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Schwarz, S., Loeffler, A. and Kadlec, K. (2017), Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. Vet Dermatol, 28: 82–e19. doi:10.1111/vde.12362

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The full details of the published version of the article are as follows:

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

AUTHORS: Stefan Schwarz, Anette Loeffler, Kristina Kadlec

JOURNAL TITLE: Veterinary Dermatology

PUBLISHER: Wiley

PUBLICATION DATE: February 2017

DOI: 10.1111/vde.12362

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

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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

39 **Abstract**
40
41 **Background –** Antimicrobial resistance has become a major challenge in veterinary
42 medicine, particularly in the context of bacterial pathogens that play a role in humans
43 and animals.
44
45 **Objectives –** This review serves as an update on acquired resistance mechanisms
46 in bacterial pathogens of human and animal origin, including examples of transfer of
47 resistant pathogens between hosts and of resistance genes between bacteria.
48
49 **Results –** Acquired resistance is based on resistance-mediating mutations or on
50 mobile resistance genes. While mutations are transferred vertically, mobile resistance
51 genes are transferred also horizontally (by transformation, transduction or
52 conjugation/mobilization), contributing to the dissemination of resistance. Mobile
53 genes specifying any of the three major resistance mechanisms – enzymatic
54 inactivation, reduced intracellular accumulation or modification of the cellular target
55 sites – have been found in a variety of bacteria from animals. Such resistance genes
56 are associated with plasmids, transposons, gene cassettes, integrative and
57 conjugative elements or other mobile elements. Bacteria, including zoonotic
58 pathogens, can be exchanged between animals and humans mainly via direct
59 contact, but also via dust and aerosols or via the food chain. Proof of the direction of
60 transfer of resistant bacteria can be difficult and depends on the location of
61 resistance genes or mutation in the chromosomal DNA or on a mobile element.
62
63 **Conclusion –** The wide variety in resistance and resistance transfer mechanisms will
64 continue to ensure the success of bacterial pathogens in the future. Our strategies to
65 counteract resistance and preserve efficacy of antimicrobial agents needs to be equally
66 diverse and resourceful.
67

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

119 production of species-specific inactivating enzymes in certain bacteria,[1] such as the
120 AcrAB-TolC system and the production of AmpC β-lactamase in Escherichia coli. In
121 addition, some bacteria, such as enterococci, are not dependent on a functional
122 folate synthesis pathway, but instead can use exogenous folates. As a consequence,
123 they are intrinsically resistant to folate pathway inhibitors, such as trimethoprim and
124 sulfonamides.[9] In contrast, acquired resistance is a strain-specific property which can
125 be based on a wide variety of resistance mechanisms present in the different
126 bacteria.[1] Such acquired resistance mechanisms can be due to mutations of cellular
127 genes or to the acquisition of novel/foreign genes, commonly referred to as
128 resistance genes. The following basic considerations are important in the context of
129 acquired resistance genes:
130
131 1. Acquired resistance genes can confer resistance to an entire class of
132 antimicrobial agents or can be specific for only a single member of an
133 antimicrobial class.
134 2. Certain acquired resistance genes can confer resistance to members of
135 different classes of antimicrobial agents.
136 3. Acquired resistance to a specific class of antimicrobial agents can be due to
137 several different resistance mechanisms.
138 4. The same acquired resistance mechanism can be encoded by different genes.
139 5. Different acquired resistance mechanisms and resistance genes can be
140 present at the same time.
141 6. Definitions of multidrug-resistance vary but a bacterium is typically referred to
142 as multidrug-resistant if it shows acquired resistance to members of at least
143 three classes of antimicrobial agents.
144
145 **Resistance mechanisms and associated resistance genes**
146
147 Acquired resistance mechanisms can be divided into one of the three major
148 categories: (i) enzymatic modification or inactivation of antimicrobial agents, (ii)
149 reduced intracellular accumulation of antimicrobial agents or (iii) alterations at the
150 target sites of the antimicrobial agents.[1, 2]
151
152 **Enzymatic inactivation** of antimicrobial agents is widespread among Gram-
153 positive and Gram-negative bacteria (Table 2). In the case of enzymatic modification,
154 bacteria produce enzymes that chemically modify the drug molecule by the
155 attachment of acetyl, adenyl or phosphate groups to specific sites of the antimicrobial
156 molecule. Such modified antimicrobial molecules can no longer bind to their target
157 site and consequently cannot maintain antimicrobial activity. This mechanism is
158 commonly used for the enzymatic inactivation of nonfluorinated phenicols, such as
159 chloramphenicol, by acetylation,[10] or of aminoglycosides by acetylation, adenylation
160 or phosphorylation.[11] Other enzymatic inactivation processes include the
161 phosphorylation of macrolides, nucleotidylation of lincosamides, and acetylation of
162 streptogramin A antibiotics.
163 In the case of enzymatic inactivation, bacteria produce enzymes that bind
164 directly to the antimicrobial molecule and disintegrate it. This is commonly done by
165 hydrolytic cleavage of specific bonds within the antimicrobial molecule. Such cleaved
166 antimicrobial molecules also do not exhibit antimicrobial activity. Examples of this
167 mode of enzymatic inactivation are the β-lactamases, which occur in Gram-positive
168 and Gram-negative bacteria and, depending on the type of β-lactamase, may exhibit
169 a more or less expanded substrate spectrum that can include penicillins,

