A comparison of serotyping, phage typing, antibiotic sensitivity, and plasmid profile analysis in a study of Salmonella dublin

by

Kathleen E. Ferris

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Interdepartmental Program: Immunobiology

Major: Immunobiology

Iowa State University
Ames, Iowa
1986

TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	4
MATERIALS AND METHODS	18
Bacterial Isolates	18
Serotyping	18
Antibiotic Sensitivity	20
Phage Typing	21
Plasmid Analysis	23
RESULTS	31
Serotyping	31
Phage Typing	31
Antibiotic Sensitivity	42
Plasmid Analysis	44
DISCUSSION	56
REFERENCES CITED	60
APPENDIX: RESULTS OF ANTIBIOTIC RESISTANCE TESTING	69
ACKNOWLEDGMENTS	73

INTRODUCTION

Salmonella dublin (*Salmonella choleraesuis* subspecies *choleraesuis* serovar dublin) has been identified as the causative agent of disease in cattle for many years (35, 58, 65, 74, 80, 85, 93, 94), and has also been associated with disease in humans and other animals (21, 69, 74, 77, 85, 92, 94). Although this serotype is not a frequent cause of salmonellosis in humans, when it does occur it tends to be much more invasive than other serotypes (74, 85). The reported number of human isolations of *S. dublin* have increased from three in 1964 to over 100 per year since 1980 (74, 85). Available information indicates that in animals this serotype has been one of the ten most frequently isolated serotypes in the United States every year since 1978 (10). Also, an increased interest in *S. dublin* has developed because of the changing distribution of this serotype in the United States. Initially, *S. dublin* was isolated only from animals west of the Rocky Mountains (21); but recently isolations have been made from humans and animals in the eastern part of the United States (9, 74, 85).

The practice of feeding antimicrobial agents to animals and the rising incidence of multiply resistant bacteria has been the subject of considerable discussion (37, 51). Therefore, there has been interest in determining patterns of antibiotic resistance exhibited by salmonellae.

The association of *S. dublin* with disease in humans and animals, especially cattle, has increased the importance of epidemiologic

studies. For many years serotyping has been used extensively for identification of salmonellae for epidemiologic purposes. However, as serotypes become more common further definitive identification is necessary. Several methods other than serotyping are available for characterizing Salmonella strains including phage typing, biotyping, tests for antibiotic resistance, and plasmid profile analysis.

Phage typing is a common procedure used in characterizing S. typhi and S. typhimurium isolates from salmonellosis in humans (3, 13, 25, 33, 34, 48). A set of six phages was used by Smith to examine S. dublin isolates but the majority of isolates were of the same phage type (78). Other problems associated with phage typing are the time required for testing isolates and the reproducibility of results (38).

Many researchers have attempted to describe strains of S. dublin based on biochemical activity (biotyping). Results indicated that this method was not useful for epidemiologic studies because the majority of isolates tested were in one group and isolates of different groups could be isolated from the same animal (30, 61, 91).

Antibiotic resistance patterns have been used in characterizing salmonellae, but do not appear to be as definitive as phage typing (38). This method is not used extensively in epidemiologic studies (38, 42).

Plasmid profile analysis is the most recent method to be used by epidemiologists and has been employed in studying several outbreaks of salmonellosis (55, 56, 72, 73, 86). No published reports were available of the plasmid types of S. dublin at the time this study was initiated. A recent study conducted by O'Brien et al. of Eco R1 plasmid digests of

twelve isolates of S. dublin (ten from cattle and two from humans) revealed that the isolates had DNA fragments of the same size (63). Also, a 50-Mdal plasmid associated with virulence for mice has been reported in isolates of S. dublin studied in Japan, Great Britain, and Belgium (44, 69, 88).

The objectives of this investigation were:

1. to examine plasmid profiles present in isolates of S. dublin obtained from cattle, swine, and man in various geographical areas of the United States;
2. to compare plasmid profiles with serotype, phage type, and antibiotic resistance.

LITERATURE REVIEW

Salmonella is a genus in the family Enterobacteriaciae. The members of this genus are straight, gram negative rods (0.7-1.5 x 2.0-5.0um) which are usually motile by peritrichous flagella (47). Colonies are usually 2-4 mm in diameter, circular, low convex, with smooth surface and entire edge (66). Most salmonellae possess mannose-sensitive, hemagglutinating and adhesive type I fimbrae (66).

Members of the genus Salmonella are facultative anaerobes, and gas is usually produced from glucose. Nitrates are reduced to nitrites; citrate is usually utilized as the sole carbon source. Methyl red test is positive; hydrogen sulfide is usually produced. Lysine and ornithine decarboxylase are usually positive; and mannitol, maltose, and sorbitol are usually fermented. Sucrose, adonitol, lactose, salicin, raffinose, inositol, and amygdalin are usually not fermented. Indol, urease, and Voges-Proskauer tests are usually negative; phenylalanine and tryptophan are not deaminated. Lipase and deoxyribonuclease are not produced (22, 47, 50).

Organisms of the genus Salmonella show at least 80% relatedness when compared by DNA/DNA hybridization analysis; the DNA base composition is 50-53 mol% G+C (48, 66). Kauffman, in 1963 and 1966, divided the genus Salmonella into four subgenera based on biochemical reactions. New serotypes belonging to subgenera II-IV are designated by their antigenic components and those in subgenus I (typical biochemically) are given names (48). In 1982, Le Minor et al. suggested a different system of

nomenclature for the salmonellae based on their biochemical reactions, into six subspecies (49). More recently, Farmer et al. used the same divisions and designated them "subgroup" rather than subspecies (23). Subspecies is the accepted term.

Salmonellae were first described by the disease produced in man and animals (66). Enteric fever was described by Bretonneau in 1822, and in 1829 Louis used the name "typhoid" to group together many of the symptoms and characteristics of enteric fever (48). Budd, in 1856, reported that typhoid was infectious and was transmitted from person to person (48). Carl Joseph Eberth, in 1880, was the first to observe the typhoid bacillus. He noted the organisms in the spleen and mesenteric lymph nodes of a patient that died of typhoid fever (39, 48). G. T. A. Gaffky, a student of Robert Koch, succeeded in isolating the organism in 1884 (39, 48). The association between the typhoid bacillus and the disease was confirmed in 1896; Pfeiffer and Kolle, and Gruber and Durham demonstrated the typhoid bacillus was agglutinated by serum from an animal immunized with the bacillus. Also, in 1896, Widal in Paris, and Grunbaum in London showed that the typhoid bacillus was agglutinated by the serum of patients affected with typhoid (48).

Research was also being done on typhoid-like disease in animals. Daniel E. Salmon and Theobold Smith, in 1885, isolated and identified Bacillus cholerae suis, the organism they believed to be the cause of hog cholera (48, 82). In 1888, Gartner reported that meat from a diseased cow was the source of an outbreak of human disease in Germany. The bacteria isolated from the meat and from a person who had died of

the infection was called Bacillus enteritidis (82). Klein, in 1889, isolated the organism causing typhoid in chickens and named it Bacillus gallinarum, and Loeffer, in 1892, isolated Bacillus typhimurium from mouse typhoid. Bacillus abortus equi was isolated from a vaginal culture by Kilborne in 1893 associated with an outbreak of abortion in mares (82).

In 1900, Lignieres established a genus to include these bacteria. He named the genus Salmonella after Dr. Salmon (48). This replaced the designation Bacillus in reference to this group of organisms.

The use of serologic procedures in the identification of salmonellae began in 1896 when Gruber and Durham demonstrated the agglutination of bacteria by specfic immune serum. Smith and Reagh, in 1903, differentiated between the somatic and flagellar antigens through the use of a non-motile variant of Salmonella cholerae-suis (48). The somatic and flagellar antigens were designated "O" and "H" by Weil and Felix in 1918 (48). They observed a thin zone of spreading produced by Bacillus proteus on an agar surface which they designated "Hauch" (breath). A non-motile variant of this organism failed to spread on the agar, and was called the "Ohne hauch" form. In 1911, Bainridge and Dudfield were able to separate different strains of salmonellae through agglutination and absorption procedures. Schutze, in 1920, showed that some members of the group could be further divided into subgroups through the use of absorbed sera. In 1922, Andrews found that the flagella of salmonellae may have two distinct forms (diphasic) (48). A surface antigen (Vi) was

described by Felix and Pitt in 1934 (48). This antigen blocked agglutination of the typhoid bacillus by somatic antisera. White developed the first antigenic scheme for Salmonella in 1926 (48). It was later expanded by Kauffman, and the Kauffman-White scheme now contains over 2000 serotypes (48).

Salmonellae are primarily intestinal parasites of vertebrates, causing enteritis and typhoid-like disease (48, 66). Most salmonellae serotypes are ubiquitous and are isolated from a wide variety of sources (48, 66). However, some of the serotypes are adapted to a particular host; these organisms tend to cause severe disease (48). Salmonella typhi, S. paratyphi A, and S. sendai are serotypes adapted to humans (48). S. cholerae-suis is adapted to swine, but can infect humans causing an unusually severe disease with about 20% fatality (66). Similarly, S. dublin is adapted primarily to cattle, but may cause serious disease in humans and other animals (74, 85). Another characteristic of these host-adapted serotypes is a tendency of survivors to become long-term carriers (48, 66).

Salmonella dublin, first reported as a distinct serotype by White in 1929, causes disease primarily in cattle (85). Cattle of all ages may be affected with an acute or subacute illness. Symptoms in acute infections in adult cattle include fever, loss of appetite, and reduced milk production often followed by diarrhea; the mortality rate is reported to be about 75% without treatment. In pregnant animals, the disease may result in abortion without other clinical symptoms. Calves infected with S. dublin exhibit many of the same symptoms seen in adult cattle

(fever, loss of appetite, and diarrhea), as well as septacemia and pneumonia. Cattle that have recovered from the disease often continue to harbor the organism and may shed the organism in their feces for many years, even following treatment with antibiotics to which the organism is sensitive. Carriers may shed the organism regularly, intermittently, or rarely (latent carriers); shedding may be brought on by stress to the animal. S. dublin may be shed in the milk of cattle following abortion or calving. Congenitally infected calves may be born to active or latent carriers of S. dublin (35, 58, 65, 80, 93, 94).

Although S. dublin is primarily isolated from cattle, other species such as sheep and swine are occasionally infected (21, 94). S. dublin is an important agent in ovine abortion with reports of morbidity rates of 10-15%, and mortality rates of 5-7% (74).

S. dublin also causes disease, sometimes quite severe, in humans. Patients suffering from chronic illness, such as leukemia, diabetes, peptic ulcer, and pernicious anemia are most likely to become infected with S. dublin (74, 85). The use of antacids is also associated with human infection (74). S. dublin tends to be more invasive in humans than are other serotypes (74, 85). Isolates from sites outside the gastrointestinal tract usually make up less that 15% of the isolates of salmonella whereas 79% of the S. dublin cases studied in 1983 in California were from extra-intestinal sites, such as the blood, lung, cerebro-spinal fluid, and peritoneal fluid (74). Only S. cholerae-suis and S. paratyphi A are associated with a higher percentage of isolates from blood than S. dublin infection (85).

Human infection by S. dublin is usually associated with the ingestion of raw milk (85); however, meat has been identified as a probable source of human infection (69). A study by Werner et al. associated human infection with the ingestion of raw milk and demonstrated the severity of the disease (92). In this study, it was found that the incidence of S. dublin infection in man demonstrated a five-fold increase from 1971 to 1975 in California. Raw milk was linked to infection in 44 of 113 cases studied; one dairy was implicated in the majority of the cases. The disease was quite severe with a hospitalization rate of 80%, and an overall fatality rate of 21%. This was different from the infections caused by most other salmonellae in which the fatality rate was less than 1% (92).

