BACTERIOPHAGE TYPING OF STAPHYLOCOCCUS ISOLATED FROM BOVINE SOURCES

by

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A B S T R A C T

ABSTRACT

Three hundred and forty-two cultures of Staphylococcus were isolated from the udder of mastitic cattle. These cultures were subjected to phage typing with 29 staphylococcal phages. Of 342 cultures, 313 or 92% were typable with the phages used. Of the typable cultures, the majority typed with phages of Group III. Antibiotic sensitivity tests using the disc technique were performed on a random sample of these isolates. Staphylococci isolated from bovine sources appear to have developed some measure of resistance to the commonly used antibiotics, although the percentage of resistant cultures is small in comparison to the percentage of antibiotic resistant cultures isolated in hospital environments.

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I N T R O D U C T I O N

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INTRODUCTION

In recent years there has been a marked increase in the incidence of staphylococci isolated from the bovine udder. Previously, streptococci constituted the largest number of bacterial isolates from mastitic milk samples submitted for routine laboratory analysis. The widespread veterinary use of antibiotics has largely eliminated the streptococci and has allowed the ubiquitous staphylococci to become predominant. This parallels the situation in medicine, where staphylococcus infection has become an increasing problem particularly in hospital environments and where staphylococci appear to have attained marked resistance to the commonly used antibiotics.

The current use of bacteriophage as a tool for the routine typing of Staphylococcus aureus may be considered to have started with the work of Burnet and Lush (2) who isolated a series of staphylococcal viruses, although Williams and Timmins (24) appear to have been the first workers to use bacteriophages as a means of distinguishing between strains of Staphylococcus aureus. These investigators tested many cultures for their susceptibility to the phages isolated by Burnet and Lush. The bases for the current techniques, however, were established by Fisk (7 & 8).

Fisk conducted an intensive study on the occurrence of bacteriophage carriers using many strains of Staphylococcus aureus. He demonstrated that many of these strains carried, bacteriophages and that these phages lysed some, but not all strains. He used this discovery in an attempt to establish a "susceptibility pattern", i.e. by a comparison of lytic patterns he could establish the similarity or dissimilarity of two strains.

Fisk recognized his lysogenic staphylococci by the method of "cross culture", in which spot inocula of a number of different staphylococcal strains were applied to a plate previously seeded with a single strain, or in some cases a mixture of strains. A resultant plaque of lysis indicated that a phage was carried either by the "inoculum" or the "seeded" culture. Fisk maintained each phage in association with the strains on which it had been propagated and freed it from the cocci immediately before use by treatment with the quaternary ammonium disinfectant, Zephiran. Typing was carried out by spotting each phage preparation on an agar culture plate previously seeded with the culture under test.

Wilson and Atkinson (26) successfully separated the phage from the cocci by filtration and demonstrated that the phage filtrates were stable. They isolated and tested numerous phages from different strains of staphylococci, and obtained still more phages by adapting the original phages

to new propagating strains. They used the "cross-culture" method of Fisk, to obtain 18 viruses. The remaining seven were isolated by an adaptation procedure. In subsequent work their methods have been modified and refined rather than altered.

Rountree (11) demonstrated that bacteriophage adsorption to the surface of staphylococcal cells is due to a nucleoprotein component situated on the surface of the cell. The tests leading to this conclusion were conducted by using intact cells, with lysates and with fractions of lysates. This investigation showed that propagation of phage was inhibited, in some strains at least, by the action of citrate and oxalate or either of these compounds individually. Because calcium and magnesium were known to be essential for certain bacterial enzyme systems, it was suggested that the citrate and oxalate removed these cations producing the inhibition. Rountree further suggested that since propagation of certain staphylococcal phages were unaffected by citrates, some alternative mechanisms of virus reproduction might be available in the staphylococcal cells.

One of the first attempts to establish bacteriophage typing by grouping of phages was carried out by Smith (16). A staphylococcal strain which acquired resistance to a phage was accepted by Smith as a new virus type. This new virus type was stable when maintained under ordinary laboratory conditions.

Rountree (13) conducted an investigation into the phenomenon of lysogeny in Staphylococcus. It was concluded that the association of bacteriophage and the lysogenic cell is intimate, and that phage may be found in each bacterial colony from a lysogenic strain. Destructive agents, such as heat, do not alter the lysogeny of the strain. From this "an intracellular method of passage of the phage in such strains is postulated such that the daughters of each staphylococcal fission each receive at least one phage particle".

Rountree proposed other mechanisms controlling phage release from lysogenic strains, and proved that lysogenic staphylococci are not necessarily resistant to the lytic action of the inherent bacterial virus.

