IN VITRO-SUSCEPTIBILITY OF HAEMOPHILUS PLEUROPNEUMONIAE TO ANTIMICROBIAL SUBSTANCES

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Usually the in vitro susceptibility of pathogenic bacteria is measured routinely by means of a standardized agar diffusion test using different commercially available antibiotic discs. The information yielded by this test is primarily for clinical use, since the results according to interpretation schemes are given in categories such as "sensitive", "intermediate" and "resistant". Such interpretation schemes, however, are based on the correlation between the diameter of the inhibition zone in vitro (indirect measure of the minimal inhibitory concentration, MIC), the expected therapeutic level in vivo and the clinical success estimated in humans. Although this kind of interpretation can in most cases also be used as indicative information for the veterinary medicine, it has to be taken into account that the pharmacokinetics of antibiotics vary among the different animal species. Thus the MIC determination in animal pathogens on the one hand and the knowledge of pharmacokinetic data on the other hand allow a better understanding of the therapeutical intervention.

It is well known that infections due to Haemophilus pleuropneumoniae do not cause serious therapeutical problems, since this bacteria proved to be very sensitive to treatment with penicillin, chloramphenicol, tetracyclines and cotrimoxazol among others (Nicolet J. and Scholl E., 1981). The in vitro susceptibility as given with an agar diffusion test also confirms the generally good sensitivity of H.pleuropneumoniae against the most antimicrobial substances (Nicolet J., 1968).

In order to have more scientific background of the in vitro susceptibility of H.pleuropneumoniae, we determined of 98 strains, collected in the past 10 years, the MIC to 12 antimicrobial substances.

The general method used was the broth microdilution technique in microtiter plates (MIC Plates - MA 1501/N, Dynatech, Cooke Laboratory Products). Schaedler broth (Difco) with 5 % Fildes enrichment (Difco) was used for testing all antibiotics and the nitrofurane, whereas Mueller-Hinton broth (Difco) supplemented with hemin and NAD (Sigma) (10 mg/l) was used for sulfamethoxazol and cotrimoxazol (Thornsberry C.et al., 1977).

The dispensation (50 ul) of diluted substances in broth and the automated inoculation (1.5 ul) of the plates were performed with the Dynatech MIC-2000 system (Cooke Laboratory Products).

The inoculum was standardized in the following way: A suspension of colonies from chocolate medium was achieved in physiological NaCl and adjusted to the turbidity of a 0.5 McFarland standard (approximately 10^8 CFU/ml). Appropriate dilution was prepared in order to obtain a final concentration of 5.10^5 CFU/ml broth. Antimicrobial substances with known activity (standards) were tested in the concentration range of 0.008 ug/ml to 16 ug/ml in two-fold dilutions.

The repartition of the MIC obtained with 98 strains of H.pleuropneumoniae are given in the following table:

	Minimal inhibitory concentration in ug/ml								
	>16	16	8	4	2	1	0,5	0,25	0,125	0,063
Penicillin G						3	86	7	2	
Ampicillin								93	5	
Cephalothin							31	67		
Streptomycin	15	77	6							
Kanamycin		55	42	1						
Spiramycin	74	22	2							
Tetracyclin							73	25		
Chloramphenicol						37	61			
Colistin					38	59	1			
Sulfamethoxazol			20	51	19	3	5			
Cotrimoxazol					1		76	19	1	1
Nitrofurane							98			

The first information we can obtain is the homogenous (unimodal) repartition of the susceptibility of the population against all substances, pointing out the absence of acquired resistance among the studied strains.

With regard to the susceptibility to the different classes of antimicrobial substances, we can report the good activity (low MIC) of β-lactam antibiotics (particularly penicillin for a gram negative bacteria), of tetracyclines, colistine, sulfonamide and cotrimoxazol. In another assay we did not find any potentiation with the MIC being given by trimethoprim alone. Noteworthy is the unusually low MIC of chloramphenicol and the high MIC of aminoglycosides (gentamicin was not tested) and macrolides (spiramycine).

In spite of our scarce knowledge about the pharmacokinetics of different antimicrobial substances in pigs, the results of the in vitro susceptibility of H.pleuropneumoniae allow the following comments:

- The use of penicillin as the drug of choice in the parenteral treatment of pleuropneumonia is, in view of its activity, its pharmacokinetics, its tolerability and its low price, absolutely justified. The use of ampicillin is only advisable if the compound is well absorbable (for example Na-Ampicillin).
- Chloramphenicol, tetracyclines or cotrimoxazol (probably trimethoprim alone) can be considered as alternative drugs (cave, chloramphenicol compounds usually available in veterinary medicine for parenteral use have a poor bioavailability). For medicated feed, cotrimoxazol (eventually trimethoprim!) and chloramphenicol (resorbable compounds) have brought the best clinical success.
- On the base of MIC values, the therapeutical use of streptomycin (also in combination with penicillin) and macrolides is not recommended.

Selected references:
Nicolet J., Path. Microbiol. 31, 215-225 (1968).
Nicolet J. and Scholl E., Haemophilus infections in Diseases of Swine, p. 368, 5th Ed. (Ed. A.D. Leman et al.), The Iowa State University Press, Ames, Iowa, USA, 1981.
Thornsberry C, Gavan T.L. and Gerlach E.H. in Cumitech 6, ed. Sherris J.C., ASM, Washington D.C., 1977.