Acta vet. scand. 1962, *3*, 226—234.

From the State Veterinary Medical Institute, Stockholm.

IN VITRO SENSITIVITY OF BOVINE AND PORCINE STRAINS OF PASTEURELLA MULTOCIDA TO ANTIBIOTICS

By

K.-A. Karlsson and *K. G. Nyström*

Several reports have been published on the *in vitro* activity of antibiotics against Pasteurella multocida (P. m.) strains isolated from various animal species (3, 8, 14, 15, 16, 17, 19). Information is also available about P. m. isolated from human beings (9). It has been pointed out (6), however, that the antibacterial spectra of antibiotics are not constant but vary from time to time and in different environments. For this reason it is important to study a large number of strains within a particular bacterial species and to observe possible variations in sensitivity to different antibiotics.

Partly in order to establish whether or not differences in cultural behaviour reflect variations in antibiotic sensitivity and partly because of the increased significance infections with P. m. in calves and pigs have obtained in this country, we have applied a uniform technique for determining the antibiotic sensitivity *in vitro* for a large number of P. m. strains isolated from these species.

It has long been observed in this laboratory that P. m. isolated from pigs practically always forms mucoid colonies while P. m. from calves regularly forms typical S-colonies when grown on horse blood agar.

MATERIAL AND METHODS

The P. m. strains have been isolated in recent years from autopsy material. Fifty strains isolated from calves and fifty strains from pigs were chosen at random. All the calf strains

formed smooth colonies and all the pig strains mucoid colonies. The calf strains originated from animals with septicaemia but without lung or pleural lesions. Most of the pig strains came from animals with pneumonia and pleuritis.

Since fermentation properties have previously been used as a basis for classification of P. m. strains (20, 21) and the fermentation patterns of strains isolated from various animal species have been compared (2, 4, 22), we have investigated some biochemical properties of the strains included in this study.

Fermentation tubes were observed for periods of 21 days. The other biochemical reactions have been carried out in the manner described by *Kauffmann* (10).

The sensitivity tests were performed by an agar diffusion technique described by *Ericsson et al.* (5, 6, 7). The strains were denoted as "sensitive", "fairly sensitive", "slighly sensitive" or "resistant". These designations correspond to Groups I, II, III and IV. The clinical evaluations of these four groups are those applied in medical practice and are listed in Table I.

This is the basis of evaluation for eleven of the antibiotics tested. The *polymyxins,* however, come into a different category. Because of their high molecular weight and other physiochemical properties they have a slow rate of diffusion. *Ericsson & Svartz-Malmberg* (6) therefore advise against using the *quantitative* variant of the disc method for this antibiotic.

Neomycin and *bacitracin* are characterized by their toxicity, and with oral therapy, effective concentrations are not possible except in the intestinal contents. Parenteral administration (e. g. in urinary infections) often involves a risk and should be reserved for treatment when especially sensitive strains are present. For these reasons Group I was excluded when evaluating the results with these agents.

Novobiocin cannot be concentrated in the urine. For this antibiotic therefore, Group II should be directly followed by Group IV.