Rountree (14) demonstrated that the typing phages fell into two very distinct serological groups, named A and B, and that a number of other characteristics were correlated with this serological grouping.

Between 1952 and 1957, a number of papers outlining the results of bacteriophage typing on staphylococcus cultures isolated from various sources have been published.

Williams and Rippon (25), published a detailed study on virus propagating techniques using broth and solid agar media. In order to insure uniformity of reaction from batch to batch, they prepared a "lytic spectrum" of phages in use routinely. This spectrum was obtained by spotting

each virus strain upon each propagating strain and noting the lytic action. Each subsequent batch prepared must conform to the established pattern. This procedure insured, therefore, that the staphylococci isolated were subjected to phage typing with bacteriophages showing like action. Any phage preparation that did not conform to the "lytic spectrum" was classed as not suitable for routine use and was discarded.

They reported that approximately 40% of the independant strains of staphylococci, that were subjected to phage typing, were not lysed by the phages at the routine test dilution. Approximately half of these were lysed by one or more viruses when typed with undiluted phage.

These investigators analysed 567 staphylococcal cultures and reported that certain phages tended to appear together in patterns. On the basis of this kind of association three main virus groups, designated "3A", "6/47" and "52" could be distinguished.

Williams and Rippon reported that no one propagating medium was superior in regard to virus titre. They pointed out that considerable latitude was permissable in the age of cultures and in the inoculation procedures.

Williams, Rippon and Dowsett (23) published a report on results obtained from staphylococcal typing by means of bacterial virus from 1349 independant strains isolated from various sources. The sources included nasal

swabs of normal persons, including mothers, and babies; boils and lesions; mastoiditis; feces and perianal swabs; superficial skin infections; osteomyelitis; bacteraemia; hospital cross infections; neonatal infections (sporadic and epidemic); pneumonia and food poisoning. In addition some of the isolates were subjected to antibiotics sensitivity tests. Of the strains tested, the majority of the cultures which showed resistance to penicillin belonged to phage Group III. Over all about a quarter of the isolates showed resistance to penicillin.

These workers demonstrated that staphylococcal cultures isolated from the same kind of infection in different patients frequently exhibit the same phage pattern. For example, Group III types almost always were the organisms found in cases of food poisoning and Group I was commonly associated with fulminating pneumonia which complicates influenza.

In the same year Rountree (12) published results of phage typing conducted in Australia. Rountree made the same analysis as Williams and Rippon (23) in 1953, using cultures submitted to their laboratory over the same period of time. As the typing technique and the source of cultures were similar to those covered in the Williams and Rippon report, it might be expected that the pattern would also be comparable. In general this was true. Rountree reported that the frequency distribution of the various phage groups

was similar to that found in England. However, there was a slightly higher incidence of Group III strains among normal nasal carriers of penicillin resistant strains, and in skin lesions there was a predominance of Group II strains.

Fusillo et.al (9) published an account of bacteriphage typing and the antibiotic sensitivity of 485 strains of Staphylococcus. They noted a significant increase in the number of strains showing penicillin resistance over the years 1943-1951, and from 1952 on, there was an accompanying increase in resistance to other antibiotics, e.g. aureomycin, terramycin, erythromycin, and carbomycin. From the data presented again the majority of staphylococci isolated from clinical material occured in phage Group III, and Fusillo found a significant correlation between his results and those obtained by Rountree (12). Invariably the larger percentage of those cultures that were resistant to a single antibiotic or to mixtures of antibiotics could be typed by the Group III phage. However, antibiotic resistance was not limited to strains typing with phages of Group III, but typed with all other phage groups as well.

Desranleau, Martin and Saint-Martin (6) published results of a study of staphylococcal infections, including some epidemiological aspects of bacteriophage typing. Desranleau et.al. employed 21 phages including phage W (Winnipeg) isolated by Dr. J.C. Colbeck, (5), formerly Director of Laboratories, Manitoba Department of Health. Results

obtained from the typing of approximately 2400 coagulase-positive staphylococci, isolated from hospital patients and from children considered as healthy carriers, indicated that the majority of the hospital isolates were typed by Group III phages. Desranleau and his associates typed 80% of the hospital strains. Strains of Group III resisted penicillin to a greater extent than did those strains typed by Group I and II phages. However, of the strains isolated from healthy carriers the majority occurred in virus Group I, Group II, and Group III in this order.

