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THE RESULTS OF SENSITIVITY TESTS ON ANIMAL PATHOGENS CONDUCTED OVER THE PERIOD 1956–1963*

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SENSITIVITY TESTING has become an important laboratory test in veterinary medicine. It is used as an aid to treatment and, in some cases, as a method of control. The need for sensitivity testing has resulted from the fact that, with the increased use of chemotherapy, the antibiotic sensitivity pattern of organisms has tended to alter. Since bacteria have the potential to mutate, new types arise that are resistant to a drug to which they were previously sensitive (4). They may so adapt themselves as to become dependent upon a particular drug for their own nutrition.

These data are reported in order to demonstrate the changing pattern of sensitivity testing on bacterial pathogens of animal origin, isolated at the bacteriology diagnostic laboratory and mastitis testing laboratory of the Ontario Veterinary College, over an eight-year period (1956–1963).

METHODS

Selection of Strains

In the diagnostic laboratory, strains were selected from isolations made from diseased animals in Ontario. The specimens used were submitted to the laboratory by the pathologists or clinicians at the Ontario Veterinary College, and those received from practitioners throughout Ontario and other provinces. A sensitivity test was made when requested by the pathologist or clinician or when the authors felt a test was warranted.

The mastitis-producing bacteria reported in this study were selected from cultures made of the milk samples submitted to the routine mastitis testing laboratory of the Ontario Veterinary College. The herd infections were usually caused by either hemolytic strains of *Staphylococcus aureus* or *Streptococcus agalactiae*. In a herd test only one strain of *S. aureus* was selected from the culture of a milk sample with a high leucocyte count and from a cow with a history of infection. If there was more than one type of colony of *S. aureus* in the herd an additional strain would be selected. Relatively few strains of *Streptococcus agalactiae* were tested as it was considered that there would be little variation in the sensitivity pattern. Most of the other organisms tested were from acute clinical cases of mastitis and were cultured from milk on initial plating.

Method of Testing

The disc method was employed for all tests reported. Discs, prepared by Baltimore Biological Laboratories (BBL) under standards set by the Department of National Health and Welfare in Canada, were used. There was no variation in the strength of the antibiotic disc employed and a number of antibiotics were used for the entire period of study. In some

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instances new antibiotic discs were incorporated for shorter periods. A colony (or colonies) of the strain selected for testing was picked from the original plate and streaked evenly over the entire surface of a 5% blood agar plate to secure confluent growth. Immediately after inoculation the discs were applied either by placing them on the plates with sterile forceps or by the use of the BBL dispenser. The plates were incubated for 18–24 hours and then examined. Where there was a definite zone around the disc the organism was classified as sensitive to that antibiotic, otherwise it was resistant. The size of the zone of inhibition was not considered significant in judging which drug or drugs might be most suitable for therapy. The concentration of the discs were: penicillin, 2 units; streptomycin, 5 mcg. (10 mcg. for mastitis); chloramphenicol, 5 mcg.; tetracycline, 5 mcg.; neomycin, 5 mcg.; nitrofurazone, 100 mcg.; and chlorhexidine, 250 mcg. In the case of the latter two drugs the high concentrations are required to secure an adequate diffusion into the medium to demonstrate any activity.

Results

Table I shows the number of strains of four organisms tested from selected sources. In Figure 1, the results of the sensitivity tests of *E. coli* isolated from bovine and porcine sources are plotted against six drugs. The results of two *Salmonella* species, *S. typhimurium* and *S. choleraesuis*, tested against streptomycin and tetracycline, are given in Figure 2. The results, using bovine and porcine strains of *Pasteurella multocida*, bovine strains of *Past. haemolytica*, and *Streptococci* of equine origin, are given in Table II.

In Table III, the results of sensitivity tests on five antibiotics against seven of the mastitis pathogens are presented according to years. The results, using other antimicrobial agents for shorter periods of time, are given in Table IV.

