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In Vitro Activities of Florfenicol against Bovine and Porcine Respiratory Tract Pathogens

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Florfenicol in vitro activities for a total of 756 bacterial isolates from respiratory tract infections of cattle and swine were comparatively investigated by the agar diffusion method and the microdilution broth method. Florfenicol showed high in vitro activity against *Pasteurella multocida*, *Mannheimia haemolytica*, *Actinobacillus pleuropneumoniae*, and *Streptococcus suis*, with all of the isolates inhibited by ≤2 μg of florfenicol per ml.

Florfenicol (FFC), a fluorinated chloramphenicol derivative, is exclusively used in veterinary medicine. So far, its use in food-producing animals—exclusive of aquaculture—has been limited to cattle and swine. FFC was licensed as an injectable drug only for the treatment of bovine and porcine respiratory tract infections in 1995 and 2000, respectively. The main target bacteria in cattle are *Pasteurella multocida*, *Mannheimia haemolytica*, and *Haemophilus somnus*, whereas those in pigs are *P. multocida*, *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, and *Streptococcus suis*. The aim of this study was to monitor the in vitro susceptibilities to FFC of bovine respiratory pathogens at 5 to 6 years after the introduction of FFC and those of porcine respiratory pathogens at the time of introduction of FFC into clinical veterinary use.

For this study, we collected a total of 756 bacterial isolates from respiratory tract infections of cattle and swine in Germany during the years 2000 and 2001. The bacteria included in this study comprised 122 *P. multocida* and 118 *M. haemolytica* isolates of bovine origin as well as 212 *P. multocida*, 45 *A. pleuropneumoniae*, 160 *B. bronchiseptica*, and 99 *S. suis* isolates of porcine origin. All isolates originated from nasal swabs or lung tissue of diseased animals and were collected on the basis of one isolate per herd. Nasal swabs were taken by veterinarians and sent to diagnostic laboratories. Lung tissue samples were obtained during necropsy at the diagnostic laboratories. Microbiological sample processing and biochemical confirmation of the species assignment followed standard procedures (4). All bacterial isolates were investigated (for their in vitro susceptibilities to FFC only) by two different methods: (a) the disk diffusion method, with disks containing 30 μg of FFC (Becton Dickinson, Heidelberg, Germany), and (b) the microdilution broth method, with microtiter plates (Sensititre, Westlake, Ohio) that contained FFC concentrations of 0.12 to 128 μg/ml in serial twofold dilutions. Performance and evaluation of the susceptibility tests followed the recommendations given in documents M31-A (8) and M31-A2 (9) of the National Committee for Clinical Laboratory Standards. The three reference strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *A. pleuropneumoniae* ATCC 27090 were used for quality control purposes (6, 8, 9).

The currently available approved FFC-specific breakpoints (9) are only valid for *P. multocida*, *M. haemolytica*, and *H. somnus* isolates from cattle. Breakpoints for susceptibility are a zone diameter of ≥19 mm and a drug MIC of ≤2 μg/ml (9). Based on these breakpoints, all bovine *P. multocida* and *M. haemolytica* isolates included in this study proved to be suscep-

TABLE 1. FFC susceptibility data for bovine *P. multocida* and *M. haemolytica* isolates

Bacterium	No. of isolates	Yr of isolation	Disk diffusion range (mm)	Microdilution			FFC-susceptible isolates (%)	Reference or source
				Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)		
P. multocida	215	1993/1994	25–53	0.25–1	0.25	0.5	100	2
	60	1996	29–54.5	0.25–1	0.5	0.5	100	3
	83	1996/1997	ND[a]	0.06–1	0.5	1	100	7
	122	2000/2001	30–47	≤0.12–1	0.25	0.5	100	This study
M. haemolytica	160	1993/1994	25–50	0.25–1	0.5	1	100	2
	26	1996	26–34	0.25–1	0.5	1	100	3
	60	1996/1997	ND	0.5–2	1	1	100	7
	118	2000/2001	27–39	0.25–2	1	2	100	This study

[a] ND, not determined.