Thatcher and Simon (19) published a report on the resistance of staphylococci and streptococci isolated from cheese to various antibiotics. The cultures were isolated from cheese specimens and subjected to sensitivity test using seven antibiotics. The sensitivity of an organism was estimated by determining the relative inhibition of each culture provided by standard antibiotic sensitivity discs (Difco) impregnated at each of three concentrations with each of the antibiotics used.

The results indicated that these isolates acquired a marked resistance to penicillin and to dihydro-streptomycin. These are the two antibiotics most commonly administered for the control of mastitis. Very little resistance was noted to antibiotics not commonly used in the treatment of dairy cattle. Thatcher and Simon suggested that the cause of the resistance found among the cheese strains was due at least

in part to exposure to antibiotics.

A thorough study on the phage typing and antibiotic resistance of staphylococci isolated in a general hospital was conducted in 1955 by Bynoe, Elder and Comtois (3). These workers used the method of virus typing recommended by Williams and Rippon (25) with a few minor modifications. At the start of the study, 22 phages were used. Later ten additional phages were included in the basic set in order to reduce the number of untypable strains. All strains were tested first against 32 phages at their routine test dilutions, (R.T.D.) which is the highest dilution giving confluent lysis. If lysis was observed no further typing tests were done and results were recorded. If however, no lysis was observed, the test was repeated using phages diluted 1:10 in order to eliminate some of the false reactions which often occurred with undiluted phage. If no lysis was observed after this treatment the culture was regarded as "not typable". The authors followed the recommendation of Williams and Rippon (25), and strains were identified according to their "phage patterns". Bynoe and co-workers (3) followed the system of grouping suggested by the International Staphylococcal Bacteriophage Typing and Reference Centre at Colindale. Although the International Committee suggested that phage 42C be placed in the "Miscellaneous" group, Bynoe and his colleagues included 42C with the Group III viruses, due to its very common association with these phages.

Each isolate was also subjected to antibiotic sensitivity tests. These tests were conducted using the serial tube-dilution method and the disc method. Six antibiotics were used for the tube-dilution method, and seven antibiotics with the disc method. Results obtained by the tube method only were reported, the disc technique having been used only as a rough check on the accuracy of the tube method.

The results of this study confirmed what many others have found, namely, that of the staphylococci isolated from hospital environments Group III strains predominated. The authors found that 90% of their cultures were typable, at R.T.D., 5% were "untypable" and the remaining 5% were typable using "strong" phage. Approximately 50% of the cultures typed fell into Group III classification. Bynoe et.al., (3) found that type 81 predominated in isolates from staphylococcal infections, e.g. carbuncles, boils, and breast abscesses, other than from infected wounds. The strains of Group III were found to be more resistant to the commonly used antibiotics, penicillin, chlortetracycline and oxytetracycline.

From the results of virus typing and antibiotic sensitivity, Bynoe and his associates concluded that "it is the increased resistance of these strains that has led to their predominence where these antibiotics are in constant use". It was, however, admitted that several

mechanisms may be responsible for the predominance of the antibiotics resistant Group III strains in the hospital staphylococcal flora.

In the same year, Thatcher and Simon (18) published a comparative study on the properties of staphylococci isolated from clinical sources and from dairy products. One of the properties investigated were that of phage typing. Thatcher and Simon reported that the cultures isolated from clinical sources belonged predominantly to Group III. Group IV (42D) was not represented. Of the cultures isolated from butter and cheese 80% were of Group IV, while the remaining 20% were untypable. Thatcher and Simon suggested that the staphylococci in cheese and butter appeared to be of minor significance as the cause of serious infections in man. The bovine forms (those lysed by Group IV)(42D) have been established as the cause of food poisoning in man (17).

MATERIALS & METHODS

MATERIALS AND METHODS: PROPAGATION OF PHAGE

At the beginning of this study, propagation of phage by the Williams and Rippon (23) modification of the Wilson and Atkinson (26) technique was employed. However, this propagation method did not yield phage of satisfactory titre for routine use and was subsequently abandoned in favour of the broth technique described by Anderson and Williams (1).

In the broth method, the phage was propagated on staphylococci in a fluid medium. On the basis of final titres, trypticase soy broth and nutrient broth with five grams of NaCl per litre were used, both were found to be equally efficient in the propagation of phage. Accordingly, all phage were propagated on nutrient broth plus 0.5% NaCl. This medium was used because of its relative uniformity and its ease of preparation.

The optimum proportion of staphylococcus propagating strain and phage in the inoculum was determined by test. In this study culturing the propagating strain in 10 ml. nutrient broth at 37°C. for three hours provided a satisfactory number of cells for the phage to act upon. Into this three-hour culture, 2 to 4 ml. of phage suspension were added, the smaller amount of phage inoculum being used when the titre of phage suspension was high.