TABLE I

Number of Strains of Bacteria Tested at Ontario Veterinary College

Organism	Species	1957	1958	1959	1960	1961	1962	1963	Total
E. coli	Bovine and Porcine	202	310	571	608	977	570	660	3898
S. typhimurium	Bovine	2	0	0	12	9	0	12	35
S. choleraesuis	Porcine	2	30	26	36	24	0	10	128
H. staphylococcus aureus	Canine	94	140	180	161	177	200	179	1131

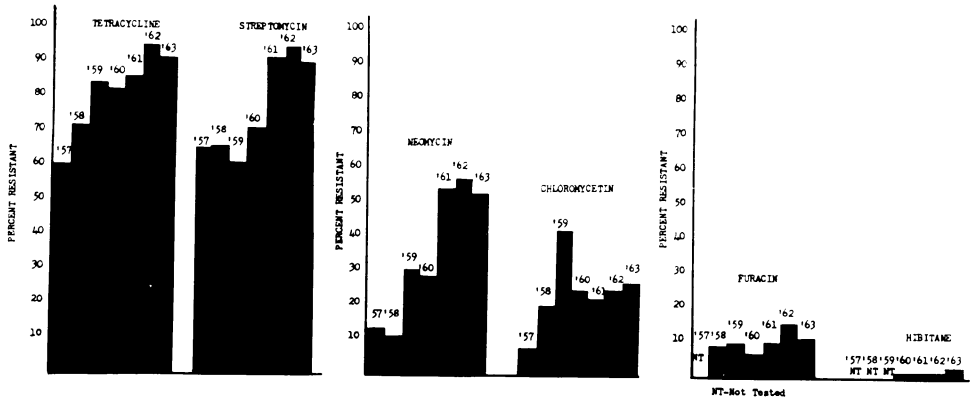

Figure 1. Results of Sensitivity Tests with *E. coli* from 1957 to 1963.

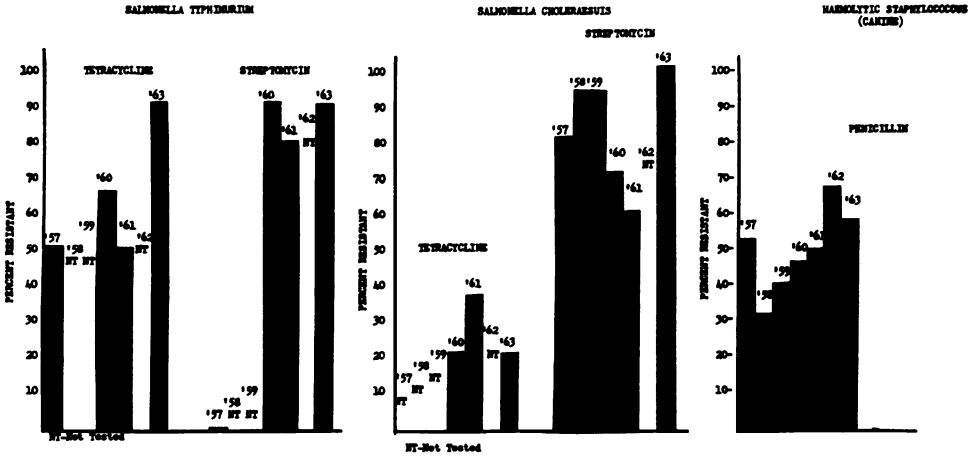

FIGURE 2. Results of Sensitivity Tests with *Salmonella typhimurium* (bovine), *Salmonella choleraesuis* (porcine) and *Haemolytic staphylococcus aureus* (canine) from 1957 to 1963.

DISCUSSION

In this report the authors recognize the limitations inherent in the disc method of assay, which was used, in comparison with the tube dilution technique. It has been reported that the size of the inoculum, the pH, composition, depth and moisture of the culture medium, the length of incubation, the rate of release of antibiotic into the agar, and the rate of diffusion in the agar, are all factors which influence the results. As the same method was followed for each test and as standardized discs from the same source were used in this report, it is justifiable to compare the results of the same species at different periods. In Canada and the United States the standardization of discs is under the control of Government agencies (1, 8) and each supplier must meet the standards of potency, quality, purity and performance as regulated by the agencies. It was not feasible to conduct the more accurate tube dilution method on such a large number of strains within the practical limits of the laboratory.

