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Antimicrobial Resistance in *Bordetella bronchiseptica*

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ABSTRACT *Bordetella bronchiseptica* is involved in respiratory tract infections mainly in dogs and pigs but may also cause infections in humans. Valid and representative data on antimicrobial susceptibility of *B. bronchiseptica* is rare. Approved antimicrobial susceptibility testing methods have been published, but very few clinical breakpoints are available. The MIC values are low for most agents but high for β-lactam antibiotics and macrolides. Information on the genetic basis of resistance is scarce. For a small number of isolates that are resistant or show elevated MICs, the molecular basis of resistance was identified. Three tetracycline resistance genes, *tet*(A), *tet*(C), and *tet*(31), coding for major facilitator superfamily efflux pumps, were identified. Two other major facilitator superfamily exporter genes confer resistance to chloramphenicol (*cmlB1*) or to chloramphenicol and florfenicol (*floR*). Two class B chloramphenicol acetyltransferase genes (*catB1* and *catB3*), which confer resistance to nonfluorinated phenicols by enzymatic inactivation, have been identified in *B. bronchiseptica*. Like the trimethoprim resistance genes *dfrA1* and *dfrB1*, which code for trimethoprim-insensitive dihydrofolate reductases, the genes *catB1* and *catB3* were located on gene cassettes and found in class 1 integrons also harboring the sulfonamide resistance gene *sul1*. In addition, the gene *sul2* has also been detected. Both *sul1* and *sul2* code for sulfonamide-insensitive dihydropteroate synthases. A gene cassette harboring the β-lactamase gene bla_{OXA-2} was also identified, whereas β-lactam resistance in *B. bronchiseptica* seems to be more likely due to reduced influx in combination with the species-specific β-lactamase encoded by bla_{BOR-1}. The resistance genes were mostly located on conjugative plasmids.

BORDETELLA BRONCHISEPTICA

B. bronchiseptica is a bacterium within the phylum *Proteobacteria* and the class *Betaproteobacteria*. It belongs to the order *Burkholderiales* and the family *Alcaligenaceae*. In the genus *Bordetella*, *B. bronchiseptica* is one of 14 approved species (http://www.bacterio.net/bordetella.html). *B. bronchiseptica* is a small, coccoid-shaped Gram-negative bacterium with a size of about 0.2 to 0.5 μm by 0.5 to 2 μm. It is motile due to peritrichous flagella. In comparison to other *Bordetella* spp., its nutritional requirements are simple, and it grows on blood agar plates at 35 to 37°C overnight. The colonies are small, grayish-white, smooth, and shiny, usually without or only with a small zone of hemolysis.

B. bronchiseptica is a commensal of the upper respiratory tract of diverse animal species, including mammals and birds. In veterinary medicine, it also plays an important role as a primary and secondary pathogen of the upper respiratory tract in several mammals but is most important and best described in dogs and in pigs. In contrast, *Bordetella pertussis*, the causative agent of whooping cough in humans, has rarely been reported in other mammals. Experimental infections showed that rhesus macaques and baboons can develop clinical disease (1), and at least one case of an epizootic of whooping cough among chimpanzees in a zoo has been described (2). *Bordetella avium* is commonly identified in birds, although *B. avium* infections have also been described in single human patients with cystic fibrosis (3, 4).

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Clinical Relevance

B. bronchiseptica is a facultative respiratory tract pathogen and causes respiratory tract infections in mammals (5). In general, clinical infections caused by *B. bronchiseptica* require additional factors (infectious or noninfectious stressors) and can be seen as multifactorial diseases (6). In addition to other bacteria or viruses, for example, transport and crowding are accompanying factors in the porcine respiratory disease complex. In the clinical scenario, *B. bronchiseptica* may be a primary pathogen and pave the way for other respiratory tract pathogens such as *Pasteurella multocida*. This is commonly the case in one of the major diseases associated with *B. bronchiseptica* in pigs: atrophic rhinitis. A mild form of atrophic rhinitis is seen when *B. bronchiseptica* is the only pathogen, whereas a progressive and much more severe form is seen when *P. multocida* is involved (7, 8). In dogs, *B. bronchiseptica* may also act as a secondary pathogen in the kennel cough complex, also known as canine infectious tracheobronchitis. In kennel cough, canine parainfluenza virus is considered the major pathogen and may pave the way for a subsequent *B. bronchiseptica* infection.

Zoonotic Potential

The vast majority of patients suffering from a clinical infection with *B. bronchiseptica* are either very young or old. Rarely, reports can be found with patients in the age group of 10 to 50 years; commonly, people in that age group and infected by *B. bronchiseptica* are immunocompromised, such as a 43-year-old man who was HIV-positive (9, 10) and an 11-year-old girl suffering from cystic fibrosis (11). However, contact with infected animals may also play a role in human *B. bronchiseptica* infections (12). The patients show respiratory symptoms, such as sinusitis, tracheobronchitis, or a pertussis-like cough (13). Septicemia and meningitis have been also described (14, 15).