After cell debris and unlysed cells were removed from the suspension by centrifuging at 5000 r.p.m. for 15 minutes the supernatant liquid was sterilized by using an ultra fine sintered glass filter. The pH of the sintered glass filter was adjusted to the alkaline side of neutrality before autoclaving in order to reduce "phage loss" due to adsorption on the filter surface. Phage loss due to adsorption was not eliminated altogether. However, if the phage titre was high this loss was almost negligible and did not interfere with the effectiveness of the phage.

With some strains this procedure produced phages with titres of 10^4 to 10^6 particles per ml. With others, the titre had to be built up by repeating the procedure through a series of passages, in each passage the phage suspension from the previous passage being used. As many as four passages were required with some phage strains. Aliquot portions of the phage suspension were stored in a frozen state.

TITRATION OF PHAGE AND PREPARATION OF ROUTINE TEST DILUTION

Three millilitres sterile nutrient broth were inoculated with the propagating strain and incubated at 37°C. for three hours. A sterile trypticase soy agar plate was then flooded with the three-hour culture, and the excess culture was removed with a sterile Pasteur pipette.

The medium was then allowed to dry at room temperature for 15 to 30 minutes with the lid of the plate tipped open slightly.

The dilutions of phage to be spotted on the plate were marked with wax pencil on the reverse side of the plate.

Serial dilutions in nutrient broth of the phage under test were prepared and loopfuls of the diluted phages were placed in the proper positions on the agar surface. These spots were allowed to dry, and the plate was then inverted. Incubation was at 30°C. overnight. The titre was considered to be the highest dilution which yielded confluent lysis.

For routine use the concentrated phage was diluted to its routine test dilution R.T.D., which is defined as the dilution that produces confluent lysis when a single drop is applied to a plate previously spread with the propagating strain of staphylococcus.

Preparation of the R.T.D. may be illustrated by an example. If the titre of phage "X" is 1/1000, for example, adding 1 ml. of phage to 999 ml. sterile broth yields a dilution of 1/1000. Similarly adding 0.01 ml. of phage to 9.99 ml. broth results in the same dilution, thereby reducing the amount of phage suspension used in a test. If clear lysis was not observed, the material was discarded and the process was repeated.

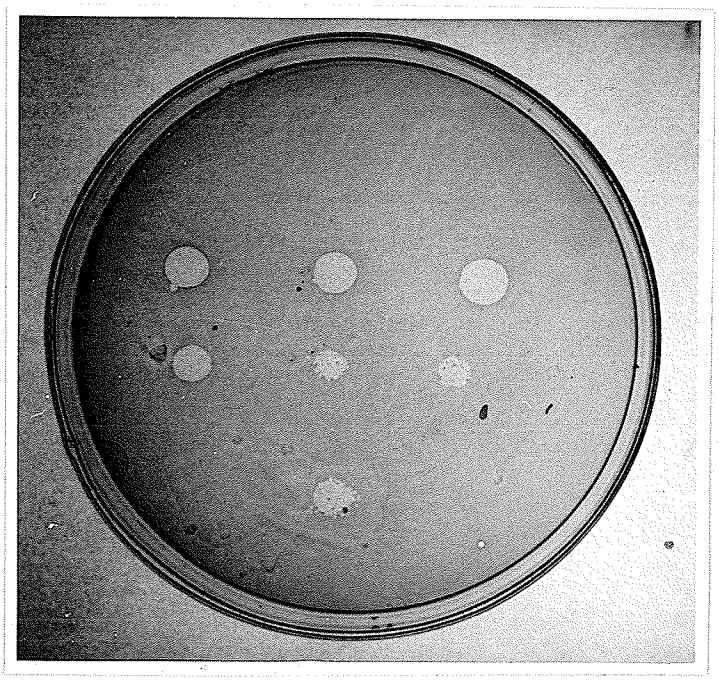

Figure 1

Phage titration with a strain of Staphylococcus aureus

Serial dilutions of the phage ranging from undiluted 1:10, 1:100, 1:1000, 1:5000, 1:10,000 and 1:20,000

reading from top row, left to right

The undiluted and the 1:10, 1:100, 1:1000 dilution showed confluent lysis. With this phage the titre was considered to be 1:1000.

The test dilutions were checked weekly for effectiveness, and were discarded if confluent lysis was not observed with its propagating strain and a new R.T.D. prepared.

PHAGE TYPING OF ISOLATES

The staphylococcal cultures subjected to phage typing were obtained through the courtesy of Dr. J. Isa, Director, Provincial Veterinary Laboratory. Samples of milk received by the Veterinary Laboratory for routine analysis for mastitis were used to isolate these haemolytic coagulase positive strains of staphylococci.

