Antimicrobial Susceptibility of *Bordetella bronchiseptica* Isolates from Porcine Respiratory Tract Infections

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MICs for 349 *Bordetella bronchiseptica* isolates from respiratory tract infections of swine were determined by broth microdilution. The lowest MIC at which 90% of isolates tested are inhibited (MIC₉₀) was that of tetracycline and enrofloxacin (0.5 µg/ml), whereas the highest MIC₉₀s were those of tilmicosin and cephalothin (32 µg/ml) as well as streptomycin (256 µg/ml).

Porcine respiratory diseases represent the leading cause of mortality in nursery and finishing units (12). *Bordetella bronchiseptica* is often involved in porcine respiratory tract infections, along with viruses and other bacteria (1). It has been shown that infections with *B. bronchiseptica* predispose pigs to secondary infections with toxigenic strains of *Pasteurella multocida* and thus play an important role in the pathogenesis of severe atrophic rhinitis (1, 8). Various antimicrobial agents are licensed and used for the control of bacteria involved in porcine respiratory diseases and atrophic rhinitis, including aminopenicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, lincosamides alone or in combination with spectinomycin, potentiated sulfonamides, fluoroquinolones, pleuromutilins, and florfenicol. In contrast to well-studied pathogens such as *P. multocida* (for a review see reference 5), comparatively little is known about the antimicrobial susceptibility of porcine *B. bronchiseptica* isolates (3, 5, 6, 9–11, 14, 16, 20).

Between 2000 and 2003, 349 *B. bronchiseptica* isolates were collected from cases of bronchopneumonia and/or atrophic rhinitis of swine in Germany. This study includes 78 isolates from 2000, 98 isolates from 2001, 91 isolates from 2002, and 82 isolates from 2003. All isolates were collected from diseased animals on the basis of one isolate per herd. The animals had not been treated with antimicrobial agents in the 3 weeks prior to sample collection. Samples included nasal swabs sent to the diagnostic laboratories by veterinarians and lung tissue obtained during postmortem inspections at diagnostic laboratories. Microbiological sample processing and biochemical confirmation of species assignment followed standard procedures (7). All bacterial isolates were investigated for their in vitro susceptibility to antimicrobial agents by the microdilution broth method with microtiter plates (Sensititre, Westlake, Ohio) that contained the antimicrobial agents in serial twofold dilutions. The layouts of the microtiter plates corresponded to those used in the German resistance monitoring program for veterinary pathogens (GERM-VET). The antimicrobial agents and concentrations tested are shown in Table 1. Performance and evaluation of the susceptibility tests followed the recom-

mendations given in document M31-A2 of the National Committee for Clinical Laboratory Standards (13). Specifically, an inoculum that corresponded to a 0.5 McFarland standard was prepared in cation-supplemented Mueller-Hinton broth and then further diluted to yield a final concentration of 10⁵ CFU/ml. After incubation for 16 to 20 h at 35°C, the wells of the microtiter plates were inspected macroscopically for growth. The reference strain *Escherichia coli* ATCC 25922 served for quality control purposes (13).

The distribution of the MICs of the *B. bronchiseptica* isolates tested in this study is shown in Table 1. A year-by-year comparison of the data obtained for each antimicrobial agent revealed virtually no variations in the MICs at which 50 and 90% of isolates tested are inhibited (MIC₅₀s and MIC₉₀s, respectively) over the 4-year period. The maximum difference seen was two dilution steps in the MIC₅₀s of cephalothin and trimethoprim and in the MIC₉₀s of trimethoprim/sulfamethoxazole.

Using National Committee for Clinical Laboratory Standards-approved *B. bronchiseptica*-specific breakpoints for florfenicol (susceptible, ≤2 µg/ml; intermediate, 4 µg/ml; resistant, ≥8 µg/ml), 10 (2.9%) isolates were classified as resistant and another 61 (17.5%) as intermediate. This confirms the results of two florfenicol-specific monitoring studies conducted in Germany in 2000 to 2001 (16) and 2002 to 2003 (4). The MICs of chloramphenicol for all florfenicol-resistant strains were also high (≥128 µg/ml). A comparison of the MICs of ampicillin and amoxicillin/clavulanic acid suggested that the presumable β-lactamases which may account for the high MICs of ampicillin are susceptible to inhibition by clavulanic acid. Different distributions of MICs were recorded for the three aminoglycoside antibiotics gentamicin, neomycin, and streptomycin. While the MICs of streptomycin for 336 (96.3%) of the isolates were ≥64 µg/ml, those of gentamicin ranged between 0.25 and 4 µg/ml, with the MICs for 343 (98.3%) isolates 1 or 2 µg/ml. In the case of neomycin, the MICs for 345 (98.9%) isolates were 2 to 8 µg/ml, while distinctly higher MICs of 64 and 128 µg/ml were seen for single isolates. With tetracycline, the MICs for 346 isolates were ≤2 µg/ml and that for the remaining 3 isolates was 64 µg/ml. Although sulfonamides were not included in the test panels, a comparison of the MICs of trimethoprim and trimethoprim/sulfamethoxazole