Prophylaxis and Therapy

On the one hand, vaccination is available for small animals, especially for dogs. Kennel cough vaccines comprising either *B. bronchiseptica* alone or *B. bronchiseptica* and canine parainfluenza virus type 2 are commercially available, the former available as an injectable vaccine and the latter as an injectable vaccine or a vaccine for intranasal application. For cats, only an intranasal vaccine against *B. bronchiseptica* is available. For rabbits, an injectable vaccine against *B. bronchiseptica* and *P. multocida* is on the market. In pigs, autogenous vaccination is used.

On the other hand, in addition to symptomatic treatment, a treatment with antimicrobial agents is a good and successful option to treat *B. bronchiseptica* infections. This prevents additional complications or additional secondary bacterial infections but does not help against other components of the multifactorial disease complexes. Thus, it is important to also reduce viral and environmental stressors. Because these respiratory diseases are highly contagious, it is also helpful to avoid contact of diseased animals with healthy animals. Such a quarantine is likely possible for pets but difficult if not impossible for pigs and rabbit breeding units, because *B. bronchiseptica* has already spread between animals before they show the first clinical signs of disease.

In human patients also, *B. bronchiseptica* infections can be treated with antimicrobial agents. However, in human medicine, the correct identification of *B. bronchiseptica* is the major problem, because this bacterium is not a common human pathogen. Of note, the very common use of β-lactams as first-choice antibiotics does not lead to therapeutic success in *B. bronchiseptica* infections. In contrast, in combination with a β-lactamase inhibitor, this treatment was successful (16). However, most patients with clinical infections have other severe underlying diseases hampering the treatment and leading to the critical situations described in the few case reports available (13, 17).

ANTIMICROBIAL SUSCEPTIBILITY OF *B. BRONCHISEPTICA*

Antimicrobial susceptibility testing prior to the treatment of clinical *B. bronchiseptica* infections is of major relevance in both human and veterinary medicine. To predict the success or failure of an antimicrobial therapy, the correct *in vitro* determination of the antimicrobial susceptibility of the *B. bronchiseptica* isolates is of utmost importance.

Antimicrobial Susceptibility Testing Methods

An internationally accepted testing procedure is available from the Clinical and Laboratory Standards Institute (CLSI) (18, 19). *B. bronchiseptica* isolates can be tested by agar disk diffusion or by determining the MIC by agar dilution or by broth micro- or macrodilution. For this, the standard procedure as described for fast-growing aerobic bacteria in CLSI document VET01-S (19) should be applied. The inoculum can be prepared by either the growth method or the direct colony suspension method and should be equivalent to a 0.5 McFarland standard. Incubation should be for 16 to

20 h at 35°C ± 2°C in ambient air. The CLSI-approved media are Mueller-Hinton agar for disk diffusion and agar dilution as well as cation-adjusted Mueller-Hinton broth for broth dilution assays. *Escherichia coli* ATCC 25922 or *Staphylococcus aureus* ATCC 25923 (disk diffusion)/ATCC 29213 (MIC determination) are recommended as quality controls (19). However, it has been reported that an increase of the incubation time to 24 h may be advantageous. The authors of this study showed that the MIC values of ten isolates determined in five replicates were more stable when read after 24 h incubation time, although the classification of the isolates as susceptible, intermediate, or resistant did not change (20).

Clinical Breakpoints

CLSI document VET01-S is currently the only antimicrobial susceptibility testing document that contains approved clinical breakpoints specific to *B. bronchiseptica* (19). However, breakpoints are only available for a few agents, namely, for ampicillin (test result can be extrapolated to amoxicillin and hetacillin), florfenicol, tildipirosin, and tulathromycin. For tulathromycin, it is worthwhile to mention that it is absolutely essential to stick to the prescribed pH value to end up with correct results for *B. bronchiseptica* as well as for other bacteria. Breakpoints are available for disk diffusion and for MICs determined by broth dilution or agar dilution for florfenicol, tildipirosin, and tulathromycin (19). In contrast, there are only MIC breakpoints for ampicillin (19, 21). Since *B. bronchiseptica* is commonly resistant to ampicillin, these breakpoints serve the diagnostic laboratory mainly to exclude ampicillin and related antimicrobial agents of the β-lactam class from treatment recommendations.

Epidemiological Cutoff Values

An interpretation of the susceptibility testing results by using epidemiological cutoff values is even more difficult. Epidemiological cutoff values for *B. bronchiseptica* had been available solely for trimethoprim-sulfamethoxazole on the EUCAST homepage and have been removed in the meantime (https://mic.eucast.org/Eucast2/). The only data still shown on the website are tetracycline MICs without giving an epidemiological cutoff value (https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=791). In contrast to other MIC values, the MICs for trimethoprim-sulfamethoxazole show a very wide range for *B. bronchiseptica* isolates, for example, comprising more than all 12 dilution steps tested from ≤0.03 mg/liter to ≥64 mg/liter (22). In comparison, tetracycline MICs of the same 349 isolates were distributed from ≤0.12 mg/liter to 2 mg/liter representing the wild-type population, with the vast majority of isolates (n = 227) having a MIC of 0.25 mg/liter (22). Three isolates showed a distinctly higher MIC of 64 mg/liter and were considered non-wild type and were later shown to harbor a specific tetracycline resistance gene (23). A very similar situation is seen on the EUCAST website, with 443 isolates distributed normally from 0.12 mg/liter to 4 mg/liter and 4 isolates showing higher MICs of 64 mg/liter (https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=791). Tetracycline MIC distributions are compared in Table 1.

