COMPARATIVE MINIMUM INHIBITORY CONCENTRATIONS OF DANOFLOXACIN AND SIX COMMONLY USED ANTIBACTERIALS AGAINST PASTEURELLA AND HAEMPOPHILUS FROM PNEUMONIC CATTLE

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INTRODUCTION

Fluoroquinolone antimicrobials exhibit inhibitory and bactericidal activity by blocking DNA gyrase (topoisomerase II), an essential bacterial enzyme.[1] These compounds possess excellent antimicrobial activity against bacterial and mycoplasmal pathogens, however few *in-vitro* studies have been conducted to evaluate the activity of fluoroquinolone antimicrobials against respiratory pathogens of veterinary significance [2,3,4] This report summarizes the results of an extensive survey to determine Minimum Inhibitory Concentrations (MIC) of danofloxacin*, a new fluoroquinolone antimicrobial, and six commonly used antibacterials against P. haemolytica, P. multocida, and H.somnus isolated from pneumonic cattle.

MATERIALS AND METHODS

The MIC survey was conducted during the period 1989-1991, at veterinary diagnostic laboratories in fourteen countries (Table 1). A total of 1,428 P. haemolytica, 889 P. multocida, and 218 H. somnus isolates were tested. All isolates were recently recovered from cases of bovine bacterial pneumonia. Prior to susceptibility testing the isolates were purified and their identity confirmed using standard biochemical and morphological tests.[5] A standardized broth micro-dilution technique was used for MIC determination. All participating laboratories were supplied with identical round-bottom well microtitre plates containing stabilized freeze-dried antimicrobials; prepared by Sensititre Ltd. (East Grinstead, Sussex, England). The plates had a shelf-life of eighteen months, when stored at room temperature. The wells were precision-dosed with danofloxacin, amoxycillin, ceftiofur, erythromycin, gentamicin, oxytetracycline or trimethoprim/sulphamethoxazole (in a ratio 1:19) over a selected range of concentrations in two-fold dilution steps. Specimens were collected, stored and plated on an agar medium according to standard microbiological laboratory practice to give discrete well separated colonies. Three to five colonies were taken from each plate and homogenized in sterile demineralized water. This suspension was standardized to a 0.5 McFarland standard; approximately 10^8 colony forming units per ml (cfu/ml). Ten μl (0.01 ml) of this suspension were pipetted into 10 ml of Mueller Hinton broth supplemented with TES buffer (Sensititre Ltd. East Grinstead, Sussex, England) for a final concentration of approximately 10^5cfu/ml. Of this final suspension 0.05 ml were inoculated in all wells of a Sensititre® MIC plate using a multi-channel pipette. The technique was modified for testing the susceptibility of H. somnus[6]. After inoculation the plates were sealed with an adhesive seal, to

* Advocin, trademark of Pfizer, Inc

prevent any evaporation of the well contents. The plates were incubated at 35° - 37°C for 18-24 hours. Bacterial growth appeared as turbidity or as a deposit of cells at the bottom of a well. Test results were only considered valid if bacterial growth was present in a control well containing no antibacterial. The MIC for each antibacterial was defined as the lowest concentration tested that prevented visible growth. The accuracy of the test procedure was monitored using reference strains E coli ATCC 25922, Ps. aeruginosa ATCC 27853, S. aureus ATCC 29213 and S. faecalis ATCC 29212 with known antimicrobial susceptibility.

Table 1: Number and Origin of Pasteurella spp. and H. somnus Isolates Tested.

	Number Tested		
Region/Country	**P. haemolytica**	**P. multocida**	**H. somnus**
Europe			
Belgium	38	16	-
Denmark	40	50	40
France	100	99	10
Germany	29	71	-
Italy	54	29	-
Netherlands	48	24	-
Spain	50	25	4
Sweden	8	24	-
Switzerland	24	50	4
United Kingdom	86	101	54
Japan	85	92	-
North America			
Canada	141	72	56
USA	684	225	50
South Africa	41	11	-
TOTALS	1,428	889	218

- None tested

RESULTS

The inhibitory activity of each of the antibacterials tested, expressed as minimum and maximum MIC, most frequently occurring (modal) MIC and MIC_{90} (concentration that inhibited at least 90% of the isolates tested) is presented in Table 2.

In general, P. haemolytica was highly susceptible to danofloxacin. Seventy percent (70%) of the strains tested were inhibited at 0.06 μg/ml, the modal MIC. The MIC_{90} was established at 0.25 μg/ml. For one strain a concentration of 16 μg/ml was required for inhibition of growth. In contrast to danofloxacin, MIC_{90} values for ceftiofur, erythromycin, gentamicin and trimethoprim were several dilutions higher. MIC_{90} values for amoxycillin and oxytetracycline were greater than 64 μg/ml, the highest concentration tested. Modal MIC values of amoxycillin, ceftiofur and trimethoprim were established at the lowest concentrations tested. Modal MIC values were close to or equal to MIC_{90} values for the other antibacterials.

Similar results were obtained for P. multocida. Ninety three percent (93%) of the strains tested were inhibited by danofloxacin at a concentration of 0.12 μg/ml. At the modal MIC, 0.03 μg/ml, 30 percent of the isolates were inhibited. For one strain the inhibitory concentration of danofloxacin was established at 8 μg/ml. MIC_{90} values for the other antimicrobials against P. multocida were several dilutions higher when compared to danofloxacin. With the exception of erythromycin and gentamicin, modal MIC values were at the lowest concentrations tested for all other antibacterials.