Each culture used was transferred from a blood plate into nutrient broth and incubated at 37°C. overnight. Trypticase soy agar plates were flooded with the broth culture and the excess fluid removed by means of a sterile Pasteur pipette. Plates were allowed to dry with the lids slightly open. The drying time, at room temperature, varied from 10 to 30 minutes depending upon the relative humidity of the room.

In order to save time and material 12 phages were tested on one plate. This was carried out as follows. A key was prepared on a piece of cardboard (5" x 5"). A circle was drawn, exactly to the size of a standard Petri dish, and this circle divided into 12 segments. Each segment was assigned a phage number. The Petri dish containing the

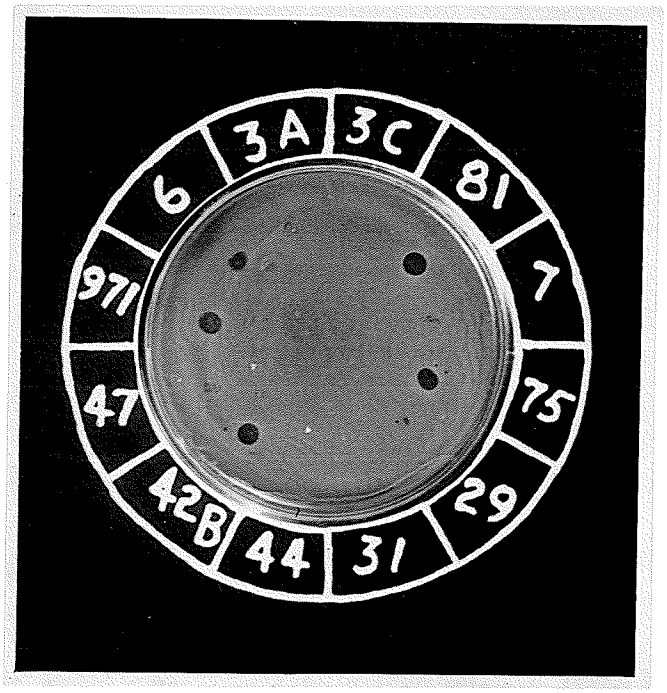

Figure 2

Phage typing of one strain of Staphylococcus aureus

Heavily seeded plate with each phage spotted as indicated

Incubated at 30°C. for 18 hours

Absence indicates no reaction with the strain of phage used.

culture to be typed was placed squarely upon the circle. A small loopful of each phage suspension at its R.T.D. was spotted in its proper position on the agar surface. Twelve additional phages were tested on a second plate, and the remaining phage on a third.

After a drying period at room temperature, incubation was at 30°C. overnight. A phage was considered to have acted upon the culture under test if there was complete lysis and in some cases if more than 50 plaques developed in the area covered by the drop of phage suspension. If the number of plaques was less than 50 or if there was no lysis the culture was considered to be "not typable" with that phage.

If no lysis was observed with any of the 30 phages at their routine dilution, the test was repeated using phages diluted 1:10. Diluting of stock phages 1:10 was done in order to eliminate false reactions that sometimes occurred with undiluted phages. The false reactions were due to some unidentified agent, present in the phage suspension which resulted in inhibition of the staphylococci (22, 25).

If no lysis was observed with any of the phages diluted 1:10, that is, with "strong phage", no further tests were performed and the culture was considered "not typable". If the lysis was observed with the 1:10 dilutions, the phages which caused this lysis were titrated against the culture

using tenfold dilutions of phage, in order to determine the maximum dilution at which lysis occurred (3). Only results obtained at the maximum test dilution were recorded, and only strong lytic reactions were taken into account, that is more than 50 plaques. As pointed out by Williams and Rippon (25), phages "appear to give pattern reactions rather than true type--specific lysis" therefore, isolates were identified according to their "lytic patterns".

At the start of this study 27 phages were used routinely. Towards the completion of this study phages 80 and 82 (52AV) were received from Dr. E.T. Bynoe, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa. These were included there-after in the basic set used routinely in typing of staphylococci. The cultures that proved "not typable" with the original 27 phages were not tested against these additional phages.

In 1958 the International Committee recommended the following system of grouping which has been followed, in general, in compiling this report, as shown in Table I.