Published Monitoring Studies

Collected information about antimicrobial susceptibility testing studies in a PhD thesis (23) shows that a direct comparison of susceptibility data is very difficult to accomplish due to different methodologies used. In the corresponding study, MIC determination followed the CLSI-approved antimicrobial susceptibility testing protocol (22). This study showed an overall favorable situation with low MIC values and no change in MICs over a period of 4 years among 349 porcine *B. bronchiseptica* isolates from pigs. More recent publications, however, were performed basically—but not exactly—according to the CLSI standard (24, 25). Commonly, in routine diagnostics as well as in some publications, such as a study of *B. bronchiseptica* isolates from Poland (26), disk diffusion is performed. While in the case of MIC determination, MIC distributions are often shown, the distribution of inhibitory zone diameters is not provided, and results are only given as percentages of isolates classified as susceptible, resistant, or (if available) intermediate. Due to the lack of approved breakpoints, these results have to be used with caution. Distributions of MIC values for florfenicol are shown in Table 2. While clinical breakpoints are available for florfenicol and often the testing range is reduced to the dilution steps of clinical interest, for tetracycline, a wider test range is applied. The tetracycline MICs show a clear bimodal distribution, with most of the isolates having an MIC value around 0.25 or 0.5 mg/liter and single isolates showing MICs of 16 mg/liter or higher (Table 1). For the florfenicol MICs, a bimodal distribution is not so clear, which is not due to the shorter testing ranges (Table 2). Isolates with an MIC in the upper range of the normal

TABLE 1 Tetracycline MIC distributions of *B. bronchiseptica* isolates

Origin	Country	Year of isolation	No. of isolates	No. of isolates with an MIC of ... mg/liter[a]												Reference
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	
Pigs	Germany	2011/2012	90	n.t.	n.t.	0	1	52	27	5	3	0	0	0	2	0	27
Companion animals[b]	Germany	2010–2012	43	n.t.	n.t.	1	14	22	4	1	0	0	0	0	1	0	24
Pigs	Germany	2010-2012	107	n.t.	n.t.	2	51	37	4	5	0	0	0	1	4	3	24
Pigs	Europe[c]	2010–2012	118	n.t.	0	0	42	52	9	7	4	1	0	0	0	3	25
Pigs	Germany	2010	43	n.t.	n.t.	0	4	24	10	4	1	0	0	0	0	0	26
Dogs (n = 8), cats (n = 5)	Germany	2010	13	n.t.	n.t.	0	0	9	2	1	1	0	0	0	0	0	60
Pigs	Germany	2009	69	n.t.	n.t.	0	9	51	3	2	1	0	0	0	0	3	29
Pigs	Germany	2008	93	n.t.	n.t.	0	49	35	5	1	0	0	1	1	1	0	30
Dogs (n = 34), cats (= 8)	Germany	2004–2006	42	0	0	0	13	18	3	6	1	0	0	0	1	0	39
Pigs	Germany	2003	82	n.t.	n.t.	8	60	11	2	1	0	0	0	0	0	0	22
Pigs	Germany	2002/2003	138	n.t.	n.t.	9	99	23	3	2	0	0	0	0	2	0	58
Pigs	Germany	2002	91	n.t.	n.t.	6	63	17	1	1	0	0	0	0	3	0	22
Pigs	Germany	2001	98	n.t.	n.t.	5	65	27	1	0	0	0	0	0	0	0	22
Pigs	Germany	2000	78	n.t.	n.t.	29	39	7	3	0	0	0	0	0	0	0	22

[a]n.t., not tested.
[b]Horses (n = 24), dogs (n = 8), rabbits (n = 8), cats (n = 2), ferret (n = 1).
[c]Belgium (n = 24), Denmark (n = 9), France (n = 12), Germany (n = 14), The Netherlands (n = 22), Poland (n = 14), Spain (n = 21), United Kingdom (n = 2).

distribution around the MIC values of 1 to 4 mg/liter have to be classified as intermediate or even as resistant according to the clinical breakpoints.

Another fact reducing the information on susceptibility of *B. bronchiseptica* is that often several agents of the same class are tested (24, 25, 27–30). Moreover, two classes licensed and used to treat respiratory tract infections are most commonly included in the panel for *B. bronchiseptica*: β-lactams and macrolides. Both classes are not useful against *B. bronchiseptica*, and *in vitro* susceptibility testing revealed high MIC values and—when breakpoints were available—100% resistant isolates (24, 25, 27–30). For other antimicrobial agents, the studies shown in Table 1 confirm the favorable situation with respect to susceptibility and resistance of *B. bronchiseptica*.

ANTIMICROBIAL RESISTANCE IN *B. BRONCHISEPTICA*

In general, little information concerning antimicrobial resistance in *B. bronchiseptica* is available from the published literature. As a facultative pathogen in a genus of bacteria that harbors human pathogens (*B. pertussis*) as well as apathogenic species (e.g., *Bordetella tumbae*), several publications focused on virulence factors and the pathogenicity of bordetellae including *B. bronchiseptica* (31). Other studies dealt with immunity and vaccination strategies (32). In fact, a *B. bronchiseptica* vaccine was one of the first antibacterial vaccines and started as a temperature-sensitive vaccine for intranasal application (33). Treatment of clinical *B. bronchiseptica* infections is easily possible with the appropriate antimicrobial agents licensed for food-producing animals. Commonly, tetracyclines are used, and more than 90% of all *B. bronchiseptica* isolates show low tetracycline MIC values (https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=791; 22, 34, 35). Overall, due to the favorable situation in terms of antimicrobial susceptibility, treatment problems are rare, and a search for alternative agents is often not necessary.