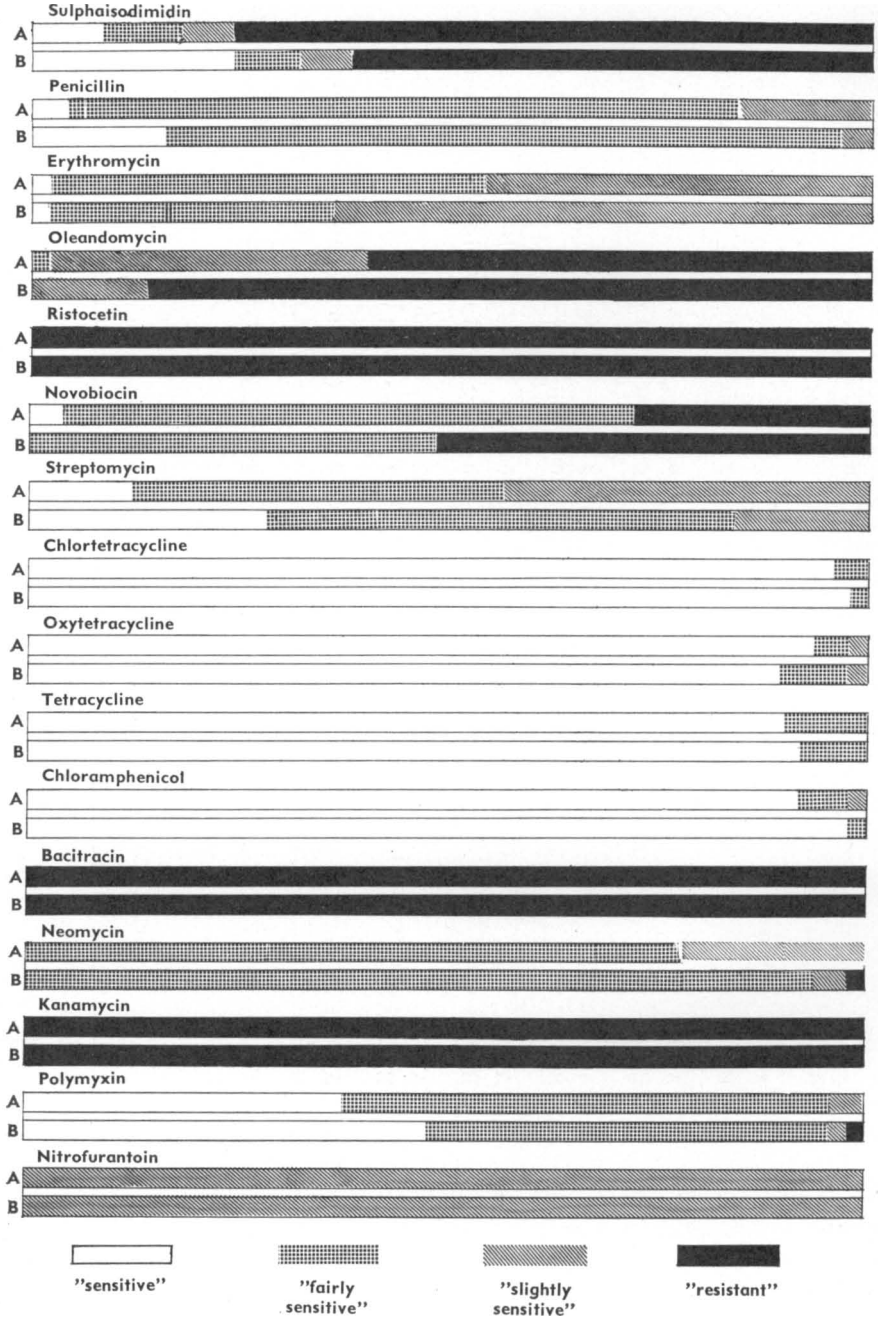
Nitrofurantoin, on the other hand, attains satisfactory therapeutic concentrations only in the urinary tract. Quantitative determination of nitrofurantoin is therefore superfluous for bacteria of Groups I and II. But for the clinician, all strains which are inhibited by a Group III nitrofurantoin concentration are fully susceptible to therapy.

Table 1. Antibiotic sensitivity of Pasteurella multocida expressed in per cent.
Material A: 50 bovine strains (smooth colonies)
Material B: 50 porcine strains (mucoid colonies)