170 cephalosporins, monobactams and/or even carbapenems.[12, 13] Other examples are
171 esterases which confer macrolide resistance or lactone hydrolases which inactivate
172 streptogramin B compounds.[14]
173
174 **Reduced intracellular accumulation** of antimicrobial agents can be achieved
175 in two ways: reduced influx or enhanced efflux (Table 3). It is known that certain
176 outer membrane proteins (OMPs), so-called porins, represent an entry point for
177 antimicrobial agents to enter the bacterial cell. As such, OmpF is involved in the
178 uptake of tetracyclines, β-lactams and chloramphenicol in E. coli, whereas OmpD is
179 involved in the uptake of carbapenems in Pseudomonas aeruginosa.[1] Reduced influx
180 of antimicrobial agents is usually the consequence of downregulation, structural
181 modification or even functional deletion of the genes coding for these porins. In such
182 cases, the outer membrane of Gram-negative bacteria can represent a permeability
183 barrier for antimicrobial agents.
184 By contrast, increased efflux describes a way by which incoming antimicrobial
185 agents are actively pumped out of the bacterial cell. This can be achieved by
186 multidrug transporters or specific transporters.[1, 2] Multidrug transporters are present
187 in virtually every bacterium and are mainly responsible for the transport of toxic
188 substances from the cell metabolism. However, studies have shown that some
189 multidrug transporters can also export antimicrobial agents. Most of them belong to
190 the resistance-nodulation-cell division (RND) family. RND transporters mainly occur
191 in Gram-negative bacteria and are composed of a cytoplasmatic and a periplasmatic
192 component which can interact with different outer membrane components. Examples
193 are AcrAB-TolC transporter in E. coli and Salmonella enterica or the MexAB-OprM
194 transporter in P. aeruginosa which can export chloramphenicol, fluoroquinolones,
195 tetracyclines, β-lactams and macrolides among others.[1, 15] It should be noted that
196 multidrug-transporters increase the MICs for their substrates, but not necessarily to a
197 level that correlates with clinical resistance.
198 Specific transporters involved in antimicrobial resistance commonly belong to
199 the following families: (i) major facilitator superfamily (MFS), (ii) ATP-binding cassette
200 (ABC) family or (iii) multidrug and toxic-compound extrusion (MATE) family.[15, 16] MFS
201 transporters often consist of 12–14 transmembrane segments, exchange a drug
202 molecule against a proton and use the proton-motive force of the membrane as an
203 energy source for the translocation. Examples of MFS transporters are the
204 tetracycline transporters Tet(K) and Tet(L) in Gram-positive bacteria and Tet (A-E, G,
205 H) in Gram-negative bacteria as well as the phenicol transporters FexA in Gram-
206 positive bacteria and FloR, CmlA and CmlB in Gram-negative bacteria.[17, 18] ABC
207 transporters use the energy of ATP hydrolysis for the translocation of substrates
208 across biological membranes. They represent a highly diverse class of transporters
209 which are not only involved in antimicrobial resistance, but also in the uptake of
210 nutrients and the secretion of proteins among other functions.[19] ABC transporters
211 involved in antimicrobial resistance are seen mainly in staphylococci and enterococci.
212 Examples are the transporters Vga(A), Vga(C), Vga(E), Lsa(E) and Sal(A) conferring
213 combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics
214 or Msr(A) involved in resistance to macrolides and streptogramin B antibiotics.[20, 21]
215 MATE proteins are also located in the cytoplasmatic membrane and act in a similar
216 way to MFS transporters. However, in contrast to MFS proteins, they are rarely
217 involved in antimicrobial resistance. Examples of MATE proteins that export
218 antimicrobial agents are NorM (hydrophilic fluoroquinolones) from Vibrio
219 parahaemolyticus and MepA (glycylcyclines) from Staphylococcus aureus.[15, 16]
220