Small and Sharp described a milkborne outbreak in Scotland in 1979 which affected at least 700 persons. The ages of those with confirmed infections ranged from three days to 87 years; about one third were under 5 years old and one sixth between 5-15 years old. The most common symptom in this outbreak was acute diarrhea, with vomiting, headache, dizziness and pyrexia reported. Sixty-two percent of the cases followed continued to excrete S. dublin for several weeks after recovery; one continued for three months. Although the vehicle of transmission was raw milk, investigators were never able to isolate the organism from the cattle believed to be the carriers (77).

Salmonella dublin has been present in animals in the United States for many years. The first summary of isolations of S. dublin was presented by Edwards in 1948 (21). At that time, and until 1968, S. dublin

was isolated only from animals in states west of the Rocky Mountains. Since 1968, S. dublin has been isolated from animals in the eastern part of the United States (9). Moreover, S. dublin has been among the ten most frequently isolated serotypes from animals in the United States each year since 1978 (10). In 1982, 194 isolations of S. dublin were reported from animal sources; the majority (173) of the isolates were from cattle. Of the total number of Salmonella isolates obtained from cattle in 1982, 13.5% were S. dublin (11). In 1983, reports of S. dublin dropped to 136; the lowest number of isolations since 1979. However, it continued to be the third most frequently identified serotype from cattle, after S. typhimurium and S. typhimurium subsp. copenhagen (83). The number of isolates of S. dublin identified from animal sources in 1984 increased to 208; 195 were from cattle. It was the second most frequently identified serotype reported from cattle in that year (Ferris and Murphy, unpublished data).

The number of S. dublin isolates reported from human infection in the United States increased from three in 1964 to about one hundred for each year from 1980-1982 (74, 85). Between 1968 and 1979, 70% of the isolates were from California (85). Although the Pacific Region states still have the highest percentage of human isolates, a greater number of isolates are being reported from other areas of the country (74).

The importance of this organism in the beef and dairy industry, as well as the possibility for human infection, has stimulated an interest in developing methods of characterizing Salmonella dublin for epidemiologic studies. Once an organism has been isolated from an animal

and identified as belonging to the genus Salmonella, there are several possible methods that may be used in characterization. The methods that are commonly used include serotyping, phage typing, biotyping, and antibiotic resistance patterns. Serotyping, one of the methods used for characterizing salmonellae, involves identifying the somatic or cell wall antigen through agglutination by specific antisera. The flagellar antigen is then identified in a similar manner. Only one flagellar antigen is present in monophasic salmonellae such as S. dublin. Methods for identification and serotyping of salmonellae have been well-defined (22).

Serotyping of salmonellae may be sufficient for epidemiologic studies especially if the serotype is relatively uncommon. However, when one of the more common serotypes is identified further tests may be needed to differentiate strains within the serotype. One additional method of differentiation is phage typing. Phage typing has been used most extensively for identifying types of S. typhi (33, 34, 48) and S. typhimurium (3, 13, 25, 48) reported in human outbreaks. A standard set of phages exists for each of these serotypes and allows strains to be differentiated on the basis of susceptibility to lysis by the various bacteriophages in the set (34). A set of phages for S. typhimurium has been used to study phage types present in animal populations (19). Smith used a set of six phages to classify 294 strains of S. dublin; he found a majority of isolates (66.9%) were of the same type (78). In 1977, a single set of phages was introduced that could be used to characterize some of the more common salmonellae included in groups A

through E (28). This original set of 50 phages was later reduced to 27 phages in an attempt to increase the practicality of phage typing (29). Although phage typing is a useful tool in epidemiologic investigations, it is a time-consuming procedure and the results are often not reproducible. Variations in lysis patterns may be recorded for an isolate tested at different times (38).

Another method used for classifying strains is biotyping. Researchers have attempted for several years to describe strains of Salmonella dublin based on biochemical activity. Neel et al., in 1953, identified six different strains of S. dublin based on carbohydrate fermentation reactions (61). A year later, an additional strain was identified by Hughes, based on an inability to ferment rhamnose (91). Hall and Taylor, in 1970, divided S. dublin into 17 biotypes based on fermentation reactions after 48 hours, 3 to 7 days, and 8 to 14 days (30). Walton used arabinose, rhamnose, trehalose, and glycerol as the basis for separating S. dublin into 7 groups, with one group being the vaccine strain. His results indicated that this grouping was of little value for epidemiology because 71% of the isolates were in one group. Also, S. dublin of different groups could be isolated from the same animal (91). The conclusion that biotyping was not useful for epidemiologic studies was supported in a paper by Brunner et al. (12).

The difficulty in devising a biotyping scheme to differentiate S. dublin strains was demonstrated in a publication by Fierer and Fleming. The similarities of biochemical reactions within the serotype S. dublin was the basis for a test that would quickly identify a culture as S.

dublin. Thirty-four strains of human and animal origin isolated in California failed to grow on Simmons' citrate and acetate agar and did not ferment arabinose in 72 hours (26).

A fourth method that has been used to characterize strains of salmonellae is the determination of antibiotic resistance patterns. Many surveys of antibiotic resistance in salmonellae have been done in recent years. A study undertaken in England of salmonellae isolated from animals in 1972 found that of the Salmonella dublin isolates, 97% were resistant to streptomycin, 78% to sulfonamides, 0.6% to tetracycline, 0.1% to furazolidone, and 0.5% to neomycin. This survey revealed that the lowest percentage of cultures sensitive to all drugs (3%) were of cattle origin (79). Also, a high incidence of multiple drug resistance in strains of cattle origin was reported in a survey of salmonellae isolated from domestic animals in Japan (87). Cherubin presented evidence which suggested that in countries in western Europe an increasing number of isolates of S. dublin were becoming resistant to antibiotics, especially chloramphenicol (16). A survey of salmonellae isolated from humans and animals in the United States indicated that resistance to tetracycline and kanamycin had increased in three serotypes (62). A recent study of salmonellae isolates from food-type animals in the United States reported that 80% of 3500 isolates tested possessed multiple resistance patterns (8).

Antibiotic sensitivity testing does not appear to be as specific as phage typing in characterizing strains of salmonellae (38); a single resistance pattern may be found in salmonellae of different phage types

(12). Therefore, it does not appear to be a valuable test for use in epidemiologic studies (38, 42).

The limitations reported in the current technics has necessitated the development of a more definitive method of analysis. A method now being used by bacteriologists is plasmid profile analysis of strains. Recent refinements in techniques for extraction and analysis of plasmid DNA (46) have made this a feasible test for laboratories to perform (46, 59, 76).

Plasmids are circular, double-stranded segments of extrachromosomal DNA that replicate independently of the chromosome (7). They have long been known to carry antibiotic resistance genes and have also been associated with genes mediating many other traits of the bacterial cell. Some of these traits are utilization of various biochemicals (12), bacteriophage resistance (17, 90), heavy metal resistance, resistance to anions and radiation, and toxin production (81).

The results of many studies indicate that some of the virulence factors of bacteria are associated with plasmids (20, 27, 31, 95). In a report by G. W. Jones, adhesive and invasive properties of virulent phenotypes of S. typhimurium were associated with the presence of an autonomous 60-megadalton plasmid (43). The presence of a 36-megadalton plasmid in S. enteritidis has been associated with increased virulence for mice (60). In a study of salmonella isolates from 50 laboratories in 48 countries, 90% of the S. typhimurium, S. enteritidis, and S. dublin, and all of the S. cholerae-suis isolates contained one serotype-specific class of plasmids (32).

Studies of S. dublin isolates have indicated that a 50-megadalton plasmid is present in all isolates tested from Japan (88) and Great Britain (44). Evidence of the association of the 50-megadalton plasmid with virulence for mice was obtained by curing a strain of S. dublin of its plasmid. The strain without the plasmid was approximately 250-fold less virulent for mice than was the parent strain which contained the plasmid (88). It was noted that the derived strain had a slightly rough appearance on DHL agar (88) which could possibly be due to the observation that a major outer membrane protein in S. dublin was dependent on the presence of the serotype-specific plasmid (32). Further evidence for the association of this plasmid with virulence was demonstrated by the insertion of transposon Tn 1 into the plasmid. The organism containing the altered plasmid was less virulent for mice, indicating an interruption of a DNA sequence coding for a virulence-associated factor (5).

A study of the molecular relationships between the plasmids thought to be associated with virulence in several salmonella serotypes was undertaken by Popoff et al. (70). A ^{3}H-labelled plasmid from S. typhimurium was used as a probe and it was found that the 54-76Kb plasmids harbored by S. abortus ovis, S. enteritidis, S. paratyphi C, S. newport, and S. dublin showed a high degree of homology. There was a 61-88% relatedness between these plasmids and the S. typhimurium probe, which may indicate a common ancestral plasmid (70).

The analysis of plasmids contained by microorganisms (plasmid profiles) has been used by epidemiologists in studying outbreaks of infections. This method was used in an outbreak of Staphylococcus aureus to identify similarities or differences in a large group of isolates of the same phage type; researchers were able to identify unique strains and those that were untypable by phage (18). Plasmid studies were used to analyze Salmonella wien isolates from epidemics among people in Europe and North Africa. The similarities in plasmids among strains indicated a possible clonal origin for the isolates (55, 56). Plasmid profile analysis proved useful in the differentiation of S. drypool isolates from two separate outbreaks (72). It was also the method used to distinguish the epidemic strain from other commonly occurring strains of S. muenchen in an outbreak associated with use of marijuana (86). In an outbreak of S. newport, plasmid analysis was used to follow the introduction and transmission of a clone by the ingestion of precooked roast beef (73).

The plasmid content of an organism can change through acquisition of a new plasmid, recombination between plasmids, or loss of a plasmid. Plasmids may be transferred from one bacterium to another (40, 41, 67). Such events are relatively rare, however, and are influenced by the bacterial environment (89). Drug resistance patterns and phage type can both be changed by the acquisition of plasmids or transposons (64). Also, a plasmid may change through the occurrence of deletion variants, such as that observed in S. johannesburg (15). Although such changes in

plasmid content can occur, the plasmid pattern of an organism may remain stable for many years over a wide geographic area (14, 64, 89).

Plasmid analysis is a useful tool in epidemiologic investigations (2, 18, 24, 55, 73). It has been found to be as reliable as phage typing in differentiating isolates in outbreaks and finding related strains (38). A single phage type can be associated with different plasmid patterns and a single plasmid pattern can be associated with different phage types; therefore both are effective in analyzing isolates. However, plasmid analysis is a simple technique and is rapid to perform (12). The importance of plasmid analysis in epidemiologic investigations was demonstrated in a study in which isolates of S. typhimurium were of the same phage type as the epidemic strain, but differed in plasmid content (90).

MATERIALS AND METHODS

Bacterial Isolates

Salmonella isolates used in this study were received from animal disease diagnostic laboratories from widespread areas in the United States by the salmonella serotyping laboratory at the National Veterinary Services Laboratories (NVSL), U. S. Department of Agriculture, Ames, Ia. The submitting laboratories were responsible for the isolation and preliminary identification of the cultures. The isolates from humans were received from the enteric diseases section of the Centers for Disease Control (CDC), Atlanta, Ga.