All three antimicrobial resistance mechanisms—enzymatic inactivation of the antimicrobial agent, reduced intracellular accumulation, and target site modifications—have been identified and described in *B. bronchiseptica* isolates.

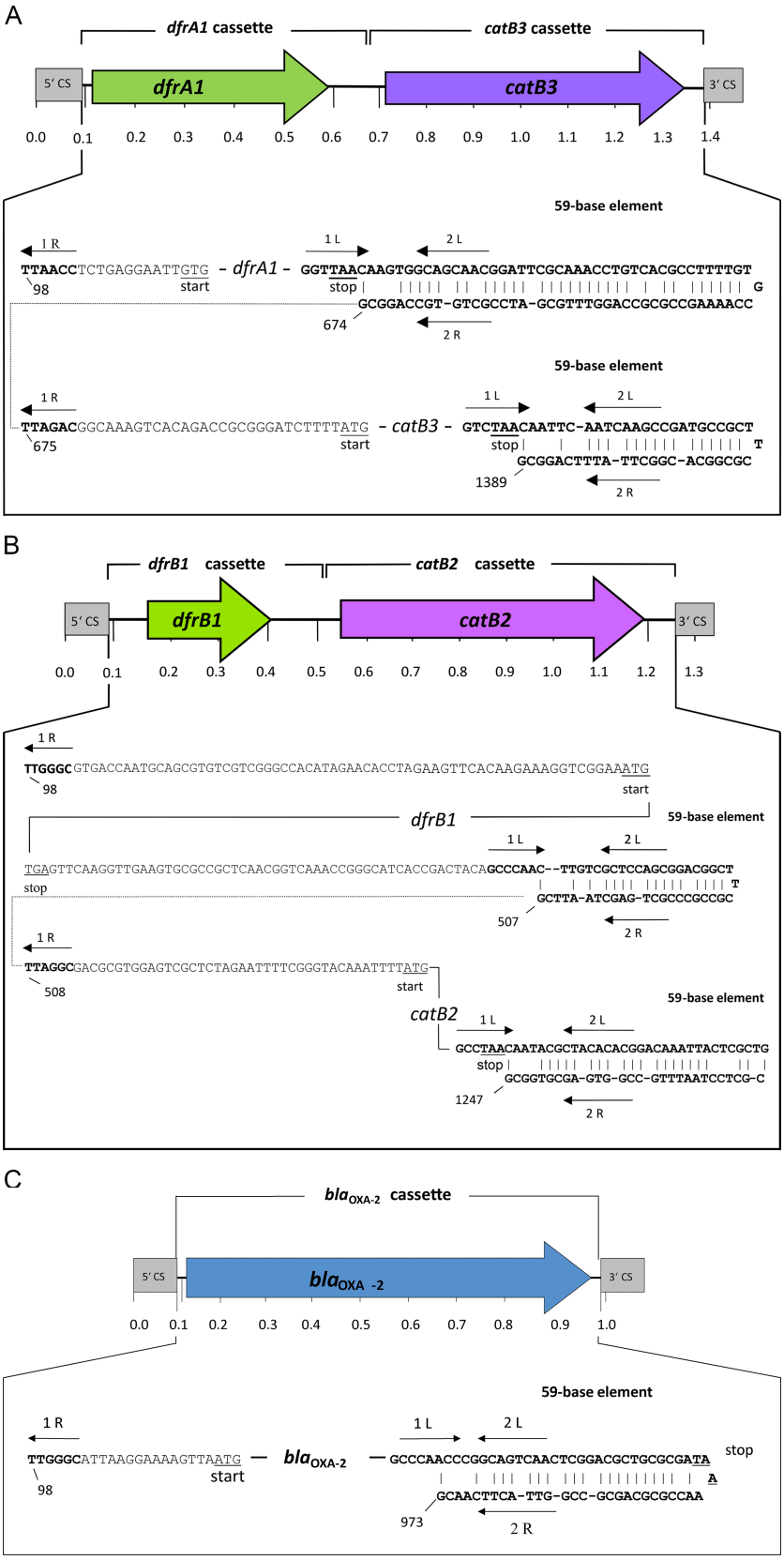
Tetracycline Resistance

High tetracycline MIC values are seen in single isolates of virtually all publications and throughout all years. In contrast to other respiratory tract pathogens, such as *Pasteurellaceae* with about 30% tetracycline-resistant isolates, only about 1% of the *B. bronchiseptica* isolates are tetracycline-resistant, e.g., 3 of 349 German porcine