221 **Alterations at the target sites** of the antimicrobial agents represent the third
222 and most variable group of resistance mechanisms (Table 4). These include
223 mutational and chemical modifications, protection of the target sites, the replacement
224 of sensitive targets by functionally analogous but insensitive ones, and
225 overproduction of sensitive targets.[22]
226 Mutational alterations of the target sites are best known for (fluoro)quinolone
227 resistance in various Gram-positive and Gram-negative bacteria. Within the genes for
228 DNA gyrase (topoisomerase II and topoisomerase IV), a specific region known as the
229 quinolone-resistance determining region (QRDR) has been defined where mutations
230 accounting for (fluoro)quinolone resistance are located. Resistance to
231 (fluoro)quinolones usually occurs in a step-wise manner by which the MIC is
232 increased with each additional mutation.[23, 24] Such a step-wise increase in resistance
233 illustrates well the advantage of using mutant prevention concentrations (MPCs) as a
234 measure for antimicrobial potency rather than MICs.[25] Because two mutations are
235 required for full (fluoro)quinolone resistance to occur, and with mutations occurring
236 randomly, the likelihood that bacteria with double mutations will persist after
237 treatment is low and measurable only in a large population of cells (i.e. in large
238 numbers of colony forming units in the laboratory). To date, MPC measurement has
239 not been applied routinely in clinical microbiology laboratories, possibly hampered by
240 practical constraints.[26]
241 Mutations in the gene fusA, which encodes the elongation factor G (EF-G),
242 have been found to account for resistance to fusidic acid in S. aureus as well as in
243 meticillin-susceptible (MSSP) and meticillin-resistant Staphylococcus
244 pseudintermedius (MRSP).[27, 28] Mutations in 16S ribosomal RNA (rRNA) have been
245 described to account for resistance to streptomycin in Mycobacterium tuberculosis, to
246 tetracyclines in Propionibacterium acnes and to spectinomycin resistance in
247 Pasteurella multocida.[1, 29] Mutations in 23S rRNA are known to cause macrolide
248 resistance in various bacteria including Mycobacterium spp., Brachyspira
249 hyodysenteriae, Campylobacter coli, Campylobacter jejuni, Haemophilus influenzae
250 and Streptococcus spp. among others.[1] In addition, mutations in the genes for
251 specific ribosomal proteins are associated with resistance to streptomycin and
252 spectinomycin.[1, 29] Mutations in the gene rpoB, which codes for the β-subunit of the
253 enzyme RNA polymerase, have been described recently to cause high-level
254 rifampicin resistance in Rhodococcus equi and in MRSP.[30, 31]
255 Chemical modification of the target site by methylation has proved to be an
256 effective way to confer combined resistance to macrolides, lincosamides and
257 streptogramin B antimicrobial agents. The corresponding Erm methylases, which
258 target the adenine residue at position 2058 in 23S rRNA, are widely distributed
259 among Gram-positive and Gram-negative bacteria.[32] To date, 46 different Erm
260 methylases have been differentiated.[33] Methylation of the adenine residue at position
261 2503, which is located in the overlapping binding region of phenicols, lincosamides,
262 oxazolidinones, pleuromutilins and streptogramin A antibiotics, results in resistance
263 to these five classes of antimicrobial agents.[34] The corresponding methylase gene,
264 cfr, has been detected in various Staphylococcus spp., Enterococcus spp., Bacillus
265 spp., Macrococcus caseolyticus, Jeotgalicoccus pinnepedialis, Streptococcus suis, E.
266 coli and Proteus vulgaris.[20, 35] Recently, the gene cfrB, which confers the same
267 resistance phenotype but is <80% identical to cfr, has been detected in Enterococcus
268 spp. and Clostridium difficile isolates.[17, 33]
269 Protection of the ribosomal target site has been noted in tetracycline resistance.
270 So far, 12 ribosome protective proteins are known which show similarities to
271 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

