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Antimicrobial Resistance in *Pasteurellaceae* of Veterinary Origin

GEOVANA B. MICHAEL,[1] JANINE T. BOSSÉ,[2] and STEFAN SCHWARZ[1]

[1]Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, D-14163 Germany; [2]Section of Pediatrics, Department of Medicine London, Imperial College London, London W2 1PG, United Kingdom

ABSTRACT Members of the highly heterogeneous family *Pasteurellaceae* cause a wide variety of diseases in humans and animals. Antimicrobial agents are the most powerful tools to control such infections. However, the acquisition of resistance genes, as well as the development of resistance-mediating mutations, significantly reduces the efficacy of the antimicrobial agents. This article gives a brief description of the role of selected members of the family *Pasteurellaceae* in animal infections and of the most recent data on the susceptibility status of such members. Moreover, a review of the current knowledge of the genetic basis of resistance to antimicrobial agents is included, with particular reference to resistance to tetracyclines, β-lactam antibiotics, aminoglycosides/aminocyclitols, folate pathway inhibitors, macrolides, lincosamides, phenicols, and quinolones.
This article focusses on the genera of veterinary importance for which sufficient data on antimicrobial susceptibility and the detection of resistance genes are currently available (*Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus*). Additionally, the role of plasmids, transposons, and integrative and conjugative elements in the spread of the resistance genes within and beyond the aforementioned genera is highlighted to provide insight into horizontal dissemination, coselection, and persistence of antimicrobial resistance genes. The article discusses the acquisition of diverse resistance genes by the selected *Pasteurellaceae* members from other Gram-negative or maybe even Gram-positive bacteria. Although the susceptibility status of these members still looks rather favorable, monitoring of their antimicrobial susceptibility is required for early detection of changes in the susceptibility status and the newly acquired/developed resistance mechanisms.

THE FAMILY *PASTEURELLACEAE* AND ITS ROLE IN ANIMAL INFECTIONS

The family *Pasteurellaceae* (order *Pasteurellales*, class *Gammaproteobacteria*) comprises a highly heterogeneous group of Gram-negative bacteria. Evaluation by sequence comparison of housekeeping genes, 16s rRNA gene sequence-based phylogenetic analysis, DNA-DNA hybridization, and analysis of the biochemical and physiological capacities has identified a number of distinct genetic and phenotypic groups (1, 2). As a consequence, the family *Pasteurellaceae* has undergone numerous reclassifications during the past years and currently (late 2017) contains 25 genera: *Actinobacillus*, *Aggregatibacter*, *Avibacterium*, *Basfia*, *Bibersteinia*, *Bisgaardia*, *Chelonobacter*, *Cricetibacter*, *Frederiksenia*, *Gallibacterium*, *Haemophilus*, *Histophilus*, *Lonepinella*,

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Correspondence: Geovana Brenner Michael, g.brenner.michael@fu-berlin.de

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Mannheimia, *Mesocricetibacter*, *Muribacter*, *Necropsobacter*, *Nicoletella*, *Otariodibacter*, *Pasteurella*, *Phocoenobacter*, *Testudinibacter*, *Ursidibacter*, *Vespertiliibacter*, and *Volucribacter* (International Committee on Sytematics of Prokaryotes, http://www.the-icsp.org/taxa-covered-family-pasteurellaceae). A new genus, *Rodentibacter*, has recently been proposed. *Rodentibacter pneumotropicus combinatio nova* (comb. nov.), which will be reclassified from [*Pasteurella*] *pneumotropica*, with NCTC 8141T (also designated CCUG 12398T) as the type strain (3). The use of square brackets enclosing the genus name indicates that there is a proposal to reclassify the species to another genus or that the species has been shown not to be a member of the genus *sensu stricto*, as is the case for [*Pasteurella*] *aerogenes*, which is now excluded from *Pasteurella sensu stricto* based on genetic analysis (4). Additional information related to reclassification of genera may be found in references 1, 2, and 5. The use of quotation marks around "*Actinobacillus porcitonsillarum*" denotes that this is currently not a validated species name (5).

Whole-genome sequencing has become an important tool for the classification of members of the family *Pasteurellaceae*, providing a better understanding of the molecular evolution of isolates and their host specificities, niche preferences, pathogenic potential, and mechanisms of transfer and uptake of mobile genetic elements involved in antimicrobial (multi)resistance. Such knowledge can also be applied to improve/develop vaccines, diagnostic tests, disease control measures, and intervention strategies (2, 6–10).

This article deals with the genera which include pathogens of veterinary importance and will focus on the genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus*, for which sufficient data on antimicrobial susceptibility and the detection of resistance genes are currently available. This article compiles the latest information on antimicrobial resistance, associated with the aforementioned genera, and the data published in previous book chapters (10, 11).

Many isolates of the genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus* are commonly found on the mucous membranes of the respiratory tract and/or genital tract of reptiles, birds, and numerous mammals, including a wide variety of food-producing animals. Some species, e.g., *Pasteurella multocida*, may be found in many different hosts, including in humans, while others, such as *Actinobacillus pleuropneumoniae* and *Mannheimia haemolytica* (formerly [*Pasteurella*] *haemolytica*), have a narrow host range, being found primarily in pigs and ruminants, respectively (5).

P. multocida is the most relevant animal-pathogenic *Pasteurella* species. Various capsular types of *P. multocida* are known, some of which preferentially occur in connection with specific diseases in animals. For example, (i) capsular type A is the causative agent of pneumonia in several animal species including, but not limited to, cattle, sheep, and pigs; mastitis in sheep; snuffles in rabbits; and fowl cholera in poultry. (ii) Capsular types B and E are the causative agents of hemorrhagic septicemia of cattle and water buffaloes in Asia and Africa, respectively. (iii) Capsular type D isolates are mainly involved in atrophic rhinitis and pneumonia in swine (12, 13). (iv) Capsular type F isolates are mainly seen in poultry but may also be involved in fatal peritonitis in calves (14).

M. haemolytica comprises 12 capsular serotypes (A1, A2, A5 to A9, A12 to A14, A16, and A17). Serotypes A1 and A6 have been the ones most commonly associated with respiratory diseases in cattle (15) or in sheep (16), respectively.

A. pleuropneumoniae is the causative agent of porcine pleuropneumonia (13), which causes huge economic losses in the swine industry worldwide. Currently, two biovars (the NAD-dependent biovar 1 and the NAD-independent biovar 2) and 16 serovars of *A. pleuropneumoniae* are distinguished (17–19). Serovars 1, 5, 9, and 11 are considered to be more virulent than other serovars (20).

Several species of the genus *Haemophilus* also represent animal pathogens, with [*Haemophilus*] *parasuis* being of major economic importance. [*H.*] *parasuis* causes Glässer's disease (also known as porcine polyserositis or infectious polyarthritis) in pigs, which is characterized by high fevers, polyserositis, polysynovitis, respiratory distress, and meningitis. More than 15 serotypes have been identified, with serotypes 1, 5, 12, 13, and 14 being thought to be the most virulent (21).

Histophilus somni (formerly [*Haemophilus*] *somnus*), *Histophilus ovis*, and *Histophilus agni* are also pathogens of cattle and sheep. *H. somni* is the etiological agent of thromboembolic meningoencephalitis in cattle. It has also been associated with various other diseases in sheep and diseases such as bronchopneumonia, necrotic laryngitis, myocarditis, arthritis, conjunctivitis, myositis, mastitis, and abortion (10).

All diseases in which *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, or *Histophilus* isolates act as primary pathogens commonly occur as peracute or acute forms and are accompanied by a high mortality rate, although subacute and chronic forms are also observed. As secondary pathogens, *P. multocida* and *M. haemo-*

lytica play a major role in the final progression to severe bronchopneumonia and pleuropneumonia in cattle, sheep, and goats (22), as well as in enzootic pneumonia in calves (23) and progressive atrophic rhinitis of swine (24). Respiratory tract infections, in which bacteria such as *P. multocida* and *M. haemolytica* isolates are involved, are often multifactorial and polymicrobial diseases, with viruses and other bacteria, such as *Mycoplasma* spp. representing the primary pathogens (13, 22–24). Under certain environmental and/or management conditions (such as transport, marketing, change of feed, climate, or ventilation) which result in stress to the animals, especially in the presence of viruses and/or *Mycoplasma* spp. which may initiate damage to the host mucosal membranes, the bacterial pathogens can rapidly proliferate, resulting in high morbidity. Under conditions of low stress, the mortality rate may be low. As the amount of stress increases, however, the mortality rate also increases. Economic losses associated with acute pneumonic episodes are primarily due to increased costs in medications and retarded growth rates rather than mortality of the affected animals. Besides these major pathogens, various other members of the family *Pasteurellaceae* have been reported to be less frequently associated with diseases in humans and animals (10).

As seen for many other bacteria, members of the family *Pasteurellaceae* also respond to the selective pressure imposed by the use of antimicrobial agents by developing or acquiring resistance genes or resistance-mediating mutations. In this regard, mobile genetic elements, such as plasmids, transposons, or integrative and conjugative elements (ICEs) that carry resistance genes, play an important role in the dissemination of antimicrobial resistance, with members of the family *Pasteurellaceae* acting as donors and/or recipients. This article provides a brief description of the most recent data on the susceptibility status of selected members of the family *Pasteurellaceae* and an update on the current knowledge regarding genes and mutations conferring antimicrobial resistance among *Pasteurellaceae* of veterinary concern and on the dissemination, coselection, and persistence of such resistance genes.

ANTIMICROBIAL SUSCEPTIBILITY OF *PASTEURELLA, MANNHEIMIA, ACTINOBACILLUS, HAEMOPHILUS,* AND *HISTOPHILUS*

Although prudent use guidelines request identification of the causative pathogen and determination of its *in vitro* susceptibility prior to the onset of antimicrobial therapy, the generally acute nature of the diseases—and in veterinary medicine, the rapid spread of the causative pathogens within animal herds—often requires an immediate therapeutic intervention in which the initial choice of the antimicrobial agents may be revised on the basis of the results of the diagnostic tests. In this regard, two major aspects need to be considered: (i) the correct performance of *in vitro* susceptibility tests and (ii) representative data on the susceptibility status of members of the *Pasteurellaceae*.

Susceptibility Testing

In vitro antimicrobial susceptibility testing (AST) is performed to predict how a bacterium may respond to an antimicrobial agent *in vivo* (clinical response) or to monitor changes in susceptibility in relation to time and geographic location. In both instances, results may be reported qualitatively, e.g., susceptible, intermediate, or resistant (S-I-R), or quantitatively, e.g., as the minimal inhibitory concentration (MIC). When performing AST for surveillance purposes, the interpretive criteria are based on the bacterial population distributions relative to inhibition zone sizes and/or MIC values. Interpretive criteria for clinical consideration require the generation of a bacterium's antibiogram in addition to knowledge of the pharmacokinetic parameters of the chosen drug in the target animal species and the pharmacodynamic parameters associated with the *in vivo* bacterium-antimicrobial agent-host interactions. In either situation, to ensure intra- and interlaboratory reproducibility, it is essential that standardized AST methods be used (25). Recently, it has been suggested that whole-genome sequencing for identification of resistance genes may be a useful adjunct to phenotypic testing, because it not only provides a reproducible means of analysis, but can provide added data for epidemiological purposes (9, 26, 27).

The results of *in vitro* AST ensure the most efficacious antimicrobial therapy by excluding antimicrobial agents to which the causative bacterial pathogen already shows resistance, or reduced susceptibility, under *in vitro* conditions. Correct performance of the tests is essential to most accurately predict the clinical response of the bacterium. Several members of the family *Pasteurellaceae* are classified as "fastidious organisms" since they require specific growth conditions, e.g., supplementation of the media with components essential for the growth of the bacteria. In this regard, performance of *in vitro* AST should follow standardized and internationally accepted rules. The *in vitro* AST of *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus* is

no exception. While many studies have reported *in vitro* susceptibility data for isolates of the these genera, obtained from animal sources in various parts of the world, there has been a notable absence of standardization in testing methods. In most cases, it is difficult to compare the results because of the use of different methods and breakpoints. The Clinical and Laboratory Standards Institute (CLSI) has published three documents, VET01-A4 (28), VET01-S (29), and VET06 (30), which provide the latest information on methods for *in vitro* susceptibility testing of *P. multocida*, *Pasteurella* spp. other than *P. multocida*, *M. haemolytica*, *A. pleuropneumoniae*, and *H. somni*. Because there is no CLSI-approved method for antimicrobial susceptibility testing of [*H.*] *parasuis*, methods recently developed and published should be followed when testing this organism (31, 32).

The CLSI documents VET01-S (29) and VET06 (30) also contain clinical breakpoints on the basis of which isolates are classified as susceptible, intermediate, or resistant. In contrast to epidemiological cutoff values, clinical breakpoints may predict the clinical outcome when the antimicrobial agent in question is dosed and administered as recommended (25, 33). Veterinary-specific clinical breakpoints applicable to bovine, canine, equine, feline, or porcine *P. multocida*, *M. haemolytica*, *H. somni*, and/or *A. pleuropneumoniae* are currently available for a number of antimicrobial agents, including ampicillin/amoxicillin, cefazolin, cefpodoxime, ceftiofur, chloramphenicol, danofloxacin, enrofloxacin, gamithromycin, florfenicol, gentamicin, penicillin G, pradofloxacin, spectinomycin, tetracycline, tiamulin, tildipirosin, tilmicosin, and/or tulathromycin (29, 34).

Susceptibility Status of Selected Pathogens of the Family *Pasteurellaceae*

In veterinary medicine, bacteria belonging to the family *Pasteurellaceae* are currently not included in most national antimicrobial susceptibility monitoring and surveillance programs. Only the German GE*RM*-Vet program, which uses CLSI AST methodology and CLSI clinical breakpoints, included *P. multocida* and *A. pleuropneumoniae* from respiratory tract infections of swine (piglets, weaners, and adult swine), as well as *P. multocida* and *M. haemolytica* from cattle (calves and adult cattle) and *M. haemolytica* from sheep and goats (35) (Table 1). Moreover, *P. multocida* from dogs and cats suffering from either respiratory tract infections or infections of mouth, ear, or skin were collected between 2004 and 2006 in the BfT-GermVet monitoring program and analyzed for their *in vitro* susceptibility (36) (Table 1). In addition, Portis and coworkers (37) published susceptibility data collected in a large-scale surveillance program over a 10-year period (2000 to 2009) from the bovine respiratory disease pathogens *M. haemolytica*, *P. multocida*, and *H. somni* in the United States and Canada (Table 1). A comparison of the percentages of resistance between the respective *P. multocida* and *M. haemolytica* isolates from North America and Germany showed that among the German isolates resistance to newer antimicrobial agents, such as ceftiofur, florfenicol, enrofloxacin, and tulathromycin, is rarely detected, if at all. In contrast, the isolates from North America showed low levels of resistance (up to 11.6%) to florfenicol, enrofloxacin, and tulathromycin. However, all isolates were susceptible to ceftiofur. It

TABLE 1 Percentages of resistance of *P. multocida*, *M. haemolytica*, *H. somni*, and *A. pleuropneumoniae* isolates from different animal sources against selected antimicrobial agents

				% resistant isolates[a]							
Bacteria	Origin	Year(s) of isolation	No. of isolates	PEN	XNL	FLO	ENR	TET	TIL	TUL	Reference
P. multocida	Cattle/USA, Canada	2009	328	3.3	0.0	11.6	2.1	40.8	23.8	4.6	37
	Cattle/Germany	2013	48	2.1	0.0	0.0	0.0	10.4	2.1	0.0	35
	Adult swine/Germany	2013	90	5.6	0.0	0.0	0.0	11.1	0.0	0.0	35
	Weaner/Germany	2013	25	0.0	0.0	0.0	0.0	8.0	0.0	0.0	35
	Piglet/Germany	2013	35	2.9	0.0	2.9	0.0	2.9	0.0	0.0	35
	Dogs, cats/Germany	2004–2006	72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36
M. haemolytica	Cattle/USA, Canada	2009	304	27.3	0.0	8.6	6.6	43.7	27.3	8.9	37
	Calf/Germany	2012–2013	63	11.1	0.0	1.6	0.0	22.2	1.6	0.0	35
	Adult cattle/Germany	2012–2013	35	8.8	0.0	0.0	0.0	0.0	2.9	2.9	35
	Sheep, goat/Germany	2012–2013	42	4.8	0.0	0.0	0.0	2.4	0.0	0.0	35
H. somni	Cattle/USA, Canada	2009	174	4.5	0.0	1.7	7.4	42.5	18.4	10.9	37
A. pleuropneumoniae	Swine/Germany	2012	102	5.9	0.0	0.0	0.0	13.7	1.0	7.8	35

[a]PEN, penicillin G; XNL, ceftiofur; FLO, florfenicol; ENR, enrofloxacin; TET, tetracycline; TIL, tilmicosin; TUL, tulathromycin.

should also be noted that none of the 72 *P. multocida* isolates from respiratory tract infections of dogs and cats investigated in the BfT-GermVet program exhibited resistance to any of the antimicrobial agents shown in Table 1. Only sulfonamide resistance was seen in 31 (43%) isolates (36).

