ANTIBIOTIC RESISTANCE TRENDS OF ANIMAL PATHOGENS IN ONTARIO*

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IN THE EARLY YEARS of penicillin therapy, it was realized that the drug had selective use against the Gram positive bacteria, but that some resistant strains of susceptible organisms were demonstrated. The full impact of resistant strains of usually susceptible bacteria was not determined until penicillin and other antibiotics received wide usage in the early 1950's.

At this time, it was necessary to evolve a method of test to determine the susceptibility of each isolate against the various drugs by *in vitro* techniques. These techniques were commonly known as "sensitivity testing", an essential practice in any modern medical or veterinary diagnostic bacteriology laboratory. Sensitivity testing of pathogenic bacterial isolates aids in the selection of a suitable agent for treatment. Sensitivity testing has become nearly as significant as demonstrating the causative organism, in the treatment of bacterial disease.

In vitro testing of microorganisms is a laboratory procedure which consists of subjecting the bacterial isolate to various antibiotic and chemotherapeutic agents of constant strength. The tube and disc methods are officially recognized. Both methods have been previously described in detail (4, 10). The discs used in the antibiotic sensitivity tests conform to the standards of the Department of National Health and Welfare in Canada. The tests reported here were conducted by the disc method.

This presentation is a review of sensitivity tests, as applied to animal pathogens, with comments relating to the significance of the results to the animal industry.

In 1965, McKay *et al* (8) reviewed the sensitivity testing results on bacteria isolated from bovine mastitis for the previous nine years. They report the testing of 5,253 strains of *Staphylococcus aureus* from separate herds selected from 29,689 cultures. At the same time, they noted that the antibiotic resistance of cultures of *S. aureus* had not varied in the degree observed in isolates from other animal and human sources in Ontario. Bacteriophage typing of 230 strains confirmed the heterogenicity of the cultures. Nineteen per cent of the strains were resistent to penicillin in 1965 with 23% resistant in both 1960 and 1962 and 24% resistant in 1964. Average percentage resistance of *S. aureus* for neomycin, tetracycline and chloramphenicol from 1957 to 1965 was 19%, 6% and 29%, respectively. Wilson (14) reported that in England 70.6% of *S. aureus* cultures were resistant to 1.5 units of penicillin in 1961, while 62% were resistant in 1958; no mention was made as to the number of strains tested or the heterogenicity of the cultures.

In Ontario during 1957–1965, all strains of *Streptococcus agalactiae* and the other streptococci were consistent in their sensitivity pattern, being sensitive to penicillin, tetracycline and chloramphenicol and resistant to streptomycin and neomycin.

Table I gives the number of cultures of four bacterial species from various sources (2). The bacterial cultures were isolated from clinical and pathological animal specimens. Many of the *Escherichia coli* cultures were isolated from young animals affected with colibacillosis, while the salmonellae isolates were derived from both adolescent and adult animals. The isolates of *S. aureus* from dogs were usually obtained from clinical cases of dermatitis and otitis.

The results of sensitivity testing of *E. coli* isolates against six drugs are given in Figure 1. These isolates were either hemolytic, non-hemolytic or mucoid in their characteristics. There were no differences

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TABLE I

NUMBER OF BACTERIAL CULTURES TESTED FOR ANTIBIOTIC SENSITIVITY AT ONTARIO VETERINARY COLLEGE 1957–65

Organism	Species	'57	'59	'61	'63	'65	Total ('57–'65)
E. coli	Bovine and Porcine	202	571	977	660	298	4823
S. typhimurium	Bovine	2	NT	9	12	6	53
S. choleraesuis	Porcine	2	26	24	10	4	144
H. Staphylococcus aureus	Canine	94	180	177	179	37	1370

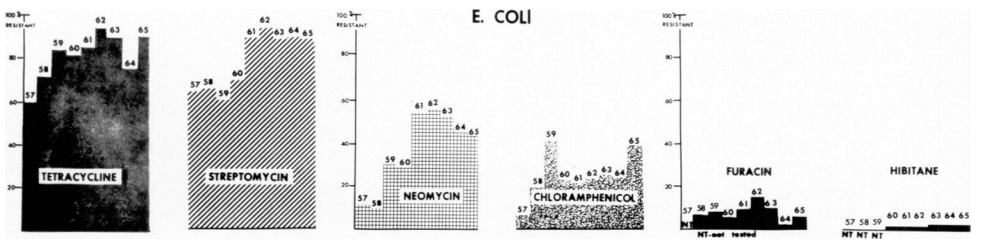

FIGURE 1. Results of sensitivity tests with *E. coli* from 1957 to 1965.

in patterns between hemolytic and non-hemolytic strains of *E. coli*.