Group characterization		I "sensitive"		II "fairly sensitive"		III "slightly sensitive"		IV "resistant"	
Likely to yield to therapy in		general infection, ordinary dosage		general infection, high dosage		organ in which the agent may be concentrated (e.g. certain urinary infections).		Therapeutic effect unlikely	
Antibiotic	Material	%	*	%	*	%	*	%	*
Sulphaisodimidine	A	8	(≦ 2.5	10	(≦ 10	6	(≦ 25	76	(> 25
	B	24	mg./ml.)	8	mg./ml.)	6	mg./ml.)	62	mg./ml.)
Penicillin	A	4	(≦ 0.1	80	(≦ 2	16	(≦ 20	0	(> 20
	B	16	IU/ml.)	80	IU/ml.)	4	IU/ml.)	0	IU/ml.)
Erythromycin	A	2	(≦ 1	52	(≦ 5	46	(≦ 50	0	(> 50
	B	2	mcg./ml.)	34	mcg./ml.)	64	mcg./ml.)	0	mcg./ml.)
Oleandomycin	A	0	(≦ 1	2	(≦ 5	38	(≦ 50	60	(> 50
	B	0	mcg./ml.)	0	mcg./ml.)	14	mcg./ml.)	86	mcg./ml.)
Ristocetin	A	0	(≦ 4	0	(≦ 16	0	(≦ 100	100	(> 100
	B	0	mcg./ml.)	0	mcg./ml.)	0	mcg./ml.)	100	mcg./ml.)
Novobiocin	A	4	(≦ 2	68	(≦ 16	see text		28	(> 16
	B	0	mcg./ml.)	48	mcg./ml.)			52	mcg./ml.)
Streptomycin	A	12	≦ 4	44	(≦ 16	44	(≦ 100	0	(> 100
	B	28	mcg./ml.)	56	mcg./ml.)	16	mcg./ml.)	0	mcg./ml.)

Chlortetracycline	A	96	(≦ 1	4	(≦ 4	0	(≦ 50	0	(> 50
	B	98	mcg./ml.)	2	mcg./ml.)	0	mcg./ml.)	0	mcg./ml.)
Oxytetracycline	A	94	(≦ 1	4	(≦ 4	2	(≦ 50	0	(> 50
	B	90	mcg./ml.)	8	mcg./ml.)	2	mcg./ml.)	0	mcg./ml.)
Tetracycline	A	90	(≦ 1	10	(≦ 4	0	(≦ 50	0	(> 50
	B	92	mcg./ml.)	8	mcg./ml.)	0	mcg./ml.)	0	mcg./ml.)
Chloramphenicol	A	92	(≦ 4	6	(≦ 16	2	(≦ 50	0	(> 50
	B	98	mcg./ml.)	2	mcg./ml.)	0	mcg./ml.)	0	mcg./ml.)
Bacitracin	A		see text	0	(≦ 0.2	0	(≦ 3	100	(> 3
	B			0	U/ml.)	0	U/ml.)	100	U/ml.)
Neomycin	A		see text	78	(≦ 16	22	(≦ 50	0	(> 50
	B			94	mcg./ml.)	4	mcg./ml.)	2	mcg./ml.)
Kanamycin	A	0	(≦ 4	0	(≦ 16	0	(≦ 100	100	(> 100
	B	0	mcg./ml.)	0	mcg./ml.)	0	mcg./ml.)	100	mcg./ml.)
Polymyxin	A	38	see	58	see	4	see	0	see
	B	48	text	48	text	2	text	2	text
Nitrofurantoin	A		see text		see text	100	(≦ 10	0	(> 10
	B					100	mcg./ml.)	0	mcg./ml.)

* Figures in brackets refer to the inhibiting concentration *in vitro*.



F i g. 1. Distribution of Sensitivity to Different Antibiotics of Pasteurella multocida.

Material A: 50 bovine strains (smooth colonies)
Material B: 50 porcine strains (mucoid colonies)

RESULTS AND DISCUSSION

The pattern of sensitivity for the P. m. strains studied is shown in Table I and Fig. I. In spite of the different sources and the cultural differences, there was a great uniformity of response to the antibiotics tested. *Mannheimer* (12)has also reported few differences in antibiotic sensitivity between smooth and mucoid P. m. strains. The tetracyclines and chloramphenicol have the greatest effects upon P. m. *in vitro*. Few studies have been reported on the *in vitro* effects of sulphonamides. According to our results, about two-thirds of the strains tested were resistant to sulphaisodimidine — a somewhat surprising result in view of several reports on successful therapeutic results in calves and pigs treated with sulphonamides. Another incongruous result was the relatively great sensitivity to penicillin for a Gram-negative organism. As for chloramphenicol, chlortetracycline, neomycin, oxitetracycline, tetracycline, penicillin, bacitracin, polymyxin, and nitrofurantoin, our results fall in with previous reports for strains of animal origin as reviewed by *Carter & Bain* (2) and the results obtained by *Henriksen & Jyssum* (9) for P. m. strains of animal and human origin. In our material, streptomycin and erythromycin were somewhat less effective against P. m. than is usually reported. Oleandomycin, ristocetin, novobiocin, and kanamycin have not been tested for activity against P. m. before. Since these antibiotics are effective primarily against Gram-positive organisms, the results were as could be expected.