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TABLE 1. Comparison of MIC data from porcine *B. bronchiseptica* isolates from 2000 to 2003

Antimicrobial agent and yr ^d	No. of isolates for which MIC (μg/ml) is ^b :															MIC (μg/ml) ^c	
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	50%	90%
Ampicillin																	
2000				0	0	0	8	2	7	56	2	0	2	1	0	8	8
2001				0	0	0	2	0	5	78	7	1	3	2	0	8	16
2002				0	0	0	1	3	6	36	41	1	0	3	0	16	16
2003				0	0	0	1	1	13	30	31	5	1	0	0	8	16
Total				0	0	0	12	6	31	200	81	7	6	6	0	8	16
Amoxicillin clavulanic acid (2:1)^d																	
2000			0	0	0	8	3	47	20	0	0	0				2	4
2001			0	0	0	2		49	45	2	0	0				2	4
2002			0	0	0	3	5	42	40	1	0	0				2	4
2003			0	0	0	1	9	31	38	2	1	0				2	4
Total			0	0	0	14	17	169	143	5	1	0				2	4
Chloramphenicol																	
2000			0	0	0	0	4	31	34	1	2	2	0	3	1	4	8
2001			0	0	0	0	0	23	67	5	1	1	1	0	0	4	4
2002			0	0	0	0	1	35	43	10	0	0	0	1	1	4	8
2003			0	0	0	0	11	30	33	3	0	0	2	2	1	2	4
Total			0	0	0	0	16	119	177	19	3	3	3	6	3	4	8
Florfenicol																	
2000								67	7	2	2	0				2	4
2001								71	26	1	0	0				2	4
2002								73	17	0	1	0				2	4
2003								67	11	0	0	4				2	4
Total								278	61	3	3	4				2	4
Tetracycline																	
2000				29	39	7	3	0	0	0	0	0	0	0	0	0.25	0.5
2001				5	65	27	1	0	0	0	0	0	0	0	0	0.25	0.5
2002				6	63	17	1	1	0	0	0	0	3	0	0	0.25	0.5
2003				8	60	11	2	1	0	0	0	0	0	0	0	0.25	0.5
Total				48	227	62	7	2	0	0	0	0	3	0	0	0.25	0.5
Gentamicin																	
2000			0	0	0	0	4	74	0	0	0	0	0	0	0	2	2
2001			0	0	0	0	2	95	1	0	0	0	0	0	0	2	2
2002			0	0	0	1	21	69	0	0	0	0	0	0	0	2	2
2003			0	0	1	1	33	45	2	0	0	0	0	0	0	2	2
Total			0	0	1	2	60	283	3	0	0	0	0	0	0	2	2
Neomycin																	
2000			0	0	0	0	0	0	32	46	0	0	0	0	0	8	8
2001			0	0	0	0	0	0	28	70	0	0	0	0	0	8	8
2002			0	0	0	0	0	30	47	13	0	0	1	0	0	4	8
2003			0	0	1	0	1	43	21	15	0	0	0	1	0	8	8
Total			0	0	1	0	1	73	128	144	0	0	1	1	0	8	8
Streptomycin																	
2000				0	0	0	0	0	0	0	0	7	52	2	17	64	256
2001				0	0	0	0	0	0	0	0	2	78	3	15	64	256
2002				0	0	0	0	0	0	0	0	1	49	28	13	64	256
2003				0	0	0	0	1	0	0	1	1	57	16	6	64	128
Total				0	0	0	0	1	0	0	1	11	236	49	51	64	256
Nalidixic acid																	
2000							1	1	14	59	1	2				8	8
2001							1	0	11	80	5	1				8	8
2002							0	3	7	69	11	1				8	16
2003							3	7	4	31	36	1				8	16
Total							5	11	36	239	53	5				8	16
Enrofloxacin																	
2000	0	1	1	8	59	7	0	2	0							0.25	0.5
2001	0	0	0	9	51	37	1	0	0							0.25	0.5