TABLE 1

ROUTINE SET OF PHAGES FOR TYPING STAPHYLOCOCCUS AUREUS

(Recommended by the International Committee--1958)

Group I	Group II	Group III	Group IV	Misc.
29	3A	6	42D	187
52	3B	7		
52A	3C	42E		
79	55	47		
80	71	53		
81		54		
82(52AV)		73		
		75		
		77		

Because it was impossible to obtain some of the phages listed above, (187 and 71) and because additional phages to those listed in this system were used, the grouping system followed was a combination of the scheme used by Dr. E.T. Bynoe, with modifications recommended by Dr. L.P. Lansdown, Manitoba Provincial Laboratory. Phage 57, or "W" as it is locally referred to, and 971 although listed in the "Miscellaneous" group in previous reports by the International Committee, seem to belong to Group III. This is one of the modifications suggested by Dr. Lansdown by personal communication and these phages have therefore been placed in Group III. The system of grouping phages used to evaluate the data that have been compiled are given in Table 2.

TABLE 2

ROUTINE SET OF PHAGES USED FOR TYPING STAPHYLOCOCCUS AUREUS

Group I	Group II	Group III	Group IV	Misc.
29	3A	6	42D	44A
52	3B	7		47A
52A	3C	42B		
79	51	42E		
	55	47		
		47B		
		47C		
		53		
		54		
		73		
		75		
		77		
		971		
		W		
		81		
		80		
		82		

ANTIBIOTIC SENSITIVITY

Forty-three staphylococcal cultures chosen at random from the cultures submitted for phage typing were tested for sensitivity to penicillin, neomycin, tetracycline, polymyxin B, erythromycin, chloromycetin, aureomycin, terramycin, dihydro-streptomycin, carbomycin, bacitracin, furadantin and Triple Sulpha by the antibiotic sensitivity disc method, (B.B.L. "Sensi-Discs"). Two concentrations of each antibiotic, (one "high" and one "low") were used in this test, with the exception of furadantin and Triple Sulpha, in which cases, only one concentration was used. The high and low concentration are presented in Table 3.

TABLE 3

CONCENTRATION OF ANTIBIOTICS USED IN SENSITIVITY TESTS

ANTIBIOTIC	HIGH	LOW
1. Tetracycline	30 mcg.	5 mcg.
2. Penicillin	10 units	2 units
3. Polymyxin B (Aerosporin)	300 units	50 units
4. Neomycin (Mycifradin)	30 mcg.	5 mcg.
5. Erythromycin	15 mcg.	2 mcg.
6. Chloromycetin (Chloramphenicol)	30 mcg.	5 mcg.
7. Aureomycin (Chlortetracycline)	30 mcg.	5 mcg.
8. Terramycin (Oxytetracycline)	30 mcg.	5 mcg.
9. Dihydro-streptomycin	10 mcg.	2 mcg.
10. Carbomycin	15 mcg.	2 mcg.
11. Bacitracin	10 units	2 units
12. Furadantin (Nitrofurantoin)	100 mcg.	-
13. Triple Sulpha[i]	1 mcg.	-

i Sulphadiazine, Sulphamethazine, Sulphamerazine

This method was chosen for two reasons. First, it was possible to test a large number of cultures with a minimum of time and materials. Second, due to recent improvements in manufacturing techniques, discs of uniform concentrations of antibiotics were available. Recent publications have indicated a good correlation between results from disc method with those obtained by the tube dilution technique (4, 15, 20, 21).

The culture under test was prepared as for phage typing. That is, the culture was grown in nutrient broth overnight, flooded on B.B.L. trypticase soy agar plates and then dried. Six antibiotics were placed on the surface, spaced as evenly as possible. Each culture required four agar plates for this test. The plates were incubated at 30°C. for 18 hours and examined for zones of inhibition. A clear zone of any size was considered as "inhibition".

A culture was considered to be "sensitive" to an antibiotic if both "high" and "low" concentration discs were surrounded by clear zone of inhibition. If a culture showed no inhibition zone around the low concentration disc but a clear zone around the disc carrying the high concentration of the antibiotic the culture was considered "moderately sensitive". A culture showing no zone of inhibition around either the "high" or the "low" disc was considered to be "resistant" to the antibiotic.

Figures 3a and 3b show results obtained using the disc method to determine antibiotic sensitivity. All degrees of sensitivity are shown, from complete "inhibition" to "not sensitive".