Serotyping

Antisera

Unabsorbed antisera used in serotyping salmonellae were obtained from CDC, Atlanta, Ga. The somatic (O) grouping antisera were diluted 1:3 with phenolized saline (0.5% phenol, 0.85% saline) and the flagellar (H) antisera were diluted 1:20 with phenolized saline. The absorbed antisera were either purchased from Difco laboratories, Detroit, Michigan, or produced by the salmonella serotyping laboratory using the following procedures outlined by Edwards and Ewing (22). The absorbed single factor "O" 9 was prepared by growing S. paratyphi A and S. reading on large plates (9 in, 250 ml of agar). The cells were harvested after incubation at 37° C for 16 h and sedimentated by centrifugation for 40 min at 34,800 x g in a Sorvall refrigerated centrifuge. The supernatant

was discarded and the cells were used to absorb O group D (9,12) S. gallinarum antisera. Two ml of antisera were added to a tube containing the cells harvested from one plate of S. paratyphi A and one plate of S. reading. The suspension of antiserum and cells was incubated in a 50° C water bath and stirred at fifteen-minute intervals for two hours. After centrifugation for 40 minutes at 34,800 x g, the antiserum was removed to a different tube and tested by using standard antigens to insure complete absorption of the factors present other than the 9 (22). The absorbed single factor "O" 46 antiserum was produced by the same procedure using cells of S. typhi and S. anatum to absorb O group D ((9),46) S. haarlem antiserum. The single factor O antisera were not diluted for use. The single factor H antisera were purchased from Difco and were diluted with phenolized saline according to the manufacturer's instructions.

Method

Serotyping was carried out according to procedures reported by Edwards and Ewing (22). The only modification was the use of a live somatic antigen rather than an alcohol-killed antigen. The somatic antigen was produced by using one ml of 0.85% saline to wash the growth from a blood agar base slant incubated at 37° C for 16 h. A slide agglutination test was used; one drop of antigen was mixed with one drop of antiserum on a glass plate and allowed to react for up to two minutes at 20° C while gently rocking the plate. Each antigen was tested against at least two different grouping antisera, and usually against

groups 18, B, C1, C2, D, and E (E1, E2, E3, and E4 pooled). If the antigen reacted with O group D, it was tested by using single factor 9 and 46 antisera in a slide agglutination test. Isolates positive for 9 and negative for 46 were members of O group D1. The flagellar antigen was prepared by adding approximately 25 ml of 0.85% saline containing 0.6% formalin to a tube of four ml trypticase soy tryptose (TST) broth inoculated with an isolate and incubated at 37° C for 16 h. Isolates possessing the somatic antigen of group D1 were tested in a tube agglutination test by using flagellar antisera g,m; 1,v; and 1,5. One ml of flagellar antigen was mixed in a 13x100 tube with 0.02 ml of diluted antisera and allowed to incubate at 50° C in a waterbath for 20 min to one hour. Antigens agglutinating with the g,m antiserum were tested with absorbed flagellar antisera f, m, s, t, p, q, u, and z51. One-half ml of antigen was mixed with 0.02 ml of diluted absorbed antiserum and allowed to incubate as before. Isolates agglutinating with only absorbed antiserum p were identified as Salmonella dublin.

Antibiotic Sensitivity

Antimicrobial sensitivity tests were conducted using discs purchased from Difco Laboratories, Detroit, Michigan. Discs used included: amikacin, 30 ug; ampicillin, 10 ug; chloramphenicol, 30 ug; gentamycin, 10 ug; kanamycin, 30 ug; neomycin, 30 ug; penicillin G, 10 units; streptomycin, 10 ug; sulfonamides, 300 ug; tetracycline, 30 ug; tobramycin, 10 ug; and trimethoprim, 5 ug.

Testing was done according to the standard method of Bauer and Kirby (6) using 150 mm Mueller-Hinton agar plates. The plates were incubated for 16-18 h at 37° C, and zone diameters were recorded in mm. A standard culture of Staphylococcus aureus was used to confirm the correct zones of inhibition with the antibiotic discs.

Phage Typing

Phage typing of isolates was conducted using twenty-seven phages (#1-27) received from M. Gershman, Orono, Maine (29). Phage #2 was not used because of difficulties in maintaining the viability at the necessary routine test dilution (RTD). An additional phage, designated #370, was used in phage typing of isolates. Phage #370 was isolated from a S. dublin strain (#84-3700) that was tested for plasmid profile and antibiotic sensitivity. Plaques caused by the lysis of culture #84-3700 were present on the antibiotic sensitivity plate after overnight incubation. The lysed area was swabbed and the swab suspended in TST broth. The broth was then filtered through a 0.45 um HA Millipore filter. Culture #84-3700 was plated on a phage agar plate and several colonies were tested with the phage lysate; one that exhibited confluent lysis was used as the propagating strain.

Two methods were used in the propagation of the phages. One was the method described by Swanstrom and Adams (84). In this method, a 15-cm petri plate containing 60 ml of nutrient agar was used. Fifteen ml of nutrient broth with 0.5% NaCl and 0.7% agar (45° C) were inoculated with one ml of broth containing approximately 9×10^7 organisms per ml of the

propagating strain and two ml of the phage. This was mixed gently and poured over the nutrient agar base. After overnight incubation, the soft agar layer was suspended in 10 ml of broth, clarified by centrifation at 60 x g for 20 min, and the supernatant filtered through a 0.45 um filter. The filtrate was then titered to find the phage content, and used at the RTD, or above if there was a higher dilution that gave confluent lysis with the propagating strain.

In a second method of phage propagation, five ml of TST broth were inoculated with the propagating stain and incubated until the growth was first visible (about $9x10^7$ organisms per ml). Phage was then added to the broth and the tubes were placed on a shaker for 2-4 hours or until the broth cleared. This was then filtered using a 0.45 um filter and the titer of the filtrate determined as in the previous procedure.

Isolates to be phage typed were grown in TST broth until the growth first became visible; two ml of broth were spread on a nutrient agar plate and allowed to dry. A drop of each phage was then applied to the plate with a Pasteur pipette. Results were recorded after overnight incubation. The results were recorded as follows: CL, confluent lysis; OL, opaque lysis (due to overgrowth); SCL, semiconfluent lysis; <SCL, less than semiconfluent lysis; +++, 60-120 plaques; ++, 20-60 plaques; +, 6-20 plaques; -, 0-5 plaques. Phage typing results were entered into an HP 3000 series 68 computer (Hewlett Packard, Cupertino, CA) with results of "+" and "-" (less than 20 plaques) entered as "-" and all other results as "+". Phagerep (Biometrics staff, NVSL, Ames, IA) was the program used in analysis of data. The code system of Audurier et

al. (4) was used in comparing phage type and plasmid group.

Plasmid Analysis

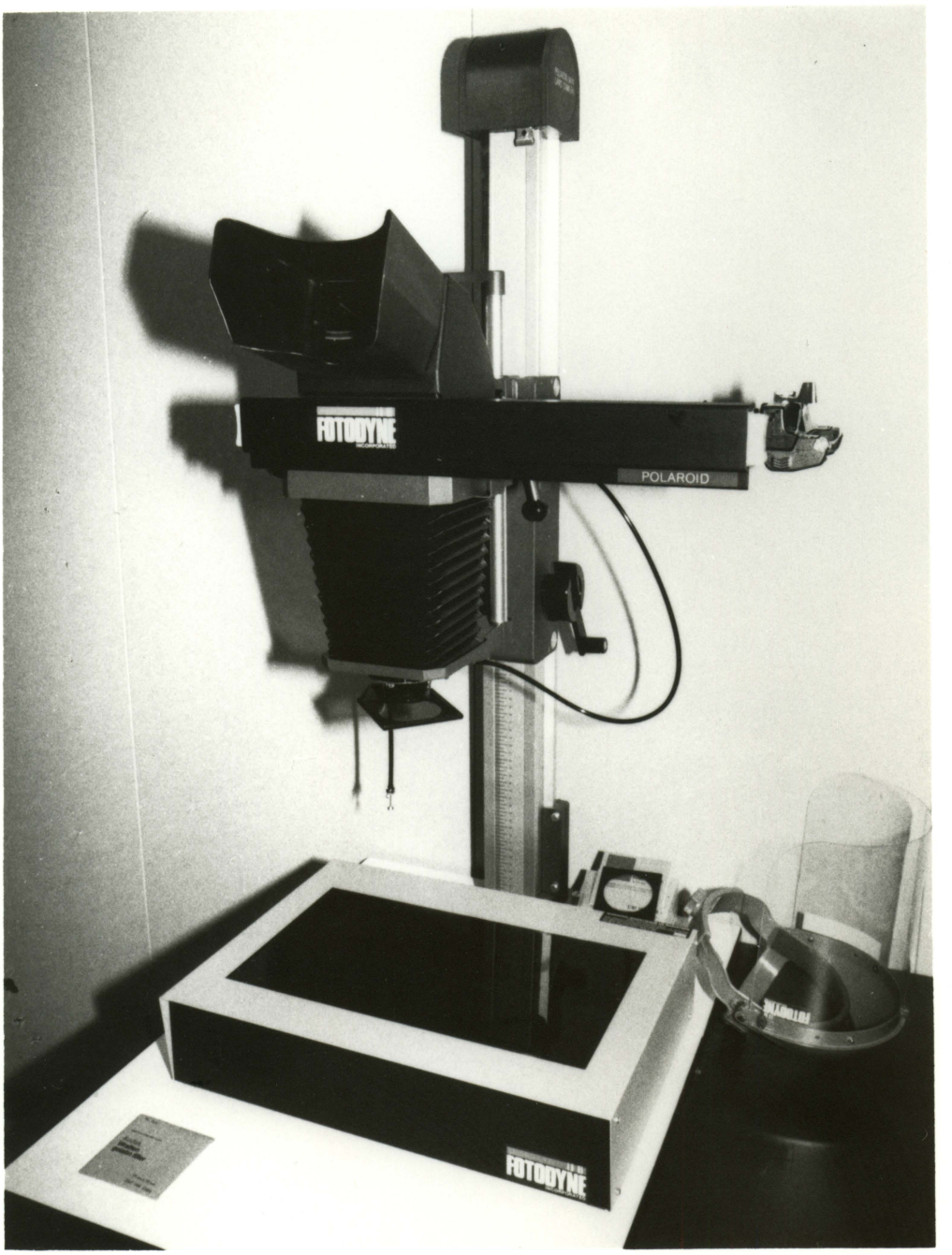
Reagents and chemicals

The TE buffer used contained 10 mM Tris-HCl, pH 8, and 1 mM disodium ethylene diamine tetraacetate (EDTA). The lysis buffer was a solution of 50 mM Tris-CL, 3% sodium dodecyl sulfate (SDS) (Sigma Chemical Company, St. Louis, Mo.), adjusted to pH 12.4 using 2 N NaOH. Tris-borate buffer contained 0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA, pH 8.4. The phenol was redistilled, crystalline, nucleic acid grade (Bethesda Research Laboratories, Inc., (BRL) Gaithersburg, MD.) Ethidium bromide (Sigma Chemical Company) was used at a concentration of 10 mg/ml. Tracking dye solution contained 0.7% bromophenol blue and 30% glycerol in Tris-borate buffer.

Gel electrophoresis

High-purity, electrophoresis grade agarose (Bethesda Research Laboratories, Inc.) was used at a concentration of 0.7% in the plasmid analysis. Horizontal gel electrophoresis was carried out on a model H5 unit (BRL). A transilluminator (300 nm) and Polaroid MP-4 (Fotodyne, Inc., New Berlin, WI.) (Figure 1) were used to visualize and photograph the gel. A Tiffen 15 orange filter and Polaroid type 55 film with 45 sec exposure time (f 4.5) were used in photographing the gel.