Drug Activity against Escherichia coli

It appears that the increasing difficulty encountered by veterinary practitioners in the prevention and treatment of colibacillosis in young animals is the result of the prevalence and emergence of resistant strains of *E. coli*. The results of sensitivity tests conducted during the period 1957–1963 have shown this to be the case. A total of 3,898 strains have been tested and the results leave little doubt that drug resistance is a phenomenon which presents a serious problem to the practitioners. In 1957, as recorded in Figure 1 approximately 60% of the strains of *E. coli* isolated from pathological or clinical cases were resistant to streptomycin. In the intervening years this percentage rose and by 1963 over 90% had become resistant. A similar pattern developed with the tetracyclines where 90% of the strains are presently resistant. Figure 1 also demonstrates that resistance to neomycin has increased from 10% in 1957 to approximately 50% in 1963. In the same period chloramphenicol has changed from 8% to approximately 22%. The introduction of nitrofurazone and chlorhexidine as chemotherapeutic agents represented a return to pure drug treatment and the design of chemicals to interfere with the metabolism of the bacterium. As an oral treatment for *E. coli* infections these drugs have been effective and have shown much promise. The results of the tests with them (Figure 1) demonstrate that less than 10% of the strains were resistant. At the present time resistance to these drugs does not appear to be developing to the same degree as with the antibiotics. When enteritis is complicated by septicemia however, treatment with

TABLE II

RESULTS OF SENSITIVITY TESTS ON SELECTED SPECIES ISOLATED FROM 1956–1963

		Antibiotic											
		Penicillin 2 units		Tetracycline 5 mcg		Streptomycin 2 mcg		Neomycin 5 mcg		Chloramphenicol 5 mcg		Nitrofurazone 100 mcg	
Organism	Origin	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant
P. multocida	Bovine	334	33.8	352	19.3	349	37.5	282	41.9	289	7.0	223	8.5
P. multocida	Porcine	144	38.9	148	13.5	147	42.2	100	47.0	105	2.9	84	4.8
P. haemolytica	Bovine	225	19.6	243	12.0	229	42.8	187	63.1	189	8.5	182	14.3
Haemolytic streptococcus	Equine	287	9.8	287	34.5	228	69.7	228	71.5	233	15.5	134	10.4

TABLE III

ANTIBIOTIC SENSITIVITY RESULTS BY YEAR ON SEVEN MASTITIS PATHOGENS
1956–1964

		Penicillin 2 units		Streptomycin 10 mcg		Neomycin 5 mcg		Chloramphenicol 5 mcg		Tetracycline 5 mcg	
Organism	Year	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant
Staph. aureus	56–57	607	6	606	2	609	0	606	3	608	2
	57–58	541	11	541	4	541	1	542	8	541	5
	58–59	591	11	587	6	589	1	591	32	589	6
	59–60	638	15	687	16	625	6	432	44	687	6
	60–61	758	23	755	18	756	25	778	53	257	19
	61–62	773	10	773	36	773	40	773	58	773	7
	62–63	649	23	649	30	649	25	649	22	649	6
	63–64	696	19	696	18	696	16	696	7	696	5
Total		5253	15	5294	19	5238	19	5067	29	4800	6