TABLE 2 Florfenicol MIC distributions of *B. bronchiseptica* isolates

Origin	Country	Year of isolation	No. of isolates	No. of isolates with an MIC of ... mg/liter[a]												Isolates in %[b]			Reference
				0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	S	I	R	
Companion animals[c]	Germany	2010–2012	43	n.t.	n.t.	0	0	1	16	11	12	2	1	0	0				24
Pigs	Germany	2010–2012	107	n.t.	n.t.	0	1	3	39	49	14	1	0	0	0	86.0	13.1	0.9	24
Pigs	Germany	2011/2012	90	n.t.	n.t.	0	0	0	1	8	79	2	0	0	0	10.0	87.8	2.2	27
Pigs	Europe[d]	2010–2012	118	n.t.	n.t.	n.t.	n.t.	n.t.	10	52	50	1	0	0	5	52.5	42.4	5.1	25
Pigs	Germany	2010	43	n.t.	n.t.	0	0	0	3	4	32	1	1	2	0	16.3	74.4	9.3	28
Pigs	Germany	2009	69	n.t.	n.t.	0	0	0	2	15	46	4	0	1	1	24.6	66.7	8.7	29
Pigs	Germany	2008	93	n.t.	n.t.	0	0	1	18	41	31	1	0	1	0	64.5	33.3	2.2	30
Dogs (n = 34), cats (n = 8)	Germany	2004–2006	42	0	0	0	0	0	0	9	32	0	0	1	0				39
Pigs	Germany	2003	82	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	67	11	0	0	4	n.t.	81.7	13.4	4.9	22
Pigs	Germany	2003	51	n.t.	n.t.	0	0	0	4	40	7	0	0	0	0	72.5	26.5	1.0	61
Pigs	Germany	2002/2003	138	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	111	25	0	0	2	n.t.	80.4	18.2	1.4	58
Pigs	Korea	1998–2003	70	n.t.	n.t.	0	0	0	remaining 67 isolates		3	0	0	0	0	95.7	4.3	0.0	59
Pigs	Germany	2002	91	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	73	17	0	1	0	n.t.	80.2	18.7	1.1	22
Pigs	Germany	2002	80	n.t.	n.t.	0	0	2	17	59	1	0	0	1	0	97.4	1.3	1.3	61
Pigs	Germany	2001	73	n.t.	n.t.	0	0	0	9	17	38	5	0	4	0	35.6	52.1	12.3	62
Pigs	Germany	2001	98	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	71	26	1	0	0	n.t.	72.5	26.5	1.0	22
Pigs	Germany	2000	87	n.t.	n.t.	0	0	0	11	18	26	26	6	0	0	33.3	29.9	36.8	62
Pigs	Germany	2000	78	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	67	7	2	2	0	n.t.	85.9	9.0	5.1	22

[a]n.t., not tested.
[b]Given for the porcine isolates, for which clinical breakpoints are available from the CLSI: S, susceptible; I, intermediate; R, resistant.
[c]Horses (n = 24), dogs (n = 8), rabbits (n = 8), cats (n = 2), ferret (n = 1).
[d]Belgium (n = 24), Denmark (n = 9), France (n = 12), Germany (n = 14), The Netherlands (n = 22), Poland (n = 14), Spain (n = 21), United Kingdom (n = 2).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dfrA1 cassette
catB3 cassette
5′ CS
dfrA1
catB3
3′ CS
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4
59-base element
1 R
1 L
2 L
TTAACCTCTGAGGAATTGTG - dfrA1 - GGTTAACAAGTGGCAGCAACGGATTCGCAAACCTGTCACGCCTTTTGT
98
start
stop
GCGGACCGT-GTCGCCTA-GCGTTTGGACCGCGCCGAAAACC
674
2 R
59-base element
1 R
1 L
2 L
TTAGACGGCAAAGTCACAGACCGCGGGATCTTTTATG - catB3 - GTCTAACAATTC-AATCAAGCCGATGCCGCT
675
start
stop
GCGGACTTTA-TTCGGC-ACGGCGC
1389
2 R
B
dfrB1 cassette
catB2 cassette
5′ CS
dfrB1
catB2
3′ CS
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3
1 R
TTGGGCGTGACCAATGCAGCGTGTCGTCGGGCCACATAGAACACCTAGAAGTTCACAAGAAAGGTCGGAAATG
98
start
dfrB1
59-base element
1 L
2 L
TGAGTTCAAGGTTGAAGTGCGCCGCTCAACGGTCAAACCGGGCATCACCGACTACAGCCCAAC--TTGTCGCTCCAGCGGACGGCT
stop
GCTTA-ATCGAG-TCGCCCGCCGC
507
2 R
1 R
TTAGGCGACGCGTGGAGTCGCTCTAGAATTTTCGGGTACAAATTTTATG
508
start
catB2
59-base element
1 L
2 L
GCCTAACAATACGCTACACACGGACAAATTACTCGCTG
stop
GCGGTGCGA-GTG-GCC-GTTTAATCCTCG-C
1247
2 R
C
blaOXA-2 cassette
5′ CS
blaOXA -2
3′ CS
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
59-base element
1 R
1 L
2 L
TTGGGCATTAAGGAAAAGTTAATG — blaOXA-2 — GCCCAACCCGGCAGTCAACTCGGACGCTGCGCGATA
stop
98
start
GCAACTTCA-TTG-GCC-GCGACGCGCCAA
973
2 R

isolates (22) and 4 of 447 isolates (https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=-1&Specium=791). Isolates collected from pretreated animals, because tetracyclines are commonly used, might show a higher resistance rate.