Figure 1. Transilluminator and Polaroid camera


FOTODYNE
POLAROID
FOTODYNE

Plasmid preparation

The method used in the analysis of plasmid profiles was a modification of procedures of Kado and Liu (46). The gel was prepared by heating 0.7% agarose in Tris-borate buffer at 100° C (in a steamer) until the agarose was dissolved. The mixture was cooled to 50° C and poured in a 11 x 14 cm tray containing a 14-well comb. The gel was allowed to solidify before use.

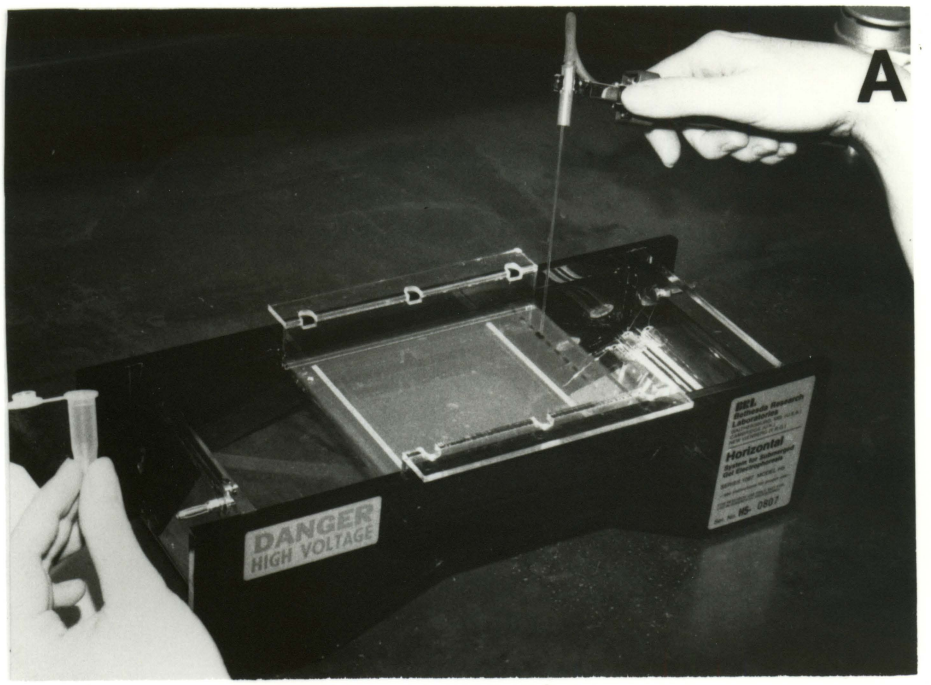
Isolates to be tested were grown 16 h at 37° C on nutrient agar plates. A small bead of cells was scraped off a plate with a toothpick (Figure 2B) and suspended in 40 ul TE in an Eppendorf tube. Lysis buffer (100 ul) was added and vortexed briefly; this mixture was placed in a 95° C water bath for five min. The tube was then immediately placed in an ice bath and 160 ul of phenol/chloroform (1:1) was added and gently mixed. The mixture was separated by centrifugation in a microcentrifuge for five min at 4° C and the supernatant was transferred to a new tube and placed on ice. Twenty ul of supernatant were mixed with 8 ul of tracking dye and placed into a well of the gel by using a capillary pipette (Figure 2A).

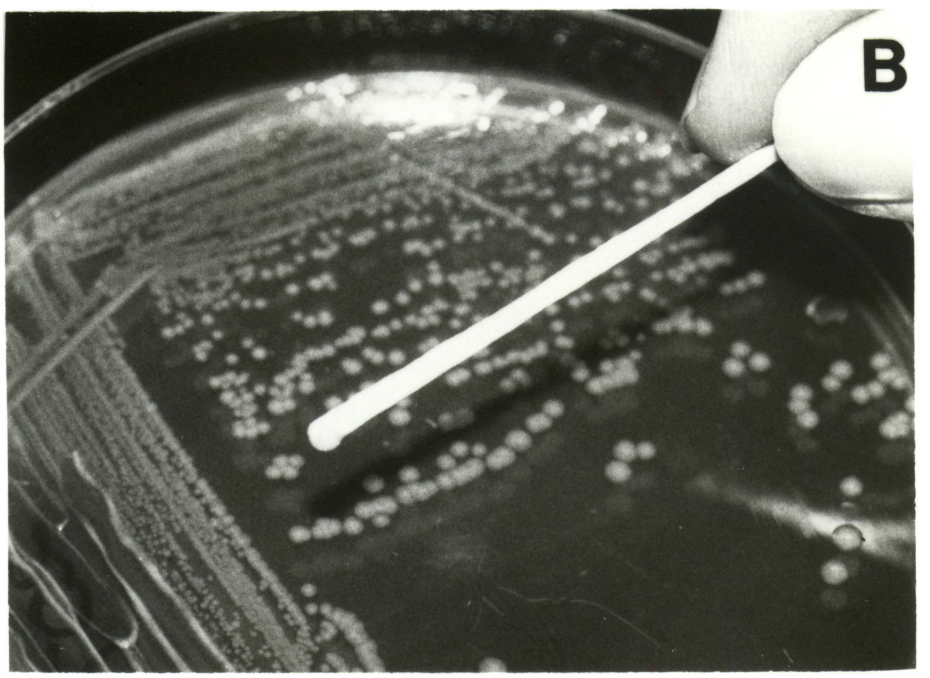
After approximately two and one half hours of electrophoresis at 12 V/cm, the gel was removed from the apparatus and stained with ethidium bromide for 10 minutes. The gel was then washed with distilled water and photographed.

The plasmid extraction procedure described above was developed after using several variations. Two variations in procedure were adopted; one involved a brief vortexing of the mixture following addition of the

Figure 2A. The plasmid preparation is placed in a well of the gel using a capillary pipette

Figure 2B. A toothpick is used to scrape a small bead of cells from a nutrient agar plate


A
DANGER
HIGH VOLTAGE
Horizontal


B

lysis buffer; the second procedure used 8 ul of tracking dye rather than 5 ul as recommended in the original procedure. Another variation involved increasing the amount of TE buffer in which cells were suspended to 100 ul in an attempt to increase the final volume of plasmid preparation available for testing The increase obtained was not significant and the resulting plasmid preparation was too dilute to visualize. Therefore, the original volume of 40 ul was used. The original amount (100 ul) of lysis buffer was used when no apparent improvement in results was detected when a larger amount (150 ul) was added.

Escherichia coli strain V517 (obtained from Dr. Thomas A. Casey, National Animal Disease Center, Ames, IA.) was used as a size reference for the plasmids (52). The micro-method described above yielded a plasmid preparation contaminated with chromosomal DNA, so an alternative method of extraction was used (53). Five ml of trypticase soy broth were inoculated with the reference strain and incubated on a shaker for 16 h at 37° C. One and one half ml of the culture were poured into an Eppendorf tube and the cells sedimented by centrifugation for one minute in a microcentrifuge. The media was removed by aspiration and the cells resuspended in 100 ul of ice-cold TEG buffer (25 mM Tris-Cl, 10 mM EDTA, 50 mM glucose, pH 8.0). This mixture was allowed to stand for five minutes at room temperature and then 200 ul of 0.2 M NaOH with 1% SDS were added. The contents were mixed by inverting the tube rapidly two or three times. After five minutes on ice, 150 ul of ice-cold potassium acetate (60 ml 5 M potassium acetate, 11.5 ml of glacial acetic acid, and 28.5 ml of H_2O) were added and the mixed gently using a vortex mixer

in an inverted position for ten seconds. This mixture was placed on ice for five minutes and then separated by centrifugation in an Eppendorf centrifuge for five minutes at 4° C. The supernatant was transferred to a fresh tube and an equal volume of phenol/chloroform (1:1) added. This was mixed on a vortex mixer and separated by centrifugation for two min in an Eppendorf centrifuge. The supernatant was transferred to a fresh tube and two volumes of ethanol were added and mixed using a vortex mixer. The mixture was allowed to stand for two min at 20° C and sedimented by centrifugation for five min at 20° C in an Eppendorf centrifuge. The supernatant was discarded and the tube inverted to allow all fluid to drain. One ml of 70% ethanol was added, mixed, and sedimented as before. The supernatant was again drained off, and the pellet resuspended in 50 ul of TE buffer (53). Ten ul of suspension were mixed with tracking dye and loaded on a gel.

RESULTS

Serotyping

One hundred isolates were identified as S. dublin on the basis of serologic reactions. Their somatic antigens agglutinated in group D antiserum and were positive for factor "9" and negative for factor "46". The flagellar antigens were monophasic and agglutinated in g,m antiserum. They were tested against factors f, m, s, t, p, q, u, and z51, and agglutination was observed only in the p antiserum. Therefore, the antigenic code of 100 isolates was 9,12: g,p which corresponds with Salmonella dublin.

Phage Typing

Phage typing results are presented in Table 1. Computer analysis was utilized to evaluate the data and group the cultures according to phage type. Some information was not available in utilizing this procedure. For example, reactions of "++" were treated the same as "CL" reactions in analyzing the data and comparing lysis patterns. This method greatly simplified the results and is commonly used in the analysis of phage typing data (4). Simplification of results was necessary in analysis of the reactions of a large number of isolates to a large number of bacteriophage because the time required to analyze data would be prohibitive.

Table 1. Results of phage typing 100 isolates of Salmonella dublin

Isolate	Phage												
Number	370	1	3	4	5	6	7	8	9	10	11	12	13
85-62	CL	-	+++	++	-	++	-	-	SCL	OL	OL	-	-
85-171	SCL	-	SCL	SCL	-	SCL	-	-	CL	SCL	+++	-	+++
85-174	-	-	CL	CL	-	CL	-	-	CL	-	-	-	CL
85-309	CL	-	+++	+++	-	++	-	-	SCL	OL	OL	-	-
85-322	++	-	+++	++	-	++	-	-	SCL	OL	OL	-	-
85-331	SCL	-	-	-	-	++	-	-	+++	-	-	-	-
85-460	CL	-	+++	+++	-	++	-	-	SCL	OL	OL	-	-
85-513	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-518	CL	+	++	++	-	++	+	+	SCL	OL	OL	+	+
85-522	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-523	CL	-	+++	+++	-	++	-	-	SCL	OL	OL	-	-
85-525	CL	+	+++	+++	+	+++	+	+	SCL	OL	OL	+	+
85-527	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-530	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-531	CL	+	+++	+++	-	+++	-	-	SCL	OL	OL	+	-
85-534	CL	++	+++	+++	+	+++	+	+	SCL	OL	OL	+	+
85-637	-	-	+++	+++	-	+++	-	-	SCL	+++	OL	-	-
85-674	SCL	++	SCL	+++	++	SCL	SCL	+++	SCL	OL	OL	+++	+++
85-763	SCL	+	SCL	SCL	-	SCL	-	-	SCL	OL	OL	-	-
85-764	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-813	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-950	+++	-	SCL	SCL	-	SCL	-	-	CL	OL	OL	-	-
85-965	SCL	-	SCL	SCL	-	SCL	-	-	CL	OL	OL	-	+++
85-970	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-991	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-992	CL	-	+++	+++	+	+++	++	++	SCL	OL	OL	++	++
85-998	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1020	SCL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1031	CL	-	+++	+++	+++	+++	+++	+++	SCL	OL	OL	+++	+++
85-1040	SCL	+	+++	+++	-	+++	-	-	SCL	OL	OL	-	-