TABLE III—*continued*

Strep. agal.	56–57	95	0	95	53	95	82	95	0	95	0
	57–58	41	0	41	46	42	57	41	0	41	0
	58–59	7	0	7	86	7	0	7	0	7	0
	59–60	47	0	47	0	47	98	33	15	47	4
	60–61	26	0	26	89	26	0	26	27	26	23
	61–62	18	0	18	89	18	0	18	28	18	11
	62–63	42	0	42	98	42	98	42	17	42	7
	63–64	43	0	43	95	43	86	43	3	43	0
Total		319	0	319	76	320	87	305	8	319	4
Strep. non-agal.	56–57	195	7	200	50	110	62	200	1	190	2
	57–58	59	3	59	46	59	49	59	5	61	5
	58–59	70	2	72	60	71	79	71	8	70	12
	59–60	120	4	119	72	120	90	79	23	120	21
	60–61	58	7	58	81	58	90	58	26	58	14
	61–62	51	4	51	77	51	90	51	32	51	24
	62–63	61	7	61	74	61	89	61	16	61	11
	63–64	50	0	50	86	50	92	50	6	50	2
Total		664	4	670	64	580	66	629	11	661	11
E. coli	56–57	115	100	115	14	115	1	115	0	105	0
	57–58	104	100	104	16	104	3	104	1	105	17
	58–59	60	100	82	27	82	5	83	4	82	31
	59–60	87	100	119	44	119	36	79	15	119	28
	60–61	154	100	154	48	154	65	154	10	154	25
	61–62	166	100	166	52	166	89	166	12	166	50
	62–63	152	100	152	51	152	55	152	3	152	32
	63–64	154	100	154	47	154	55	154	5	154	33
Total		992	100	1046	39	1046	45	1007	96	1037	29
Past. multocida	56–57	24	8	24	0	24	0	24	0	24	0
	57–58	12	25	12	0	12	0	12	9	3	0
	58–59	5	0	5	40	5	40	5	0	5	0
	59–60	12	8	12	17	12	58	9	0	12	0
	60–61	10	0	11	27	11	70	11	0	11	0
	61–62	20	20	20	30	20	70	20	5	20	5
	62–63	6	17	6	17	6	67	6	0	6	0
	63–64	9	11	9	0	9	12	9	0	9	0
Total		98	12	98	13	98	37	96	1	90	1

TABLE III—*concluded*

Organism	Year	Penicillin 2 units		Streptomycin 10 mcg		Neomycin 5 mcg		Chloramphenicol 5 mcg		Tetracycline 5 mcg	
		No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant	No. tested	% resistant
Pseudo. sp.	56–57	25	100	54	9	54	26	28	86	53	91
	57–58	21	100	38	42	32	72	21	100	3	100
	58–59	18	100	36	35	31	81	24	88	27	95
	59–60	40	100	40	33	40	88	20	100	40	100
	60–61	31	100	31	42	28	93	31	100	31	100
	61–62	38	100	38	52	38	100	38	100	38	100
	62–63	21	100	21	18	21	100	21	100	21	100
	63–64	29	100	29	7	29	93	29	100	29	100
Total		223	100	287	30	273	77	212	97	242	97
C. pyrogenes	56–57	8	0	8	0	8	13	8	0	—	—
	57–58	8	0	8	0	8	25	8	0	—	—
	58–59	—	—	—	—	—	—	—	—	—	—
	59–60	2	0	2	0	2	0	2	0	2	0
	60–61	3	0	3	0	3	0	3	33	3	0
	61–62	12	0	12	33	12	0	12	0	12	0
	62–63	12	0	12	42	12	84	12	8	12	8
	63–64	22	0	22	23	22	95	22	0	22	0
Total		67	0	67	21	67	76	67	3	51	2
Serratia marcescens Total		14	100	14	72	14	21	14	14	14	86
Yeasts Total		13	100	13	100	13	100	13	100	13	100

these drugs is usually unsuccessful and must be used in combination with other drugs.

Streptomycin and Tetracycline Activity against the Salmonella Sp.

It appears from Figure 2 that in 1957 *Salmonella typhimurium* infection in the bovine was quite sensitive to streptomycin. Since that year a high degree of resistance (90% in 1963) to this drug has been found. In 1957 one-half of the strains tested were resistant to tetracycline. This figure rose to 90% in 1963. Unfortunately only 45 strains of *S. typhimurium* were available for comparison during the years 1957–1963. In swine similar results were obtained, over 90% of the strains of *Salmonella cholerasuis* tested were resistant to streptomycin in 1963. These results are shown in Figure 2. During this same period the organisms have not developed resistance to the tetracyclines to the same extent, with only 20% of the strains being resistant. Thus tetracycline would appear to be effective in the treatment of salmonellosis in swine. A total of 128 strains of *Salmonella cholerasuis* were tested. In most cases a good response to treatment is obtained by the use of neomycin, chloramphenicol, nitrofurazone, chlorhexidine, or a combination of these drugs in salmonellosis in all animal species. It is the carrier-stage of this disease which is the most difficult to treat and control.