The first two *B. bronchiseptica* isolates considered tetracycline-resistant were isolated in the United Kingdom from cats (35). The gene *tet*(C) was identified in these feline isolates. This gene codes for a specific efflux protein of the major facilitator superfamily (MFS) conferring resistance in various Gram-negative bacteria by active efflux of tetracyclines. To date, the *tet*(C) gene has been also identified in a porcine isolate (Kadlec and Schwarz, unpublished). Another MFS *tet* gene, *tet*(A), was identified for the first time in *B. bronchiseptica* in porcine isolates from Germany (23). Later, *tet*(A) was also identified in all eight tetracycline-resistant isolates from another study of German isolates (24). A third MFS gene, *tet*(31), has been confirmed in *B. bronchiseptica* (36). All three genes have also been described in other Gram-negative bacteria but not in other common respiratory tract pathogens, except *tet*(C) in *Chlamydia suis* (37).

Sulfonamide Resistance

Among the three sulfonamide resistance genes *sul1*, *sul2*, and *sul3*, so far only *sul1* and *sul2* have been identified in *B. bronchiseptica*. All *sul* genes code for an alternative dihydropteroate synthase that is insensitive to sulfonamides. As in other bacteria, *sul1* was in *B. bronchiseptica* a part of the 3′-conserved segment of class 1 integrons that were located on plasmids (38). The gene *sul2* has also been identified in *B. bronchiseptica* isolates obtained from dogs and cats in the BfT-GermVet study (39). This gene is commonly seen in close proximity to *strA* and *strB* (40). In a study by Prüller and coworkers (24), the *sul2*-positive *B. bronchiseptica* isolates were also *strA* and *strB* positive, but an analysis of the linkage of these genes was not performed. Complete class 1 integrons are common in other Gram-negative bacteria but have not been described in respiratory tract pathogens of the family *Pasteurellaceae* so far. In contrast, the gene cluster *sul2-strA-strB* has been seen in *Pasteurellaceae* and is also common in several other Gram-negative bacteria (40–42).

Trimethoprim Resistance

Up to now, three trimethoprim resistance genes have been described in *B. bronchiseptica* (24, 38). All three genes (*dfrA1*, *dfrA7*, and *dfrB1*) code for alternative dihydrofolate reductases. Among the various *dfr* genes that have been described in Gram-negative bacteria, about 30 genes code for class A dihydrofolate reductases, such as the genes *dfrA1* and *dfrA7*, and only seven code for class B dihydrofolate reductases, namely, *dfrB1*, *dfrB2*, *dfrB3*, *dfrB4*, *dfrB5*, *dfrB6*, and *dfrB7* (43). The acquisition of such a resistance gene and thereby the replacement of the naturally occurring trimethoprim-sensitive dihydrofolate reductase by an alternative trimethoprim-insensitive enzyme leads to very high trimethoprim MIC values (>256 mg/liter) compared to wild-type isolates with low MIC values of <0.12 mg/liter. This observation has also been made in *B. bronchiseptica* (38). However, trimethoprim alone is usually neither tested nor used for treatment. These *dfr* genes are commonly located on gene cassettes, a fact that has been also described for *dfrA1* and *dfrB1* in *B. bronchiseptica* (38). The *dfr*-carrying gene cassettes described in *B. bronchiseptica* are shown in Fig. 1. In the study that described the identification of *dfrA7*, the authors also detected a *sul1* gene, a hint about the presence of a class 1 integron, but did not confirm the location of *dfrA7* in a gene cassette (24).

Aminoglycoside and Aminocyclitol Resistance

In general, the streptomycin MIC values of *B. bronchiseptica* isolates are high, as described for 150 isolates, 132 of which had MICs of 32 to 128 mg/liter and the remaining 18 of which had distinctly higher MICs of ≥1,024 mg/liter. In 17/18 streptomycin-resistant isolates (tentatively classified as resistant by MICs of ≥1,024 mg/liter), the genes *strA* and *strB* were detected (24). These genes often occur together and code for phosphotransferases, namely for the aminoglycoside-3′-phosphotransferase and the aminoglycoside-6′-phosphotransferase,

FIGURE 1 Schematic presentation of the class 1 integrons described so far in *B. bronchiseptica* isolates. The reading frames of the antimicrobial resistance genes are shown as arrows, and the conserved segments of the class 1 integron are shown as boxes. The beginning and the end of the integrated cassettes are shown in detail below. The translational start and stop codons are underlined. The 59-base elements are shown in bold type, and the putative IntI1 integrase binding domains 1L, 2L, 2R, and 1R are indicated by arrows. The numbers refer to the positions of the bases in the EMBL database entries with the following accession numbers: (a) AJ844287, (b) AJ879564, and (c) AJ877267 (41, 50).

respectively. Thus, they confer resistance by the inactivation of streptomycin. For neomycin, the majority of *B. bronchiseptica* isolates showed MICs of 1 to 8 mg/liter (22, 24). In four isolates with distinctly higher MICs of ≥128 mg/liter, no resistance gene was detected (24). MICs of gentamicin are commonly around 2 mg/liter (22, 24), and isolates exhibiting high MICs have not yet been observed. For the aminocyclitol spectinomycin, data from the German BfT-GermVet study revealed that all 42 isolates from cats and dogs had very high MICs of ≥512 mg/liter (39).