Isolate	Phage													
Number	14	15	16	17	18	19	20	21	22	23	24	25	26	27
85-62	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	CL	-	CL	SCL	-
85-171	+++	SCL	SCL	SCL	CL	SCL	CL	-	CL	SCL	-	CL	SCL	-
85-174	-	CL	CL	-	CL	CL	CL	-	+++	CL	-	CL	CL	-
85-309	OL	SCL	SCL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
85-322	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
85-331	-	CL	SCL	-	CL	-	SCL	-	SCL	SCL	-	CL	-	-
85-460	OL	SCL	SCL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
85-513	OL	SCL	SCL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
85-518	OL	SCL	CL	OL	CL	CL	SCL	-	CL	SCL	+	CL	CL	-
85-522	OL	SCL	SCL	OL	CL	CL	CL	-	CL	SCL	-	CL	SCL	-
85-523	OL	SCL	SCL	OL	CL	CL	CL	-	CL	SCL	-	CL	SCL	-
85-525	OL	SCL	CL	OL	CL	CL	SCL	+	CL	SCL	+	CL	SCL	+
85-527	OL	SCL	SCL	OL	SCL	SCL	CL	-	CL	SCL	-	CL	SCL	-
85-530	OL	SCL	SCL	OL	CL	SCL	CL	-	CL	SCL	-	CL	SCL	-
85-531	OL	SCL	SCL	OL	SCL	SCL	CL	-	CL	SCL	-	CL	SCL	-
85-534	OL	SCL	SCL	OL	CL	SCL	CL	++	CL	SCL	++	CL	SCL	+
85-637	OL	CL	CL	OL	CL	CL	SCL	-	+++	CL	-	CL	CL	-
85-674	OL	CL	SCL	OL	CL	SCL	CL	++	SCL	SCL	SCL	CL	SCL	++
85-763	OL	SCL	SCL	OL	SCL	SCL	SCL	+	+++	SCL	-	CL	SCL	-
85-764	OL	SCL	SCL	OL	SCL	SCL	SCL	-	+++	SCL	-	CL	SCL	-
85-813	OL	SCL	CL	OL	SCL	SCL	SCL	+	+++	SCL	-	CL	SCL	-
85-950	OL	SCL	SCL	OL	SCL	SCL	CL	-	+++	SCL	-	CL	SCL	-
85-965	OL	CL	SCL	OL	SCL	SCL	SCL	+++	+++	CL	-	CL	SCL	+
85-970	OL	SCL	CL	OL	SCL	SCL	SCL	-	+++	CL	-	CL	CL	-
85-991	OL	SCL	SCL	OL	SCL	SCL	SCL	+	+++	SCL	-	CL	SCL	-
85-992	OL	SCL	SCL	SCL	SCL	SCL	SCL	++	+++	SCL	-	CL	SCL	++
85-998	OL	SCL	SCL	OL	SCL	SCL	SCL	+	+++	SCL	-	CL	SCL	-
85-1020	OL	SCL	SCL	OL	SCL	SCL	SCL	-	+++	SCL	-	CL	SCL	-
85-1031	OL	SCL	SCL	OL	SCL	SCL	SCL	SCL	SCL	SCL	+++	CL	SCL	+++
85-1040	OL	SCL	SCL	OL	SCL	SCL	SCL	++	SCL	SCL	-	CL	SCL	+

Table 1 (Continued)

Isolate	Phage												
Number	370	1	3	4	5	6	7	8	9	10	11	12	13
85-1046	CL	-	++	+++	-	++	-	-	SCL	OL	OL	-	-
85-1047	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1099	CL	+	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1129	SCL	+	SCL	+++	-	SCL	-	-	SCL	OL	OL	-	-
85-1140	SCL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1148	SCL	-	++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1264	CL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	+++	+++
85-1266	SCL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1271	+++	++	+++	+++	++	+++	++	+++	SCL	SCL	SCL	+	+
85-1439	CL	+++	+++	SCL	+++	SCL	++	+++	SCL	SCL	SCL	++	++
85-1520	CL	-	-	+	-	-	-	-	++	OL	OL	-	-
85-1521	CL	++	+++	+++	++	+++	++	+++	++	OL	OL	+	++
85-1522	CL	+++	SCL	+++	+++	SCL	+++	+++	SCL	+++	SCL	++	+++
85-1555	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1614	SCL	+	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1628	SCL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1671	++	-	CL	CL	-	CL	+	+	CL	-	-	-	CL
85-1715	CL	-	++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1743	CL	-	++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1794	CL	-	++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1816	CL	OL	OL	OL	OL	SCL	SCL	OL	SCL	SCL	SCL	SCL	SCL
85-1849	SCL	-	+++	+++	-	SCL	-	-	SCL	OL	OL	-	-
85-1879	CL	-	++	+++	-	+++	-	-	SCL	OL	OL	-	-
85-1903	CL	-	-	+	-	++	-	-	+++	OL	OL	-	-
85-1981	CL	-	++	+++	-	++	-	-	SCL	OL	OL	-	-
85-1996	SCL	-	SCL	SCL	-	SCL	-	-	SCL	OL	OL	-	-
84-1042	SCL	-	-	+	-	++	-	-	SCL	OL	OL	-	-
84-1541	CL	+++	+++	+++	+++	+++	+++	SCL	SCL	OL	OL	++	+++
84-2235	CL	-	++	+++	-	+	-	-	+++	OL	OL	-	-

Isolate	Phage													
Number	14	15	16	17	18	19	20	21	22	23	24	25	26	27
85-1046	OL	SCL	SCL	OL	SCL	SCL	SCL	-	+++	SCL	-	CL	SCL	-
85-1047	OL	SCL	SCL	OL	SCL	SCL	SCL	-	+++	CL	-	CL	SCL	-
85-1099	OL	SCL	SCL	OL	SCL	SCL	SCL	++	+++	SCL	-	CL	SCL	+
85-1129	OL	SCL	SCL	OL	SCL	SCL	SCL	++	+++	CL	-	CL	SCL	+
85-1140	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	CL	-	CL	SCL	-
85-1148	SCL	CL	CL	OL	SCL	CL	SCL	-	+++	CL	-	CL	CL	-
85-1264	SCL	CL	CL	SCL	SCL	CL	SCL	++	SCL	SCL	+++	CL	CL	+++
85-1266	SCL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
85-1271	OL	SCL	CL	SCL	SCL	CL	SCL	-	SCL	SCL	+	CL	CL	++
85-1439	SCL	SCL	CL	SCL	SCL	CL	SCL	+++	+++	SCL	++	CL	CL	++
85-1520	OL	+++	CL	OL	SCL	CL	++	-	++	SCL	-	SCL	SCL	-
85-1521	OL	+++	CL	OL	++	CL	++	++	+	SCL	+	-	CL	+
85-1522	SCL	SCL	SCL	SCL	SCL	SCL	SCL	++	+++	SCL	++	CL	SCL	++
85-1555	SCL	SCL	CL	OL	SCL	SCL	SCL	+	SCL	CL	-	CL	SCL	+
85-1614	SCL	SCL	CL	OL	SCL	SCL	SCL	+	SCL	SCL	-	CL	SCL	+
85-1628	SCL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
85-1671	-	SCL	CL	+	SCL	CL	CL	+	+++	SCL	-	CL	CL	++
85-1715	CL	SCL	CL	OL	SCL	CL	SCL	-	+++	CL	-	CL	CL	-
85-1743	SCL	SCL	SCL	OL	SCL	SCL	+++	-	+++	SCL	-	CL	SCL	-
85-1794	SCL	SCL	CL	OL	SCL	CL	SCL	-	+++	CL	-	CL	SCL	-
85-1816	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	SCL	CL	SCL	SCL
85-1849	OL	SCL	SCL	OL	SCL	SCL	CL	-	SCL	+++	-	CL	SCL	-
85-1879	OL	SCL	CL	OL	SCL	CL	SCL	-	+++	SCL	-	CL	CL	-
85-1903	OL	SCL	SCL	OL	SCL	SCL	+++	-	SCL	SCL	-	CL	SCL	-
85-1981	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
85-1996	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	CL	-	CL	SCL	-
84-1042	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
84-1541	SCL	SCL	SCL	OL	CL	SCL	SCL	+++	SCL	SCL	+++	CL	SCL	+++
84-2235	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	+++	-	CL	SCL	-

Table 1 (Continued)

Isolate	Phage												
Number	370	1	3	4	5	6	7	8	9	10	11	12	13
84-2338	SCL	-	-	+	-	-	-	-	+++	OL	OL	-	-
84-2357	-	-	++	+++	-	SCL	-	-	SCL	OL	OL	-	-
84-2608	CL	-	-	+	-	-	-	-	++	OL	OL	-	-
84-2684	-	-	SCL	CL	-	++	-	-	SCL	-	-	-	SCL
84-2818	SCL	-	-	-	-	+	-	-	++	-	-	-	-
84-2819	CL	-	+++	+++	-	-	-	-	+++	OL	OL	-	-
84-2821	SCL	-	-	++	-	-	-	-	+++	OL	OL	-	-
84-2932	SCL	-	-	++	-	++	-	-	+++	OL	OL	-	-
84-2956	CL	-	+	++	-	-	-	-	+++	OL	OL	-	-
84-3155	+++	-	+++	SCL	-	+++	-	-	SCL	OL	OL	-	-
84-3211	SCL	-	+++	+++	-	+	-	-	+++	OL	OL	-	-
84-3509	++	-	+++	+++	-	-	-	-	+++	OL	OL	-	-
84-3698	-	-	SCL	CL	-	+++	-	-	SCL	-	-	-	SCL
84-3700	CL	-	++	+++	-	+	-	-	+++	OL	OL	-	-
84-3729	CL	-	++	++	-	-	-	-	+++	OL	OL	-	-
84-3788	+++	-	+++	+++	-	+	-	-	SCL	OL	OL	-	-
84-3947	CL	-	++	+++	-	-	-	-	+++	OL	OL	-	-
84-3950	CL	-	+++	++	-	++	-	+	SCL	OL	OL	+	+
84-3952	SCL	+	+++	++	-	++	-	+	SCL	OL	OL	+	+
84-4013	CL	-	+++	++	-	++	-	-	SCL	OL	OL	-	+
84-4266	CL	-	-	-	-	-	-	-	SCL	OL	OL	-	-
84-4576	-	-	-	-	-	-	-	-	-	-	-	-	-
84-4617	+++	-	+++	++	-	++	-	-	SCL	OL	OL	-	-
84-4637	++	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
84-4638	++	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
84-4640	++	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
84-4641	++	-	+++	++	-	+++	-	-	SCL	OL	OL	-	-
84-4642	CL	-	+++	+++	++	++	-	-	SCL	OL	OL	-	-
84-4645	CL	-	++	++	-	++	-	-	+++	OL	OL	-	-