323 element harbours a tra gene complex which specifies the transfer apparatus. If a
324 conjugative element provides its transfer apparatus to nonconjugative elements,
325 mainly plasmids that co-reside in the same donor cell, such nonconjugative plasmids
326 can move over to the recipient cell. This process is referred to as mobilization.
327 Conjugation and mobilization of various mobile genetic elements are believed to play
328 key roles in the dissemination of antimicrobial resistance in bacteria.[1, 2, 55]
329 Furthermore, dissemination is thought to be particularly efficient amongst bacteria of
330 the same species or clonal lineage. Barriers to HGT gene transfer, which protect
331 bacteria against "foreign" DNA from other bacterial species or lineages, have been
332 identified and are now widely described in many bacterial species.[56] Barrier systems
333 described in staphylococci, including S. pseudintermedius, include restriction-
334 modification systems, competence genes and Clustered Regularly Interspaced Short
335 Palindromic Repeats (CRISPR) systems, and these have been linked to the
336 successful spread of certain lineages and their ability to protect themselves from
337 foreign DNA.[57] However, their role in preventing acquisition of resistance genes, at
338 least in S. pseudintermedius, is questionable based on finding them distributed
339 randomly amongst multidrug-resistant and -susceptible isolates.[28]
340
341 There are several mobile genetic elements (MGEs) which can harbour antimicrobial
342 resistance genes and which are essential to horizontal gene transfer. All of them are
343 double-stranded DNA molecules. Plasmids are the most abundant MGEs. They can
344 vary distinctly in their sizes between < 2 kb and > 200 kb. Plasmids replicate
345 autonomously and independently from the chromosomal DNA. They can carry
346 antimicrobial resistance genes, heavy metal resistance genes, virulence genes and
347 genes for a number of other properties, including metabolic functions. Plasmids can
348 harbour transposons and gene cassettes/integrons.
349
350 Transposons differ distinctly in size and structure. In contrast to plasmids, they are
351 replication-deficient and as such must integrate for their replication either into
352 plasmids or the chromosomal DNA. They move by transposition, either into specific
353 sites or into various sites in plasmids or in the chromosomal DNA. The importance of
354 large transposons in the emergence of the extremely drug resistant phenotypes was
355 recently highlighted by the identification of a Tn5405-like element carrying up to five
356 antimicrobial resistance genes in all of 11 fully sequenced multidrug-resistant MRSP
357 isolates of four different lineages.[28]
358
359 Gene cassettes are the smallest MGEs which commonly carry only one gene, mostly
360 an antimicrobial resistance gene, and a recombination site, known as the 59-base
361 element. They can neither replicate nor transpose. They move by site-specific
362 recombination and are commonly found in integrons. The integrase of the integron
363 catalyses the integration and excision of the gene cassette using the 59-base
364 element. As gene cassettes usually do not have an own promoter, the cassette-
365 borne gene is transcribed from a promoter in the 5′-conserved region of the integron.
366 Gene cassettes are rarely found at secondary sites outside of an integron.[1, 2, 55]
367
368 Integrative and conjugative elements (ICEs) are large elements of >20 kb which
369 integrate site-specifically into the chromosomal DNA. They can excise from the
370 chromosomal DNA, form a circular intermediate and transfer themselves via a
371 replicative cycle into new host cells where they integrate again into the chromosomal
372 DNA. In terms of antimicrobial multidrug-resistance, the SXT element of Vibrio
373 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,

425 respectively.[65] Depending on the virulence of the resistant bacteria, they may cause
426 clinical diseases with limited treatment options. Transfer of bacteria – including
427 resistant strains – can be exchanged between humans and animals in both directions
428 by either contact, inhalation of dust and aerosols that contain bacteria, or via the food
429 chain.[65]
430 Direct contact is likely the quickest and easiest way by which bacteria are
431 transferred in either direction between humans and animals, particularly for those
432 such as staphylococci which reside on body surfaces. Anyone who shares close
433 contact with pets or companion animals may be affected.[66] In this regard, it is
434 important to consider the current role of dogs and cats as actual family members in
435 many households in industrialized countries. A study published in 2014 revealed the
436 presence of approximately 11.5 million cats, 6.9 million dogs, 6.1 million other pet
437 animals (e.g. rabbits, guinea pigs, hamsters) and 3.4 million pet birds in German
438 households.[67] Pet owners often have extensive contact with their pets, especially to
439 cats and dogs which may be allowed lick their owners' faces and hands or to sleep in
440 their owners' beds.[67, 68] Based on this close contact, a transfer of bacteria between
441 pets and people is unavoidable and not surprising.[66, 69-72] As "family members", cats,
442 dogs and other pet animals often enjoy not only an extensive support in terms of food
443 supply and housing, but also broad medical care. In Germany, pet owners spent
444 almost €4.8 billion for pet supplies in 2013, of which €3.75 billion accounted for pet
445 food and €1.05 billion for equipment.[67] For medical care of their pets, Germans spent
446 approximately €2.1 billion in 2013.[67] These data clearly show that pet owners have
447 considerable interest in maintaining the health of their pets. As many infectious
448 diseases in cats and dogs are caused by bacteria, particularly those infecting the skin
449 of dogs,[73] this also involves the application of antimicrobial agents. A wide range of
450 antimicrobial agents has been licensed for use in cats and dogs. In addition,
451 antimicrobial agents approved for use in human medicine may also be applied to
452 nonfood-producing animals under the Animal Medicinal Drug Use Clarification Act
453 (AMDUCA) in the USA or similar regulations in other countries.[74] Although such
454 applications should be kept to a minimum, it means that antimicrobial agents of last
455 resort in human medicine, such as carbapenems, glycopeptides, oxazolidinones or
456 lipopeptides, may be used in small animal medicine. However, no data are available
457 to allow quantification of the use of these last resort agents for cats and dogs.
458
459 **Animal transmission to companion animal owners**
460 There have been numerous examples of the transfer of resistant bacteria,
461 especially staphylococci and E. coli, between pets and people, beginning with the
462 landmark report of the possible zoonotic spread of MRSA by a cat to hospitalized
463 people.[75] Reports of interspecies transmission of MRSA include: livestock-associated
464 (LA-) MRSA ST398-t034 transferred from a colonized veterinarian to his dog,[70]
465 healthcare-associated MRSA ST225-t014 transferred from a family member (who
466 suffered from an infected decubitus ulcer) to the family dog,[70] MRSA ST80-t131
467 isolated from a woman who suffered from multiple recurrent skin abscesses and her
468 husband, children and a cat living in the same household (where the patient's
469 disease resolved completely after topical decolonization of all family members
470 including the MRSA-positive cat),[76] and the likely horse-to-human transmission of a
471 LA-MRSA ST398-t011.[77] MRSA colonization of persons in contact with infected or
472 colonized horses has been reported from the investigation of several outbreaks.[78]
473 Aside from MRSA, indistinguishable isolates of S. pseudintermedius ST33 have been
474 reported from a dog and its owner.[69]
475