In addition to these data from GE*RM*-Vet (35), Bft-GermVet (36), and the aforementioned large-scale surveillance program (37), numerous other studies have dealt with the *in vitro* susceptibility of bovine, porcine, and avian *Pasteurellaceae* in different countries (e.g., 31, 38–43). Major problems arising from a comparison of the results of these studies are that (i) isolates have not been selected according to a defined sampling plan, (ii) different AST methods have been used, (iii) different interpretive criteria have been used for the evaluation of the results, and/or (iv) isolates may have not been investigated for their relatedness to prevent the inclusion of multiple members of the same clone in the test collection.

Additional studies that have determined the susceptibility to antimicrobial agents of *Pasteurellaceae* isolates from animal sources in different countries include the following. VetPath is a pan-European antibiotic susceptibility monitoring program collecting pathogens from diseased (but not antimicrobial-treated) cattle, pigs, and poultry. Two studies dealing with respiratory tract pathogens, including *P. multocida* and *M. haemolytica* from cattle and *P. multocida* and *A. pleuropneumoniae* from pigs, and covering the years 2002 to 2006 (38) and 2009 to 2012 (39) have been published. The results of these studies showed that, for most antimicrobial agents and pathogens, the percentages of resistance remained largely unchanged in the period from 2009 to 2012 compared to those of the period from 2002 to 2006. Moreover, these data also showed that resistance to amoxicillin/clavulanic acid, ceftiofur, enrofloxacin, florfenicol, tulathromycin, tiamulin, and tilmicosin was absent or <2%. A study conducted in Australia investigated *P. multocida* and *A. pleuropneumoniae* isolates from pigs with respiratory tract infections for their antimicrobial susceptibility (40). This study illustrated that Australian isolates of swine bacterial respiratory pathogens also exhibited low levels of resistance to antimicrobial agents commonly used in the pig industry. A study from Canada in which *A. pleuropneumoniae* isolates were tested for their resistance pheno- and genotypes revealed that all isolates were susceptible to ceftiofur, florfenicol, enrofloxacin, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, and tilmicosin. A low level of resistance was observed toward tiamulin, penicillin, and ampicillin as well as danofloxacin, whereas the majority of the tested isolates were resistant to chlortetracycline (88.4%) and oxytetracycline (90.7%) (41). A large-scale study of 2,989 *M. haemolytica* isolates from feedlot cattle in Canada revealed that 87.8% of the isolates were pan-susceptible, whereas the percentages of resistant isolates varied between 0.0 and 4.5% for the antimicrobial agents tested (42). It should also be noted that a literature review of antimicrobial resistance in pathogens associated with bovine respiratory disease has recently been published (43).

MOLECULAR MECHANISMS OF ANTIMICROBIAL RESISTANCE IN *PASTEURELLA, MANNHEIMIA, ACTINOBACILLUS, HAEMOPHILUS,* AND *HISTOPHILUS*

The following subsections provide an update of the current status of resistance genes and resistance-mediating mutations known to occur in *Pasteurellaceae*. The focus will be on members of the genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus*, which infect animals and for which data on the genetic basis of resistance are available (Table 2). As far as information is available, the association of the resistance genes with mobile genetic elements and their potential to spread across species and genus borders will be discussed.

Resistance to Tetracyclines

Tetracycline resistance is a highly heterogeneous property with more than 40 resistance genes known to date (http://faculty.washington.edu/marilynr/). In bacteria of the genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus*, at least nine tetracycline resistance genes (*tet* genes), representing two resistance mechanisms (tetracycline exporters and ribosome protective proteins) have been detected (Table 2).

Tetracycline resistance mediated by specific exporters

Among the *tet* genes coding for membrane-associated proteins of the major facilitator superfamily which specifically export tetracyclines from the bacterial cell, the genes *tet*(A), *tet*(B), *tet*(C), *tet*(G), *tet*(H), *tet*(L), and *tet*(K) have been identified in bacteria of the aforementioned five genera, but *tet*(K) is commonly found on small plasmids only in human pathogens. Studies from the late 1970s and 1980s reported transferable

TABLE 2 Antimicrobial resistance genes and mutations identified in *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus* isolates of veterinary importance

Antimicrobial agent	Resistance gene or resistance-mediating mutation(s)	Protein specified by the resistance gene or mutation position	Detected in[a]					Reference[b]/Accession number[c]
			Pas	*Man*	*Act*	*Hae*	*His*	
Tetracycline	*tet*(A)	12-TMS efflux protein	–	–	+	–	–	72, 81
	tet(B)	12-TMS efflux protein	+	+	+	+	–	53, 66, 75, 81
	tet(C)	12-TMS efflux protein	–	–	+	–	–	81
	tet(G)	12-TMS efflux protein	+	+	+	–	–	64, 76, 81
	tet(H)	12-TMS efflux protein	+	+	+	–	+	48, 49, 57, 61
	tet(L)	14-TMS efflux protein	+	+	+	–	–	79, 81
	tet(M)	Ribosome protective protein	+	–	+	–	–	49, 81
	tet(O)	Ribosome protective protein	+	–	+	–	–	65, 81
Penicillins	*bla*$_{CMY-2}$	β-Lactmase	+	–	–	–	–	90
	bla$_{OXA-2}$	β-Lactmase	+	+	–	–	+	55, 57
	bla$_{PSE-1}$	β-Lactmase	+	–	–	–	–	76
	bla$_{ROB-1}$	β-Lactmase	+	+	+	+	–	85, 87, 88, 93
	bla$_{TEM-1}$	β-Lactmase	+	–	–	–	–	89
Streptomycin	*strA*	Aminoglycoside-3″-phosphotransferase	+	+	+	+	+	55–57, 121, 194
	strB	Aminoglycoside-6-phosphotransferase	+	+	+	–	+	55–57, 121
Streptomycin/ spectinomycin	*aadA1*	aminoglycoside-3″-adenyltransferase	+	–	–	–	–	76
	aadA14	aminoglycoside-3″-adenyltransferase	+	–	–	–	–	132
	aadA25	aminoglycoside-3″-adenyltransferase	+	+	–	–	+	55, 57
Spectinomycin	Mutation in 16S rRNA	C1192G	+	–	–	–	–	143
	Mutation in *rpsE* coding for ribosomal protein S5	3-bp deletions resulting in S32I and loss of F33 or loss of K33	+	–	–	–	–	143
Kanamycin/neomycin	*aphA1*	Aminoglycoside-3′-phosphotransferase	+	+	+	+	+	55–57, 123; JN202624
	aphA3	Aminoglycoside-3′-phosphotransferase	+	–	–	–	–	135
Gentamicin	*aacC2*	Aminoglycoside 3-*N*-acetyltransferase	–	–	+	+	–	95; JN202624
	aacC4	Aminoglycoside 3-*N*-acetyltransferase	–	–	+	–	–	81
	aadB	Aminoglycoside-2″-adenyltransferase	+	+	+	–	+	55, 57, 81

Sulfonamides	*sul2*	Sulfonamide-resistant dihydropteroate synthase	+	+	+	+	+	55–57, 95; JN202624
Trimethoprim	*dfrA1*	Trimethoprim-resistant DHFR	+	–	–	–	–	76
	dfrA14	Trimethoprim-resistant DHFR		–	+	–	–	122
	dfrA20	Trimethoprim-resistant DHFR	+	–	–	–	–	62
Macrolides	*erm*(T)	rRNA methylase	–	–	–	+	–	154
	erm(A)	rRNA methylase	–	–	+	–	–	70
	erm(C)	rRNA methylase	–	–	+	–	–	70
	erm(42)	rRNA methylase	+	+	–	–	+	57, 155, 157
	Mutation in 23S rRNA	A2058G, A2059G	+	+	–	+	–	153, 159
	mrs(E)-*mph*(E)	Macrolide efflux protein and phospotransferase	+	+	–	–	+	57, 155, 157
Lincosamides	*lnu*(C)	*O*-nucleotidyltransferase	–	–	–	+	–	161
Chloramphenicol	*catA1*	Type A chloramphenicol acetyltransferase	+	+	–	–	–	163
	catA3	Type A chloramphenicol acetyltransferase	+	+	+	+	–	95, 163; JN202624
	catB2	Type B chloramphenicol acetyltransferase	+	–	–	–	–	76
Chloramphenicol/ florfenicol	*floR*	12-TMS efflux protein	+	+	+	+	+	57, 73, 174, 179, 180
(Fluoro) quinolones	Mutation in *gyrA*	G75S, S83F, S83I, S83R, S83V, S83Y, A84P, D87G, D87H, D87N, D87Y, D492G, D492V, G627E	+	+	+	+	–	9, 55, 187, 189–192
	Mutation in *gyrB*	V211I, D254G	+[d]	–	–	+	–	192
	Mutation in *parC*	S73I, S73R, S80L, G83C, I84S, S85R, S85Y, E89K, Q227H, L379I, C578Y	+	+	+	+	–	55, 187, 189, 190, 192
	Mutation in *parE*	P440S, S459F, E461D, E461K, D479E, T551A	–	–	+	+	–	190, 192
	qnrA1	Quinolone resistance protein	–	–	–	+	–	191
	qnrB6	Quinolone resistance protein	–	–	–	+	–	191
	aac(6′)-Ib-cr	Quinolone resistance protein	–	–	–	+	–	191
Streptothricin	*sat2*	Streptothricin-acetyl-transferase	+	–	–	–	–	130

[a]*Pas*, *Pasteurella*; *Man*, *Manheimia*; *Act*, *Actinobacillus*; *Hae*, *Haemophiluis*; *His*, *Histophilus*; +, present; –, absent.
[b]At least a reference or an accession number is included for each genus in which a specific gene or mutation is found. Additional information and references may be found in the text of this chapter.
[c]Accession number was provided in case of unpblished data.
[d]The mutations in *gyrB* were detected in *Pasteurella* isolates after passage on subinhibitory concentrations of fluoroquinolones.

tetracycline resistance among *P. multocida* and *M. haemolytica* isolates of animal origin (44–47), although the type of *tet* gene involved was not determined in any of these studies. In 1993, Hansen and coworkers identified a novel type of *tet* gene, designated *tet*(H) (48). The *tet*(H) gene was first detected on plasmid pVM111, which originated from a *P. multocida* isolate obtained from a turkey in the late 1970s in the United States. (44). In a subsequent study, Hansen et al. found the *tet*(H) gene to be the predominant *tet* gene among *P. multocida* and *M. haemolytica* from infections of cattle and pigs in North America (49). Since the *tet*(H) gene was located either in chromosomal DNA or on plasmids, they speculated about the involvement of a transposable element in the spread of *tet*(H) (49). The corresponding transposon, Tn*5706*, was identified in 1998 on plasmid pPMT1 from a bovine *P. multocida* isolate (50). Tn*5706* is a small, nonconjugative composite transposon of 4,378 bp and represents the first known resistance-mediating transposon identified among members of the genus *Pasteurella* (51). The *tetR-tet*(H) gene region in Tn*5706* is bracketed by inverted copies of the two closely related insertion sequences, IS*1596* and IS*1597* (50) (Fig. 1). Truncated Tn*5706* elements, in which these insertion sequences were deleted in part or completely, have been found on small plasmids in isolates of *M. haemolytica*, *P. multocida*, and [*P.*] *aerogenes* (52, 53) and more recently as part of ICEs in isolates of *P. multocida*, *H. somni*, and *M. haemolytica* (54–57). The *tet*(H) gene has also been found in bacteria outside of the family *Pasteurellaceae*, namely in *Moraxella* spp. and *Acinetobacter radioresistens* (58), both obtained from salmon farms in Chile. The *A. radioresistens* isolate also harbored the insertion sequence IS*1599* (58), which is closely related to the Tn*5706*-associated insertion sequences IS*1596* and IS*1597*.

The *tetR-tet*(H) gene region of the *P. multocida* plasmid pVM111 is bracketed by the *sul2* sulfonamide resistance gene and the *strA* and *strB* streptomycin resistance genes and thus represents part of a novel resistance gene cluster (59) (Fig. 1). The *tet*(H) gene is occasionally present in tetracycline-resistant *A. pleuropneumoniae* (9, 41, 60–63). Two *tet*(H)-carrying plasmids, p9956 and p12494 (5,674 bp and 14,393 bp, respectively), have been isolated from porcine *A. pleuropneumoniae* and sequenced completely (61). Structural analysis showed that they differed distinctly from one another and from the *tet*(H)-carrying plasmids previously found in *Pasteurella* spp. and *Mannheimia* spp. (Fig. 1). In plasmid p12494, a 100-bp insertion in the *tetR* gene was detected which resulted in the loss of 60 amino acids at the C-terminus. MIC determination of the corresponding *A. pleuropneumoniae* isolate, in the presence and absence of tetracycline, suggested that this *tet*(H) gene was constitutively expressed (61).

The *tet*(B) gene has been identified as the dominant *tet* gene among porcine [*P.*] *aerogenes* isolates (53) and has also been detected in porcine *P. multocida* isolates from the United States and Germany (64) and Spain (65), as well as in a bovine *M. haemolytica* isolate from France (66). In all but two cases, the *tet*(B) gene was located in one or two copies in the chromosome. The gene *tet*(B) is part of the nonconjugative transposon Tn*10* (67, 68) and represents the most widely spread *tet* gene among *Enterobacteriaceae* (69). Hybridization studies using SfuI-digested whole cellular DNA of *tet*(B)-carrying *P. multocida* and [*P.*] *aerogenes* isolates suggested that there are complete copies of Tn*10* in the majority of the isolates investigated (53, 64). In the 4.8-kb plasmid pPAT2, recovered from [*P.*] *aerogenes* in Germany, the *tetR-tet*(B) genes proved to be part of a largely truncated Tn*10* element (53), whereas in the 5.1-kb *P. multocida* plasmid from Spain, pB1001, the *tet*(B) gene was found without *tetR* (65).