Isolate	Phage													
Number	14	15	16	17	18	19	20	21	22	23	24	25	26	27
84-2338	OL	SCL	SCL	OL	SCL	SCL	+++	-	CL	+++	-	CL	SCL	-
84-2357	OL	SCL	SCL	OL	SCL	SCL	SCL	-	+++	SCL	-	CL	OL	-
84-2608	SCL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	+++	-	CL	SCL	-
84-2684	-	SCL	SCL	-	SCL	SCL	CL	-	SCL	SCL	-	CL	SCL	-
84-2818	-	SCL	-	-	SCL	-	SCL	-	SCL	+++	-	CL	-	-
84-2819	OL	SCL	CL	OL	SCL	SCL	SCL	-	CL	+++	-	CL	CL	-
84-2821	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	+++	-	CL	SCL	-
84-2932	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	+++	-	CL	SCL	-
84-2956	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
84-3155	OL	SCL	SCL	OL	+++	SCL	SCL	-	SCL	+++	-	SCL	SCL	-
84-3211	OL	+++	SCL	OL	SCL	SCL	SCL	-	SCL	+++	-	SCL	SCL	-
84-3509	OL	-	SCL	OL	-	SCL	SCL	-	SCL	+	-	SCL	SCL	-
84-3698	-	SCL	SCL	-	SCL	SCL	CL	-	SCL	SCL	-	CL	SCL	-
84-3700	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	+++	-	CL	CL	-
84-3729	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	+++	-	CL	SCL	-
84-3788	OL	+++	SCL	OL	++	SCL	SCL	-	CL	+++	-	SCL	SCL	-
84-3947	OL	SCL	CL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	CL	-
84-3950	OL	SCL	SCL	OL	CL	SCL	SCL	+	CL	SCL	+	CL	SCL	-
84-3952	OL	SCL	SCL	OL	SCL	SCL	SCL	++	SCL	SCL	+	CL	SCL	++
84-4013	OL	SCL	SCL	OL	SCL	SCL	SCL	+	CL	SCL	-	CL	SCL	-
84-4266	OL	SCL	SCL	OL	CL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
84-4576	-	-	-	-	-	-	-	-	-	-	-	-	-	-
84-4617	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
84-4637	+++	SCL	CL	OL	CL	CL	CL	-	CL	SCL	-	CL	CL	-
84-4638	+++	SCL	CL	OL	CL	CL	CL	-	CL	SCL	-	CL	CL	-
84-4640	+++	SCL	CL	OL	CL	CL	CL	-	CL	SCL	-	CL	CL	-
84-4641	+++	SCL	CL	OL	CL	CL	CL	-	CL	SCL	-	CL	CL	-
84-4642	OL	SCL	SCL	OL	CL	SCL	CL	-	CL	SCL	-	CL	SCL	-
84-4645	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-

Table 1 (Continued)

Isolate	Phage												
Number	370	1	3	4	5	6	7	8	9	10	11	12	13
84-4651	CL	-	+++	-	-	-	-	-	SCL	OL	OL	-	-
84-4702	CL	-	+++	+++	-	++	-	-	SCL	OL	OL	-	-
84-4777	SCL	-	+++	+++	-	+++	-	-	CL	SCL	+++	-	-
84-4795	CL	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
84-4964	-	-	+++	SCL	-	+++	-	-	+++	-	-	-	+++
83-225	+++	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
83-1101	+++	-	SCL	SCL	-	SCL	-	-	CL	OL	OL	-	+++
84-254	CL	-	+++	++	-	++	-	-	SCL	OL	OL	-	-
84-340	CL	-	+++	+++	-	++	-	-	SCL	OL	OL	-	-
84-341	CL	+++	+++	++	++	+++	++	-	SCL	OL	OL	-	-
84-594	+++	-	+++	+++	-	+++	-	-	SCL	OL	OL	-	-
84-757	CL	-	+++	++	-	++	-	-	SCL	OL	OL	-	-

OL= opaque lysis

Cl= confluent lysis

SCL= semiconfluent lysis

<SCL= less than semiconfluent lysis

+++= 60-120 plaques

++= 20-60 plaques

+= 6-20 plaques

-= 0-5 plaques

Isolate	Phage													
Number	14	15	16	17	18	19	20	21	22	23	24	25	26	27
84-4651	OL	SCL	SCL	OL	SCL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
84-4702	OL	SCL	SCL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
84-4777	SCL	SCL	SCL	OL	SCL	SCL	CL	-	CL	SCL	-	CL	SCL	-
84-4795	OL	SCL	CL	OL	CL	CL	CL	-	CL	SCL	-	CL	CL	-
84-4964	-	+++	SCL	-	+++	SCL	CL	-	SCL	+++	-	CL	SCL	-
83-225	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
83-1101	+++	SCL	SCL	OL	SCL	SCL	CL	-	SCL	SCL	-	CL	SCL	-
84-254	OL	SCL	SCL	OL	CL	SCL	SCL	-	SCL	SCL	-	CL	SCL	-
84-340	OL	SCL	SCL	OL	SCL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
84-341	OL	SCL	SCL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-
84-594	OL	SCL	SCL	OL	CL	SCL	CL	-	CL	SCL	-	CL	SCL	-
84-757	SCL	CL	CL	OL	CL	SCL	SCL	-	CL	SCL	-	CL	SCL	-

Phage types and the number of isolates exhibiting each phage lysis pattern are shown in Table 2. The phage type is designated by its octal code which assigns a number to each set of three phage lysis reactions (4). Reactions of -, -, - to phages 370, 1, and 3 was given a code number of 0. Code number 1 corresponded to results of +, -, -; #2 was -, +, -; #3 was +, +, -; #4 was -, -, +; #5 was +, -, +; #6 was -, +, +; and #7 was assigned to results of +, +, +. This system was used for each culture tested and each lysis pattern was assigned a 9-digit code number corresponding to the reactions obtained in testing with the 27 phages.

Twenty-six different patterns of lysis were observed in phage testing (Table 2). Fifty-two percent (52/100) of isolates tested possessed lysis pattern #16 characterized by the octal code 554367333. Sixteen isolates exhibited unique phage lysis patterns (phage types 1, 5, 6, 7, 8, 9, 10, 11, 12, 15, 17, 19, 20, 21, 24, and 26) and nine patterns were possessed by less than ten isolates each (types 2, 3, 4, 13, 14, 18, 22, 23, and 25). The reactions of those cultures belonging to phage type 18 differ from those in type 16 in the sensitivity to lysis by one phage. Phage type 4 was sensitive to lysis by all of the phages used in testing, and one isolate, #84-4576 was resistant to lysis by all of the phages used.

Table 2. Results of phage typing of 100 isolates of Salmonella dublin

Phage Type	Octal Code	Number of Isolates
1	000000000	1
2	454367333	2
3	454055333	4
4	777777777	6
5	777377722	1
6	777367337	1
7	775367333	1
8	754367773	1
9	577777777	1
10	574367333	1
11	557777737	1
12	554377733	1
13	554377333	2
14	554367733	3
15	554367737	1
16	554367333	52
17	554055337	1
18	514367333	7
19	514323313	1
20	504367333	1
21	154367333	1
22	114367333	2
23	144367333	2
24	144045231	1
25	104367333	5
26	104044231	1
	total	100

Antibiotic Sensitivity

One hundred cultures were tested for resistance to twelve different antibiotics. Antibiotics used included: amikacin (AN), ampicillin (AM), chloramphenicol (C), gentamicin (GM), kanamycin (K), neomycin (N), penicillin G (P), streptomycin (S), sulfonamides (SD), tetracycline (TE), tobramycin (TM), and trimethoprim (TMP). Zone diameters (mm) were interpreted according to guidelines provided by Difco Laboratories for use with sensitivity discs. Intermediate range reactions (diameters between resistant and susceptible) were recorded as sensitive in reporting the antibiotic resistance patterns shown in Table 3.

Fourteen patterns of antibiotic resistance were observed (Table 3) with 79 isolates (79%) belonging to one of three major patterns of resistance. Resistance to eight of the antibiotics used (AM, C, K, N, P, S, SD, TE) was observed in 31 (31%) of the isolates tested (pattern #2). In pattern #4, 27 of the isolates tested were resistant to a total of six different antibiotics (AM, K, N, P, S, TE). Twenty-one of the cultures tested did not demonstrate zones of resistance to any of the antibiotics used, although the majority (82%) of them possessed zones in the intermediate range to one or two of the antibiotics. One culture was resistant to all of the antibiotics used except amikacin (pattern #1). It was the only culture tested that was resistant to gentamicin, tobramycin, and trimethoprim. Pattern #3 included isolates resistant to all the antibiotics in pattern #2 except chloramphenicol.

Table 3. Antibiotic resistance patterns of 100 *Salmonella dublin* isolates

Antibiotic combinations	Number of isolates
1. AM, C, GM, K, N, P, S, SD, TE, TM, TMP	1
2. AM, C, K, N, P, S, SD, TE	31
3. AM, K, N, P, S, SD, TE	2
4. AM, K, N, P, S, TE	27
5. C, K, N, S, SD, TE	2
6. AM, K, N, P, S	7
7. AM, K, P, S, TE	1
8. AM, P, S, TE	1
9. AM, P, S	1
10. K, N	1
11. P, SD	2
12. K	1
13. P	2
14. sensitive [a]	21
Total isolates	100

AM=ampicillin	N=neomycin	TE=tetracycline
C=chloramphenicol	P=penicillin	TM=tobramycin
GM=gentamicin	S=streptomycin	TMP=trimethoprim
K=kanamycin	SD=sulfamides	

[a] Sensitive to all antibiotics tested.

Isolates possessing resistance pattern #5 were resistant to six antibiotics (C, K, N, S, SD, TE), but were the only isolates out of the first nine resistance groups that were not resistant to ampicillin and penicillin. Patterns #6 and 7 included isolates resistant to five antibiotics: AM, K, N, P, and S in pattern 6; and AM, K, P, S, and TE in pattern 7. One isolate possessed pattern 8, resistance to four antibiotics (AM, P, S, TE). Pattern 9 (resistance to AM, P, and S) and pattern 10 (resistance to K and N) were each exhibited by one isolate. Two isolates were resistant only to penicillin and sulfonimides, one to kanamycin, and two to penicillin.

The percentage of isolates in which zones in the resistant, intermediate, and sensitive ranges were demonstrated is presented in Table 4. The percentage of resistance was over 70% for five of the antibiotics used: ampicillin, 71%; kanamycin, 73%; neomycin, 71%; penicillin, 75%; and streptomycin, 73%. Sixty-five percent of the isolates were resistant to tetracycline, 34% to chloramphenicol, and 38% to sulfonamides. All isolates tested were sensitive to amikacin, and only one was resistant to gentamicin, tobramycin, and trimethoprim.

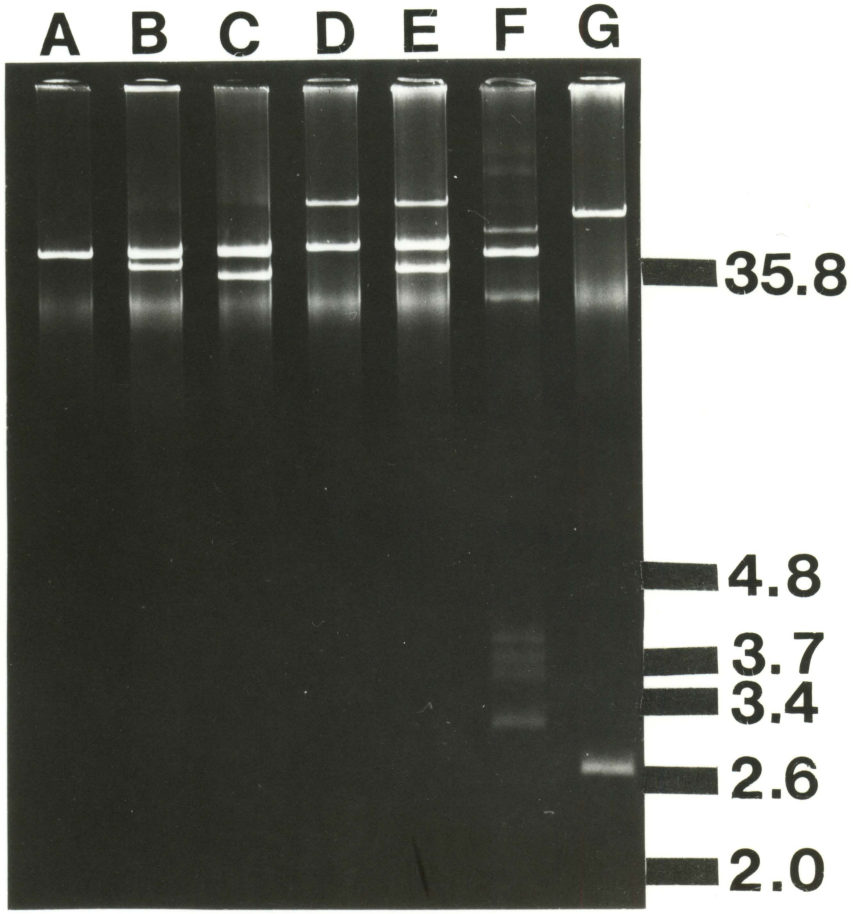
Plasmid Analysis

Plasmid profile analysis was performed on 100 isolates of S. dublin. The isolates were grouped according to the large plasmids they possessed. These groups were designated A-G (figure 3). Group A isolates possessed a 38 Mdal plasmid, while group B was made up of isolates that possessed a 35.5 Mdal plasmid in addition to the 38 Mdal plasmid.