In veterinary practice the relatively large doses required make the cost of particular antibiotics a major factor in their choice. For this reason sulphonamides, penicillin, and streptomycin are most commonly used on calves and pigs. These three agents had different degrees of effect on the different strains of P. m. tested here. Determination of the antibiotic sensibility *in vitro* of a particular strain would obviously increase the chances of successful therapy.

Fermentation reactions are listed in Table 2. Only in xylose and lactose did the calf and pig strains show a consistent difference. Many more calf strains fermented lactose; many more pig strains fermented xylose. A comparative study of the biochemical properties of various Pasteurella species has been carried out by *Knapp* (11). *Carter & Byrne* (1) obtained much the same results as we did for the xylose-fermenting properties of calf and pig strains. The relatively large number of lactose-

Table 2. Biochemical reactions of Pasteurella multocida.

	Bovine strains (smooth colonies)		Porcine strains (mucoid colonies)	
	+	—	+	—
Arabinose	0	50	0	50
Rhamnose	0	50	0	50
Xylose	16	34	45	5
Glucose	50	0	50	0
Lactose	20	30	2	48
Sucrose	50	0	50	0
Maltose	49	1	46	4
Adonitol	0	50	0	50
Mannitol	50	0	50	0
Sorbitol	49	1	50	0
Dulcitol	0	50	2	48
Inositol	0	50	0	50
Salicin	0	50	0	50
Hydrogen Sulphide	0	50	0	50
Gelatin Liquefaction	0	50	0	50
Nitrate Reduction	50	0	50	0
Methyl-Red Test	0	50	0	50
Voges-Proskauer Test	0	50	0	50
Urea Hydrolysis	0	50	0	50
Growth in Liquid Ammonium-Citrate Medium	0	50	0	50
Indol Production	50	0	50	0

fermenting calf strains is somewhat unusual judging from published reports (12, 13). *Smith* (22) found that the majority of P. m. strains isolated from calves and pigs did not ferment maltose, but practically all our strains did so.

REFERENCES

1. *Carter, G. R. and Byrne, J. L.:* A serological study of the hemorrhagic septicemia Pasteurella. Cornell Vet. 1953, *2*, 223.
2. *Carter, G. R. and Bain, R. V. S.:* Pasteurellosis (Pasteurella multocida) a review stressing recent developments. Vet. Rev. 1960, *6*, 105.
3. *Coles, E. H.:* The sentivity of bacteria of animal origin to streptomycin. Amer. J. vet. Res. 1948, *9*, 152.
4. *Das, M. S.:* Studies on Pasteurella septica (Pasteurella multocida). Observations on some biophysical characters. J. comp. Path. 1958, *63*, 288.

