, Thomson R, Malone-Lee J *et al*. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection inhumans? *J Hosp Infect* 1988; 12: 29–34.
76. Sing A, Tuschak C, Hörmansdorfer S. Methicillin-resistant *Staphylococcus aureus* in a family and its pet cat. *N Engl J Med* 2008; 358: 1200–1201.
77. van Duijkeren E, Ten Horn L, Wagenaar JA *et al*. Suspected horse-to-human transmission of MRSA ST398. *Emerg Infect Dis* 2011; 17: 1137–1139.
78. van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM *et al*. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. *Vet Microbiol* 2010; 141: 96–102.
79. Soares Magalhães RJ, Loeffler A, Lindsay J *et al*. Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dogs and cats: a case-control study. *Vet Res* 2010; 41: 55.
80. Lefebvre SL, Weese JS. Contamination of pet therapy dogs with MRSA and *Clostridium difficile*. *J Hosp Infect* 2009; 72: 268–269.
81. Paterson GK, Harrison EM, Murray GG *et al*. Capturing the cloud of diversity reveals complexity and heterogeneity of MRSA carriage, infection and transmission. *Nat Commun*. 2015; 6: 6560.
82. Schaufler K, Semmler T, Wieler LH *et al*. Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410-anothersuccessful pandemic clone? *FEMS Microbiol Ecol* 2016; 92. pii: fiv155.
83. Ewers C, Bethe A, Stamm I *et al*. CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? *J Antimicrob Chemother* 2014; 69: 1224–1230.
84. Schmiedel J, Falgenhauer L, Domann E *et al*. Multiresistant extended-spectrum β-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol*. 2014; 14: 187.
85. Weiß S, Kadlec K, Feßler AT *et al*. Identification and characterization of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus pettenkoferi* from a small animal clinic. *Vet Microbiol* 2013; 167: 680–685.

86. Groves MD, Crouch B, Coombs GW *et al*. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* Isolated from Australian veterinarians. *PLoS One* 2016; 11: e0146034.
87. Cuny C, Nathaus R, Layer F *et al*. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. *PLoS One* 2009; 4: e6800.
88. Feßler AT, Olde Riekerink RG, Rothkamp A *et al*. Characterization of methicillin-resistant *Staphylococcus aureus* CC398 obtained from humans and animals on dairy farms. *Vet Microbiol* 2012; 160: 77–84.
89. Wendlandt S, Kadlec K, Feßler AT *et al*. Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers. *J Antimicrob Chemother* 2013; 68: 2458–2463.
90. Richter A, Sting R, Popp C, *et al*. Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals. *Epidemiol Infect* 2012; 140: 2223–2232.
91. Wendlandt S, Kadlec K, Fessler AT *et al*. Transmission of methicillin-resistant *Staphylococcus aureus* isolates on broiler farms. *Vet Microbiol* 2013; 167: 632–637.
92. von Salviati C, Laube H, Guerra B *et al*. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. *Vet Microbiol* 2015; 175: 77–84.
93. Laube H, Friese A, von Salviati C *et al*. Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. *Vet Microbiol* 2014; 172: 519–527.
94. Rule AM, Evans SL, Silbergeld EK. Food animal transport: a potential source of community exposures to health hazards from industrial farming (CAFOs). *J Infect Public Health* 2008; 1: 33–39.
95. Zurek L, Ghosh A. Insects represent a link between food animal farms and theurban environment for antibiotic resistance traits. *Appl Environ Microbiol* 2014; 80: 3562-3567.
96. van de Giessen AW, van Santen-Verheuvel MG, Hengeveld PD *et al*. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. *Prev Vet Med* 2009; 91: 270-273.
97. The Healthline website. Bacterial Gastroenteritis. Available at: http://www.healthline.com/health/bacterial-gastroenteritis#Treatment6. Accessed Feb 14, 2016.
98. Bywater RJ, Casewell MW. An assessment of the impact of antibiotic resistance in different bacterial species and of the contribution of animal sources to resistance in human infections. *J Antimicrob Chemother* 2000; 46: 643–645.
99. Michael GB, Freitag C, Wendlandt S *et al*. Emerging issues in antimicrobial resistance of bacteria from food-producing animals. *Future Microbiol* 2015; 10: 427–443.
100. Köck R, Ballhausen B, Bischoff M *et al*. The impact of zoonotic MRSA colonization and infection in Germany. *Berl Münch Tierärztl Wochenschr* 2014; 127: 384–398.
101. Wu G, Day MJ, Mafura MT *et al*. Comparative analysis of ESBL-positive *Escherichia coli* isolates from animals and humans from the UK, The Netherlands and Germany. *PLoS One* 2013; 8: e75392.
102. Kadlec K, Wendlandt S, Feßler AT *et al.* Methods for the detection of antimicrobial resistance and the characterization of *Staphylococcus aureus* Isolates from food-producing animals and food of animal origin. In: Chen C-Y,