Table 4. Results of antibiotic sensitivity in 100 isolates of *Salmonella dublin*

	Percentages		
Antibiotic	Resistant	Intermediate	Sensitive
Amikacin	0	0	100
Ampicillin	71	0	29
Chloramphenicol	34	0	66
Gentamicin	1	0	99
Kanamycin	73	0	27
Neomycin	71	2	27
Penicillin G	75	16	9
Streptomycin	73	3	24
Sulfonamides	38	9	52
Tetracycline	65	3	32
Tobramycin	1	0	99
Trimethoprim	1	0	99

Figure 3. Plasmid profiles of groups A-G using 0.7% agarose gel. Lane A= group A, lane B= group B, lane C= group C, lane D= group D, lane E= group E, lane F= group F, and lane G= group G. Sizes in Mdal determined with Escherichia coli strain V517


A
B
C
D
E
F
G
35.8
4.8
3.7
3.4
2.6
2.0

Group C organisms possessed a 38 Mdal plasmid as well as a plasmid slightly smaller (32.5 Mdal) than the one found in group B organisms. Isolates belonging to group D possessed a 52 Mdal plasmid in addition to a 39 Mdal plasmid, and group E organisms possessed 52 Mdal, 39 Mdal, and 34.5 Mdal plasmids. One isolate possessed the plasmids found in group F. This organism possessed five large plasmids (70 Mdal, 62 Mdal, 41 Mdal, 38 Mdal, and 29 Mdal) and four small plasmids (4.0 Mdal, 3.6 Mdal, 3.3 Mdal, and 3.1 Mdal). One isolate (Group G) possessed a large plasmid (48.5 Mdal) and one small plasmid (2.8 Mdal), but lacked the 38-39 Mdal plasmid common the the other S. dublin isolates.

Of the 100 isolates tested, 28 demonstrated the plasmid profile of group A. Twenty-eight isolates were classified as group B, 7 were group C, 34 were members of group D, and one isolate was classified in each of the groups E, F, and G. All of the isolates of human origin were members of group A. Small plasmids were present in four of the group B cultures and four of the group C cultures (Figure 4). No apparent correlation between the presence of small plasmids and the reaction of these cultures in phage typing and antibiotic resistance determinations could be made.

No apparent association could be made in the geographical distribution of the different groups of plasmid profiles (Figure 5). This could be due in part to the small number of isolates received from some of the eastern states. An attempt was made to trace the origin of the calf

Figure 4. Plasmid profiles of seven isolates of Salmonella dublin showing presence of small plasmids (0.7% agarose gel). Lanes A-D= group B isolates, and lanes E-G= group C isolates. Sizes in Mdal determined with Escherichia coli strain V517

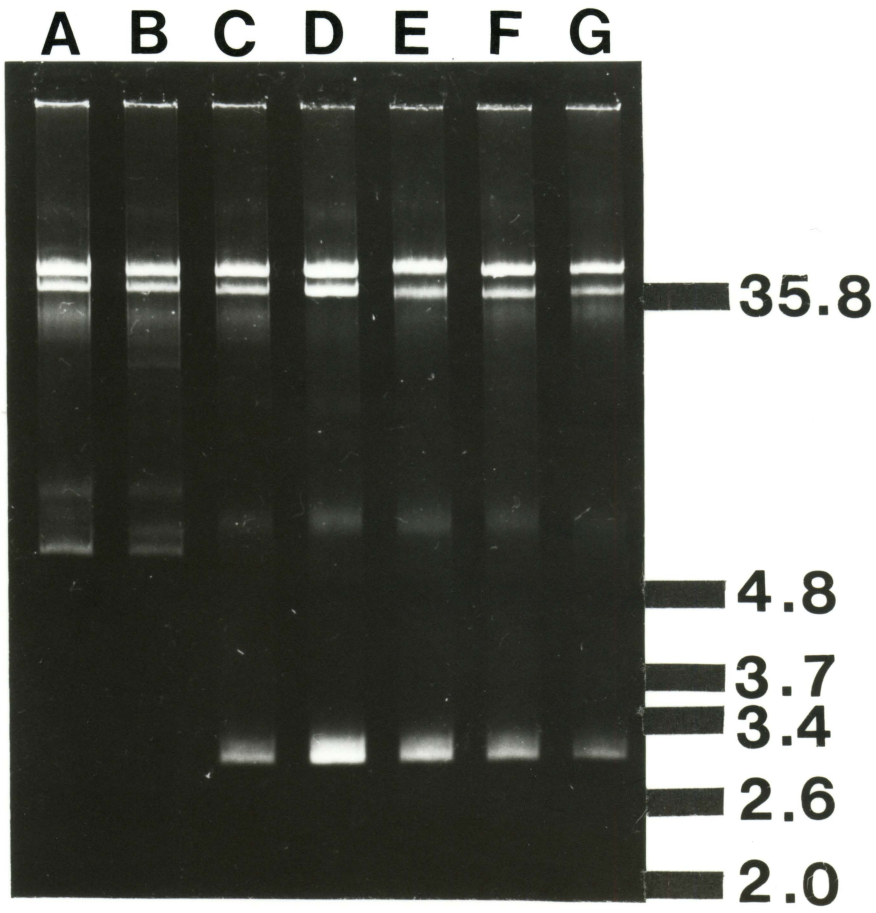
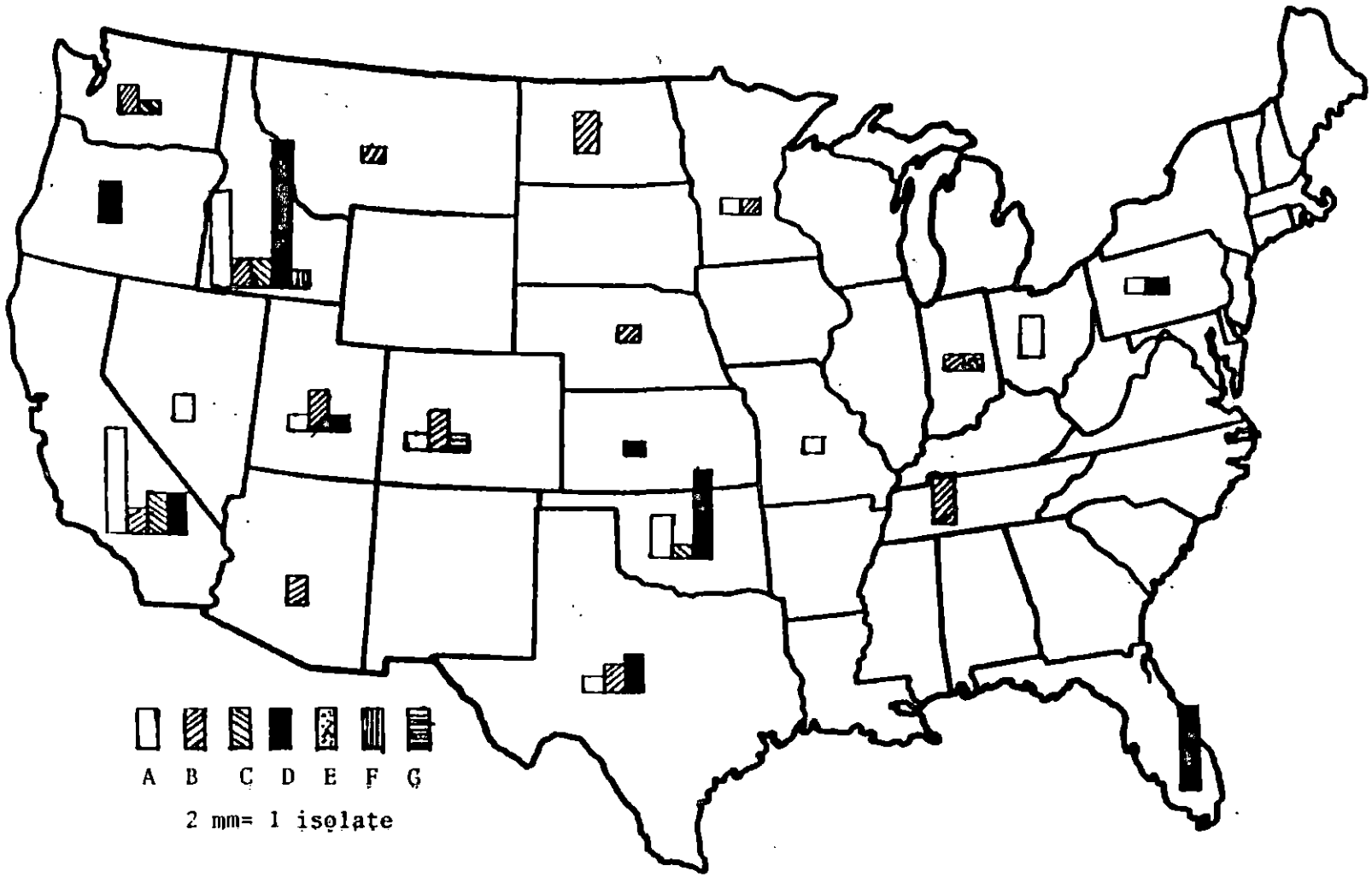

A B C D E F G
35.8
4.8
3.7
3.4
2.6
2.0

Figure 5. Number of plasmid groups of 100 Salmonella dublin isolates identified in different states


A B C D E F G
2 mm= 1 isolate

from which the culture with the group F plasmid pattern was isolated. However, this was not possible because the animal was sold at a sale barn and no records were available indicating the origin of the animal.

There was no apparent correlation between phage lysis pattern and plasmid profile except in the case of culture #84-4576 (Group F) which was resistant to lysis by all of the phages used. This was the only phage lysis pattern that appeared to be associated with a particular plasmid profile. A comparison of phage lysis results and plasmid analysis is shown in Table 5. Of those cultures that shared the major lysis pattern, 17 (33%) possessed plasmid pattern A, 12 (23%) were classified as plasmid group B, 3 (6%) were group C, and 20 (38%) were group D.

There was a strong association between plasmid profile and patterns of resistance to antibiotics. Culture #84-4576 (group F) was resistant to the greatest number of antibiotics (11), and was sensitive only to amikacin. Table 6 compares the antibiotic resistance pattern to the plasmid profile. All of the cultures that were sensitive to all of the antibiotics used were members of group A. Of 34 isolates of plasmid group D, 30 (88%) were resistant to eight of the antibiotics tested (AM, C, K, N, P, S, SD, TE). The isolate with plasmid pattern E also possessed this antibiotic resistance pattern. Of the 28 cultures of plasmid group B, 23 (82%) were resistant to six antibiotics (AM, K, N, P, S, TE). Also, the plasmid group G isolate possessed this pattern. Four of the 7 group C isolates had resistance to five antibiotics (AM, K, N, P, S).