476 Typically, such reports are based on evidence from genetic typing studies which
477 identify indistinguishable isolates from animals and in-contact humans. However, the
478 direction of inter-host transmission can rarely be proven definitively, but rather, is
479 often deduced from epidemiological characteristics. Even an MRSA outbreak
480 investigation in a small animal hospital using whole genome sequencing of multiple
481 isolates from each sample had to conclude that directions of transmission could only
482 be suspected.[79] For MRSA isolated from dogs and cats, for example, a
483 predominantly human-to-animal direction of transmission is assumed because most
484 isolates belong to MRSA clonal lineages that are also prevalent in human healthcare
485 facilities and thus likely represent a “spill-over” to pets.[69, 70, 80, 81]
486
487 Evidence for transmission of Gram-negative pathogens between animals and
488 humans is only just beginning to emerge, but already includes some highly drug-
489 resistant nosocomial pathogens, such as E. coli ST410 and other multidrug-resistant
490 Extended Spectrum Beta Lactamase-producing (ESBL) E. coli.[82-84] Escherichia coli
491 isolates, which belonged to the same phylogenetic group (B2 or D) and exhibited the
492 same Amplified Fragment Length Polymorphism patterns, were detected among
493 family members and their dogs.[68]
494
495 **People with occupational contact to animals**
496 In addition to pet and companion animal owners, people who have occupational
497 contact with animals also are at risk for acquisition of bacteria from animals. Notably,
498 these include veterinarians, but also veterinary students, farmers, abattoir workers
499 and other animal caretakers. These people often work in an environment where they
500 care for sick animals and in which antimicrobial agents are applied. Besides direct
501 contact with animals, dust and aerosols, especially on farms and in abattoirs, may
502 also play a role as vehicles that transport resistant bacteria and are inhaled by
503 animals and humans.
504
505 There are a number of published reports which suggest occupational
506 transmission in various settings. In a small animal clinic, multidrug-resistant
507 Staphylococcus epidermidis ST5 was shown to be present at various locations in the
508 stationary area and the quarantine ward, as well as in feline patients and in the nose
509 of one veterinary nurse.[85] A study from Australia revealed that veterinarians often
510 carry multidrug-resistant MRSA isolates.[86] A study conducted in Germany showed
511 that 97 (85.8%) of 113 swine farmers but only five (4.3%) of their 116 family
512 members were positive for LA-MRSA.[87] Likewise, 22 (44.9%) of 49 swine
513 veterinarians but only four (9.1%) of their 44 family members were positive for LA-
514 MRSA in another report.[87] These observations suggest that the human-to-human
515 transfer of LA-MRSA occurs distinctly more rarely than the animal-to-human transfer.
516 A study involving 26 dairy farms in the Netherlands revealed that the same LA-MRSA
517 types, based on pulsed-field gel electrophoresis (PFGE) type, spa type and
518 resistance patterns, were detected not only among dairy cattle and their contact
519 personnel (e.g. milkers), but occasionally also among other animals living on the
520 same farm.[88]
521
522 LA-MRSA isolates with the molecular characteristics ST398-t011-dt11a and
523 ST9-t1430-dt10a, both with very similar PFGE patterns and resistance phenotypes,
524 were detected among poultry and workers in a Dutch poultry abattoir.[89] The analysis
525 of turkey flocks and their carers revealed that almost 60% of the farm personnel were
526 colonized by LA-MRSA that exhibited the same spa type and SCCmec type as the