Yan X, Jackson C, eds. *Antimicrobial Resistance and Food Safety*, Amsterdam: Elsevier, 2015; 207–232.
103. Sobral D, Schwarz S, Bergonier D *et al*. High throughput multiple locus variable number of tandem repeat analysis (MLVA) of *Staphylococcus aureus* from human, animal and food sources. PLoS One. 2012; 7: e33967.
104. Harrison EM, Paterson GK, Holden MT *et al*. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mecA* homologue *mecC*. *EMBO Mol Med* 2013; 5: 509–15.
105. Falgenhauer L, Schmiedel J, Ghosh H *et al*. Resistance plasmids in ESBL-encoding *Escherichia coli* isolates from humans, dogs and cats. *Berl Münch Tierärztl Wochenschr*. 2014; 127: 458–463.
106. McCarthy AJ, Loeffler A, Witney AA *et al*. Extensive horizontal gene transfer during *Staphylococcus aureus* co-colonization in vivo. *Genome Biol Evol* 2014; 6: 2697–2708.
107. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*. Genetic basis. *Microbiol Rev* 1987; 51: 88–134.
108. Schwarz S, Shen J, Wendlandt S *et al*. Plasmid-mediated antimicrobial resistance in staphylococci and other firmicutes. *Microbiol Spectr* 2014; 2.
109. Schwarz S, Feßler AT, Hauschild T *et al*. Plasmid-mediated resistance to protein biosynthesis inhibitors in staphylococci. *Ann N Y Acad Sci* 2011; 1241: 82–103.
110. Wang Y, Lv Y, Cai J *et al*. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. *J Antimicrob Chemother* 2015; 70: 2182–2190.
111. Hillier A, Lloyd DH, Weese JS et al. Guidelines for the diagnosis and antimicrobial therapy of canine superficial bacterial folliculitis (Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases). *Vet Dermatol* 2014; 25: 163–175, e43.
112. Mueller RS, Bergvall K, Bensignor E et al. A review of topical therapy for skin infections with bacteria and yeast. *Vet Dermatol* 2012; 23: 330–341.
113. Maaland MG, Guardabassi L, Papich MG. Minocycline pharmacokinetics and pharmacodynamics in dogs: dosage recommendations for treatment of meticillin-resistant Staphylococcus pseudintermedius infections. *Vet Dermatol* 2014; 25: 182–190.
114. Bush K, Courvalin P, Dantas G *et al*. Tackling antibiotic resistance. *Nat Rev Microbiol* 2011; 9: 894–896.
115. McNulty CA, Lecky DM, Farrell D *et al*. Overview of e-Bug: an antibiotic and hygiene educational resource for schools. *J Antimicrob Chemother* 2011; 66 Suppl 5: v3–12.
116. e-Bug website. Available at: www.e-Bug.eu. Accessed Feb 14, 2016.
117. Centers of Disease Control and prevention website. Get SmartPrograms & Observances. Available at: http://www.cdc.gov/getsmart/. Accessed Feb 14, 2016.
118. European Centre for Disease Prevention and Control website. European Antibiotic Awareness Day. Available at: http://ecdc.europa.eu/en/EAAD/Pages/Home.aspx. Accessed Feb 14, 2016.
119. Association of Medical Microbiology and Infectious Disease Canada website. Antibiotic Awareness Week. Available at: https://www.ammi.ca/topics/antibiotic-awareness-week/. Accessed Feb 14, 2016.