Studies of tetracycline-resistant *A. pleuropneumoniae* from pigs (9, 60, 70, 71) also revealed the presence of the gene *tet*(B), which appears to be the predominant *tet* gene in isolates, including those from Spain, Switzerland, Japan, Korea, Australia, and the United Kingdom (9, 60, 62, 63, 72, 73). This gene was also detected in the "*A. porcitonsillarum*" reference strain CCUG46996, and a single field isolate of this bacterium (62). Recently, whole-genome sequencing revealed that some United Kingdom isolates of *A. pleuropneumoniae* carry *tet*(B) in their chromosome as part of a transposon insertion disrupting the *comM* gene, whereas other isolates have Tn*10* insertions carrying *tet*(B) as part of the 56-kb ICE*Apl1*, located in a copy of tRNA-Leu (9, 74). Neither of the ICEs so far identified in *P. multocida* (ICE*Pmu1*) and *M. haemolytica* (ICE*Mh1*) carry *tet*(B) (54–56). It would be interesting to determine if the previously reported chromosomally encoded *tet*(B) genes in these species are part of ICEs related to ICE*Apl1* or other distinct ICEs. As more whole-genome sequences become available, it is likely that more ICEs will be identified in isolates of the *Pasteurellaceae*.

Among the Spanish *A. pleuropneumoniae* isolates, the *tet*(B) gene was found mainly on small plasmids, which were indistinguishable by their HindIII and DraI restriction patterns (60). One of these, p11745, a 5,486-bp plasmid, was sequenced completely. Again,

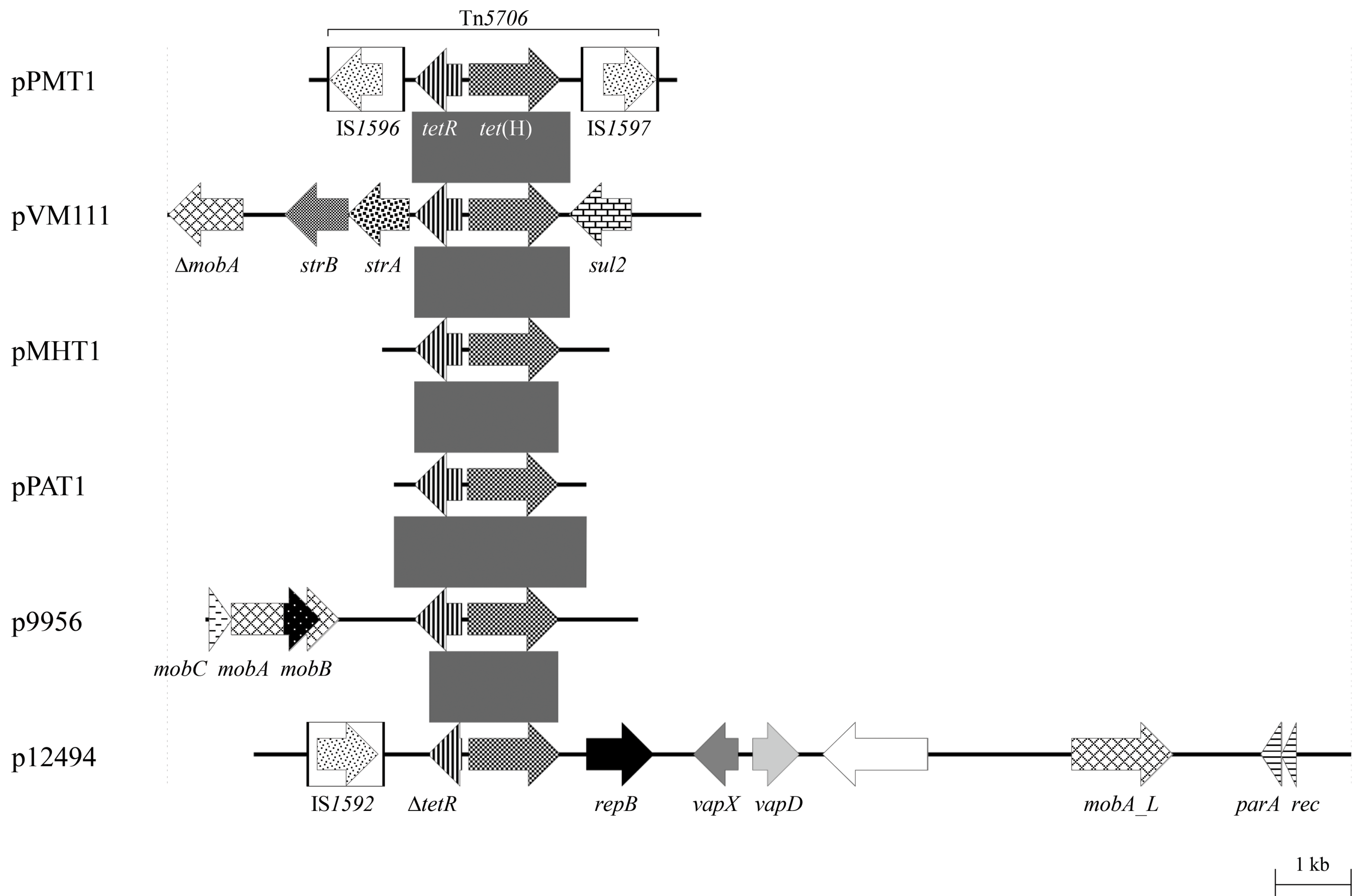

FIGURE 1 Schematic representation of the structure and organization of genes found in *tet*(H)-carrying plasmids from *P. multocida*, *M. haemolytica*, [*P.*] *aerogenes*, and *A. pleuropneumoniae*. Comparison of the maps of the partially sequenced plasmids pPMT1 (accession no. Y15510) and pVM111 (accession nos. AJ514834 and U00792), both from *P. multocida*, pMHT1 (accession no. Y16103) from *M. haemolytica*, and pPAT1 (accession no. AJ245947) from [*P.*] *aerogenes* (accession no. Z21724) and the completely sequenced plasmids p9956 (accession no. AY362554; 5,674 bp) and p12494 (accession no. DQ517426; 14,393 bp), both from *A. pleuropneumoniae*. Genes are shown as arrows, with the arrowhead indicating the direction of transcription. The following genes are involved in antimicrobial resistance: *tetR-tet*(H) (tetracycline resistance), *sul2* (sulfonamide resistance), and *strA* and *strB* (streptomycin resistance); plasmid replication: *repB*; mobilization functions: *mobA*, *mobB*, *mobC*, and *mobA_L*; recombination functions: *rec*; DNA partition: *par*; virulence: *vapD* and *vapX*; unknown function: the open reading frame indicated by the white arrow. The Δ symbol indicates a truncated functionally inactive gene. The white boxes in the maps of pPMT1 and p12494 indicate the limits of the insertion sequences IS*1592*, IS*1596*, and IS*1597*; the arrows within these boxes indicate the reading frames of the corresponding transposase genes. Gray shaded areas indicate the *tetR-tet* (H) gene region common to all these plasmids with ≥95% nucleotide sequence identity. A distance scale in kilobases is shown at the bottom of the figure.

as with pB1001 from *P. multocida*, neither the *tetR* repressor gene nor other parts of Tn*10* were detectable in p11745 (60) (Fig. 2). Although both of these plasmids, isolated in Spain, encode a replication gene, *rep*, upstream of the *tet*(B) gene, there is little sequence similarity outside of the *tet*(B) gene (Fig. 2). In addition, genes for mobilization, *mobC* and *mobA/L*, are found in p11745, but not in pB1001. The 4,597-bp plasmid pHPS1019, isolated from [*H.*] *parasuis* in China (accession number HQ622101), encodes the same *rep*

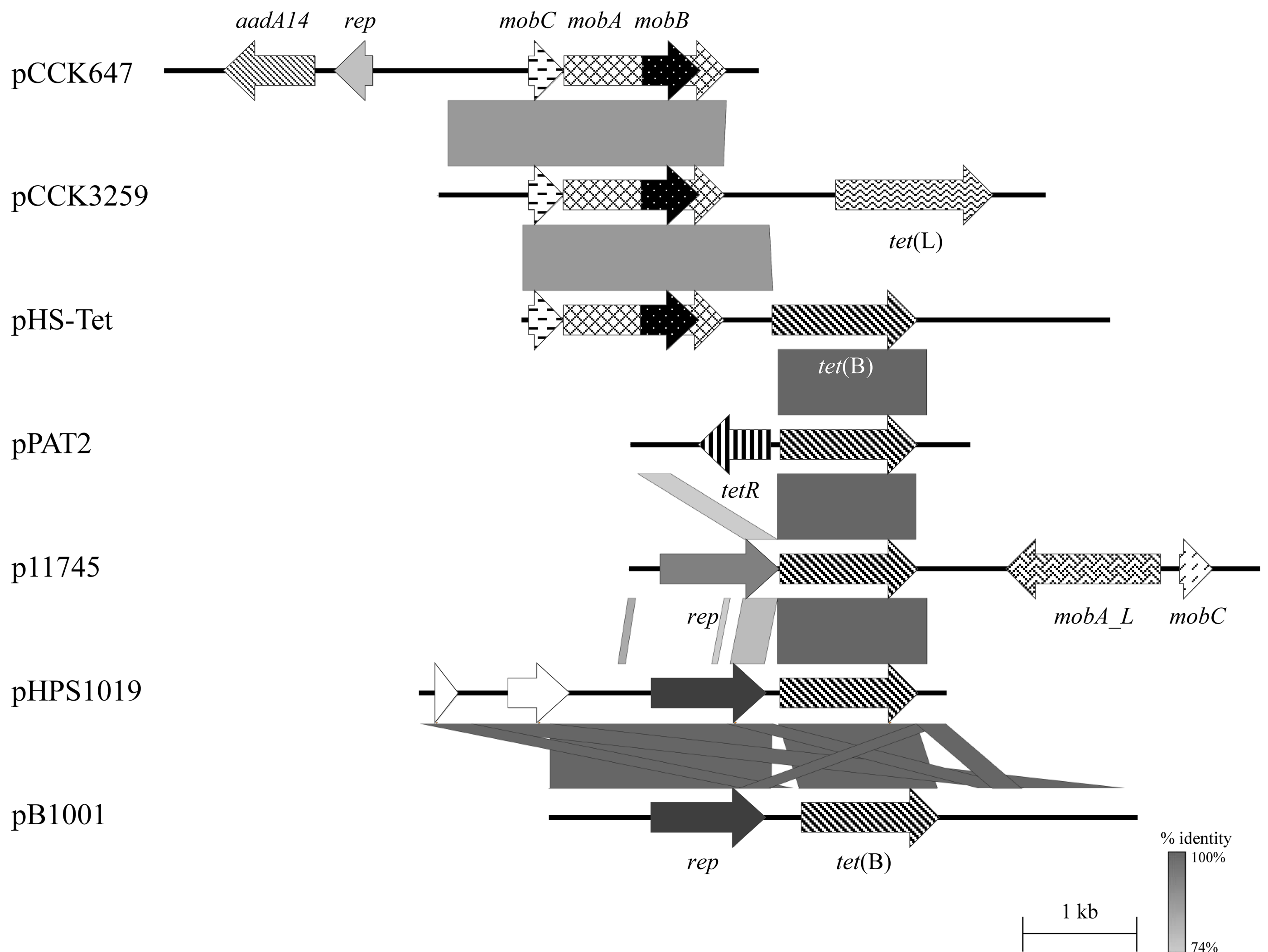

FIGURE 2 Schematic representation of the structure and organization of *aadA14*-, *tet*(L)-, and *tet*(B)-carrying plasmids from *P. multocida*, *M. haemolytica*, [*H.*] *parasuis*, [*P.*] *aerogenes*, and *A. pleuropneumoniae*. Comparison of the maps of the *aadA14*-carrying streptomycin/spectinomycin resistance plasmid pCCK647 (accession no. AJ884726; 5,198 bp) from *P. multocida*, the *tet*(L)-carrying tetracycline resistance plasmid pCCK3259 (accession no. AJ966516; 5,317 bp) from *M. haemolytica*, and the *tet*(B)-carrying tetracycline resistance plasmids pHS-Tet (accession no. AY862435; 5,147 bp) from [*H.*] *parasuis*, pPAT2 (accession no. AJ278685; partially sequenced) from [*P.*] *aerogenes*, p11745 (accession no. DQ176855; 5,486 bp) from *A. pleuropneumoniae*, pHPS1019 (accession no. HQ622101; 4,597 bp) from [*H.*] *parasuis*, and pB1001 (accession no. EU252517; 5,128 bp) from *P. multocida*. Genes are shown as arrows, with the arrowhead indicating the direction of transcription. The following genes are involved in antimicrobial resistance: *tetR*-*tet*(B), *tet*(B), and *tet*(L) (tetracycline resistance) and *aadA14* (streptomycin/spectinomycin resistance); plasmid replication: *rep*; mobilization functions: *mobA*, *mobB*, and *mobC*; unknown function: the open reading frames indicated by white arrows. Gray-shaded areas indicate the regions common to plasmids, and the different shades of gray illustrate the percentages of nucleotide sequence identity between the plasmids, as indicated by the scale at the bottom of the figure. A distance scale in kilobases is shown.

and *tet*(B) genes as pB1001 and also shares an extended region of identity downstream of the *tet*(B) gene (Fig. 2). In these two plasmids, there are direct repeats flanking the *tet*(B) gene, extending from within the 3′ terminus of *rep* for 484 bp in pB1001 and 198 bp in in pHPS1019. Another distinct *tet*(B) plasmid, pHS-Tet, was isolated from [*H.*] *parasuis* in Australia (75). This 5,147-bp plasmid, also without an accompanying *tetR* repressor gene, carries different mobilization genes than those in p11745 (Fig. 2).

The gene *tet*(G) has been found on the chromosome of six epidemiologically related *M. haemolytica* isolates from cattle (64) and on plasmid pJR1 from avian *P. multocida* (76). Surprisingly, the *tet*(G) structural gene in plasmid pJR1 was found in the absence of a corresponding *tetR* repressor gene, which is considered to be essential for the tetracycline-inducible expression of *tet*(G). It should be noted that plasmid pJR1 has not been transferred into susceptible recipient strains for phenotypic confirmation of the activity of the resistance genes found on this plasmid (76). Previously, *tet*(G) was shown to be part of the multiresistance gene cluster present in *Salmonella enterica* serovar Typhimurium DT104 (77), which has since been shown to be part of an integrative mobilizable element, SGI1 (78). It is currently not known whether a related integrative mobilizable element is present in the chromosomes of the six *M. haemolytica* isolates carrying *tet*(G).

The *tet*(L) gene, which is commonly found in Gram-positive cocci and *Bacillus* spp., was detected on small plasmids of *M. haemolytica* and *Mannheimia glucosida*, but also in the chromosomal DNA of single *M. haemolytica* and *P. multocida* isolates, all originating from cattle in Belgium (79). One such plasmid of 5,317 bp from *M. haemolytica*, designated pCCK3259, was sequenced completely (Fig. 2). Besides the *tet*(L) gene, it contains only the *mobABC* operon responsible for mobilization of the plasmid (79). In Gram-positive bacteria, the *tet*(L) gene is inducibly expressed via translational attenuation (80). The corresponding regulatory region, however, was absent in plasmid pCCK3259, whereas all elements required for constitutive expression of the *tet*(L) gene were detected in the upstream sequence (79). Two different-sized *tet*(L)-carrying plasmids (5.6 and >12 kb) have also been identified in porcine *A. pleuropneumoniae* (60). The smaller one of these plasmids, p9555, was sequenced completely and revealed striking structural similarities to plasmid pCCK3259 from *M. haemolytica* (60). Again, the regulatory region of the *tet*(L) gene was missing in plasmid p9555. While plasmid p9555 could be transferred into *Escherichia coli*, where it expressed tetracycline resistance (60), plasmid pCCK3259 did not replicate in *E. coli* but did in a *P. multocida* host (79).

More recently, isolates of *A. pleuropneumoniae* from Japan and South Korea were shown to harbor the *tet*(A) gene, as well as other tetracycline resistance genes (72, 81). In both reports, detection of resistance genes was by PCR, and localization to plasmid or chromosome was not determined. Of the 65 isolates tested by Kim et al. (81), 62 were resistant to tetracycline, with 21 of them carrying *tet*(A). Eleven of these isolates harboured two or more tetracycline resistance genes, as for example one isolate with *tet*(A), *tet*(B), *tet*(C), *tet*(G), and *tet*(L); another with *tet*(A), *tet*(B), *tet*(C), and *tet*(G); and another with *tet*(A), *tet*(B), and *tet*(M)/*tet*(O) (81).