Salmonella typhimurium and *Salmonella choleraesuis*, isolated from cattle and swine, were tested against tetracycline and streptomycin (Figure 2). Also, in the same figure the sensitivity for penicillin of cultures of *S. aureus* isolated from dogs are recorded.

Table II summarizes the results as reported by Hemsley (6) of testing a number of *E. coli* isolates from poultry against a group of chemotherapeutic agents. In 1965, submissions to the laboratory consisted of 7,670 cases, a case consisting of one or more birds from a flock. These cases comprised 33,076 birds. Clinical diagnosis in many of the isolates was chronic respiratory disease, colibacillosis or colisepticemia in turkeys.

Table III summarizes the results, as reported by Miniats (9), of sensitivity tests conducted on a number of hemolytic *E. coli* isolates recovered from early weaned pigs in an experimental herd. *E. coli* is a frequent pathogen in weanling pigs, producing colibacillosis evidenced by diarrhea (11). Stress factors such as change in feed, castration, movement to new pens, increase the numbers of hemolytic *E. coli* in the intestinal tract.

The data in Table IV demonstrates the pattern of resistance demonstrated in *Salmonella typhimurium* isolates against

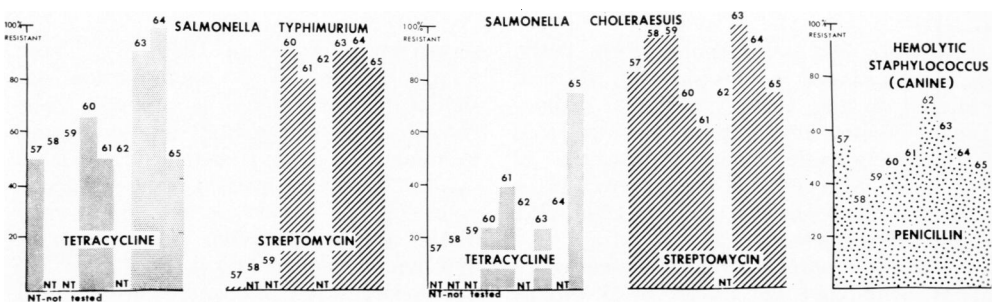

FIGURE 2. Results of sensitivity tests with *Salmonella typhimurium* (Bovine), *Salmonella choleraesuis* (Porcine) and *Haemolytic staphylococcus aureus* (Canine) from 1957 to 1965.

TABLE II

RESULTS OF *In Vitro* SENSITIVITY TESTS OF *E. Coli* ISOLATES—AVIAN ORIGIN

Generic Name	Potency	No. Sensitive/ No. tested	% Sens.
Chlorhexidine	250 μg.	213/213	100.0
Polymyxin B	50 units	213/213	100.0
Colistin	2 μg.	164/165	99.4
Nitrofurazone	100 μg.	211/213	99.1
Ampicillin	30 μg.	123/126	97.6
Framycetin	50 μg.	59/62	95.2
Framycetin	30 μg.	97/111	87.4
Chloramphenicol	5 μg.	169/213	79.3
Neomycin	5 μg.	163/213	76.5
Nalidixic acid	30 μg.	155/213	72.8
Furaltadone	50 μg.	39/51	58.8
Dihydrostreptomycin	10 μg.	107/213	50.2
Oxytetracycline	30 μg.	23/114	20.2
Tetracycline	5 μg.	15/189	7.9
Oxytetracycline	5 μg.	13/213	6.1
Chlortetracycline	5 μg.	8/213	3.8
Ampicillin	2 μg.	4/114	3.5
Bacitracin	2 units	0/100	0.0
Erythromycin	2 μg.	0/100	0.0
Phenethicillin	2 μg.	0/52	0.0
Tylosin	30 μg.	0/31	0.0
Triple Sulfas*	0.25 mg	0/213	0.0
Sulfachloropyridazine	2 mg.	0/160	0.0

*Equal amounts of sulfadiazine, sulfamerazine, sulfamethazine.