120. Goh S, Loeffler A, Lloyd DH *et al*. Oxacillin sensitization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* by antisense peptide nucleic acids in vitro. *BMC Microbiol* 2015; 15: 262.
121. Lloyd DH. Alternatives to conventional antimicrobial drugs: a review of future prospects. *Vet Dermatol* 2012; 23: 299–304.
122. Nobrega FL, Costa AR, Kluskens LD *et al*. Revisiting phage therapy: new applications for old resources. *Trends Microbiol* 2015; 23: 185–191.
123. Tsonos J, Vandenheuvel D, Briers Y *et al*. Hurdles in bacteriophage therapy: deconstructing the parameters. *Vet Microbiol* 2014; 171: 460–469.
124. Fischetti VA. Lysin therapy for *Staphylococcus aureus* and other bacterial pathogens. *Curr Top Microbiol Immunol* 2016 Jan 5. [Epub ahead of print]
125. Schuch R, Lee HM, Schneider BC *et al*. Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus aureus*-induced murine bacteremia. *J Infect Dis* 2014; 209: 1469–1478.
126. Thandar M, Lood R, Winer BY *et al*. Novel engineered peptides of a phage lysin as effective antimicrobials against multidrug resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2016 Feb 8. pii: AAC.02972-15. [Epub ahead of print]
127. Brandenburg K, Schürholz T. Lack of new antiinfective agents: Passing into the pre-antibiotic age? *World J Biol Chem* 2015; 6: 71–77.
128. Lüthje P, Brauner A. Novel strategies in the prevention and treatment of urinary tract infections. Pathogens. 2016; 5. pii: E13.
129. Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 2005; 3: 238–250.
130. Abedon ST. Ecology of anti-biofilm agents II: Bacteriophage exploitation and biocontrol of biofilm bacteria. *Pharmaceuticals (Basel)* 2015; 8: 559–589.
131. Brackman G, Coenye T. Quorum sensing inhibitors as anti-biofilm agents. *Curr Pharm Des* 2015; 21: 5–11.
132. de la Fuente-Núñez C, Reffuveille F, Fernández L *et al*. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Curr Opin Microbiol* 2013; 16: 580–589.
133. Bikard D, Euler CW, Jiang W *et al*. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol* 2014; 32: 1146–1150.

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.