Tetracycline resistance mediated by ribosome protective proteins

Another two tetracycline resistance genes, *tet*(M) and *tet*(O), both coding for ribosome protective proteins, have been identified in *P. multocida* and *A. pleuropneumoniae*. The gene *tet*(M) is associated with the conjugative transposon Tn*916* (82). It is considered to be the most widespread *tet* gene among Gram-positive and Gram-negative bacteria (69; http://faculty.washington.edu/marilynr/). It has been detected by hybridization in the chromosomal DNA of two bovine *P. multocida* isolates (49, 66). The gene *tet*(O), previously identified in *Campylobacter* spp. and streptococci, was detected in chromosomal DNA of a porcine *A. pleuropneumoniae* isolate in 2004 (71). More recently, the *tet*(O) gene was also detected in eight porcine *A. pleuropneumoniae* isolates from Spain (60), five from Japan (72), four from Korea (73), and seven from Canada (41). In the isolates from Spain, the *tet*(O) gene was located on small plasmids of ca. 6 kb. Sequencing of a 2,489-bp region, including the complete *tet*(O) gene, of one of these plasmids (p13142) revealed 99% sequence identity to the *tet*(O) gene of *Campylobacter jejuni* (60).

Resistance to β-Lactam Antibiotics

In general, resistance of *Pasteurellaceae* to β-lactam antibiotics is based on the production of a β-lactamase or the presence of penicillin-binding proteins with low affinity to β-lactams (10, 11, 83). However, the latter mechanism has only been reported for the human pathogen *Haemophilus influenzae* (10, 11, 83). Other mechanisms, such as reduced outer membrane permeability or multidrug efflux systems that can efficiently export β-lactams from the bacterial cell (10, 11, 83), have rarely—if at all—been identified in *Pasteurellaceae*.

So far, five β-lactamase (*bla*) genes have been identified among *Pasteurellaceae*: bla_{ROB-1} (84–88), bla_{TEM-1} (89), bla_{PSE-1} (76), bla_{CMY-2} (90), and bla_{OXA-2} (55, 57). It is interesting to note that the complete bla_{OXA-2} gene, identified as part of ICE*Pmu1*, was found to be nonfunctional in *P. multocida* but functional in *E. coli* (55). According to the existing classification schemes of β-lactamases, the ROB-1 and TEM enzymes are assigned to the Ambler class A because of their structure and to the Bush class 2b on the basis of their substrate profile (91). Members of this class can hydrolyze penicillins and first-generation cephalosporins (narrow spectrum of activity) but are sensitive to inhibition by β-lactamase inhibitors such as clavulanic acid. The PSE-1 β-lactamase also belongs to the Ambler class A but to the Bush class 2c. This enzyme, also known as CARB-2 β-lactamase, can hydrolyze carbenicillin and is also inactivated by clavulanic acid.

Although initially identified in *H. influenzae* from a human infection (92), ROB-1 β-lactamases are widely distributed and have been detected in porcine *A. pleuropneumoniae* isolates (62, 93–95), porcine "*A. porcitonsillarum*" isolates (62), bovine and porcine *P. multocida*, [*P.*] *aerogenes*, and *M. haemolytica* isolates (84, 85, 96), as well as porcine [*H.*] *parasuis* isolates (88, 97). Comparisons confirmed that the similar sized bla_{ROB-1}-carrying plasmids from *P. multocida* and *M. haemolytica* (85) were structurally closely related. The sequences of the small bla_{ROB-1}-carrying plasmids pB1000 from [*H.*] *parasuis* and pB1002 from *P. multocida* (65, 88) are also structurally related to the bla_{ROB-1}-carrying plasmid pAB2 from bovine *M. haemolytica* (98) and the APP7_A plasmid from *A. pleuropneumoniae* (accession number CP001094) (Fig. 3), as well as to the *tet*(B)-carrying tetracycline resistance plasmid pHS-Tet from [*H.*] *parasuis* (75). Comparative analysis confirmed that all so far sequenced bla_{ROB-1} genes from *M. haemolytica*, *A. pleuropneumoniae*, "*A. porcitonsillarum*," and [*H.*] *parasuis* code for an identical β-lactamase protein of 305 amino acids. Mutations in the bla_{ROB-1} gene, which resulted in resistance to extended-spectrum cephalosporins and β-lactamase inhibitors, have been produced *in vitro* (99). Only a single report has described the detection of a TEM-1 β-lactamase in a *P. multocida* isolate from a human dog-bite wound (89). Similarly, PSE-1 β-lactamase has been found in a single avian *P. multocida* isolate (76). In these studies, the TEM-1 β-lactamase was confirmed by isoelectric focusing and sequence analysis of part of the bla_{TEM-1} gene, whereas the bla_{PSE-1} gene was completely sequenced. The bla_{CMY-2} gene, detected by Chander and coworkers (90) in an apparently ceftiofur-resistant *P. multocida* isolate from a pig, was only detected by PCR. Since the bla_{CMY-2} gene was also detected in ceftiofur-susceptible isolates in the same study and no functional analysis has been conducted, the role of bla_{CMY-2} in ceftiofur resistance of *P. multocida* remains questionable.

β-Lactam resistance among *Pasteurellaceae* is often associated with small plasmids. These range in size between 4.1 and 5.7 kb in *P. multocida* (65, 84, 89), 4.1 and 5.2 kb in *M. haemolytica* (85, 87, 98, 100–104), 2.5 and 15.1 kb in *A. pleuropneumoniae* (93–95, 105–107), 8.7 and 13.4 kb in "*A. porcitonsillarum*" (62, 108), and 2.7 and 4.6 kb in [*H.*] *parasuis* (88, 97). Complete sequences indicate that bla_{ROB-1}-carrying plasmids of ≥6 kb encode additional resistance genes, as shown in Table 3. Most of the β-lactam resistance plasmids detected among *Pasteurellaceae* have been identified phenotypically by transformation or conjugation experiments. Recently, some β-lactam-resistant *A. pleuropneumoniae* isolates from South Korea were found to be negative by PCR for both bla_{ROB-1} and bla_{TEM-1} (73, 81). The mechanism of this resistance has not yet been investigated.

Resistance to Aminoglycosides and Aminocyclitols

Resistance to aminoglycoside and aminocyclitol antibiotics is usually mediated by enzymes that inactivate the drugs by adenylation, acetylation, or phosphorylation. Moreover, mutations in chromosomal genes have also been identified to mediate resistance to selected members of these classes of antimicrobial agents (109).

Resistance to aminoglycosides and aminocyclitols by enzymatic inactivation

Streptomycin and/or spectinomycin resistance mediated by enzymatic inactivation

The first aminoglycoside resistance genes detected in *Pasteurella* and *Mannheimia* were those mediating streptomycin resistance. In 1978, Berman and Hirsh published a report on plasmids coding for streptomycin resistance along with sulfonamide resistance, or sulfonamide and tetracycline resistance, in *P. multocida* from turkeys (44). Streptomycin resistance is commonly associated with small nonconjugative plasmids of less than 15 kb in *P. multocida* (44–47, 59, 65, 110–114), [*P.*] *aerogenes* (53), *M. haemolytica* (103, 115), *Mannheimia varigena* (116), "*A. porcitonsillarum*" (62), *A. pleuropneumoniae* (94, 95, 107, 117–122), and *Avibacterium paragallinarum* (123). In one case, streptomycin resistance was mediated by a conjugative

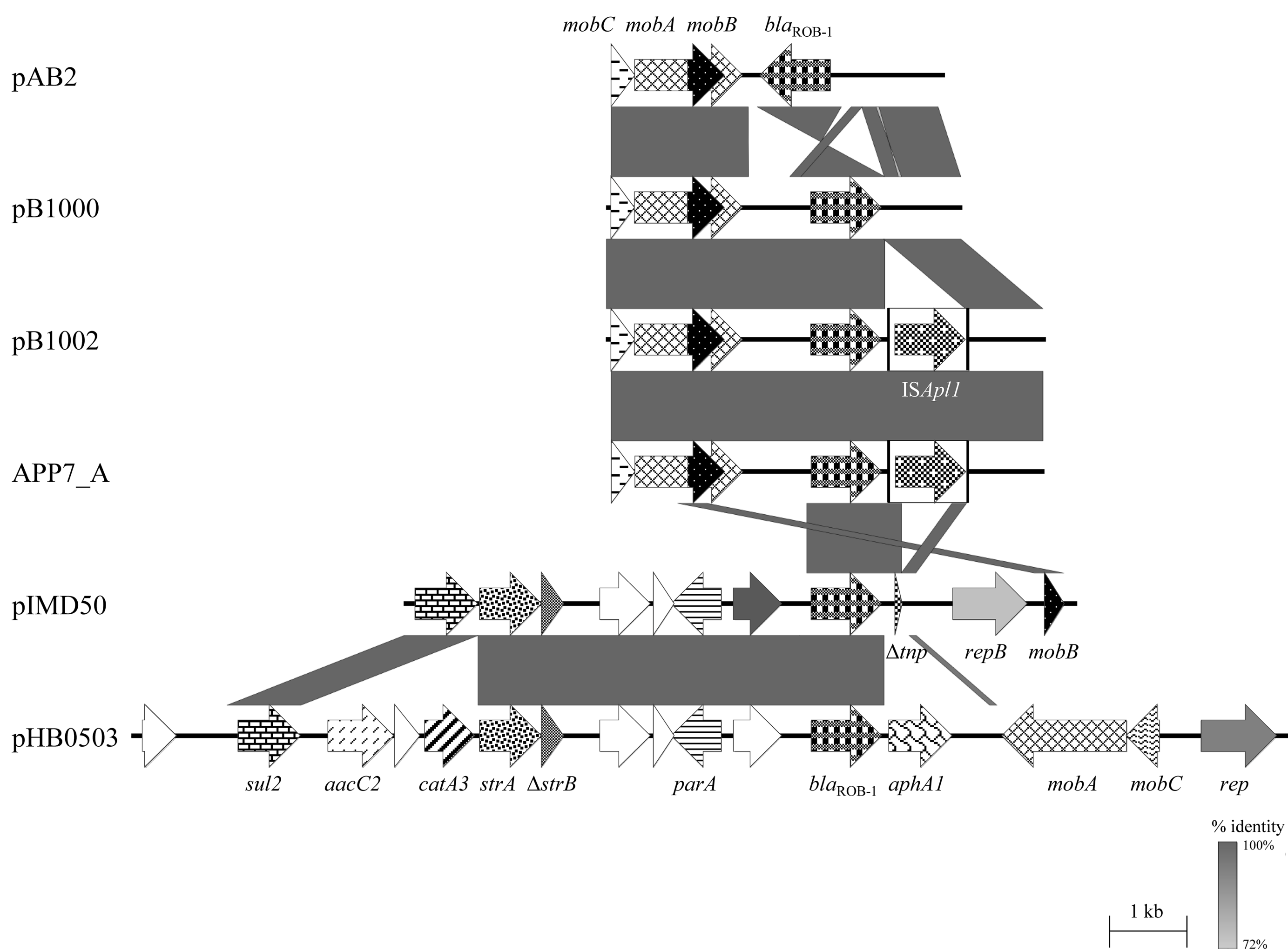

FIGURE 3 Schematic representation of the structure and organization of the bla_{ROB-1}-carrying resistance plasmids from *M. haemolytica*, [*H.*] *parasuis*, *P. multocida*, *A. pleuropneumoniae*, and "*A. porcitonsillarum*." Comparison of the maps of bla_{ROB-1}-carrying resistance plasmids pAB2 (accession no. Z21724; 4,316 bp) from *M. haemolytica*, pB1000 (accession no. DQ840517; 4,613 bp) from [*H.*] *parasuis*, pB1002 (accession no. EU283341; 5,685 bp) from *P. multocida*, APP7_A (accession no. CP001094; 5,685 bp) from *A. pleuropneumoniae*, pIMD50 (accession no. AJ830711; 8,751 bp) from "*A. porcitonsillarum*," and pHB0503 (accession no. EU715370; 15,079 bp) from *A. pleuropneumoniae*. It should be noted that another three pIMD50-related bla_{ROB-1}-carrying resistance plasmids from "*A. porcitonsillarum*" have been sequenced completely: pKMA5 (accession no. AM748705), pKMA202 (accession no. AM748706), and pKMA1467 (accession no. AJ830712). Genes are shown as arrows, with the arrowhead indicating the direction of transcription. The following genes are involved in antimicrobial resistance: *sul2* (sulfonamide resistance), *strA* and *strB* (streptomycin resistance), bla_{ROB-1} (β-lactam resistance), *aacC2* (gentamicin resistance), *catA3* (chloramphenicol resistance), and *aphA1* (kanamycin/neomycin resistance); plasmid replication: *rep*; mobilization functions: *mobA*, *mobB*, and *mobC*; resolvase function: *res*; DNA partition: *parA*; unknown function: open reading frames indicated by white arrows. The prefix Δ indicates a truncated functionally inactive gene. Gray-shaded areas indicate the regions common to plasmids, and the different shades of gray illustrate the percentages of nucleotide sequence identity between the plasmids, as indicated by the scale at the bottom of the figure. A distance scale in kilobases is shown.

TABLE 3 Subset of resistance plasmids identified in *Pasteurella*, *Mannheimia*, and *Actinobacillus* of veterinary importance