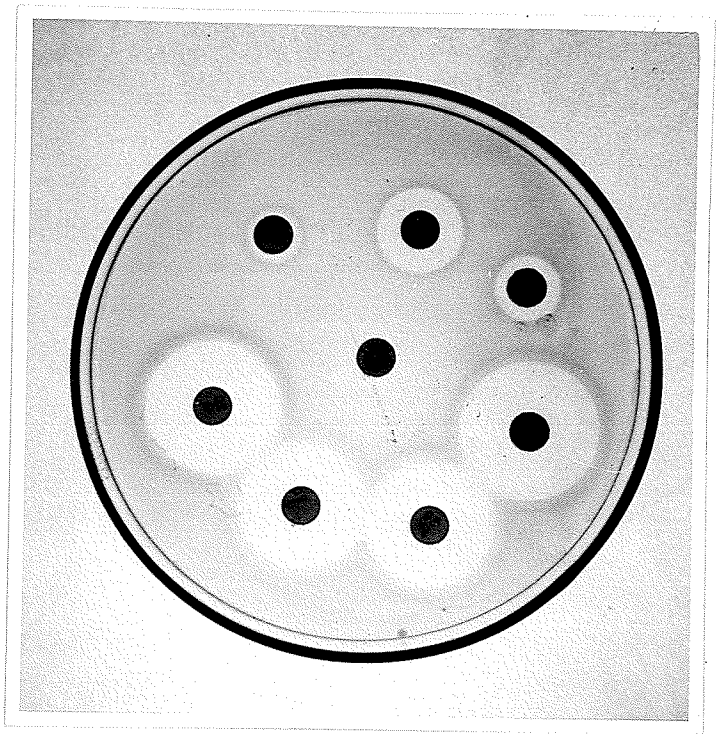

Figure 3a

Reaction of a strain of Staphylococcus aureus to "high" concentrations of certain antibiotics and of Triple Sulpha to a Staphylococcus culture.

From top left corner reading clockwise

polymxin B, dihydro-streptomycin, penicillin, aureomycin, terramycin, tetracycline, chloramphenicol and Triple Sulpha(centre).

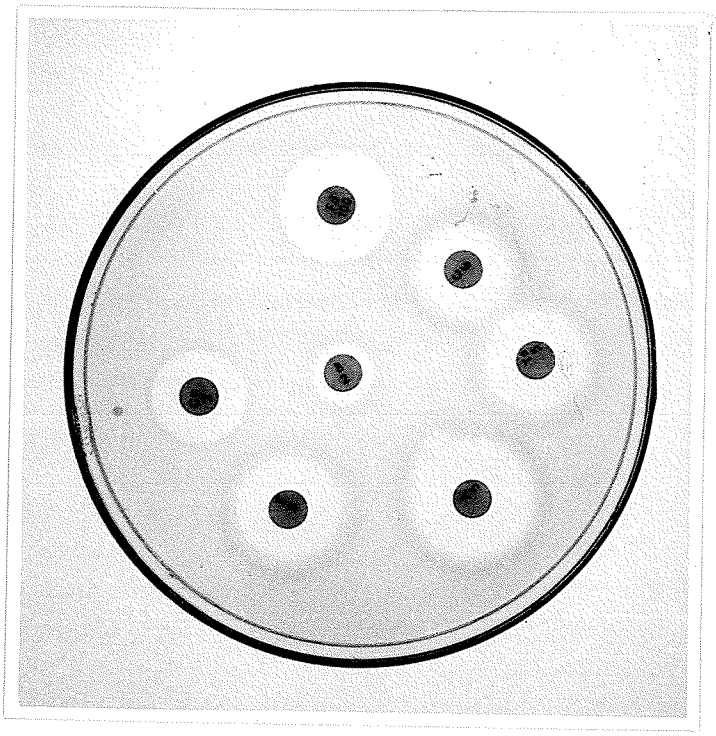

Figure 3b

Reaction of "low" concentration of the same antibiotics and certain others to the same culture.

From top centre reading clockwise

furadantin, chloramphenicol, terramycin, aureomycin, tetracycline, carbomycin and penicillin(centre).

R E S U L T S

RESULTS

PHAGE TYPING

In Table 4A are shown the results of phage typing of 342 haemolytic, coagulase positive Staphylococcus aureus cultures. Only 29 were untypable with the phages used, and 84 of the typable cultures reacted to more than one phage group. By far the largest number of cultures were typed by phages of Group III (Table 4). Forty-three or 16% were typable only with "strong" phage and 270 or 84% were typable using phages at R.T.D.

Of the 220 isolates typable by phage Group III approximately 81% have in addition to phages of Group III one or more of the 80/81/82 series (Table 4C).

Of the 84 isolates showing cross-group reactions, (the Mixed group), all have Group III phages in common. No cross-grouping between other groups, e.g. I/II, were found. In this breakdown, Group II was most commonly associated with Group III phages (Table 4B).