527 turkeys.[90] A study on the transmission of LA-MRSA on broiler farms in the
528 Netherlands revealed the presence of MRSA ST398-t034-dt10q with
529 indistinguishable PFGE and resistance patterns among the broilers, dust samples
530 from the broiler house and the farmer.[91] The emission of bacteria from pig fattening
531 and broiler chicken farms to the surrounding area was confirmed by the detection of
532 ESBL-/AmpC-producing E. coli in air samples from inside as well as outside the farm
533 buildings.[92, 93] Another study showed that food animal transport in open crates
534 resulted in the dissemination of bacteria, including resistant enterococci, into the
535 environment.[94] In addition, indirect transmission via insects or rats can occur on
536 farms.[95, 96]
537
538 **Transmission via the food chain**
539
540 Transfer of resistant bacteria via the food chain usually occurs by ingestion of
541 raw or insufficiently heated, contaminated food. In this regard, it is worth noting that
542 (i) the number of ingested bacteria must be sufficiently high to survive the passage
543 through the acidic environment in the stomach, which varies according to the type of
544 foodborne pathogen and (ii) the virulence of most food-borne pathogens is more
545 relevant than their antimicrobial resistance due to the fact that antimicrobial agents
546 are not recommended for use in uncomplicated self-limiting cases of intestinal
547 infections.[97] However, when resistant bacteria are ingested, they may transfer
548 antimicrobial resistance genes to members of the intestinal microbiota of the host.
549 Unfortunately, there are little if no data which provide reliable information about the
550 extent at which bacteria transfer their resistance genes during transient colonization
551 of a new host.
552
553 **Proof of transfer of resistant bacteria and resistance genes**
554
555 In view of the many opportunities for exchange of resistant bacteria and resistance
556 genes amongst human and animal hosts and the respective selection pressures, a
557 key question is: what proportion of resistance problems in human medicine is caused
558 by bacteria of animal origin? One study has assessed the impact of antimicrobial
559 resistance in different bacterial species and of the contribution of animal sources to
560 resistance in human infections.[98] Based on the results of a questionnaire sent to
561 recognized experts in the UK and elsewhere, the authors concluded that bacteria
562 from animal sources, mainly nontyphoid Salmonella enterica serovars, E. coli O157,
563 Campylobacter spp. and vancomycin-resistant enterococci, might account for 3.88%
564 of the human antibiotic resistance problem.[98] It should be noted that this survey was
565 conducted at a time when LA-MRSA and ESBL-producing E. coli were not yet
566 recognized as emerging zoonotic problems.[99] Nevertheless, this survey suggested
567 strongly that most of the resistance problems encountered in human medicine as well
568 as in veterinary medicine are self-made problems in either sector. Only a minority
569 results from the transfer of zoonotic bacteria.
570
571 A study on zoonotic MRSA colonization and infection in Germany showed that
572 zoonotic transmission of LA-MRSA CC398 from livestock to humans occurs
573 predominantly in people with occupational livestock contact, whereas dissemination
574 in the general population is limited so far.[100] LA-MRSA CC398 currently causes about
575 2% of all human MRSA infections in Germany, but up to 10% in regions
576 characterized by a high density of livestock farming.[100] Likewise, a study investigating
577 629 ESBL-producing E. coli from people in the Netherlands, Germany and UK, which