Phenicol Resistance

B. bronchiseptica isolates resistant to florfenicol that also exhibited high MICs to chloramphenicol, but also isolates that were susceptible to florfenicol but had high chloramphenicol MIC values have been described (22, 44). Among the latter isolates, two phenicol resistance mechanisms were identified. The *catB1* and *catB3* genes code for class B chloramphenicol acetyltransferases which inactivate only nonfluorinated phenicols, such as chloramphenicol. As described for other Gram-negative bacteria, these genes were located on gene cassettes and integrated into class 1 integrons (Fig. 1) (38). Another chloramphenicol resistance mechanism was identified in one *B. bronchiseptica* isolate. The isolate harbored a novel gene, *cmlB1*, coding for an MFS exporter. Database searches revealed that the gene is still very rare. Only one additional database entry was found which described the *cmlB1* gene in the whole-genome sequence of an *Acinetobacter pittii* isolate (45). No phenotype was described for this *A. pittii* isolate. In contrast to *cmlA* genes, *cmlB1* was not part of a gene cassette. It was located on a large nonconjugative plasmid and also conferred chloramphenicol resistance after transfer to *E. coli* (44).

Most of the *B. bronchiseptica* isolates that were resistant to florfenicol and had high chloramphenicol MICs harbored the widely distributed resistance gene *floR*. This gene also codes for an MFS exporter and was located in the chromosomal DNA of 7/10 florfenicol-resistant *B. bronchiseptica* isolates (44). The remaining three isolates showed distinctly lower florfenicol and chloramphenicol MIC values and were no longer classified as florfenicol-resistant when an efflux inhibitor was added. The inhibitor PaβNA indicates the presence of a not further specified exporter of the resistance-nodulation-cell division type (44).

β-Lactam Resistance

In *B. bronchiseptica*, the species-specific β-lactamase gene bla_{BOR-1} has been described (46). Involvement of a β-lactam hydrolyzing enzyme in the decreased susceptibility of *B. bronchiseptica* to β-lactam antibiotics is underlined by the fact that β-lactam MICs are lower in the presence of the β-lactamase inhibitor clavulanic acid. Among 150 isolates from pigs, cats, and dogs, 147 were positive in a PCR for bla_{BOR-1} (24). In addition to this class A β-lactamase gene, the class D β-lactamase gene bla_{OXA-2} has been described in *B. bronchiseptica* (47). As previously reported in *Enterobacteriaceae*, bla_{OXA-2} was located on a gene cassette and integrated into a class 1 integron (Fig. 1). In addition, it was shown that low membrane permeability could contribute to the β-lactam resistance of *B. bronchiseptica* (47).

Fluoroquinolone Resistance

Fluoroquinolones are usually highly active against *B. bronchiseptica*. An early study in which fluoroquinolones were evaluated for their activity against porcine respiratory bacterial pathogens revealed that ciprofloxacin was the most active quinolone against nine strains of *B. bronchiseptica* with mean MICs of 0.58 mg/liter (48). In another study, all 78 canine *B. bronchiseptica* isolates were reported to be susceptible to enrofloxacin (49). In a study of feline *B. bronchiseptica* isolates, all 43 strains tested were susceptible to marbofloxacin and enrofloxacin (MIC_{90}, 0.5 mg/liter), while 93% and 84% of the strains were susceptible, respectively, to ciprofloxacin and difloxacin, with MIC_{90} values of 1 and 8 mg/liter, respectively (50). Testing of 42 *B. bronchiseptica* isolates from dogs and cats for their susceptibility to pradofloxacin revealed MICs in the range between 0.12 mg/liter and 1 mg/liter with both MIC_{50} and MIC_{90} values of 0.25 mg/liter (51). Porcine *B. bronchiseptica* isolates (n = 349; 2000 to 2003) ranged in their MICs between ≤0.015 mg/liter and 2 mg/liter with MIC_{50} and MIC_{90} values of 0.25 and 0.5 mg/liter, respectively (22). The marbofloxacin MIC values of 504 *B. bronchiseptica* isolates collected in various European countries between 1994 and 2013 ranged between 0.06 mg/liter and 2 mg/liter with both MIC_{50} and MIC_{90} values of 0.5 mg/liter (52).

LOCATION OF RESISTANCE GENES ON MOBILE GENETIC ELEMENTS

Most of the resistance genes described so far were located on plasmids. In all cases, the corresponding resistance plasmid was the only plasmid harbored by the respective field isolates. Most of these plasmids were conjugative and could be successfully transferred to *E. coli* (23, 35, 38, 44). The easy transfer into *E. coli* and