5. *Ericsson, H., Erlandsson, P., Tunevall, G. and Wallmark, G.:* Bestämning av bakteriers kemoresistens. Svenska Läkartidn. 1953, *50*, 476.
6. *Ericsson, H. and Svartz-Malmberg, G.:* Determination of bacterial sensitivity in vitro and its clinical evaluation. Antibiotica et Chemotherapia 1957, *6*, 41.
7. *Ericsson, H.:* Rational use of antibiotics in hospitals. Scand. J. clin. Lab. Invest. 1960, *12*, Suppl. 50.
8. *Gorzynski, G. A. and Neter, E.:* Study on the efficacy of viomycin, chloramphenicol and polymyxin B on Pasteurella multocida.
9. *Henriksen, S. D. and Jyssum, K.:* A study of some Pasteurella strains from the human respiratory tract. Acta path. microbiol. scand. 1961, *51*, 354.
10. *Kauffmann, F.:* Enterobacteriaceae. 1954, 2nd ed. Ejnar Munksgaard, Publisher, Copenhagen.
11. *Knapp, W.:* Pasteurella pseudotuberculosis unter besonderer Berücksichtigung ihrer humanmedizinischen Bedeutung. Erg. Microbiol. Immunitätsf. exp. Therap. 1959, *32*, 196.
12. *Magnusson, H.:* Om pasteurellos hos ren jämte ett bidrag till kännedomen om pasteurellans biologiska egenskaper. Skand. VetTidskr. 1913, *3*, 159.
13. *Mannheim, W.:* Ueber die Erkennung mukoider Stämme von Pasteurella multocida (P. septica) aufgrund ihrer Hyaluronidase-Sensibilität und Ihre Eignung zum biologischen Nachweis des Enzyms. Zbl. Bakt. I Orig. 1961, *183*, 180.
14. *McLean, I. W., Jr., Schwab, J. L., Hilegas, A. B. and Schlingman, A. S.:* Susceptibility of micro-organisms to chloramphenicol (chloromycetin). J. clin. Invest. 1949, *28*, 953.
15. *Muysson, J. R. and Carter, G. R.:* The antibiotic sensitivity in vitro of Pasteurella hemolytica and four serological types of Pasteurella multocida. Canad. J. Microbiol. 1959, *5*, 122.
16. *Neter, E. and Gorzynski, G. A.:* Relative efficacy upon Pasteurella multocida of various antibiotics. Aureomycin, terramycin, bacitracin and polymyxin B. Proc. Soc. exp. Biol. 1950, *74*, 328.
17. *Neter, E., Gorzynski, G. A. and Cass, W. A.:* Aureomycin and terramycin treatment of Pasteurella multocida infection and neomycin's in vitro effects on P. multocida. Proc. Soc. exp. Biol. 1951, *76*, 493.
18. *Nørrung, V.:* Pasteurella infections in calves. Nord. Vet.-Med. 1960, *12*, 425.
19. *Prier, J. E.:* The in vivo and in vitro effect of aureomycin upon Pasteurella multocida. Vet. Med. 1950, *45*, 243.
20. *Roberts, R. S.:* An immunological study of Pasteurella septica. J. comp. Path. 1947, *57*, 261.
21. *Rosenbusch, C. T. and Merchant, I. A.:* Study of the hemorrhagic septicemia Pasteurellae. J. Bact. 1939, *37*, 69.

22. *Smith, J. E.:* Studies on Pasteurella septica II. Some cultural and biochemical properties of strains from different host species. J. comp. Path. 1958, *68*, 315.

SUMMARY

An agar diffusion technique was used to study the sensitivity to 16 antibiotics of 50 Pasteurella multocida strains isolated from calves (smooth colonies) and 50 strains isolated from pigs (mucoid colonies). The tetracyclines (chlortetracycline, oxitetracycline, and tetracycline) were most effective *in vitro*. There were no major differences between calf and pig strains in antibiotic sensitivity.

ZUSAMMENFASSUNG

Die Empfindlichkeit vom Kalb und Schwein isolierter Pasteurella multocida-Stämme für verschiedene Antibiotika.

Die Aktivität von 16 Antibiotika gegenüber zwei Sammlungen von je 50 Pasteurella multocida-Stämmen, die von Kälbern (S-Kolonien) bezw. Schweinen (mukoide Kolonien) isoliert worden waren, wurde mittels einer Agardiffusionstechnik studiert. Die Tetracycline (Chlortetracyclin, Oxytetracyclin, Tetracyclin) und Chloramphenicol erwiesen sich *in vitro* gegen diese Bakterienart als am wirksamsten. In der Empfindlichkeit für die untersuchten Antibiotika liessen sich zwischen den beiden Gruppen von Stammen keine grösseren Abweichungen nachweisen.

SAMMANFATTNING

Känsligheten av Pasteurella multocida stammar isolerade från kalv och svin för olika antibiotica.

Aktiviteten av 16 antibiotika mot två kollektiv om vardera 50 Pasteurella multocida stammar, som isolerats från respektive kalv (S-kolonier) och svin (mukoida kolonier) har studerats medelst en agardiffusionsteknik. Tetracyklinerna (klortetracyklin, oxitetracyklin, tetracyklin) och kloramfenikol visade sig vara mest effektiva *in vitro* mot denna bakterieart. Inga större avvikelser mellan de båda kollektivens känslighet för de testade antibiotika har framkommit.

(Received May 11. 1962).