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TABLE 1—Continued

Antimicrobial agent and yr ^a	No. of isolates for which MIC (μg/ml) is ^b :															MIC (μg/ml) ^c	
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	50%	90%
2002	0	0	5	13	18	53	2	0	0							0.5	0.5
2003	1	1	4	5	32	36	2	1	0							0.25	0.5
Total	1	2	10	35	160	133	5	3	0							0.25	0.5
Trimethoprim																	
2000								32	13	22	4	3	4			4	16
2001								25	9	24	38	1	1			8	16
2002								40	24	12	11	3	1			4	16
2003								63	5	10	4	0	0			2	8
Total								160	51	68	57	7	6			4	16
Trimethoprim/sulfamethoxazole (1:19) ^e																	
2000		31	6	4	3	1	4	14	10	1	1	2	1			0.12	4
2001		36	20	8	0	0	3	11	15	4	0	1	0			0.12	4
2002		47	8	1	0	1	4	14	10	3	2	1	0			0.06	4
2003		52	7	1	1	6	7	2	6	0	0	0	0			0.06	1
Total		166	41	14	4	8	18	41	41	8	3	4	1			0.12	4
Tilmicosin																	
2000				0	0	0	0	2	12	32	22	10				8	32
2001				0	0	0	0	1	1	8	54	34				16	32
2002				0	0	0	0	0	10	5	51	25				16	32
2003				0	0	0	1	0	10	13	35	23				16	32
Total				0	0	0	1	3	33	58	162	92				16	32
Ceftiofur																	
2000	0	0	0	0	0	0	0	0	0	5	73					16	16
2001	0	0	0	0	0	0	0	0	0	1	97					16	16
2002	0	0	0	0	0	0	0	0	1	78	12					8	16
2003	0	0	0	0	0	0	0	1	2	57	22					8	16
Total	0	0	0	0	0	0	0	1	3	141	204					16	16
Cephalothin																	
2000			0	0	0	0	0	0	2	68	7	0	1	0	0	8	16
2001			0	0	0	0	0	0	0	57	36	3	2	0	0	8	16
2002			0	0	0	0	0	0	0	30	38	22	0	1	0	16	32
2003			0	0	0	0	0	0	0	18	11	50	1	2	0	32	32
Total			0	0	0	0	0	0	2	173	92	75	4	3	0	8	32

^a n = 78, 98, 91, and 82 for 2000, 2001, 2002, and 2003, respectively.

^b MICs equal to or lower than the lowest concentration tested are given as the lowest concentration, whereas MICs equal to or higher than the highest concentration tested are given as the highest concentration.

^c 50% and 90%, MIC₅₀ and MIC₉₀, respectively.

^d The MICs of amoxicillin/clavulanic acid (2:1) are expressed as MICs of amoxicillin.

^e The MICs of trimethoprim/sulfamethoxazole (1:19) are expressed as MICs of trimethoprim.

suggested that sulfonamides had some effect against isolates for which MICs of trimethoprim were elevated. The overall MICs of both cephalosporins tested for the *B. bronchiseptica* isolates in this study were high: ceftiofur, MIC₉₀ of ≥16 μg/ml; and cephalothin, MIC₉₀ of 32 μg/ml. A similar situation was seen with tilmicosin, with MIC₅₀ and MIC₉₀ of 16 and 32 μg/ml, respectively. In contrast, a low MIC₅₀ and MIC₉₀ of 0.25 and 0.5 μg/ml, respectively, were recorded for enrofloxacin.

Comparison of the results of this study with those of other studies is often problematic for several reasons: (i) different methodologies were used for susceptibility testing, including agar dilution (9, 10, 14, 17, 18), E-test (17, 18), and disk diffusion (6, 15, 19, 20); (ii) different antimicrobial agents were tested (6, 9, 10, 14); (iii) the evaluation of the results followed different guidelines (6, 9, 10, 14, 19); and/or (iv) isolates from animals other than pigs were tested (15, 17, 18). However,

three studies from the United States (2, 3, 11) were suitable for comparisons with our data. In the first study (11), the range of MICs as well as the MIC₉₀s of various antimicrobial agents were determined in 1988 for 48 porcine *B. bronchiseptica* isolates collected in the United States. The results for ampicillin, gentamicin, chloramphenicol, cephalothin, and trimethoprim/sulfamethoxazole corresponded closely to those of the present study, whereas the values for tetracycline were lower in the current study of German isolates (11). The second study described the in vitro susceptibility to tilmicosin of porcine respiratory tract pathogens collected between 1994 and 1998 in the United States (3). There was a close similarity between their observed range and MIC₉₀ of tilmicosin and those found in the present study. The third study dealt with the in vitro susceptibility of porcine respiratory tract pathogens to ceftiofur and revealed that *B. bronchiseptica* isolates are rather insensitive to

ceftiofur; the MICs for these isolates were ≥ 8 $\mu\text{g/ml}$ (2). This was in good accordance with our observation that the ceftiofur MIC for 345 (98.9%) of the 349 *B. bronchiseptica* isolates was ≥ 8 $\mu\text{g/ml}$.

The classification of *B. bronchiseptica* isolates as susceptible, intermediate, or resistant based on the MIC data presents some problems. Interpretive criteria that can be used explicitly for *B. bronchiseptica* are currently only available for florfenicol, but not for the other antimicrobial agents tested in this study. Nevertheless, the data presented in this study allow a reliable estimate of the resistance status of German *B. bronchiseptica* isolates from porcine respiratory diseases based on testing a large number of isolates and using internationally accepted methods. In addition to other data such as pharmacokinetic and pharmacodynamic parameters or clinical efficacy, the MIC data of this study may help to establish breakpoints for antimicrobial agents for which no breakpoints approved for *B. bronchiseptica* are currently available.

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