Plasmid designation	Plamsid size (kb)	Plasmid sequencing	Resistance genotype	Bacterial source	Accession number	Reference
pARD3079	4.0	Complete	*sul2*	*A. pleuropneumoniae*	AM748707	108
pKMA2425	3.1	Complete	*sul2*	*A. pleuropneumoniae*	AJ830714	108
pKMA757	4.5	Complete	*sul2*	*"A. porcitonsillarum"*	AJ830713	108
ABB7_B	4.2	Complete	*sul2, strA*	*A. pleuropneumoniae*	NC_010941	Unpublished
pFZG1012	Unknown	Partial	*sul2, strA*	*[H.] parasuis*	HQ015158	Unpublished
pYC93	4.2	Complete	*sul2, strA*	*[H.] parasuis*	HM486907	194
pB1005	4.2	Complete	*sul2, strA*	*P. multocida*	FJ197818	65
pHN06	5.4	Complete	*sul2, strA*	*P. multocida*	NC_017035	114
Unnamed	5.4	Complete	*sul2, strA*	*P. multocida*	CP003314	114
pCCK1900	10.2	Complete	*sul2, strA, strB, floR*	*P. multocida*	FM179941	178
pMS260	8.1	Complete	*sul2, strA, strB*	*A. pleuropneumoniae*	AB109805	121
pTYM1	4.2	Complete	*sul2, strA, ΔstrB*	*A. pleuropneumoniae*	AF303375	94
pPSAS1522	4.2	Complete	*sul2, strA, ΔstrB*	*A. pleuropneumoniae*	AJ877041	108
pYFC1	4.2	Complete	*sul2, strA, ΔstrB*	*M. haemolytica*	M83717	103
pPASS2	4.7	Partial	*sul2, strA, ΔstrB*	*[P.] aerogenes*	Not provided	125
pPASS1	5.5	Partial	*sul2, strA, ΔstrB*	*[P.] aerogenes*	Not provided	125
pIG1	5.4	Complete	*sul2, strA, ΔstrB*	*P. multocida*	U57647	150
pB1003	5.1	Complete	*sul2, strA, ΔstrB*	*P. multocida*	EU360945	65
pPMSS1	4.2	Partial	*sul2, strA, ΔstrB*	*P. multocida*	Not provided	125
pB1003	5.0	Complete	*sul2, strA, ΔstrB*	*P. multocida*	EU360945	65
pKMA505	8.6	Complete	*sul2, strA, ΔstrB*	*"A. porcitonsillarum"*	NC_007094	108
pYMH5	5.0	Complete	*sul2, Δsul2, strA, ΔstrB, aphA1*	*A. paragallinarum*	EF015636	123
pIMD50	8.8	Complete	*sul2, strA, ΔstrB,* bla_{ROB-1}	*"A. porcitonsillarum"*	NC_007095	108
pKMA5	9.5	Complete	*sul2, strA, ΔstrB,* bla_{ROB-1}	*"A. porcitonsillarum"*	NC_009623	108
pKMA1467	11.1	Complete	*sul2, strA, ΔstrB,* bla_{ROB-1}	*"A. porcitonsillarum"*	NC_007096	108
pOV	13.6	Complete	*sul2, strA, ΔstrB,* bla_{ROB-1}	*P. multocida*	JX827416	Unpublished
pKMA202	13.4	Complete	*sul2, strA, ΔstrB,* bla_{ROB-1}	*"A. porcitonsillarum"*	AM748706	108
pM3389T	6.1	Complete	*sul2, ΔstrA, dfrA14*	*A. pleuropneumoniae*	KP197005	122
pM3224T	6.1	Complete	*sul2, ΔstrA, dfrA14, strB*	*A. pleuropneumoniae*	KP197004	122
pMHSCS1	5.0	Complete	*sul2, catA3, strA, ΔstrB*	*Mannheimia* taxon 10[a]	AJ249249	125
pMVSCS1	5.6	Complete	*sul2, catA3, strA, ΔstrB*	*M. varigena*	NC_003411	116
pPASCS1	5.6	Partial	*sul2, catA3, strA, ΔstrB*	*[P.] aerogenes*	Not provided	125
pPASCS2	6.0	Partial	*sul2, catA3, strA, ΔstrB*	*[P.] aerogenes*	Not provided	125
pPASCS3	6.1	Partial	*sul2, catA3, strA, ΔstrB*	*[P.] aerogenes*	Not provided	125
pCCK13698	15.0	Complete	*sul2, catA3, ΔstrA, floR*	*B. trehalosi*	AM183225	183
pFZ51	15.7	Complete	*sul2, aacC2, catA3, strA, ΔstrB,* bla_{ROB-1}*, aphA1*	*[H.] parasuis*	JN202624	Unpublished
pHB0503	15.1	Complete	*sul2, aacC2, catA3, strA, ΔstrB,* bla_{ROB-1}*, aphA1*	*A. pleuropneumoniae*	EU715370	95
pVM111	9.8	Partial	*sul2, tetR-tet*(H)*, strA, strB*	*P. multocida*	AJ514834	49, 59

pCCK154	11.0	Partial	*sul2, dfrA20*	*P. multocida*	AJ605332	146
pJR1	6.8	Complete	*sul2, tet*(G)*, catB2*	*P. multocida*	AY232670	76
p9956	5.7	Complete	*tetR-tet*(H)	*A. pleuropneumoniae*	AY362554	60
pMHT1	4.4	Partial	*tetR-tet*(H)	*M. haemolytica*	Y16103	64
pPAT1	5.5	Partial	*tetR-tet*(H)	[*P.*] *aerogenes*	AJ245947	52
pPMT1	6.8	Partial	*tetR-tet*(H)	*P. multocida*	Y15510	50
pB1018	6.0	Complete	*tetR-tet*(H)	*P. multocida*	JQ319774	Unpublished
p12494	14.4	Complete	Δ*tetR-tet*(H)	*A. pleuropneumoniae*	DQ517426	61
p9555	5.7	Complete	*tet*(L)	*A. pleuropneumoniae*	AY359464	60
pCCK3259	5.3	Complete	*tet*(L)	*M. haemolytica*	NC_006976	79
p11745	5.5	Complete	*tet*(B)	*A. pleuropneumoniae*	DQ176855	60
pHS-Tet	5.1	Complete	*tet*(B)	[*H.*] *parasuis*	AY862435	75
pTetHS016	3.4	Complete	*tet*(B)	[*H.*] *parasuis*	KC818265	195
pHPS1019	4.6	Complete	*tet*(B)	[*H.*] *parasuis*	HQ622101	Unpublished
pB1001	5.1	Complete	*tet*(B)	*P. multocida*	EU252517	65
pPAT2	4.8	Partial	*tetR-tet*(B)	[*P.*] *aerogenes*	AJ278685	53
p13142	6.0	Partial	*tet*(O)	*A. pleuropneumoniae*	AY987963	60
pB1006	6.0	Complete	*tet*(O)	*P. multocida*	FJ234438	65
pJR2	5.3	Complete	*aadA1*, bla_{PSE-1}	*P. multocida*	AY232671	76
pCCK343	5.4	Complete	*aadA1, dfrA1, sat2*	[*P.*] *aerogenes*	FR687372	130
pCCK647	5.2	Complete	*aadA14*	*P. multocida*	NC_006868	132
Unnamed	Unknown	Partial	bla_{ROB-1}	*A. pleuropneumoniae*	Not provided	105
APP7_A	5.7	Complete	bla_{ROB-1}	*A. pleuropneumoniae*	CP001094	Unpublished
pB1000	4.6	Complete	bla_{ROB-1}	[*H.*] *parasuis* *P. multocida*	DQ840517 GU080062, GU080067	88 65
pAB2	4.3	Complete	bla_{ROB-1}	*M. haemolytica*	Z21724	98
pPH51	4.1	Partial	bla_{ROB-1}	*M. haemolytica*	X52872	85
pYFC2	4.2	Partial	bla_{ROB-1}	*M. haemolytica*	Not provided	103
pB1002	5.7	Complete	bla_{ROB-1}	*P. multocida*	EU283341	65
pJMA-1	2.7	Complete	bla_{ROB-1}	[*H.*] *parasuis*	KP164834	97
pCCK411	5.3	Complete	bla_{ROB-1}, *aphA3*	*P. multocida*	FR798946.1	135
pFS39	7.6	Complete	bla_{ROB-1}, *erm*(T)	[*H.*] *parasuis*	KC405064	154
pFAB-1	4.3	Partial	bla_{TEM-1}	*P. multocida*	Not provided	89
pHPSF1	6.3	Complete	*floR*	[*H.*] *parasuis*	KR262062.1	180
pM3446F	7.7	Complete	*floR*	*A. pleuropneumoniae*	KP696484	181
pCCK381	10.8	Complete	*floR*	*P. multocida*	NC_006994	174
pMH1405	7.7	Complete	*floR*	*P. multocida*	NC_019260[a]	179
p518	3.9	Complete	*floR, strA,* Δ*strB*	*A. pleuropneumoniae*	KT355773	182
pQY431	7.8	Complete	*aacA-aphD*	[*H.*] *parasuis*	KC405065	Unpublished
FJS5863	7.8	Complete	*aacA-aphD*, bla_{ROB-1}	[*H.*] *parasuis*	HQ015159	Unpublished
pHN61	6.3	Complete	*lnu*(C)	[*H.*] *parasuis*	FJ947048	161

[a]Initially identified as *M. haemolytica*.

multiresistance plasmid of approximately 113 kb in an avian *P. multocida* isolate, but the resistance genes were not investigated (47). Many of these plasmids carry additional resistance genes such as the sulfonamide resistance gene *sul2*, the kanamycin/neomycin resistance gene *aphA1*, the chloramphenicol resistance gene *catA3* (Fig. 4), and/or the ampicillin resistance gene $bla_{\mathrm{ROB-1}}$ (Fig. 3) (Table 3).

The predominant streptomycin resistance gene in bacteria of the genera *Pasteurella*, *Mannheimia*, and *Actinobacillus* is *strA*. It codes for an aminoglycoside-3″-phosphotransferase of 269 amino acids and is sometimes found together with the gene *strB*, which codes for an aminoglycoside-6-phosphotransferase of 278 amino acids. Both genes are part of transposon Tn*5393* from *Erwinia amylovora* (124). In the streptomycin-resistant *Pasteurella*, *Mannheimia*, and *Actinobacillus* isolates, *strA* is usually complete, whereas various truncated *strB* genes have been identified (116, 121, 125). Isolates that carry a functionally active *strA* gene, but a largely truncated *strB* gene, have been shown to be highly resistant to streptomycin (125), suggesting that *strA* is the relevant gene for the expression of streptomycin resistance. Further support for this hypothesis comes from the observation that plasmid pSTOJO1, which carries an intact *strB* gene but a functionally inactive *strA* gene that is disrupted by the integration of a *dfrA14* gene cassette, does not express streptomycin resistance in *E. coli* (126). Studies of the prevalence and distribution of the *strA*-*strB* genes showed that the *strA* gene—in combination with a complete or truncated copy of *strB*—occurs on plasmids or in the chromosomal DNA of a wide range of commensal and pathogenic bacteria from humans, animals, and plants (127–129).

An *aadA1* gene coding for an aminoglycoside-3″-adenyltransferase that mediates resistance to both the aminoglycoside streptomycin and the aminocyclitol spectinomycin has been detected on the 5.2-kb plasmid pJR2 from avian *P. multocida* (76) and the 5.4-kb plasmid pCCK343 from porcine [*P.*] *aerogenes* (130). In pJR2, the *aadA1* gene is part of a gene cassette, along with $bla_{\mathrm{PSE-1}}$, that is inserted into a relic of a class 1 integron. The *intI1* gene coding for the integrase in the 5′-conserved segment of this integron is truncated, but without affecting the promotor, and the *sul1* gene in the 3′ conserved fragment is missing completely (76). In plasmid pCCK343, a gene cassette array comprised of *dfrA1-sat2-aadA1* is part of a truncated class 2 integron (130). In this case, the streptothricin acetyltransferase gene, *sat2*, conferred resistance to the streptothricin antibiotic nourseothricin (MIC, >256 mg/liter), in addition to the streptomycin and spectinomycin resistance (MICs of >256 mg/liter for both) conferred by the aminoglycoside adenyltransferase gene, *aadA1*. Attempts to identify the *aadA* genes in bovine isolates of *P. multocida* and *M. haemolytica* from Germany that are highly resistant to streptomycin and spectinomycin using PCR were unsuccessful (131). In all these isolates, streptomycin resistance was based on an *strA* gene, whereas a spectinomycin resistance gene could not be identified.

A novel streptomycin-spectinomycin resistance gene, designated *aadA14*, was identified on a small 5.2-kb plasmid from a bovine *P. multocida* capsular type F isolate from Belgium (132). The corresponding isolate was obtained from a case of fatal peritonitis in a calf (14). The 261-amino acid AadA14 adenyltransferase protein exhibited only 51.4 to 56.0% identity to the so far known AadA proteins and hence proved to be only distantly related to AadA proteins previously found in other bacteria.

Two ICEs from *Pasteurellaceae*, ICE*Pmu1* and ICE*Mh1*, have been shown to contain genes associated with resistance to streptomycin and other aminoglycosides and aminocyclitols (55, 56). In addition to other resistance genes, the 92-kb ICE*Mh1* contains *strA* and *strB*, conferring resistance to streptomycin (MIC, 256 mg/liter), as well as *aphA1*, conferring resistance to kanamycin/neomycin (MICs, ≥512 mg/liter and 64 mg/liter, respectively) (56). The 82-kb ICE*Pmu1* carries 12 resistance genes, including *strA* and *strB*, *aadA25*, *aadB*, and *aphA1*, with the latter three genes conferring resistance to streptomycin (MIC, ≥256 mg/liter) and spectinomycin (MIC, ≥512 mg/liter), gentamicin (MIC, 128 mg/liter), and kanamycin/neomycin (MICs, ≥128 mg/liter and ≥32 mg/liter), respectively, in *P. multocida* strain 36950 (54, 55).

Kanamycin and neomycin resistance mediated by enzymatic inactivation

Kanamycin/neomycin resistance has been associated with the gene *aphA1*, also known as *aph(3′)-Ia*, which codes for an aminoglycoside-3′-phosphotransferase that mediates resistance to kanamycin and neomycin. This gene has been identified on transposon Tn*903* (133). Subsequently, it was detected together with the streptomycin resistance genes *strA-strB* and the sulfonamide resistance gene *sul2* on the broad-host-range plasmid pLS88 from human host-specific *Haemophilus ducreyi* (134). A pLS88-related plasmid has also been identified in the *A. paragallinarum* strain A14 (123) (Fig. 4).

FIGURE 4 Schematic representation of the structure and organization of selected *sul2*-based (multi-)resistance plasmids from *A. pleuropneumoniae*, "*A. porcitonsillarum*," *A. paragallinarum*, *H. ducreyi*, [*H.*] *parasuis*, *M. haemolytica*, *Mannheimia* unnamed taxon 10, *M. varigena*, and *P. multocida*. Comparison of the maps of the plasmids pKMA2425 (accession no. AJ830714; 3,156 bp) from *A. pleuropneumoniae*, pARD3079 (accession no. AM748707; 4,065 bp) from *A. pleuropneumoniae*, pKMA757 (accession no. AJ830713; 4,556 bp) from "*A. porcitonsillarum*," ABB7_B (accession no. NC_010941; 4,236 bp) from *A. pleuropneumoniae*, pIG1 (accession no. U57647) from *P. multocida*, pYFC1 (accession no. M83717) from *M. haemolytica*, pFZG1012 (accession no. HQ015158; partially sequenced) from [*H.*] *parasuis*, pLS88 (accession no. L23118; 4,772 bp) from *H. ducreyi*, pYMH5 (accession no. EF015636; 4,772 bp) from *A. paragallinarum*, pM3224T (accession no. KP197004; 6,050 bp) from *A. pleuropneumoniae*, pMS260 (accession no. AB109805; 8,124 bp) from *A. pleuropneumoniae*, pMVSCS1 (accession no. AJ319822; 5,621 bp) from *M. varigena*, pMHSCS1 (accession no. AJ249249; 4,992 bp) from *Mannheimia* unnamed taxon 10, pFZ51 (accession no. JN202624; 15,672 bp) from [*H.*] *parasuis*, and pKMA757 (accession no. AJ830713; 4,556 bp) from "*A. porcitonsillarum*." The map of another *sul2*-based multiresistance plasmid, pIMD50 (accession no. AJ830711) from "*A. porcitonsillarum*," is displayed in Fig. 3. Genes are shown as arrows, with the arrowhead indicating the direction of transcription. The following genes are involved in antimicrobial resistance: *sul2* (sulfonamide resistance), *strA* and *strB* (streptomycin resistance), *catA3* (chloramphenicol resistance), *aphA1* (kanamycin/neomycin resistance), and bla_{ROB-1} (β-lactam resistance); plasmid replication: *rep*, *repA*, *repB*, and *repC*; mobilization functions: *mobA*, *mobB*, *mobC*, *mobA′*, *mobB′*, and *mobC′*; unknown function: open reading frames indicated by white arrows. The prefix Δ indicates a truncated functionally inactive gene. Gray-shaded areas indicate the regions common to plasmids and the different shades of gray illustrate the percentages of nucleotide sequence identity between the plasmids, as indicated by the scale at the bottom of the figure. A distance scale in kilobases is shown.

Further studies on kanamycin/neomycin-resistant [*P.*] *aerogenes*, *P. multocida*, and *M. glucosida* isolates identified the *aphA1* gene mostly in chromosomal DNA (135). In a single *P. multocida* isolate, this gene was found on the 5,955-bp plasmid pCCK3152, along with complete *strA* and *sul2* genes and a truncated *strB* gene (135). The 15.1-kb multiresistance plasmid pHB0503 (95) and the 15.7-kb plasmid pFZ51 (accession number JN202624), both carrying *sul2*, *aacC2*, *catA3*, *strA*, *ΔstrB*, and bla_{ROB-1}, along with *aphA1*, have been identified in Chinese *A. pleuropneumoniae* and [*H.*] *parasuis* isolates, respectively. Kang et al. (95) reported that the *A. pleuropneumoniae* isolate harboring pHB0503 was resistant to streptomycin (MIC, 512 mg/liter), kanamycin (MIC, 256 mg/liter), and gentamicin (MIC, 512 mg/liter), as well as to penicillin (MIC, 256 mg/liter), sulfadimidine (MIC, 1,024 mg/liter), and chloramphenicol (MIC, 16 mg/liter), whereas the [*H.*] *parasuis* isolate harboring pFZ51 has not been characterized (Fig. 4).