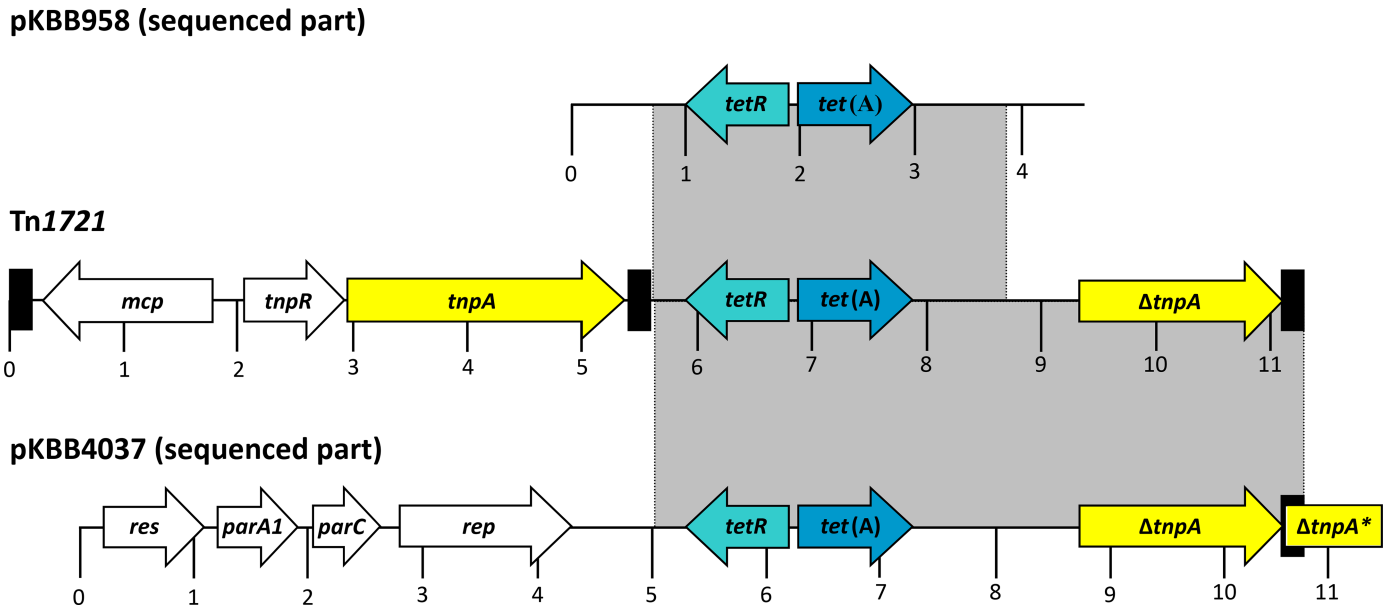

FIGURE 2 Comparison of Tn*1721* (GenBank accession no. X61367) and the sequenced parts of the resistance plasmids pKBB958 (GenBank accession no. AM183165) and pKBB4037 (GenBank accession no. AJ877266) from *B. bronchiseptica*. A distance scale in kb is given below each map. The genes *tetR*, *tet*(A), *mcp*, *tnpR*, *tnpA*, Δ*tnpA*, *res*, *parA1*, *parC*, and Δ*tnpA** are presented as arrows, with the arrowhead indicating the direction of transcription. The Δ symbol indicates a truncated, functionally inactive gene. The black boxes represent the terminal or internal 38-bp repeats of Tn*1721*. The gray shaded areas indicate the homologous parts between the *B. bronchiseptica* plasmids and Tn*1721* (26).

also the good maintenance in *E. coli* is in contrast to the transfer of plasmids isolated from other respiratory tract pathogens, namely *Pasteurellaceae*.

In addition to plasmids, *B. bronchiseptica* makes use of gene cassettes as mobile genetic elements. Trimethoprim, chloramphenicol, and β-lactam resistance genes have been already described as part of gene cassettes in *B. bronchiseptica* (Fig. 1). While very common in *Enterobacteriaceae*, other respiratory tract pathogens do not often carry class 1 integrons.

Moreover, for the tetracycline resistance gene *tet*(A), remnants of the small nonconjugative transposon Tn*1721* occurring commonly in *E. coli* and other *Enterobacteriaceae* were identified by sequence analysis (Fig. 2). The fact that different parts of transposon Tn*1721* were present on the two further analyzed plasmids indicates that different genetic events led to the final plasmid structure and that, very likely, a Tn*1721*-located gene *tet*(A) was acquired more than once by *B. bronchiseptica*. Tn*1721* has been described in *E. coli* but not in *Pasteurellaceae*. In *Pasteurella*, the genes *tet*(B) and *tet*(H) are the most common tetracycline resistance genes (41, 53). The streptomycin resistance genes *strA* and *strB* are often located on plasmids and are associated with the transposon Tn*5393* (54). Although not described so far, it is very likely that such a location is also present in *B. bronchiseptica*. The genes *strA* and *strB* are also found in *Pasteurellaceae*: plasmids and integrative and conjugative elements carrying *strA*, *strB*, and/or *sul2* have been described (41, 42, 53, 55, 56). However, these genes seem to be ancient and have also been found in streptomycin-resistant bacteria from permafrost (57). Thus, it is not astonishing that these genes are present in a wide variety of bacterial genera.

CONCLUDING REMARKS

B. bronchiseptica is in general susceptible to most antimicrobial agents, which therefore can be used to treat clinical infections. In addition to taxonomy and the identification of novel species, as well as pathogenicity and immunization, in which *B. bronchiseptica* offers a lot of lessons to learn, antimicrobial resistance in *B. bronchiseptica* appears to be of less interest judging from the number of published studies. *B. bronchiseptica* has proved to be able to acquire resistance genes from other bacterial genera, especially from *E. coli*. The future will show whether *B. bronchiseptica* will gain further resistance genes directed against important or critically important antimicrobial agents.

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