TABLE III

RESULTS OF *In Vitro* SENSITIVITY TESTS ON HEMOLYTIC *E. Coli* ISOLATES—EARLY WEANED PIGS IN AN EXPERIMENTAL HERD

Antibacterial Agent		No. strains tested	No. strains sensit.	No. strains resist.	% sens.
Chlorohexidine	2.5 mcg.	165	157	8	95.2
Nitrofurazone	1.00 mg.	251	198	53	78.8
Chloramphenicol	5.0 mcg.	251	197	54	78.4
Neomycin	5.0 mg.	251	116	135	46.2
Sulfachloropyridazine	2.0 mg.	251	74	177	29.5
Tylosin	1.5 mcg.	51	4	47	7.9
Tetracycline	5.0 mcg.	251	1	250	0.4
Dihydrostreptomycin	2.0 mg.	251	0	251	0.0
Triple Sulfa	0.25 mg.	19	0	19	0.0
Sulfadiazine	1.0 mg.	40	0	40	0.0

antibiotics and sulfonamides from cattle of various ages in Ontario (3). It is of interest to note that in 1965, the Salmonella isolates resistant to one or more chemotherapeutic agents, represented 32 or 71.1%. Also in 1963 there were 11% of isolates fully sensitive and in 1965, 28% were fully sensitive.

Table V reports the results of sensitivity tests conducted on strains of *S. typhimurium* isolated from humans affected with salmonellosis in Ontario during a seven-month period in 1966 (3). These human isolates of *S. typhimurium* are similar to those found in animals. Thus the organism can readily be transmitted to humans by animals or their products. A point of particular interest is the resistance pattern which has developed against antibiotics and sulfonamides. In a group of 419 human salmonella isolates, 136 or 32% were resistant to one or more antimicrobial agents.

TABLE IV

RESULTS OF SENSITIVITY TESTS *S. Typhimurium* ISOLATES—BOVINE ORIGIN

	1963	1964	1965
Fully Sensitive	1	—	13
Res. to A T Su S	4	9	7
Res. to T Su S	4	—	8
Res. to A Su	—	1	—
Res. to A T Su S N	—	2	—
Res. to T S	—	—	13
Res. to C T Su S	—	—	3
Res. to Su	—	—	1
Not tested	—	—	2
TOTAL	9	12	47

A—Ampicillin—10 μg. S—Streptomycin—2 μg.
T—Tetracycline—5 μg. C—Chloramphenicol—5 μg.
N—Neomycin—5 μg. Su—Sulfadiazine—0.25 mgm.

TABLE V

RESULTS OF SENSITIVITY TESTS
S. Typhimurium ISOLATES—HUMAN ORIGIN—1966

No. tested 419. No. fully sens. 283

Resistant To:			
T Su A S	22	T S	21
T S Su N A K	2	S	61
Su N A S K	1	Su S	6
T S Su	2	T Su	3
T B A Col	1	Su	3
T N S K	1	T Su A	3
N S	1	T	8
		A S	1

Total Resistant Isolates 136 (32%)

C	—Chloramphenicol	5 μg.
A	—Ampicillin	2 μg.
N	—Neomycin	5 μg.
Col	—Colistin	10 μg.
T	—Tetracycline	5 μg.
Su	—Sulphadiazine	0.25 mgm.
B	—Polymyxin B	50 units
K	—Kanamycin	5 μg.
S	—Streptomycin	10 μg.

DISCUSSION

In the figures and tables that have been presented, one distinct observation can be made. It is noted, in particular, that enteric bacterial organisms recovered from the intestinal tract elicit a pronounced resistance to antibiotics, sulfonamides and to a lesser degree other chemotherapeutic agents, e.g. nitrofurans and chlorohexidine. In 1957, as shown in Figure 1, 60% of the strains of *E. coli* were resistant to streptomycin, while in 1965 over 90% showed resistance. A similar resistance pattern has developed for chloramphenicol. The use of nitrofurans and chlorohexidine as chemotherapeutic agents does not appear to have produced an appreciable increase in resistant bacteria. Less than 10% of the bacterial strains tested against these agents demonstrated resistance as determined by sensitivity testing.

The salmonellae have shown a pronounced resistance to many antibiotics and sulfonamides since 1957. In many instances as illustrated in Tables IV and V salmonellae recently isolated show resistance to as many as six antibiotics or sulfonamides. This may be attributed to the relationship of salmonellae and *E. coli* in the intestinal tract.

For some time, it has been considered

by research investigators that this resistance trend in bacteria was a slow progressive change whereby microorganisms by mutation and selection would gradually become resistant to recently introduced antibiotic and chemotherapeutic agents over periods of time. This mechanism is slow and cumbersome. On this basis, it was hypothesized by many investigators that new antibiotics possibly semi-synthetics such as ampicillin, could be introduced rapidly enough to replace those which were ineffective. Consequently, antibiotic therapy would continue as formerly.