ANTIBIOTIC SENSITIVITY

The antibiotic sensitivity of 43 bovine Staphylococcus aureus cultures picked at random from the 342 cultures subjected to phage typing is presented in Table 5. These cultures showed a high percentage of resistant strains to some antibiotics, e.g. Polymyxin B and Triple Sulpha, and a high percentage of sensitive strains to others,

e.g. bacitracin and furadantin. However, to the more commonly used antibiotics there is considerable resistance, (penicillin 23% and dihydro-streptomycin 16%), although many Staphylococcus aureus isolates are susceptible to these antibiotics.

TABLE 4A

PHAGE-GROUPING OF STAPHYLOCOCCUS AUREUS

ISOLATED FROM MASTITIC CATTLE

	Phage Group						
	I	II	III	IV	Misc.	Untypable	Mixed
No.	2	5	220	0	2	29	84
Percent	0.6	1.5	64.1	0	0.6	8.5	24.7

TABLE 4B

PHAGE-GROUPING OF STAPHYLOCOCCUS AUREUS

CULTURES OF "MIXED GROUP"

	I/III	II/III	IV/III	Misc./III
No.	20	56	0	18
Percent[i]	23.8	66.7	0	21.4

[i] As some isolates reacted with phage types from more than one group, the sum of percentages exceeds 100.

TABLE 4C

ASSOCIATION OF 80/81/82 WITH 220 CULTURES

TYPED BY GROUP III PHAGES

	GROUP III only	GROUP III 80/81/82
No.	41	179
Approx. Percent	19	81

TABLE 5

THE SENSITIVITY OF FORTY-THREE BOVINE STAPHYLOCOCCUS CULTURES ISOLATED FROM MASTITIC MILK SAMPLES TO DIFFERENT ANTIBIOTICS

ANTIBIOTIC	SENSITIVE		MODERATELY SENSITIVE.		RESISTANT	
	No.	Approx Percent	No.	Approx Percent	No.	Approx. Percent
1. Polymixin B	1	2	5	12	37	86
2. Neomycin	32	74	10	23	1	3
3. Erythromycin	31	72	10	23	2	5
4. Penicillin	32	74	1	3	10	23
5. Terramycin	33	76	8	18	2	6
6. Carbomycin	30	69	11	25	2	6
7. Bacitracin	38	88	4	9	1	3
8. Tetracycline	32	74	9	21	1	3
9. Aureomycin	35	81	8	19	0	0
10. Dihydro-Streptomycin	4	10	32	74	7	16
11. Chloromycetin	25	58	16	37	2	5
12. Furadantin	42	97	0	0	1	3
13. Triple Sulpha	2	8	0	0	39	92

D I S C U S S I O N

DISCUSSION

The data obtained demonstrates the nature of bovine staphlococcal infection in Manitoba. Others have shown that the majority of Staphylococcus aureus cultures isolated from milk and dairy products type with Group IV phage (42D) (18), Price, Neave, Rippon and Williams (10) found that in addition to various Group IV strains, staphylococci of Group III were also common. The data presented appears to warrant the assumption that Group IV staphylococci represents a portion of the normal flora found in milk and dairy products, while staphylococci causing mastitis belong to Group III, and appear to be the same as those causing infections in humans. It may also be assumed that these Group III organisms may be transferred from man to animals. It was found that these staphylococcal isolates were not insensitive to the typing phages used although the phages had been selected for testing human strains.

The results have indicated that there is a change in the flora of the bovine udder when a staphylococcal infection occurs. In the normal animal, Group IV strains predominate (17), but when staphylococcal infection occurs, these strains are replaced by the infectious Group III strains. It is interesting to note also, that although these isolates were taken from known mastitis cases, no Group IV strains were found. Many strains showed weak reactions with phage 42D, but none showed reactions strong enough to be considered as phage 42D types.

In the treatment of such infections, penicillin and streptomycin are still the drugs of choice by many veterinarians. The percentage of resistant strains in cattle is far removed from the reported percentage found in hospital environments (3). Although 74% of the strains are sensitive to penicillin, and 84% (both sensitive and moderately sensitive) of the isolates tested showed some degree of sensitivity to dihydro-streptomycin. The fact that approximately 23% and 16% respectively were found to be resistant appears to indicate that probably these strains are developing in dairy cattle as the result of the wide use of these antibiotics. In hospital environments, infections are more likely to be transmitted from patient to patient (3) and a higher percentage of antibiotic resistant strains is to be expected. In the dairy herds spread over Manitoba resistant strains have not been introduced on quite the same scale, but the level is at the point where some thought should be given to limiting the use of chemotherapeutic techniques by veterinarians and farmers.

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