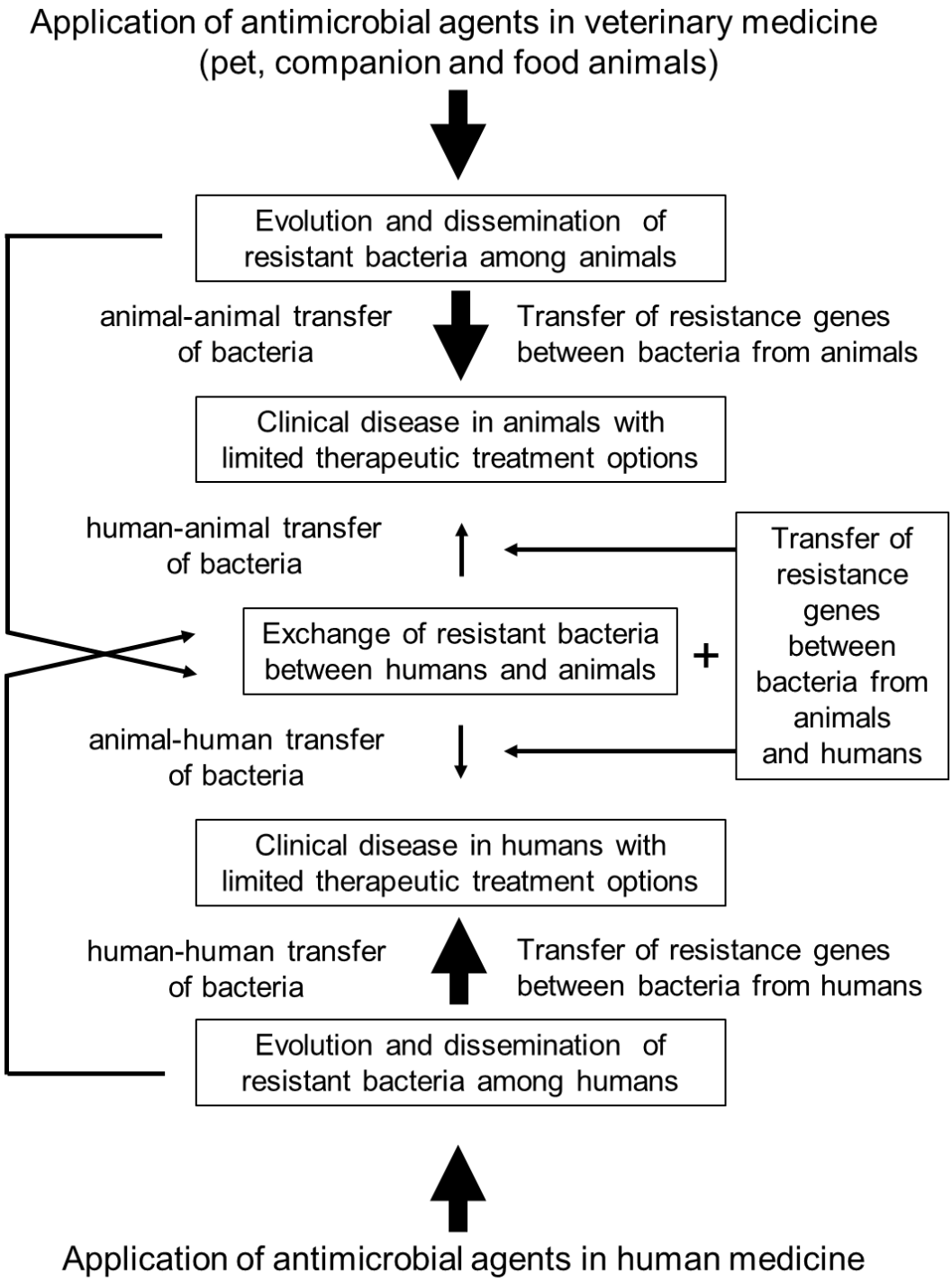

Table 1. CLSI-approved clinical breakpoints available for skin and soft tissue infections as well as wounds in animals[8]

Animal species	Target bacteria	Antimicrobial agent	Clinical breakpoints (mg/L)*		
			S	**I**	**R**
Dog	*E. coli*	Ampicillin	≤ 0.25	0.5	≥ 1
	S. pseudintermedius	Ampicillin	≤ 0.25	—	≥ 0.5
	Streptococcus spp., *S. canis* (group G, β-hemolytic group)	Ampicillin	≤ 0.25	—	—
	E. coli, *Staphylococcus* spp., *Streptococcus* spp.	Amoxicillin-clavulanate	≤ 0.25/0.12	0.5/0.25	≥ 1/0.5
	E. coli, *S. aureus*, *S. pseudintermedius*, *Streptococcus* spp. (β-hemolytic group)	Cephalothin	≤ 2	4	≥ 8
	E. coli, *P. multocida*, *S. aureus*, *S. pseudintermedius*, *Streptococcus* spp. (β-hemolytic group)	Cefazolin	≤ 2	4	≥ 8
	E. coli, *P. mirabilis*, *P. multocida*, *S. aureus*, *S. pseudintermedius*, *S. canis* (group G, β-hemolytic group)	Cefpodoxime	≤ 2	4	≥ 8
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Difloxacin	≤ 0.5	1-2	≥ 4
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Enrofloxacin	≤ 0.5	1-2	≥ 4
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Marbofloxacin	≤ 1	2	≥ 4
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Orbifloxacin	≤ 1	2-4	≥ 8
	E. coli, *S. pseudintermedius*	Pradofloxacin	≤ 0.25	0.5-1	≥ 2

	Staphylococcus spp., *Streptococcus* spp. (β-hemolytic group)	Clindamycin	≤ 0.5	1-2	≥ 4
	S. pseudintermedius	Doxycycline	≤ 0.12	0.25	≥ 0.5
	Staphylococcus spp.	Tetracycline	≤ 0.25	0.5	≥ 1
Cats	*E. coli*, *Staphylococcus* spp., *Streptococcus* spp.	Amoxicillin-clavulanate	≤ 0.25/0.12	0.5/0.25	≥ 1/0.5
	Enterobacteriaceae, *P. aeruginosa*, *Staphylococcus* spp., *Streptococcus* spp.	Enrofloxacin	≤ 0.5	1-2	≥ 4
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Marbofloxacin	≤ 1	2	≥ 4
	Enterobacteriaceae, *Staphylococcus* spp., *Streptococcus* spp.	Orbifloxacin	≤ 1	2-4	≥ 8
	E. coli, *S. aureus, S. pseudintermedius, S. felis*	Pradofloxacin	≤ 0.25	0.5-1	≥ 2
	P. multocida, *S. canis*	Pradofloxacin	≤ 0.25	—	—