A second kanamycin/neomycin resistance gene, *aphA3* [also known as *aph(3′)-III*], was detected on the 5.1-kb plasmid pCCK411 in single isolates of *P. multocida* and [*P.*] *aerogenes* (135). In addition to the *aphA3* gene, this plasmid also carried a *mobABC* operon for mobilization. A not further specified aminoglycoside-3′-phosphotransferase gene mediating kanamycin resistance was also found, together with a bla_{ROB-1} gene, on the 6-kb plasmid pTMY2 from *A. pleuropneumoniae* (94). Two virtually identical 7,777-bp plasmids from [*H.*] *parasuis*, FJS5863 (accession number HQ015159), and pQY431 (accession number KC405065) contain the *aacA-aphD* gene encoding a bifunctional aminoglycoside *N*-acetyltransferase and aminoglycoside phosphotransferase, in addition to the bla_{ROB-1} gene, but no functional studies of these plasmids have been published.

Gentamicin resistance mediated by enzymatic inactivation

Although *P. multocida*, *A. pleuropneumoniae*, and "*A. porcitonsillarum*" isolates with MICs of gentamicin of ≥32 mg/liter have been detected, most attempts to detect specific resistance genes, such as *aadB* [*ant(2″)-Ia*], *aacC2* [*aac(3)-IIc*], or *aacC4* [*aac(3)-IVa*], have failed (62; Kehrenberg and Schwarz, unpublished data). Moreover, attempts to transfer gentamicin resistance from *P. multocida* donors were also unsuccessful (Kehrenberg and Schwarz, unpublished data). Recently, PCR analysis revealed that among 12 gentamicin-resistant *A. pleuropneumoniae* isolates from South Korea, 11 produced amplicons specific for *aadB* [*ant(2″)-Ia*], and one for *aacC4* [*aac(3)-IVa*] (81). In addition, a 15.1-kb plasmid from *A. pleuropneumoniae* (pHB0503) has been found carrying *aacC2*, encoding an aminoglycoside-acetyltransferase (95). This plasmid conferred an MIC of gentamicin of 512 mg/liter in the original serovar 4 clinical isolate (from China) and an MIC of 256 mg/liter when transformed into M62, the reference strain of *A. pleuropneumoniae* serovar 4 (95). The 15.7-kb plasmid pFZ51 (accession number JN202624) from [*H.*] *parasuis* also carries *aacC2* but has not been functionally characterized (Fig. 4).

Resistance to aminocyclitols by mutations

Ribosomal mutations conferring spectinomycin resistance have been described in a variety of bacteria (136–140). All these mutations were present in a specific region of helix 34 in 16S rRNA. This region encompassed the cross-linked positions 1063 to 1066 and 1090 to 1093, which are known to be involved in the binding of spectinomycin to the ribosome. Moreover, the *rpsE* gene coding for the ribosomal protein S5 is also relevant for the drug binding, and mutations in *rpsE* have been described to affect spectinomycin binding (141, 142). The analysis of high-level spectinomycin-resistant *P. multocida* isolates (MICs of spectinomycin ≥4,096 mg/liter), in which no enzymatic inactivation of the drug could be detected, revealed four types of mutations: (i) a C1192G transversion (no additional mutations in positions which have been associated with spectinomycin resistance were found) in 16S rRNA in all six or (ii) in five of the six rRNA operons, (iii) the aforementioned transversion in only two of the six operons accompanied by a 3-bp deletion in *rpsE* that resulted in a change of the amino acids 32-SF-33 to 32-I, and (iv) a 3-bp deletion in *rpsE* that resulted in the loss of 23-K without additional rRNA mutations (143). Molecular modeling suggested that both types of mutations in the S5 protein have a negative impact on spectinomycin binding to the ribosome.

Resistance to Folate Pathway Inhibitors

Sulfonamides and trimethoprim are competitive inhibitors of different enzymatic steps in folic acid metabolism. Sulfonamides represent structural analogs of *p*-aminobenzoic acid and inhibit the enzyme dihydropteroate synthase (DHPS), which—in the initial step of folic acid metabolism—catalyzes the synthesis of dihydropteroic acid from dihydropteridin and *p*-aminobenzoic acid. Trimethoprim inhibits the enzyme dihydrofolate reductase (DHFR), which—in a later step

of folic acid metabolism—reduces dihydrofolic acid to tetrahydrofolic acid. Resistance to both drugs is commonly mediated by replacement of susceptible DHPS or DHFR enzymes by those with reduced affinity to sulfonamides or trimethoprim, respectively. Moreover, overproduction of susceptible targets, or mutations in chromosomal DHPS or DHFR genes, which alter substrate specificity, may also cause resistance (109). However, overproduction of a trimethoprim-sensitive DHFR, encoded by the gene *folH* (144), or a short insertion into the chromosomal gene *folP* encoding a DHPS (145), have so far only been reported to confer resistance to trimethoprim or sulfonamides, respectively, in human host-specific *H. influenzae*.

Sulfonamide resistance mediated by altered DHPSs

Sulfonamide resistance among *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* isolates is commonly mediated by a type 2 DHPS with reduced affinity to sulfonamides. The corresponding gene, *sul2*, is frequently found on small plasmids with sizes ranging from 3.1 to 15.7 kb (Table 3). The *sul2*-encoded DHPS proteins commonly consist of 271 amino acids. However, several variants ranging in size between 263 and 289 amino acids have also been reported (11, 55, 56, 125, 134, 146). Sequence analysis showed that single-base pair insertions downstream of codon 225 in the DHPS of the *H. ducreyi* plasmid pLS88 (134) resulted in a shortened C-terminus that differs from all so far known DHPS variants. A mutation in the translational stop codon of the *sul2* gene from plasmids pYFC1 of *M. haemolytica* (103) and pTYM1 of *A. pleuropneumoniae* (94) led to an extension of 12 amino acids at the C-terminus. In plasmid pCCK154 from *P. multocida*, the loss of a single "A" at position 793 within the *sul2* gene caused a frameshift mutation which led to the substitution of 6 codons and extended the reading frame by 18 codons (146). Finally, a recombination in the 3′ end of the *sul2* reading frame changed the final three codons and extended the *sul2* reading frame by one codon in plasmid pVM111 from *P. multocida* (59). Plasmids carrying *sul2* have also been identified in bacteria other than *Pasteurellaceae*, e.g., *E. coli* (147) and *Photobacterium damselae* subsp. *piscicida* (148). In addition to their location on small plasmids, *sul2* genes have also been detected on broad-host-range conjugative (e.g., pGS05) or nonconjugative (e.g., RSF1010, pLS88) plasmids (134, 147, 149) and on the ICE*Pmu1* (55).

Various studies revealed that the *sul2* gene is often linked to the *strA*-*strB* genes (65, 103, 108, 122, 125, 134, 150). PCR assays were developed to confirm the linkage of *sul2* and *strA* in both orientations (125). In some cases, the *strA* gene followed by a truncated Δ*strB* gene was detected upstream of *sul2* (125, 150). However, in most *P. multocida*, [*P.*] *aerogenes*, and *Mannheimia* isolates studied, these genes were found in the orientation *sul2*-*strA*, whereas a truncated Δ*strA* may also be found upstream of *sul2* (65, 103, 125, 134). Detailed studies of the noncoding spacer between *sul2* and *strA* revealed different lengths (121, 125) and showed that this region might represent a hot spot for recombination events. A *catA3* gene, coding for chloramphenicol resistance, was found to be inserted between *sul2* and *strA* via illegitimate recombination. Such *sul2*-*catA3*-*strA* clusters have been found on various plasmids as well as in the chromosomal DNA of [*P.*] *aerogenes*, *M. haemolytica*, *M. varigena*, and *Mannheimia* taxon 10 isolates (116, 125). Further insertion of *aacC2* between *sul2* and *catA3* was identified in pHB0503 from *A. pleuropneumoniae* (95). In plasmid pVM111 from an avian *P. multocida* isolate (44, 48), a Tn*5706*-like *tetR*-*tet*(H) segment, responsible for tetracycline resistance, was also found to be inserted between *sul2* and *strA* via illegitimate recombination (59). The resulting *sul2*-*tetR*-*tet*(H)-*strA* cluster, however, has not yet been detected on plasmids other than pVM111 or in the chromosomal DNA of *Pasteurella*, *Mannheimia*, or *Actinobacillus* isolates (Fig. 1). In addition to insertions between *sul2* and *strA*, two recent *A. pleuropneumoniae* plasmids, pM3224T and pM3389T, were found to have insertions of *dfrA14* (encoding trimethoprim resistance) disrupting the *strA* gene, located either downstream (pM3224T) (Fig. 4) or upstream (pM3389T) of *sul2* (122).

Several small plasmids that carry only the sulfonamide resistance gene *sul2*, but no other resistance genes, have been sequenced completely. These include the plasmids pKMA2425 (3,156 bp) and pARD3079 (4,065 bp), both from *A. pleuropneumoniae*, as well as pKMA757 (4,556 bp) from "*A. porcitonsillarum*" (108) (Fig. 4). A 4,236-bp *A. pleuropneumoniae* plasmid, ABB7_B (accession number NC_010941), carries *sul2* and *strA*. All of these plasmids share the same mobilization genes (*mobABC*), except pKM2425, which contains only *mobC* and a truncated copy of *mobA* (Fig. 4).

Other *sul* genes, such as *sul1* and *sul3*, which also code for dihydropteroate synthetases with reduced affinity to sulfonamides, have not yet been detected among *Pasteurellaceae* species (62; Kehrenberg and Schwarz, unpublished data).

Trimethoprim Resistance Mediated by Altered DHFRs

A novel trimethoprim resistance gene was detected on the 11-kb plasmid pCCK154 from bovine *P. multocida* (146). This plasmid was transferable into *E. coli*, where it replicated and expressed high-level resistance to sulfonamides and trimethoprim. Sequence analysis identified the gene *sul2* for sulfonamide resistance and a novel gene, designated *dfrA20*, for trimethoprim resistance. The *dfrA20* gene codes for a trimethoprim-resistant DHFR of 169 amino acids, which is only distantly related to the DHFRs of Gram-negative bacteria, but upon cluster analysis appears to be related to those found in the Gram-positive genera *Staphylococcus*, *Bacillus*, and *Listeria* (146).

A different trimethoprim resistance gene, *dfrA1*, was identified on plasmid pCCK343 recovered from a porcine intestinal [*P.*] *aerogenes* isolate (130). This 5,415-bp plasmid contains a backbone sequence with homology to pHS-Tet, including the *mobC* gene (but not *mobAB*), with insertion of a partially truncated class 2 integron containing *dfrA1-sat2-aadA1*. In addition to conferring trimethoprim resistance (MIC, >256 mg/liter), the presence of the streptothricin acetyltransferase gene, *sat2*, and the aminoglycoside adenyltransferase gene, *aadA1*, conferred resistance to the streptothricin antibiotic nourseothricin (MIC, >256 mg/liter) and to streptomycin and spectinomycin (MICs of >256 mg/liter each), respectively. The truncated integron showed high identity with a sequence found in an *E. coli* ICE, AGI-5 (130).

Earlier studies of trimethoprim-resistant bovine *M. haemolytica* isolates from France revealed that this resistance was not associated with plasmids, and also was not transferable by conjugation. Hybridization experiments with gene probes specific for the genes *dfrA1* to *dfrA5* did not yield positive results (151). Similar negative PCR results were obtained for *A. pleuropneumoniae* and "*A. porcitonsillarum*" isolates in Switzerland, in which none of 27 different *dfrA* or *dfrB* genes—including *dfrA20*—could be identified using primers designed to detect groups of *dfr* genes (62). Recently, whole-genome sequencing was used to identify *dfrA14* in trimethoprim-resistant *A. pleuropneumoniae* isolates from the United Kingdom (122). This gene may have been responsible for trimethoprim resistance in the Swiss isolates of "*A. porcitonsillarum*" and *A. pleuropneumoniae* (62), if there was a failure to amplify a product using the primers designed to detect *dfrA5/dfrA14/dfrA25*. Alternatively, these isolates possess another mechanism for trimethoprim resistance that remains to be elucidated. The study by Bossé et al. (122) highlights the value of whole-genome sequencing for determination of the genetic basis of resistance, not only providing information regarding the presence of specific resistance genes and/or mutations, but also facilitating localization of the gene(s) within the chromosomal DNA or on plasmids.

In the United Kingdom isolates, two distinct mobilizable trimethoprim resistance plasmids were identified (122) in which the *dfrA14* gene was inserted into *strA*, as has been reported for enterobacterial plasmids pSTOJO1 and pCERC1 (126, 152). Differences in the gene order of flanking regions, with pM3224T carrying *sul2-ΔstrA-dfrA14-ΔstrA-strB* (Fig. 4) and pM3389T carrying *ΔstrA-dfrA14-ΔstrA-sul2*, suggest separate recombination of the *ΔstrA-dfrA14-ΔstrA* cassette (likely of enterobacterial origin) into different *Pasteurellaceae* plasmids.

Resistance to Macrolides

Many Gram-negative bacteria are believed to be innately resistant to macrolides due to permeability barriers or multidrug efflux pumps. However, chemical modification of the ribosomal target site by rRNA methylases or mutations in ribosomal proteins have also been described (109).

Macrolide Resistance Mediated by rRNA Methylases

Studies of the presence of macrolide resistance genes in bacteria of the genus *Actinobacillus* led to the identification of the rRNA methylase genes *erm*(A) and *erm*(C) in *A. pleuropneumoniae* (70). Mating experiments showed that these genes were transferred into *Moraxella catarrhalis* and/or *Enterococcus faecalis* (70). PCR-directed analysis of bovine *P. multocida* and *M. haemolytica* which exhibited MICs of erythromycin of ≥16 mg/liter did not detect any of the three genes *erm*(A), *erm*(B), or *erm*(C) (Kehrenberg and Schwarz, unpublished data). Matter and coworkers obtained similar results for *A. pleuropneumoniae* and "*A. porcitonsillarum*" isolates with MICs of tilmicosin of ≥32 mg/liter, in which none of the genes *erm*(A), *erm*(B), or *erm*(C) could be detected by PCR (62). Australian isolates of *A. pleuropneumoniae* with MICs of erythromycin of ≥16 mg/liter and/or MICs of tilmicosin of ≥32 mg/liter (40) also failed to amplify specific products for *erm*(A), *erm*(B), *erm*(C), *erm*(42), *mph*(E), *mef*(A), *msr*(A), or *msr*(E) (153). Furthermore, whole-genome sequencing of *A. pleuropneumoniae* HS 3572 (MIC of erythromycin of 16 mg/liter; MIC of tilmicosin of 32 mg/liter; MIC of tulathromycin of 16 mg/liter) revealed neither

specific macrolide resistance genes nor any known point mutations previously associated with macrolide resistance (153). In [*H.*] *parasuis*, a 7,577-bp plasmid, pFS39, has been reported to carry the *erm*(T) gene, previously only identified in Gram-positive bacteria, along with $bla_{\text{ROB-1}}$ (154). The [*H.*] *parasuis* isolate harboring pFS39 had MICs of 64 mg/liter for both erythromycin and lincomycin (154). A novel monomethyltransferase gene, *erm*(42), has been identified in the chromosome of *M. haemolytica* and *P. multocida* isolates with high levels of resistance to multiple macrolides (155–157). The sequence of this monomethyltransferase gene was found to be divergent from previously reported *erm* genes and was only detected by whole-genome sequencing of the resistant isolates.