578 were collected during the years 2005-2009 and examined by DNA microarray and
579 multi-locus sequence typing (MLST), showed that the majority of the human isolates
580 differed distinctly from isolates of animal origin due to diversity in virulence and
581 antimicrobial resistance genes.[101] It was concluded that attempts to minimize the
582 human-to-human transfer of ESBL-producing E. coli are essential to limit the
583 dissemination of these bacteria among humans. ESBL-producing E. coli from
584 animals may play a role as a reservoir of virulence and antimicrobial resistance
585 genes rather than directly causing infections in humans.[101]
586
587 The methodological attempts to prove the transfer of resistant bacteria or resistance
588 genes strongly depend on the location of the resistance gene. For bacteria such as
589 MRSA, where the meticillin resistance genes mecA or mecC are located on a
590 chromosomally integrated SCCmec cassette, molecular strain typing methods can be
591 applied. These include pattern-based techniques, such as PFGE, or sequence-based
592 methods such as MLST, single locus sequence typing via spa and dru typing, as well
593 as multiple loci VNTR analysis (MLVA).[102, 103] In addition, the presence of the
594 relevant resistance genes can be detected by PCR. Whole-genome sequencing with
595 subsequent SNP analysis can also be used as the ultimate proof.[81, 104] The results of
596 these methods can enable definite proof of clonality and transference of resistance
597 genes.
598
599 If a resistance gene is located on a MGE (e.g. plasmid-borne ESBL genes in E. coli)
600 strain typing methods like PFGE, MLST or PCR-directed typing methods can still be
601 applied. In addition, it is necessary also to characterize the resistance plasmid in
602 question (e.g. by pMLST, replicon typing, restriction analysis or even whole plasmid
603 sequencing).[105] In the transfer of resistance plasmids, different scenarios are
604 conceivable. Scenario 1 describes a situation where the transferred strain and its
605 resistance plasmid multiply stably in the new host. In such a case, the
606 aforementioned methods enable the verification of the transferred strain and the
607 resistance plasmid.[106] In scenario 2, the transferred strain cannot replicate in the new
608 host, but transfers its resistance plasmid to bacteria of the new host. In this case, the
609 transferred strain is not detectable any more, but the resistance plasmid may be
610 detected in the new host bacteria. Scenario 3 describes a situation in which the
611 transferred strain cannot replicate in the new host and the transferred plasmid cannot
612 replicate in the new host bacteria but undergoes recombination with plasmids already
613 residing in these new host bacteria. In this case, neither the original bacterial strain
614 nor the original plasmid are detectable and the confirmation of transfer is not
615 possible.
616
617 Another problem is the confirmation of the direction of transfer. In staphylococci, for
618 instance, structurally closely related small mobilizable plasmids that carry the
619 tetracycline resistance gene tet(K), the chloramphenicol resistance gene catpC221 or
620 the MLSB resistance gene erm(C) are prevalent in various staphylococcal species
621 from both humans and animals.[107-109] Because tetracyclines, chloramphenicol and
622 macrolides have been used in human and veterinary medicine for more than 60
623 years, it is impossible to determine in retrospect where and when these resistance
624 genes first developed and which transfer events across species and host boundaries
625 have taken place since then. In contrast, the recently identified phenicol and
626 oxazolidinone resistance gene optrA is likely to have developed in enterococci of
627 animal origin in China under the selective pressure imposed by the use of florfenicol
628 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]

680
681 The search for new antimicrobial agents – natural and synthetic – should be stimulated
682 by making the development of new agents more attractive to the pharmaceutical
683 industry (e.g. by expanding the time of patent protection or lowering the administrative
684 hurdles in the approval process). Public–private partnerships, which take the
685 development of new antimicrobial agents forward, should be encouraged. As
686 mentioned for florfenicol, more efforts also should be made to develop chemical
687 modifications which provide antimicrobial derivatives that evade known resistance
688 mechanisms.
689
690 Revival of "old" antimicrobial agents, including those discarded, not fully developed or
691 even rejected, should be re-investigated. Combinations of antimicrobial agents with an
692 inhibitor (e.g. an efflux inhibitor) should be explored for their ability to restore the activity
693 of old antimicrobial agents.[120]
694
695 Control of the use of antimicrobial agents: As the selective pressure imposed by the
696 use of antimicrobial agents is a major driving force in the development of antimicrobial
697 resistance, the nontherapeutic use of antimicrobial agents, for example, as growth
698 promoters, must be discontinued worldwide. Antimicrobial agents in humans and
699 animals should be made available by prescription only. Over-the-counter sales of
700 antimicrobial agents should be forbidden worldwide. Monitoring of the consumption of
701 antimicrobial agents in both human and veterinary medicine, including antimicrobial
702 use in small animal practice, should be implemented.
703
704 Alternatives to antimicrobial agents: Novel nonantibiotic approaches for prevention of
705 and protection against infectious diseases should be explored.[121] These include the
706 development of vaccines (especially for animal diseases), phage therapy[122, 123] and
707 phage lysin therapy,[124-126] adjuvants, antivirulence therapies (including synthetic
708 polypeptides that neutralize bacterial pathogenicity factors),[127] pre- and probiotics,
709 immunostimulants, antimicrobial peptides (such as cathelicidins, defensins and
710 dermicins),[128, 129] anti-biofilm therapies[130-132] and reprogrammed nucleases that target
711 antimicrobial resistance genes.[133]
712