Sensitivity Tests against Penicillin

Penicillin has achieved an enviable record in the treatment of many bacterial infections in animals and most of the gram-positive infections continue to be successfully controlled by penicillin therapy. The sensitivity pattern of many organisms, including *Streptococci* and *Pasteurella* has remained unchanged (Table II). One exception to this has been *Staphylococcus aureus*, where the penicillinase-producing strains from some species become predominant. The percentage of resistant staphylococci found in 1,131 isolations from dogs, as presented in Figure 2, decreased during the last year of test.

Mastitis Pathogens

Table III shows that the antibiotic resistance of *Staphylococcus aureus* from mastitis has not changed to the marked degree that has been observed in strains of this organism isolated from other animal and human sources. Other reports on the sensitivity of mastitis strains have indicated higher resistance to penicillin than observed in this work. In England, Wilson (9) reported 70.6% of *Staphylococcus aureus* resistant to 1.5 units penicillin in 1961, while 62% were resistant in 1958. In Ireland, Pearson (5) reported that 90% were resistant. The authors did not state the number of strains tested nor whether many of the strains came from a selected number of herds. As described previously, most of the 5,253 strains listed in this series came from separate herds and were selected from 29,689 isolates. Unreported results on bacteriophage typing of 230 strains have confirmed the heterogenicity of the cultures.

Donaldson *et al.* (3), in a study of penicillinase-producing strains, reported that their average, minimal, inhibitory concentration was 6.05 ug/ml. This was low when compared to human strains and does suggest that if strains were tested against a greater concentration even more might be sensitive. Although 19% of the

TABLE IV

ANTIBIOTIC SENSITIVITY PATTERNS OF SELECTED DRUGS OVER VARYING PERIODS

Organism	Antibiotic	Years Tested	No. Tested	% Resistant
Staph. aureus	kanomycin (10 mcg)	1960–61	491	7
E. coli	kanomycin (10 mcg)	1960–61	61	12
Staph. aureus	nitrofurazone (100 mcg)	1961–64	1740	1
Strep. agal.	" (100 mcg)	1961–64	103	6
Strep. non-agal.	" (100 mcg)	1961–64	152	14
E. coli	" (100 mcg)	1961–64	342	2
Staph. aureus	dimethoxyphenol (5 mcg) penicillin	1963–64	96	0

strains were resistant to penicillin in the last year of the test, it was not the highest average of any year. The average of 6% resistant to tetracycline is comparable to that found by Wilson (9) in 1961. The year 1960–61 showed a marked increase in resistant strains, a trend that did not continue. An increase in resistance shown against chloramphenicol (1960–62), could have resulted from an increased use of this antibiotic in mastitis therapy at that time. In consideration of all the tests made on *Staphylococcus aureus* during the last year reported, it is obvious that the apparent failure to cure the mammary gland of staphylococcus infections is not due to its *in vitro* resistance to antibiotics. The concentrations used in the laboratory assay can be obtained readily in the milk by intramammary injection. The test level of penicillin is, however, higher than can be obtained by the intramuscular injection by at least 9 million units of penicillin. No strains of *Streptococcus agalactiae* were found resistant to penicillin. This finding has been reported by workers throughout the world as well as by Stableforth and Galloway (6). The relatively few strains of *Streptococcus agalactiae* tested in comparison to *Staphylococcus aureus* is a result of our method of selecting strains. Streptococci, other than *Streptococcus agalactiae*, were comparable to *Streptococcus agalactiae* in that they were usually resistant to streptomycin and neomycin, and sensitive to penicillin, tetracycline and chloramphenicol. The *in vitro* results on streptococci and staphylococci indicates an imperative need for further evaluation of the use of antibiotics in the treatment of bovine mammary gland infections. The significant difference observed by Branden *et al.* (2) in the treatment of mastitis with the same antibiotic but incorporated in either a slow or a fast release base suggests one area of investigation.