Forty-four percent of the H. somnus isolates were inhibited by danofloxacin concentrations of 0.06 μg/ml, the modal MIC. The MIC_{90} was 0.25 μg/ml; and at this concentration 91% of the strains were inhibited. Two strains required a concentration of 1 μg/ml for inhibition. When compared with danofloxacin, MIC_{90} results for amoxycillin and ceftiofur were similar, but for the other antibacterials were several dilutions higher. With the exception of gentamicin, modal MIC values for the other antibacterials were at the lowest concentration tested.

Table 2: Minimum Inhibitory Concentration (μg/ml)

Antimicrobial	P. haemolytica[1]				P. multocida[2]				H. somnus[3]			
	min	max	mode	mic_{90}	min	max	mode	mic_{90}	min	max	mode	mic_{90}
Danofloxacin	≤.008	16	.06	.25	≤.008	8	.03	.12	≤.008	1	.06	.25
Amoxycillin	≤.50	>64	≤.50	>64	≤.50	>64	≤.50	4	≤.50	32	≤.50	≤.50
Ceftiofur	≤.12	>16	≤.12	4	≤.12	>16	≤.12	1	≤.12	16	≤.12	.50
Erythromycin	≤.50	>64	4	8	≤.50	>64	2	8	≤.50	8	≤.50	2
Gentamicin	≤.25	>32	2	2	≤.25	>32	2	4	≤.25	32	1	2
Oxytetracycline	≤.50	>64	> 64	>64	≤.50	>64	≤.50	32	≤.50	64	≤.50	4
Trimethoprim/ Sulphamethoxazole[4]	≤.25	>16	≤.25	2	≤.25	>16	≤.25	4	≤.25	>16	≤.25	2

(1) n = 1428, except ceftiofur n = 264, gentamicin n =1355, trimethoprim/sulphamethoxazole n = 1372
(2) n = 889, except ceftiofur n = 251, erythromycin n = 870, gentamicin n = 828, trimethoprim/sulphamethoxazole n = 853
(3) n = 218, except ceftiofur n = 54, erythromycin n = 180, gentamicin n = 153, trimethoprim/sulphamethoxazole n = 179
(4) value of trimethoprim shown. Trimethoprim/sulphamethoxazole in ratio 1:19.

DISCUSSION

The objective of the study was to determine and compare the *in-vitro* susceptibility of a large number of P. haemolytica, P. multocida, and H. somnus strains isolated from pneumonic cattle to danofloxacin and six other commonly used antibacterials. Danofloxacin was very effective in inhibiting bacterial growth at low concentrations; a characteristic shared with other fluoroquinolones.[2,7] With the exception of amoxycillin and ceftiofur activity against H. somnus, MIC_{90} values for other antibacterials tested were ten to hundred fold higher compared to danofloxacin.

SUMMARY

Using a standardized broth micro-dilution procedure, the MIC of danofloxacin, a novel fluoroquinolone, and those of amoxycillin, ceftiofur, erythromycin, gentamicin, oxytetracycline and trimethoprim/sulphamethoxazole were determined against P. haemolytica, P. multocida, and H. somnus obtained from pneumonic cattle. Danofloxacin MICs ranged from ≤.008 μg/ml to 16 μg/ml with MIC_{90} values of 0.25 μg/ml, 0.12 μg/ml and 0.25 μg/ml for P. haemolytica, P. multocida, and H. somnus, respectively. MIC values of the other antibacterials were often many fold higher than those of danofloxacin.

RESUMEN

Las concentraciones minimas inhibitorias (CMI) de danofloxacin, una novedosa fluoroquinolona, y de los antibacterianos amoxicilina, eritromicina, gentamicina, oxitetraciclina y trimetoprim/sulfametoxazol fueron determinadas frente a cepas de P. haemolytica, P. multocida y H. somnus aisladas de bovinos con neumonia. Se utilizo para esto un procedimiento de microdilucion en caldo estandarizado. Las CMI de danofloxacin quedaron comprendidas dentro de un rango de ≤0.008 μg/ml a 16 μg/ml, con valores de CMI_{90} de 0.25 μg/ml, 0.12 μg/ml y 0.25 μg/ml para P. haemolytica, P. multocida y H. somnus, respectivamente. Los valores de CMI de los otros antibacterianos evaluados generalmente fueron mucho mas altos que los de danofloxacin.

RÉSUMÉ

Les auteurs comparent les CMI de la danofloxacine, une nouvelle fluoroquinolone, à celles de l'amoxycilline, du ceftiofur, de l'érythromycine, de la gentamycine, de l'oxytétracycline et du triméthoprime/sulphaméthoxazole contre P. haemolytica, P. multocida et H. somnus isolés chez des bovins atteints de pneumonie. Ces résultats furent déterminés en utilisant une micro version de la méthode classique de détermination des CMI en milieu liquide. Les CMI de la danofloxacine furent comprises entre ≤ 0,008 μg/ml et 16 μg/ml avec des CMI_{90} de 0,25 μg/ml, 0,12 μg/ml et 0,25 μg/ml pour P. haemolytica, P. multocida et H. somnus respectivement. Les CMI des autres antibactériens furent souvent beaucoup plus élevées que celles de la danofloxacine.

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