Macrolide Resistance Mediated by Mutations in Ribosomal Proteins and 23S rRNA

Mutations in ribosomal proteins L4 or L22 have been reported in human macrolide-resistant *H. influenzae* (158). In isolates of *P. multocida* and *M. haemolytica* reported to be highly resistant (MICs >64 mg/liter) to multiple macrolides including erythromycin, tilmicosin, tildipirosin, tulathromycin, and gamithromycin, mutations were found in one of two locations (either A2058G or A2059G) in all six copies of the 23S rRNA (159). Similarly, genome sequencing revealed an A2059G transition in all six copies of the 23S rRNA gene in one isolate of [*H.*] *parasuis* (HS 315) with MICs of erythromycin of 64 mg/liter, tilmicosin of ≥128 mg/liter, and tulathromycin of 64 mg/liter; however, other isolates with MICs of erythromycin of 64 mg/liter, but lower MICs of tilmicosin and tulathromycin compared to HS 315, did not show this, or any other known, mutation associated with macrolide resistance (153).

Macrolide Resistance Mediated by Active Efflux or Inactivation by Phosphotransferases

It was shown that some isolates of *M. haemolytica*, *P. multocida*, and *H. somni* had genes, *mrs*(E) and *mph*(E), encoding macrolide efflux and phospotransferase proteins, respectively (57, 155–157). These two genes, found in tandem and expressed from the same promoter, were also predicted to be chromosomally encoded (155, 156). When found in combination with *erm*(42), the presence of the *mrs*(E)-*mph*(E) operon resulted in the highest level of resistance to all macrolides tested (tulathromycin, gamithromycin, tilmicosin, and clindamycin), whereas those with just the *mrs*(E)-*mph*(E) genes were not resistant to clindamycin and had lower MICs for tilmicosin but higher MICs for gamithromycin and tulathromycin compared to isolates with only the *erm*(42) gene (155, 156, 160). All three genes [*erm*(42), *mrs*(E), and *mph*(E)] were identified as part of ICE*Pmu1* (55), which might explain their dissemination across strain, species, and genus boundaries.

Resistance to Lincosamides

Resistance mechanisms to lincosamides may involve 23S rRNA methyltransferase, efflux proteins, inactivation enzyme genes, and mutations (e.g., 23S rRNA, L4 and/or L22 genes). Lincosamide transferase genes have been commonly detected among Gram-positive bacteria, and the *lnu*(F) gene has been detected in *Salmonella* and *E. coli* (http://faculty.washington.edu/marilynr/ermweb4.pdf). However, an *lnu*(C) gene, located on the 6.3-kb plasmid pHN61, was identified in a porcine [*H.*] *parasuis* isolate from China (161). The high MICs of lincomycin (32 mg/liter) and clindamycin (8 mg/liter) and the low MIC of erythromycin (0.25 mg/liter) indicates that *lnu*(C) mediates resistance to lincosamides but not to macrolides. The replication and mobilization region of pHN61 shares sequence similarity with that in pHB0503 from an *A. pleuropneumoniae* isolate.

Resistance to Phenicols

Resistance to nonfluorinated phenicols may be due to enzymatic inactivation by chloramphenicol acetyltransferases, whereas resistance to fluorinated and/or nonfluorinated phenicols can be mediated by phenicol-specific exporters (109, 162). Other mechanisms, such as permeability barriers, have rarely been detected among *Pasteurellaceae*.

Chloramphenicol resistance mediated by enzymatic inactivation

Resistance to chloramphenicol is usually due to enzymatic inactivation of the drug by chloramphenicol acetyltransferases. Two types of chloramphenicol acetyltransferases, A and B, specified by a number of different *catA* and *catB* genes, are currently known (162). The *catA* and *catB* genes are often located on plasmids, transposons, or gene cassettes. Plasmids mediating chloramphenicol resistance have been identified in porcine *P. multocida* isolates (113), bovine *P. multocida* and *M. haemolytica* isolates (163), porcine [*P.*] *aerogenes* (125), bovine *Mannheimia* spp. (116, 125), and porcine *A. pleuropneumoniae* (95, 106, 117, 164, 165). As previously seen with other resistance plasmids, those mediating chloramphenicol resistance are commonly less than 15 kb in size and occasionally carry additional resistance genes (Table 3). Initial molecular studies

of chloramphenicol resistance among *P. multocida* and *M. haemolytica* isolates included the detection of the three most frequently occurring *cat* genes among Gram-negative bacteria—*catA1*, *catA2*, and *catA3* (formerly known as *catI*, *catII*, and *catIII*)—by specific PCR assays (163). The *catA3* gene was detected on small plasmids of 5.1 kb, whereas the *catA1* gene was located on plasmids of either 17.1 or 5.5 kb (163). In porcine [*P.*] *aerogenes* and bovine *Mannheimia* isolates, *catA3* genes have been identified as parts of chromosomal or plasmid-borne resistance gene clusters (116, 125). In these gene clusters, the *catA3* gene was always inserted between the sulfonamide resistance gene *sul2* and the streptomycin resistance gene *strA* (116, 125) (Fig. 4). In pHB0503, a 15.1-kb plasmid isolated from *A. pleuropneumoniae*, this gene arrangement has been disrupted by insertion of *aacC2* and a truncated copy of an ATPase gene between *sul2* and *catA3* (95). A similar 15.7-kb plasmid from [*H.*] *parasuis*, pFZ51, contains the same resistance gene cluster as pHB0503 (accession number JN202624; Fig. 4, Table 3).

Although the *catA2* gene is commonly found in *H. influenzae* (166, 167), this gene has not yet been detected in chloramphenicol-resistant *Pasteurella*, *Mannheimia*, or *Actinobacillus*. However, a *catB2* gene, which codes for a different type of chloramphenicol acetyltransferase than the aforementioned *catA* genes (162), was found on plasmid pJR1 from avian *P. multocida* (76). The *catB2* gene is part of a gene cassette and thus needs the integron-associated promotor for its expression. In plasmid pJR1, the *catB2* cassette was located outside of an integron structure. Gene cassettes located at secondary sites outside of integrons may be expressed if a suitable promotor is available (126). However, such a promotor has not been identified in pJR1 (76).

Chloramphenicol and florfenicol resistance mediated by active efflux

So far, resistance to florfenicol, a structural analogue of thiamphenicol, has rarely been detected among *Pasteurellaceae*, except in Korea, where 34% of *A. pleuropneumoniae* isolates collected between 2006 and 2010 and 43% of isolates collected between 2010 and 2013 were resistant to florfenicol (73, 81). All of the isolates characterized by Yoo et al. (73) had MICs of florfenicol of between 8 and ≤64 mg/liter and carried the *floR* gene, which codes for an exporter of the major facilitator family that mediates the efflux of phenicols from the bacterial cell. Monitoring studies to specifically determine the MIC values of florfenicol among bovine and porcine respiratory tract pathogens showed that all *P. multocida*, *M. haemolytica*, and *A. pleuropneumoniae* isolates collected between 1994 and 2005 were susceptible to florfenicol (168–171; Schwarz, unpublished data). Data from the monitoring programs GE*RM*-Vet and BfT-GermVet, conducted in Germany, also confirmed that all *P. multocida* from pigs, poultry, dogs, and cats were susceptible to florfenicol (36, 172, 173), whereas 8.6% of *M. haemolytica* and 11.6% of *P. multocida* isolated from cattle in Canada and United States in 2009 were resistant (37).

The first report of a florfenicol-resistant bovine *P. multocida* isolate was published in 2005 (174). This isolate of capsular type A originated from a calf that had died from pneumonia in the United Kingdom. Florfenicol resistance was based on the presence of the gene *floR*. This gene was located on the 10,874-bp plasmid pCCK381 (Fig. 5). Interestingly, a florfenicol-resistant *S. enterica* subsp. *enterica* serovar Dublin isolate recovered from the same calf carried a plasmid indistinguishable from pCCK381. This observation, and the fact that plasmid pCCK381 was able to replicate and express florfenicol resistance in different *E. coli* hosts (174), strongly suggested that the plasmid pCCK381 can be exchanged between bacteria of different genera. Sequence analysis confirmed that this plasmid consists of three segments that show extended similarity to plasmids pDN1 from *Dichelobacter nodosus* (175), pMBSF1 from *E. coli* (176), and pRVS1 from *Vibrio salmonicida* (177). All these plasmids have been found either in bacteria such as *E. coli* from cattle and pigs, which have previously been shown to carry the *floR* gene along with a variable length ORF with homology to a *lysR* regulator gene, or in bacteria which cause diseases in fish and ruminants, such as cold-water vibriosis (*V. salmonicida*) or infectious pododermatitis (*D. nodosus*), for the control of which florfenicol is approved in several non-European Union countries. Although it is not possible to determine in retrospect where and when plasmid pCCK381 evolved, structural analysis suggested that this plasmid is most likely the result of interplasmid recombination processes (174). It is also interesting to note that pCCK381 has been isolated from both porcine and bovine isolates of *P. multocida* in Germany (178).

More recently, a number of smaller plasmids with *floR* as the only resistance gene have been identified in [*H.*] *parasuis* (pHPSF1), *M. haemolytica* (pMH1405), and *A. pleuropneumoniae* (pM3446F and p518) (179–182). Plasmids pMH1405 and pM3446F, both 7.7 kb, share extensive regions of identity with pCCK381, including the mobilization and replication genes, whereas the 6.3-kb pHPSF1 contains different *mob* and *rep* genes.

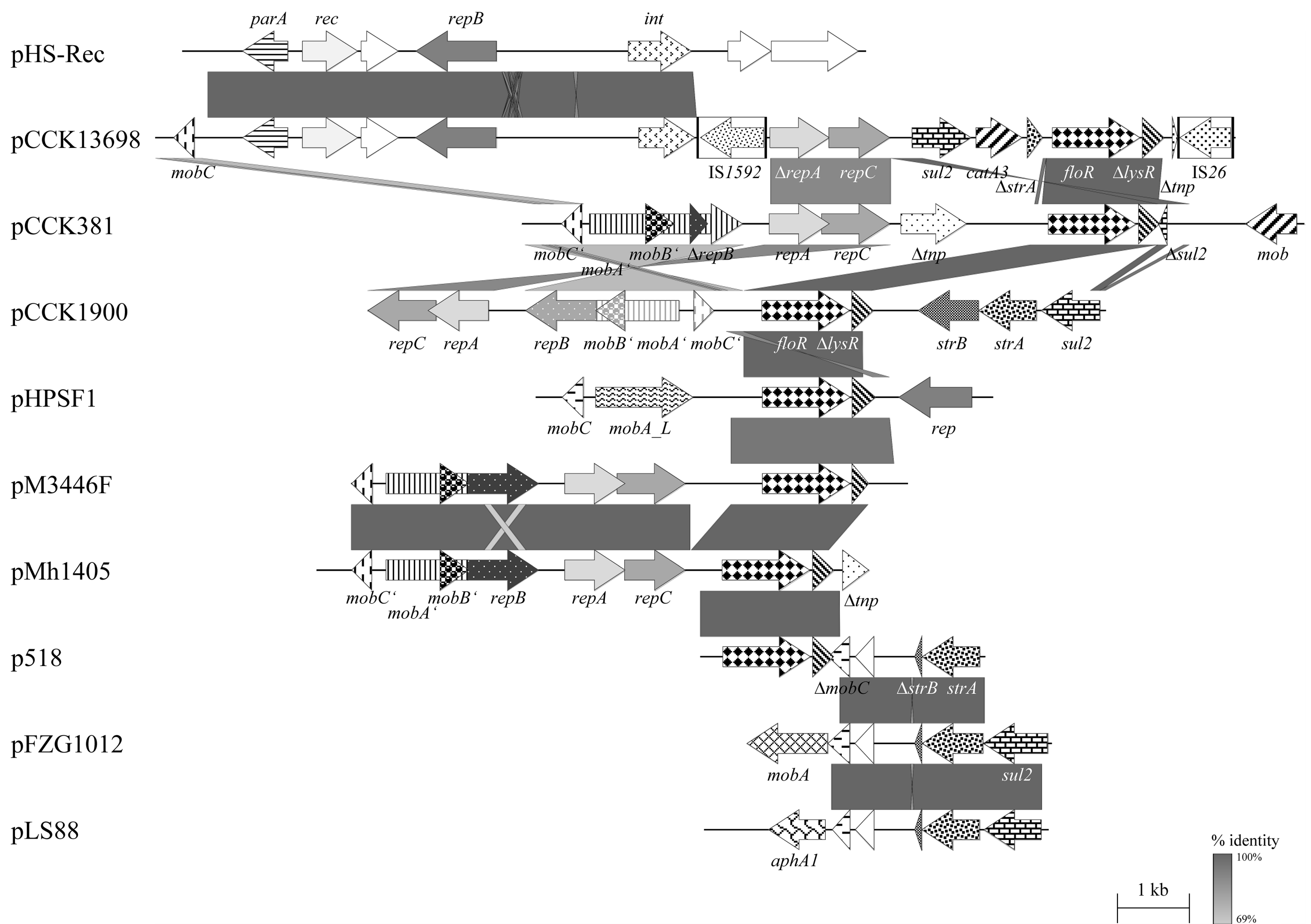

FIGURE 5 Schematic representation of the structure and organization of selected *floR*-based (multi-)resistance plasmids from *B. trehalosi* compared to an in-part-related plasmid from [*H.*] *parasuis*, and *A. pleuropneumoniae*, [*H.*] *parasuis*, *P. multocida*, and *sul2*-based (multi-)resistance plasmids from *H. ducreyi* and [*H.*] *parasuis*. Comparison of the maps of plasmids pCCK13698 (accession no. AM183225) from *B. trehalosi* and its in-part-related plasmid pHS-Rec (accession no. AY862436; 9,462 bp) from [*H.*] *parasuis*, pCCK381 (accession no. AJ871969; 10,874 bp) from *P. multocida*, pCCK1900 (accession no. FM179941; 10,226 bp) from *P. multocida*, pHPSF1 (accession no. KR262062; 6,328 bp) from [*H.*] *parasuis*, pM3446F (accession no. KP696484; 7,709 bp) from *A. pleuropneumoniae*, pMh1405 (accession no. NC_019260; 7,674 bp) from *M. haemolytica*, p518 (accession no. KT355773; 3,937 bp) from *A. pleuropneumoniae*, pFZG1012 (accession no. HQ015158; partially sequenced) from [*H.*] *parasuis*, and pLS88 (accession no. L23118; 4,772 bp) from *H. ducreyi*. Genes are shown as arrows, with the arrowhead indicating the direction of transcription. The following genes are involved in antimicrobial resistance: *sul2* (sulfonamide resistance), *strA* and *strB* (streptomycin resistance), *catA3* (chloramphenicol resistance), *floR* (chloramphenicol/florfenicol resistance), and *aphA1* (kanamycin/neomycin resistance); plasmid replication: *rep*, *repA*, *repB*, and *repC*; mobilization functions: *mobA*, *mobB*, *mobC*, and *mob*; transposition functions: *tnp*; recombinase or integrase functions: *rec* and *int*; DNA partition: *parA*; unknown function: open reading frames indicated by white arrows. The prefix Δ indicates a truncated functionally inactive gene. The boxes in the map of pCCK13698 indicate the limits of the insertion sequences IS*1592* and IS*26*; the arrows within these boxes indicate the reading frames of the corresponding transposase genes. Gray-shaded areas indicate the regions common to plasmids, and the different shades of gray illustrate the percentages of nucleotide sequence identity between the plasmids, as indicated by the scale at the bottom of the figure. A distance scale in kilobases is shown.