General Comments

Antibiotic resistance is a combination of mutation and selection, with the latter being more important. The increased resistance of many organisms such as *E. coli* and *Staph. aureus* to certain antibiotics closely follows their introduction into the clinical field. The administration of low levels of antibiotics, as occurs in the antibiotic supplemented feed (4) or the use of sub-inhibitory doses in clinical infections, contribute to the selection of resistant strains.

The *in vitro* testing of organisms provides a useful guide to successful treatment in most cases. Discrepancies arise which may be due to one or more of the following factors: (1) pus or dead tissue may prevent the drug from reaching a sensitive organism; (2) two or more organisms may be involved in a mixed infection, of which one organism is resistant to the drug (as often occurs in middle ear infections in dogs), and an initial favourable clinical response may be followed by a relapse; (3) the pH of the environment may inhibit an effective drug from working (streptomycin is 50 times more effective in alkaline than in acid urine); (4) in urinary infections a large fluid intake may dilute a drug to a concentration below its effective level; (5) the organism tested with the drug was not the pathogen involved in the disease; and (6) a wrong diagnosis.

Summary

The antibiotic sensitivity testing results on bacteria isolated from animals over an eight-year period are presented. Strains of *E. coli*, and *Salmonella* species show evidence of marked increased resistance. A total of 1,131 strains of *S. aureus* isolated from dogs developed increased resistance to penicillin from 31 to 68% while the resistance of *S. aureus* from bovine mastitis had a 13% increase in resistance. Mastitis strains of *S. aureus* remained highly sensitive to tetracycline and chloramphenicol. Tests conducted on other bacteria did not reveal any marked change.

Résumé

On donne les résultats des épreuves de sensibilité antibiotique que présentent certaines espèces de bactéries prélevées sur des animaux pendant une période de huit ans. Les cultures des epèces bactériologiques *Coli E* et *Salmonella* montrent des signes évidents de résistance accrue. Les 1131 échantillons de l'espèce *S. aureus*, prélevée sur des chiens, ont développé

une résistance à la pénicilline plus forte et variant de 31 à 68 p. cent, tandis que l'espèce *S. aureus*, provenant de la mammite bovine, n'indiquait qu'une augmentation de résistance de 13 p. cent. Ces dernières bactéries montraient une sensibilité élevée à la tétracycline et au chloramphenicol. Des épreuves effectuées sur d'autres espèces bactériologiques n'ont pas abouti à des changements concluants.

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BOOK REVIEW

Feline Medicine and Surgery. First Edition. Edited by E. J. Catcott. Published by American Veterinary Publications, Inc., Santa Barbara, California. 512 pages. 434 illustrations. 1964.

When thirty-one authorities provide material for a textbook, differences of opinion are to be expected. On page 54, Clark concedes that intraperitoneal administration of whole blood has advantages; whereas, on page 78, Ott discourages this route for transfusion. In addition, Hamblin states that the incidence of heart disease in cats is very low, (page 218); whereas Mullenax, quoting Joshua, states that heart murmurs are common in middle-aged and older cats (page 389).

There are inaccuracies in the book such as: "epileptiform convulsions are not uncommon in young animals with tapeworm infestation" (page 126), and "cats need 100 grams of calcium per day" (page 298).

The book is an excellent source of references, some of which must have been difficult to locate. This makes the book an excellent tool for those who are particularly interested in diseases of the cat.

The paper used is excellent and the print very legible. Photographs are numerous and generally illustrate the text. A small percentage of the photographs are difficult to interpret, usually because of poor focus.

In general, the quality of the material is excellent. It would be difficult to find one author, sufficiently conversant with feline medicine, who could have produced a comparable book. By applying the knowledge available in this text, veterinarians should be able to elevate the practice of feline medicine from its present level of mediocrity. *J. E. B. Graham.*