* S (susceptible), I (intermediate), R (resistant)

Table 2. Examples of resistance to antimicrobials by enzymatic inactivation (modified from ref. 1)

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene
chemical modification	*aac*, *aad (ant)*, *aph*	acetyl-, adenyl-, phosphotransferases	aminoglycosides	various Gram+, Gram–, aerobic bacteria	T, GC, P, C
	aad (ant)	adenyltransferases	aminocyclitols	various Gram+, Gram–, aerobic bacteria	T, GC, P, C
	catA, *catB*	acetyltransferases	chloramphenicol	various Gram+, Gram–, aerobic, anaerobic bacteria	P, T, GC, C
	vat(A-E)	acetyltransferases	streptogramin A	*Staphylococcus, Enterococcus*	P, C
	mph(A-E)	phosphotransferases	macrolides	*Escherichia, Shigella, Staphylococcus*	P, T, C
	lnu(A), *lnu*(B)	nucleotidyltransferases	lincosamides	*Staphylococcus*	P
	tet(X), *tet*(37)	oxidoreductases	tetracyclines	*Bacteroides*	T, P
hydrolytic cleavage	*blaZ, bla*$_{TEM}$*, bla*$_{SHV}$*, bla*$_{CTX-M}$*, etc.*	β-lactamases	β-lactam antibiotics	various Gram+, Gram–, aerobic, anaerobic bacteria	P, T, GC, C
	ere(A), *ere*(B)	esterase	macrolides	*E. coli*, *Staphylococcus*	P, GC

	vgb(A), *vgb*(B)	lactone hydrolases	streptogramin B	*Staphylococcus*	P

[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)

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene
efflux via multidrug transporters	*mexA-mexB-oprM*, *acrA-acrB-tolC*	multidrug efflux in combination with specific OMP's	chloramphenicol, β-lactams, macrolides, fluoroquinolones, tetracyclines, etc.	*Pseudomonas*, *E. coli*, *Salmonella*	C
	emrE	4-TMS multidrug efflux protein	tetracyclines, nucleic acid binding compounds	*E. coli*	C
	blt, *norA*	12-TMS multidrug efflux protein of the major facilitator superfamily	chloramphenicol, fluoroquinolones, nucleic acid binding compounds	*Bacillus, Staphylococcus*	C
efflux via specific exporters	*tet*(A-E, G, H, I, J, K, L, Z), *tetA*(P), *tet*(30)	12-, 14-TMS efflux system of the major facilitator superfamily	tetracyclines	various Gram+ and Gram– bacteria	P, T, C
	floR	12 TMS efflux system of the major facilitator superfamily	phenicols	various Gram– bacteria	T, P, C
	cmlA, *cmlB*	12 TMS efflux system of the major facilitator superfamily	chloramphenicol	various Gram– bacteria	T, P, GC, C

fexA	14 TMS efflux system of the major facilitator superfamily	phenicols	*Staphylococcus*	T, P, C
mef(A)	efflux system of the major facilitator superfamily	14-, 15-membered macrolides	*Streptococcus*, other Gram+ bacteria	T, P, C
msr(A)	efflux system of the ABC transporter family	macrolides and streptogramin B	*Staphylococcus*	P
vga(A), *vga*(C), *vga*(E), *lsa*(E), *sal*(A)	efflux system of the ABC transporter family	streptogramin A, lin	*Staphylococcus*, *Enterococcus*	P
optrA	efflux system of the ABC transporter family	phenicols, linezolid, tedizolid	*Enterococcus, Staphylococcus*	P, C

[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)