The smallest plasmid, the 3.9-kb p518, shares the *floR*-*lsyR* region (high sequence identity) with pMh1405, and the remaining region, containing Δ*mobC* and *strA*-Δ*strB* sequences, with [*H.*] *parasuis* plasmid pFZG1012 (Fig. 5).

The *floR* gene has also been found on multiresistance plasmids pCCK13698 and pCCK1900 (178, 183), and it is part of ICE*Pmu1* and ICE*Pmu1*-related ICEs detected in *M. haemolytica* and *H. somni* (55). The

14,969-bp pCCK13698 plasmid was identified in a bovine isolate of *Bibersteinia trehalosi* (184) which originated from a calf that had died from a respiratory tract infection in France (183). The 10.2-kb pCCK1900 plasmid was recovered from a porcine *P. multocida* isolate in Germany (178). Both plasmids differ structurally from plasmid pCCK381 and from each other (Fig. 5). While in pCCK13698, the *floR-lysR* sequence is located downstream of the complete genes *sul2* and *catA3*, and a truncated *strA* streptomycin resistance gene (183), in plasmid pCCK1900, the *floR-lysR* genes are found downstream of (and in the opposite orientation to) complete *sul2*, *strA*, and *strB* genes (Fig. 5). Moreover, plasmid pCCK13698 shares almost 6.8 kb of identity with the [*H.*] *parasuis* plasmid pHS-Rec (75) and harbors two insertion sequences, IS*1592* and IS*26* (183). In contrast, pCCK1900 appears to have arisen by insertion of the *floR-lysR* genes into the broad-host-range plasmid RSF1010 (178). Such analysis reveals a high level of involvement of the region *floR-lysR* in interplasmid recombination events. In ICE*Pmu1*, *floR* is found as part of "resistance region 1," a 15,711-bp sequence bracketed by copies of IS*Apl1*, an insertion sequence initially identified in *A. pleuropneumoniae* (185). This region carries *aphA1*, *strA*, *strB*, *sul2*, *floR*, and *erm*(42), with the *floR-lysR* genes found to be bracketed by a truncated and a complete IS*CR2* element (55).

Resistance to (Fluoro)quinolones

Quinolones are broad-spectrum antimicrobial agents that inhibit bacterial DNA gyrase and topoisomerase IV. Resistance is commonly due to mutational alterations in the genes coding for the different subunits of both enzymes but is also due to active efflux or protection of the enzymes by Qnr proteins (109).

(Fluoro)quinolone resistance mediated by target site mutations

Very little is known about (fluoro)quinolone resistance in *Pasteurella*, *Mannheimia*, *Actinobacillus*, or *Haemophilus*. Studies of *Pasteurella* spp. from humans and animals (36, 172, 173, 186) and *A. pleuropneumoniae* (62) identified virtually all isolates to be highly susceptible to the fluoroquinolones tested. The first report describing the analysis of the quinolone resistance determining region (QRDR) of the proteins encoded by the genes *gyrA* and *parC* in *P. multocida* isolates identified a Ser83Ile alteration in GyrA in an isolate that had a nalidixic acid MIC of 256 mg/liter, whereas Asp87Gly alterations were detected in isolates with nalidixic acid MICs of 4 and 12 mg/liter (187). None of these isolates exhibited resistance to fluoroquinolones. Whole-genome sequencing of the multiresistant bovine *P. multocida* isolate 36950, exhibiting an enrofloxacin MIC of 2 mg/liter, led to the identification of two base pair exchanges in the QRDR of *gyrA*, which resulted in amino acid alterations GGT → AGT (Gly75Ser) and AGC → AGA (Ser83Arg). In addition, a single base pair exchange in the QRDR of *parC*, TCA → TTA, which resulted in a Ser80Leu exchange, was also seen (55).

More recently, fluoroquinolone-resistant clinical isolates of *P. multocida*, with MICs of 0.5 mg/liter for both enrofloxacin and ciprofloxacin, were found to have Asp87Asn or Ala84Pro mutations in GyrA (188). In isolates further selected for resistance by passage on subinhibitory concentrations of fluoroquinolones, multiple mutations in *gyrA*, *gyrB*, and *parC*, but not *parE*, were found to be associated with high-level fluoroquinolone resistance (MICs, >4 mg/liter). Similarly, in *M. haemolytica*, resistance to nalidixic acid was associated with at least one amino acid substitution in one or both of GyrA and ParC, whereas all of the isolates with fluoroquinolone MICs ≥8.0 mg/liter had two mutations in GyrA and one additional change in ParC (189). A study of enrofloxacin-resistant *A. pleuropneumoniae* isolates in Taiwan (190) identified various mutations in *gyrA*, *parC*, and *parE*, with resistant isolates carrying at least one mutation in the QRDR of *gyrA*, resulting in amino acid changes at codon 83 or 87. Functional efflux pumps were also found to contribute to enrofloxacin resistance in these isolates, as demonstrated by addition of an efflux pump inhibitor (190). In a recent study comparing antimicrobial resistance profiles and the presence of resistance genes in whole-genome sequences of *A. pleuropneumoniae* (9), only a slightly elevated enrofloxacin MIC of 0.25 mg/liter was detected in a limited number of isolates, and in all cases this resistance was associated with the GyrA Ser83Phe substitution described by Wang et al. (190).

In a survey of 115 [*H.*] *parasuis* isolates collected from South China between 2008 and 2010, 20 were identified with high levels of resistance to nalidixic acid (MICs ≥128 mg/liter) as well as resistance to enrofloxacin and other fluoroquinolones (MICs ≥4 mg/liter), and these were shown to have at least one mutation in the *gyrA* gene causing changes at codon 83 or 87 (191). In another survey, of 138 [*H.*] *parasuis* isolates from China between 2002 and 2009, 60% were found to be resistant to enrofloxacin, and sequencing of PCR products demonstrated 10 point mutations in the quinolone QRDR regions of *gyrA*, *gyrB*, *parC*, and *parE* contributing to varying degrees of enrofloxacin resistance (192).

However, the mutation causing the amino acid change Asp87Asn was common to all resistant isolates. This study further showed, by site-directed mutagenesis, that point mutations leading to three amino acid changes (Asp87Asn in GyrA, Ser73Arg in ParC, and Thr551Ala in ParE) were specifically involved in fluoroquinolone resistance (192).

(Fluoro)quinolone resistance mediated by other mechanisms

The AcrAB efflux pump has been shown in *Enterobacteriaceae* to be able to export fluoroquinolones from the cell (193). Although the use of efflux inhibitors has demonstrated a contribution of efflux pump(s) to fluoroquinolone resistance in *A. pleuropneumoniae* and *P. multocida* (188, 190), the specific proteins involved were not identified. A PCR survey of 115 Chinese [*H.*] *parasuis* isolates detected quinolone resistance genes *qnrA1*, *qnrB6*, and *aac(6′)-Ib-cr* in three, one, and three isolates, respectively (191). To date, this is the only report of acquired quinolone resistance genes in the *Pasteurellaceae*.

DISSEMINATION, COSELECTION, AND PERSISTENCE OF RESISTANCE GENES IN *PASTEURELLA*, *MANNHEIMIA*, *ACTINOBACILLUS*, *HAEMOPHILUS*, AND *HISTOPHILUS*

Molecular analysis of isolates of *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* revealed that, in many cases, antimicrobial resistance genes are associated with plasmids. Most of the resistance plasmids identified to date are <15 kb in size and are nonconjugative, though many carry *mob* genes (Table 3) (Fig. 1 to 5) and have been shown to be mobilizable. For these plasmids to be horizontally transferred by conjugation, the genes encoding the conjugation machinery must be supplied *in trans*. The only conjugative elements so far identified in members of the *Pasteurellaceae* are ICEs, including those recently described in *P. multocida*, *M. haemolytica*, *H. somni*, and *A. pleuropneumoniae* (55–57, 74). These ICEs are likely, along with others yet to be identified, be the source of the genes required for conjugal transfer of the smaller mobilizable plasmids identified in these species.

The number of plasmids for which complete sequences are available continues to grow. Currently, there are complete sequences for 60 plasmids carrying antimicrobial resistance genes (Table 3). In addition, the resistance gene regions of several other plasmids (also listed in Table 3) have been sequenced. Despite the relatively small size of these plasmids, many of them carry two or more resistance genes (Table 3, Fig. 3 to 5). To date, there are no sequences for resistance plasmids from *Histophilus* isolates in GenBank (last accessed October 27, 2017). Sequence analysis has provided insights into (i) the genes involved in antimicrobial resistance of *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* and their organization; (ii) the structural relationships between the resistance plasmids; and (iii) mechanisms resulting in the formation of multiresistance plasmids and plasmid-borne multiresistance gene clusters.

Some of the genes accounting for antimicrobial resistance in *Pasteurella*, *Mannheimia*, *Haemophilus*, *and Actinobacillus*, such as the tetracycline resistance gene *tet*(H) and the β-lactamase gene $bla_{\mathrm{ROB-1}}$, appear to be more commonly found among *Pasteurellaceae*. In contrast, other resistance genes, such as the streptomycin resistance genes *strA-strB*, have been detected in a wide range of Gram-negative bacteria. The fact that these genes are associated with the transposon Tn*5393* (124), and have also been found on two mobilizable broad-host-range plasmids, pLS88 (134) and RSF1010 (147), might explain their widespread occurrence, which is likely due to horizontal gene transfer.

The location of resistance genes on mobile genetic elements allows their spread into bacteria of other species and genera. This has been confirmed by the detection of highly similar plasmids in different host bacteria, such as the *tet*(H)-carrying plasmid pMHT1, which was found in *P. multocida*, *M. haemolytica*, *M. varigena*, and *M. glucosida* (64), and the *sul2-strA*-carrying plasmid pPMSS1, which was identified in *P. multocida*, [*P.*] *aerogenes*, and *M. haemolytica* (125). Several of these small plasmids carry *mob* genes which allow mobilization in the presence of a conjugative element. Mobilization has also been confirmed for the 8.1-kb *sul2-strA*-carrying plasmid pMS260 from *A. pleuropneumoniae* (121). This plasmid, which closely resembles the broad-host-range plasmid RSF1010 (147), proved to be mobilizable into a wide variety of respiratory tract pathogens including *P. multocida*, *Bordetella bronchiseptica*, and *Pseudomonas aeruginosa*, as well as other isolates of *A. pleuropneumoniae* (119). Moreover, most of the small resistance plasmids found in *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* are able to replicate and express their resistance properties in *E. coli*. On the other hand, most resistance plasmids originating from the *Enterobacteriaceae* and harboring either *catA3*, *strA-strB*, *tet*(B), or *sul2*, usually cannot replicate

in *Pasteurella*, *Mannheimia*, *Actinobacillus*, or *Haemophilus* hosts. With the exception of the RSF1010-like plasmid pMS260 from *A. pleuropneumoniae* (121), analysis of the regions flanking the *sul2* and *strA-strB* genes in plasmids of *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* revealed sequences similar not to those of *Enterobacteriaceae* plasmids, but rather to those of plasmids known to occur in *Pasteurellaceae*. This observation suggests that, most likely, recombination events between endogenous plasmids of *Pasteurellaceae* and resistance plasmids from other bacterial sources, which may be replication-deficient in *Pasteurellaceae*, have occurred. As a result, the horizontally acquired resistance genes became inserted into new plasmidic replicons which then have been stably maintained in *Pasteurellaceae*. Studies of the plasmid-borne *sul2-strA* gene cluster in *Pasteurella* and *Mannheimia* showed that the noncoding spacer region between the two resistance genes may represent the target site for further recombination events (116, 125). So far, a *catA3* gene and the entire *tetR-tet*(H) region, respectively, have been found to be integrated between the genes *sul2* and *strA* in different plasmids (59, 116).

Based on the observation that many of the resistance plasmids so far detected in *Pasteurella*, *Mannheimia*, and *Actinobacillus* carry more than one resistance gene, coselection of these resistance genes is likely. Once such a plasmid is transferred to a new host, all resistance genes located on it are also transferred. As a consequence, a new host bacterium gains resistance to two or more antimicrobial agents, or classes of antimicrobial agents, by the acquisition of a single small plasmid. In these cases, selective pressure imposed by the use of one such antimicrobial agent, e.g., tetracyclines, is sufficient to favor the exchange of the multiresistance plasmid. The location of different resistance genes on the same plasmid also enables their persistence, particularly if the resistance genes are organized in a cluster. A multiresistance gene cluster in which the genes *sul2*, *catA3*, and *strA* are organized as a transcriptional unit has been identified on several plasmids, as well as in chromosomal DNA of [*P.*] *aerogenes* and several *Mannheimia* spp. (116, 125). It is highly unlikely that individual genes from such a cluster are lost. A study of the location of chloramphenicol resistance genes in *Pasteurella* and *Mannheimia* isolates from Germany showed that the dominant chloramphenicol resistance gene *catA3* was located in a *sul2-catA3-strA* cluster in all cases, except for a single bovine *M. glucosida* isolate (125). Although chloramphenicol has been prohibited for use in food-producing animals in the European Union and the United States for many years, aminoglycosides, including streptomycin, as well as sulfonamides are still used. Thus, streptomycin and/or sulfonamides might present the selective pressure that ensures the maintenance of the entire cluster. Thus, clusters such as that containing the genes *sul2-catA3-strA* may play an important role in maintaining resistance genes without direct selective pressure.

Analysis of the resistance genes, their location, and their organization provides insight into (i) the gene pool to which bacteria of the genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* have access; (ii) horizontal transfer processes which play a key role in the dissemination of the resistance genes between and beyond bacteria of the four aforementioned genera; and (iii) integration and recombination events which are of major importance for the development of novel resistance plasmids and the formation of multiresistance gene clusters in *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* isolates.

CONCLUDING REMARKS

Isolates of several genera in the family *Pasteurellaceae* cause a number of economically important diseases in cattle, swine, and other food-producing animals. Due to the multifactorial and polymicrobial nature of some of the infections in which *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Histophilus* isolates are involved, prevention is—except in confined production systems with high biosecurity—a cumbersome task that often yields unsatisfying results. Despite hygienic measures, improved management and the use of vaccines, antimicrobial agents are indispensable tools for the control of infections in which bacteria of the family *Pasteurellaceae* are involved. Increasing numbers of resistant, or multiresistant, isolates are reducing the efficacy of antimicrobial agents currently approved for use in animals. It is anticipated that in the near future, there will be no new classes of antimicrobial agents approved for use in veterinary medicine. Thus, veterinarians will have to rely on currently available antimicrobial agents. To retain their efficacy, prescription and administration of antimicrobial agents should be undertaken with discretion supported by an accurate diagnosis, a careful choice of the antimicrobial agent(s), and the most appropriate dosing regimen. Imprudent use of antimicrobials bears a high risk of selecting resistant bacteria.

Many of the resistance genes known to be present in *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* are associated with plasmids or transposons and

thus may be exchanged horizontally, not only between bacteria of the family *Pasteurellaceae*, but also with other Gram-negative bacteria. This update on the molecular basis of antimicrobial resistance illustrates that *Pasteurella*, *Mannheimia*, *Actinobacillus*, and *Haemophilus* have obviously acquired a number of resistance genes from other Gram-negative, or maybe even Gram-positive, bacteria. Knowledge of the location and colocation of the resistance genes on mobile genetic elements, as well as the conditions for their coselection and persistence, will be valuable information for veterinarians and will assist them in selecting the most efficacious antimicrobial agents for control of isolates of the family *Pasteurellaceae*. Even though the current susceptibility status of *Pasteurellaceae* from infections in animals looks rather favorable, continuous monitoring of the antimicrobial susceptibility of *Pasteurellaceae* is required for early detection of changes in the susceptibility status and for analyzing the respective isolates for newly acquired/ developed resistance genes and resistance-mediating mutations.

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