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TABLE 2. FFC susceptibility data for porcine *P. multocida, A. pleuropneumoniae*, *B. bronchiseptica*, and *S. suis* isolates

Bacterium	No. of isolates	Yr of isolation	Disk diffusion range (mm)	Microdilution			Reference or source
				Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	
P. multocida	119	1996	24.5–36.5	0.25–1	0.5	0.5	3
	212	2000/2001	28–43	≤0.12–2	0.25	0.5	This study
A. pleuropneumoniae	92	1994	ND[a]	0.2–1.56	0.39	0.78	12
	68	1995–1997	ND[a]	0.1–0.78	0.39	0.39	13
	45	2000/2001	30–40	≤0.12–2	0.25	0.5	This study
B. bronchiseptica	160	2000/2001	0–36	1–32	4	8	This study
S. suis	99	2000/2001	22–40	0.25–2	1	2	This study

[a] ND, not determined.

tible to FFC by each of the two methods. The results obtained from the bovine *P. multocida* and *M. haemolytica* isolates collected in 2000 and 2001 are displayed in Table 1 together with the corresponding data for bovine *P. multocida* and *M. haemolytica* isolates collected either prior to the introduction of FFC into clinical use in Germany (2) or after 1 or 2 years of clinical use in Germany (3) and in The Netherlands (7). A comparison of the data from 2000 and 2001 with those from previous years revealed that the drug MIC values at which 50% of the isolates were inhibited (MIC_{50}) and MIC_{90} values for bovine *P. multocida* isolates from 2000 and 2001 were the same as those for the isolates from 1993 and 1994, whereas the drug MIC_{90} value for the *M. haemolytica* isolates from 2000 and 2001 was one dilution step higher than the corresponding values from 1993, 1994, and 1996. This increase in the MIC_{90} value is based exclusively on the presence of 17 isolates collected in 2000 for which the drug MIC was 2 μg/ml. However, *M. haemolytica* isolates for which the drug MIC was elevated to this level have not been observed in 2001. These comparisons confirmed that after more than 5 years of veterinary use, *P. multocida* and *M. haemolytica* isolates from bovine respiratory tract infections in Germany are still susceptible to FFC and that no development of resistance has been detected so far in these target bacteria.

Approved breakpoints for the assignment of the in vitro susceptibility data of porcine *P. multocida*, *A. pleuropneumoniae*, *B. bronchiseptica*, and *S. suis* isolates to the susceptible, intermediate, and resistant categories are currently not available. Since there have been only few studies on the FFC susceptibility of porcine respiratory tract pathogens (3, 5, 12, 13; J. A. Jackson, G. W. Davis, K. F. Lechtenberg, T. L. Katz and P. W. Lockwood, poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., Vol. III, p. 186, 1998, and J. A. Jackson, M. T. Rodibaugh, J. W. Harker, S. A. Bales, T. L. Katz, and P. W. Lockwood, poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., Vol. III, p. 187, 1998), the results of this study may contribute to the definition and approval of FFC-specific breakpoints for porcine *P. multocida*, *A. pleuropneumoniae*, *B. bronchiseptica*, and *S. suis* isolates. The analysis of porcine *P. multocida* isolates from Germany collected in 1996 (3) revealed the same FFC MIC_{90} value as that for the isolates from 2000 and 2001 (0.5 μg/ml) (Table 2). Another study performed in the United States (Jackson et al., poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., p. 187) revealed drug MIC_{90} values of 0.5 and 0.25 μg/ml for porcine *P. multocida* isolates from nasal swabs and lung tissues, respectively, which also corresponded closely to the data of the present study. FFC MIC_{90} values for *A. pleuropneumoniae* isolates from Japan (0.39 or 0.78 μg/ml) (12, 13) and the United States (0.5 μg/ml) (Jackson et al., poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., p. 186) were similar to the corresponding data for the German isolates from 2000 and 2001 (0.5 μg/ml). The FFC MIC_{90} value for *S. suis* isolates collected in Germany in 2000 and 2001 was the same as that for *S. suis* isolates from the United States (2 μg/ml) (Jackson et al., poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., p. 186, and Jackson et al., poster presentation, Proc. 15th Int. Pig Vet. Soc. Congr., p. 187). Another study from France (5) showed that all *S. suis* isolates from swine ($n = 110$) and from humans ($n = 25$) were susceptible to FFC; however, no MIC data or zone diameters were provided. The MICs for *B. bronchiseptica* isolates from 2000 and 2001 were (in general) higher than those for the other porcine pathogens (Table 2). A total of nine independent isolates for which the FFC MICs were 16 or 32 μg/ml were detected. PCR assays for the genes *floR* (1), *cmlA* (11), and *cfr* (10) confirmed that none of these genes was present in any of the nine *B. bronchiseptica* isolates.

In conclusion, continuous monitoring of in vitro susceptibilities to FFC is an essential requirement for the determination of the actual susceptibility status and for the early recognition of potential resistance developments among bacterial pathogens involved in respiratory tract infections of cattle and swine. Based on the low MICs seen in the present and earlier studies, no resistance development has been documented in *P. multocida*, *M. haemolytica*, *A. pleuropneumoniae*, and *S. suis* isolates from bovine and porcine respiratory tract infections since the introduction of FFC into veterinary use.

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