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1102 **Figure 1.** Schematic presentation of the dissemination of resistant bacteria and
1103 resistance genes among different hosts with particular reference to the exchange
1104 between humans and animals. The thickness of the different arrows shall indicate the
1105 likelihood of the various transfer ways.
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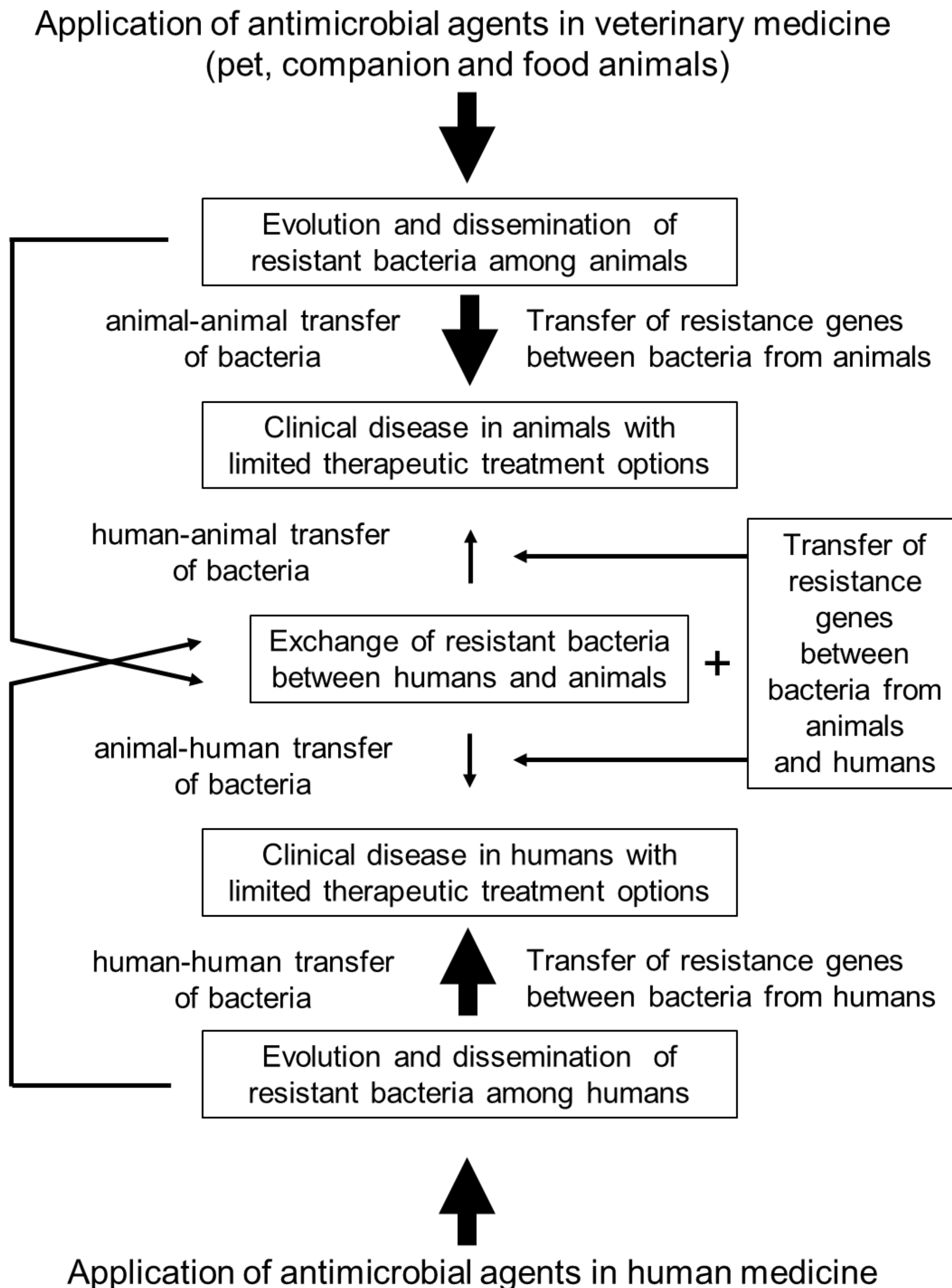

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1113 **Table 1.** CLSI-approved clinical breakpoints available for skin and soft tissue
1114 infections as well as wounds in animals[8]
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Animal species	Target bacteria	Antimicrobial agent	Clinical 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	–	–

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1117 * S (susceptible), I (intermediate), R (resistant)

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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*$_{\text{TEM}}$*, bla*$_{\text{SHV}}$*, bla*$_{\text{CTX-M}}$*, 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.