A rather startling finding was made by Harada *et al.* (5) in 1960, and by Watanabe (13) that transfer of infective drug resistance can be made between organisms of the same or different species. This finding was reconfirmed by Anderson and Lewis (1) in 1965, by Smith and Halls (12) in 1966 in England, and again by Kabins and Cohen (7) in the United States, also in 1966. The transfer of infective drug resistance applies particularly to Gram negative bacteria and especially to those of the intestinal tract. These organisms comprise the *Enterobacteriaceae* group. This transfer of drug resistance from one bacterial organism to another is infectious and occurs by conjugation. Donor and recipient bacterial strains have been recognized. A resistance transfer factor (RTF) + genetic material (R determinant) = resistance factor (R factor) which confers drug resistance into recipient bacteria. As an example, *E. coli* which may serve as a pathogen or commensal in the intestinal tract could readily transfer a spectrum of antibiotic or sulfonamide resistance to *S. typhimurium*, a companion bacterium in the *Enterobacteriaceae* family. Resistance to such agents as ampicillin, triple sulfas, tetracyclines and streptomycin could be transferred at the same time. Enteric bacteria have been isolated which carried resistance factors mediating resistance to as many as six drugs. This transfer of infectious drug resistance can readily be demonstrated in the laboratory.

The infective hazards of intense farming, combined with the widespread use of antibiotics and other chemotherapeutic agents, both for the treatment of actual infections such as colibacillosis and for so-called prevention as used in medicated feeds favour the spread of bacterial infection and infectious drug resistance at the same time. For the veterinary practitioner, the immediate importance of infectious drug resistance is that it provides a mechanism for the rapid spread of inherited antibiotic resistance between organisms of the same or different genera. This poses a serious threat to the effective treatment of enterobacterial diseases. Increased microbic resistance has darkened the future of antibiotic treatment. The time has clearly arrived for a re-evaluation of the whole question of the use of antibiotics and other drugs in the rearing of livestock and poultry.

Summary

The results of antibiotic sensitivity tests conducted on animal pathogens isolated from clinical and pathological specimens over a ten year period are reviewed. Strains of *E. coli* and salmonella elicit a pronounced increase in resistance to a number of antibiotic or chemotherapeutic agents at the time of isolation, regardless of source or species of origin. Infectious drug resistance of members of the *Enterobacteriaceae* family appears to be a contributing factor to this resistance upsurge. This type of resistance, with its capacity for rapid dissemination presents a potential threat to the efficacy of treatment of bacterial infections of the gastro-intestinal tract.

Résumé

Etude de la compilation des résultats d'épreuves de sensibilité effectués, au cours d'une décennie, sur des agents pathogènes isolés de spécimens cliniques ou pathologiques. Les souches d'*E. coli* et de Salmonella, au moment de leur isolement, manifestent une augmentation appréciable de résistance aux antibiotiques ou aux agents chimiothérapeutiques, indépendamment des espèces dont elles sont isolées. Il semble que la résistance d'autres membres de la famille des *Entérobactériaceae* aux agents anti-infectieux constitue une des raisons de cette résistance. Cette transposition de résistance, compte tenu de son potentiel de dissémination rapide, représente une menace pour l'efficacité du traitement des infections bactériennes du tractus gastro-intestinal.

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ABSTRACTS OF PAPERS TO BE PRESENTED AT THE 19TH ANNUAL C.V.M.A. MEETING

Contrast Radiography of the Gastro-Intestinal Tract—*K. R. Gadd*

The indications, techniques, and materials used in contrast radiography of the gastro-intestinal tract will be discussed. Radiographic interpretation of specific congenital and acquired diseases of the alimentary tract will be reviewed with the aid of slides.

Dermatology—*W. J. Lennox*

This paper will deal with skin problems in small animals which are related to endocrine disturbances. Differential diagnosis and treatment will be discussed.

Thoracic Surgery—*A. J. Cawley*

The Seminar on Thoracic Surgery is intended to expose participants to the important anatomical structures of the canine chest and to outline operative procedures within the thorax. With the aid of illustrations, surgery of the lungs, heart, great vessels, diaphragm and thoracic cage will be discussed. In general, the principles of thoracic surgery will be dealt with and specific surgical techniques of the various thoracic viscera will be demonstrated.

Examination of Urinary Sediment—*J. A. Love*

The identification of the normal and abnormal constituents of urinary sediment along with the techniques employed will be dealt with in this paper. The origin of the abnormalities, their diagnostic and prognostic significance will be discussed. The talk will be illustrated.

Cash Basis in Practice—*J. I. Robinson*

The advantages of establishing a "pay now" basis in practice and some methods of achieving it will be discussed.

The Importance of the Front Office—*G. R. Cormack*

The management of the client from the initial contact through the various phases (receptionist, waiting room, examining room, physical examination, progress reports, fees, discharge of patient and follow-up) will be discussed.