Resistance mechanism	Resistance gene(s)	Gene product	Resistance phenotype	Bacteria involved	Location of the resistance gene
methylation of the target site	*erm*(A-46)	rRNA methylase	macrolides, lincosamides, streptogramin B	various Gram+ and Gram– bacteria	P, T, C
methylation of the target site	*cfr, cfrB*	rRNA methylase	phenicols, lincosamides, linezolid, pleuromutilins, streptogramin A	various Gram+ and Gram– bacteria	P, C
protection of the target site	*tet*(M, O, P, Q, S, T)	ribosome protective proteins	tetracyclines	various Gram+ and Gram– bacteria	T, P, C
	fusB	ribosome protective protein	fusidic acid	*Staphylococcus*	P
replacement of a sensitive target by an alternative drug-resistant target	*mecA, mecC*	penicillin-binding proteins with altered substrate specificity	penicillins, cephalosporins, carbapenems, monobactams	*Staphylococcus*	C
	sul1, sul2, sul3	sulfonamide-insensitive dihydropteroate synthase	sulfonamides	various Gram– bacteria	P, I
	dfrA, dfrB	trimethoprim-insensitive dihydrofolate reductase	trimethoprim	various Gram+ and Gram– bacteria	P, GC, T, C

	mupA, ileS2	mupirocin-insensitive isoleucyl-tRNA synthase	mupirocin	*Staphylococcus*	P
	vanA-E	alternative peptide-glycan precursors	glycopeptides	*Enterococcus*, *Staphylococcus*	T, P, C
mutational modification of the target site	—	mutations in the genes for topoisomerase II and IV	fluoroquinolones	various Gram+ and Gram– bacteria	C
	—	mutation in the gene coding for ribosomal protein S12	streptomycin	several Gram+ and Gram– bacteria	C
	—	mutation in the gene for the ribosomal protein L3	tiamulin	*E. coli*	C
	—	mutation in the 16S rRNA	tetracyclines	*Propionibacterium*	C
	—	mutations in the 23S rRNA	oxazolidinones	*Staphylococcus*	C
	—	mutation in the fusA gene	fusidic acid	*Staphylococcus*	C
mutational modification of regulatory elements	—	mutations in the *marRAB soxR* or *acrR* genes	fluoroquinolones	*E. coli*	C

[a] 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.

Ziele – Dieser Artikel vermittelt eine aktuelle Übersicht über erworbene Resistenzmechanismen von Bakterien, die an Hautinfektionen von Tieren beteiligt sind. Zusätzlich enthält er Beispiele für den Transfer resistenter Infektionserreger zwischen verschiedenen Wirten und für den Transfer von Resistenzgenen zwischen Bakterien von Tieren und Menschen.

Ergebnisse – Erworbene Resistenz basiert auf resistenzvermittelnden Mutationen oder mobilen Resistenzgenen. Während Mutationen vertikal weitergegeben werden, erfolgt der Transfer mobiler Resistenzgene auch horizontal (mittels Transformation, Transduktion oder Konjugation/Mobilisierung) und trägt dadurch zur Verbreitung antimikrobieller Resistenzen bei. Bisher wurden mobile Resistenzgene, die einen der drei Resistenzmechanismen – enzymatische Inaktivierung, reduzierte intrazelluläre Akkumulation oder Modifizierung der zellulären Angriffsstellen – vermitteln bei einer Vielzahl von Bakterien nachgewiesen. Solche Resistenzgene liegen als Bestandteil von Plasmiden, Transposons, Genkassetten, integrativen und konjugativen Elementen oder anderer mobiler Elemente vor. Bakterien, einschließlich zoonotischer Infektionserreger, können zwischen Tieren und Menschen hauptsächlich durch direkten Kontakt, aber auch über Staub und Aerosole sowie Lebensmittel ausgetauscht werden. Der Nachweis der Transferrichtung von resistenten Bakterien kann sich schwierig gestalten und hängt von der Lokalisation der Resistenzgene oder Mutationen in der chromosomalen DNA oder auf mobilen Elementen ab.

Schlussfolgerungen – Die große Vielfalt an Resistenz- und Transfermechanismen wird auch in Zukunft den Erfolg bakterieller Infektionserreger sichern. Unsere Strategien der Resistenzentwicklung entgegen zu wirken und die Wirksamkeit antimikrobieller Wirkstoffe zu erhalten muss ähnlich vielfältig und erfindungsreich sein.