Table 5. Comparison of results of phage typing and plasmid analysis of 100 isolates of *Salmonella dublin*

Phage Type	Number of	Plasmid Type						
Octal Code	Isolates	A	B	C	D	E	F	G
1-000000000	1						1	
2-454367333	2			1				1
3-454055333	4				3	1		
4-777777777	6	2	3		1			
5-777377722	1				1			
6-777367337	1			1				
7-775367333	1	1						
8-754367773	1	1						
9-577777777	1		1					
10-574367333	1	1						
11-557777737	1				1			
12-554377733	1		1					
13-554377333	2	1	1					
14-554367733	3	2	1					
15-554367737	1		1					
16-554367333	52	17	12	3	20			
17-554055337	1		1					
18-514367333	7	2	1		4			
19-514323313	1		1					
20-504367333	1	1						
21-154367333	1		1					
22-114367333	2			1	1			
23-144367333	2	1	1					
24-144045231	1				1			
25-104367333	4		1	1	2			
26-104044231	1		1					

Table 6. Results of comparison of antibiotic sensitivity and plasmid analysis of 100 isolates of Salmonella dublin

Resistance pattern	Total	Plasmid type A	B	C	D	E	F	G
1.	1						1	
2.	31				30	1		
3.	2		2					
4.	27		23	2	1			1
5.	2	1			1			
6.	7		1	4	2			
7.	1		1					
8.	1		1					
9.	1			1				
10.	1	1						
11.	2	2						
12.	1	1						
13.	2	2						
14.	21	21						

DISCUSSION

This thesis describes the characterization of field isolates of S. dublin using serotype, plasmid profile, phage type, and antibiotic resistance pattern. Seroagglutination tests were conducted on 100 isolates of S. dublin obtained from animals in widespread areas of the Unites States. After isolates were determined to be S. dublin based on their serologic reactions, phage types, antibiotic sensitivities, and plasmid profiles were determined.

The largest number of subgroups (26) were obtained by using phage typing, but the majority of isolates tested (52%) exhibited the same phage lysis pattern (#16). This percentage was slightly lower than the 67% reported by Smith (78); however, phage type #16 contained more isolates than were present in any of the subgroups identified using either plasmid profile or antibiotic resistance. It could be argued that different results might be obtained through the use of phages isolated from S. dublin; however, the phages used by Smith were isolated from S. dublin. It is of interest that phage #370 was isolated from S. dublin yet only 7 of the 100 cultures tested were resistant to lysis by this phage.

Phage typing was the most time-consuming of the methods used. In addition to the time required in setting up the test, the propagation, maintenance, and titering of the phages required time.

Isolates were divided into 14 groups based on patterns of resistance to 12 antibiotics. The antibiotic-resistance groups corresponded closely with the plasmid profiles and could possibly be used for screening

isolates in epidemiological investigations. Researchers have found that this is not a definitive test in epidemiologic studies because the majority of isolates show the same resistance pattern (38, 42). However, this was not the case in this study and testing for resistance could be employed in further defining isolates of the same phage or plasmid type. The relationship of resistance groups to plasmid profile can be an indicator of the information coded for by the plasmids observed in the plasmid profile. Methods, such as transformation of competent cells, are available to confirm the presence of antibiotic-resistance determinants on plasmids.

The method used in the determination of plasmid profile was rapid, easy to perform, and reproducible; i.e., thirteen isolates could be prepared for testing in approximately one hour. It was also effective in destroying the chromosomal DNA. One limitation of the method used was that the resulting plasmid preparation was not suitable for use with restriction endonucleases because the concentration of plasmids was low and the pH was high. Therefore, other methods of extraction should be used on isolates to be tested with restriction endonucleases. Also, suitable photographs of the plasmid profile could not be obtained when a mini-gel apparatus was used because of the low concentration of plasmids in the plasmid preparation.

The majority of isolates tested (90%) for plasmid profile possessed plasmids which were characteristic of Group A, B, or D. Isolates were fairly evenly divided between the three groups (A=28%, B=28%, D=34%); this could be a useful subgrouping. Also, variations in the three main

groups occurred occassionally; i.e., Groups C, E, F, and small plasmids in Groups B and C. These variations provide distinctive characterization of the isolates. Plasmid profile analysis would be a useful second step after serotyping, and might be sufficient to differentiate isolates. However, if there were some question of identity after determination of plasmid profile, further means of differentiation such as phage typing could be used.

There was a rather large difference between the plasmid sizes found in the analysis of these isolates and those reported by other researchers (44, 88). There was no indication of the standard that was used in the determination of plasmid size in previous reports, so the difference in size could be the result of using a different standard. The 38-39 Mdal plasmid is probably a serotype-specific plasmid as reported in the literature. This plasmid was present in 99% of the isolates examined. It appeared slightly larger (39 Mdal) in plasmid groups D and E, and a restriction endonuclease digest of the plasmids would be helpful in comparing the 38 and 39 Mdal plasmids. Also, DNA homology tests could be used to prove identity.

These results also would tend to support the clonal theory of the spread of bacterial pathogens (64), and are similar to the results seen in other studies (44, 88). The clonal theory suggests that a common origin exists for similar bacterial isolates obtained from various sources, located in different areas and isolated at different times (64).

Many possibilities for further research on Salmonella isolates exist. One possible study could be the confirmation that antibiotic-resistance determinants are carried on the plasmids other than the serotype-specific plasmid through the transformation of competent cells. Another possibility for further study could be the development of a DNA probe through the use of the serotype-specific plasmid that would enable the identification of cultures as S. dublin that were untypable by serologic methods ("rough" or nonmotile cultures). This would be especially useful in identification of the causative agent in an outbreak and could be helpful in tracing the source of infection.

In conclusion, plasmid profile analysis would be useful in subgrouping isolates of S. dublin for epidemiological purposes. However, further testing such as phage typing could be needed in special cases for further differentiation of strains.

REFERENCES CITED

1. Anderson, D. M. 1980. Plasmid studies of Salmonella typhimurium phage type 179 resistant to ampicillin, tetracycline, sulphonamides, and trimethoprim. J. Hyg. Camb. 85:293-300.

2. Anderson, E. S., E. J. Threlfall, J. M. Carr, M. M. McConnell, and H. R. Smith. 1977. Clonal distribution of resistance plasmid-carrying Salmonella typhimurium, mainly in the Middle East. J. Hyg. Camb. 79:425-448.

3. Anderson, E. S., L. R. Ward, M. J. DeSaxe, and J. D. H. DeSa. 1977. Bacteriophage-typing designations of Salmonella typhimurium. J. Hyg. Camb. 78:297-300.

4. Audurier, A., F. Chalons, and M. Toucas. 1979. A code for reporting and comparing results in phage typing. Ann. Microbiol. (Inst. Pasteur) 130A:345-349.

5. Baird, G. D. and E. J. Manning. 1984. Molecular evidence for plasmid involvement in the virulence of Salmonella dublin. Pages 147-153 in H. Errebo Larsen, ed. Agriculture priority aspects of salmonellosis research. Commission of the European Communities, Brussels, Luxembourg.

6. Bauer, A. W., W. M. M. Kirby, and J. C. Sherris. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496.

7. Bennett, P. M. and J. Grinsted. 1984. Introduction. Page 1 in P. M. Bennett and J. Grinsted, ed. Methods in microbiology, Vol. 17, Plasmid Technology. Academic Press, London.

8. Blackburn, Billie O., Linda K. Schlater, and Merrill R. Swanson. 1984. Antibiotic resistance of members of the genus Salmonella isolated from chickens, turkeys, cattle, and swine in the United States during October 1981 through September 1982. Am. J. Vet. Res. 45:1245-1249.

9. Blackburn, Billie O., Kathleen Sutch, and Rube Harrington, Jr. 1980. The changing distribution of Salmonella dublin in the United States. Pages 445-451 in Proceedings of the 84th annual meeting of The United States Animal Health Association. Carter Printing Co., Richmond, VA.

10. Blackburn, Billie O., Kathleen Sutch, and Rube Harrington, Jr. 1982. Salmonella and Arizona serotypes from animals and related sources reported during fiscal year 1981. Pages 454-471 in Pro-

ceedings of the 86th Annual Meeting of the United States Animal Health Association. Carter Printing Co., Richmond, VA.

11. Blackburn, Billie O. and Kathleen Sutch. 1983. Salmonella and Arizona serotypes from animals and related sources reported during fiscal year 1982. Proceedings of the 87th Annual Meeting of the United States Animal Health Association. Carter Printing Co., Richmond, VA.

12. Brunner, Felix, Anita Margadant, Raffaele Peduzzi, and Jean-Claude Piffaretti. 1983. The plasmid pattern as an epidemiologic tool for Salmonella typhimurium epidemics: Comparison with the lysotype. J. Infect. Dis. 148:7-11.

13. Callow, B. R. 1959. A new phage typing scheme for Salmonella typhimurium. J. Hyg. Camb. 57:346.

14. Casalino, M., A. Comanducci, M. Nicoletti, and F. Maimone. 1984. Stability of plasmid content in Salmonella wien in late phases of the epidemic history. Antimicrob. Agents Chemother. 25:499-501.

15. Chau, P. Y., Julia Ling, E. J. Threfall, and S. W. K. Im. 1982. Genetic Instability of R plasmids in relation to the shift of drug resistance patterns in Salmonella johannesburg. J. Gen. Microbiol. 128:239-245.

16. Cherubin, Charles E. 1981. Antibiotic resistance of Salmonella in Europe and the United States. Rev. Infect. Dis. 3:1105-1126.

17. Coetzee, J. N., D. E. Bradley, and R. W. Hedges. 1982. Phages 1 alpha and I2-2: Incl plasmid-dependent bacteriophages. J. Gen. Microbiol. 128:2797-2804.

18. Collins, James K., J. S. Smith, and Michael T. Kelly. 1984. Comparison of phage typing, plasmid mapping, and antibiotic resistance patterns as epidemiolgic markers in a nosocomial outbreak of methicillin-resistant Staphylococcus aureus infections. Diagn. Microbial. Infect. Dis. 2:233-245.

19. Coloe, P. J., Jennifer Taplin, and W. Fromm. 1984. The distribution of specific phage types of Salmonella typhimurium in chickens in Australia. J. Hyg. Camb. 92:177-182.

20. Crosa, J. H., M. H. Schiewe, and S. Falkow. 1977. Evidence for plasmid contribution to the virulence of the fish pathogen Vibrio anguillarum. Infect. Immun. 18:509-513.

21. Edwards, P. R., D. W. Bruner, and A. B. Moran. 1948. The genus Salmonella: Its occurrence and distribution in the United States. Ky. Agric. Exp. Stn. Bull. 525. 60 pp.

22. Edwards, P. R. and W. H. Ewing. 1972. The genus Salmonella. Pages 146-208 in Identification of Enterobacteriaceae. Third edition. Afton Press, Afton, MN.

23. Farmer, J. J. III, Don J. Brenner, and George K. Morris. 1984. The Salmonella-arizona group of Enterobacteriaceae: nomenclature, classification, and reporting. Clin. Microbiol. Newsl. 6:63-66.

24. Farrar, W. Edmund, Jr. 1983. Molecular analysis of plasmids in epidemiologic investigation. J. Infect. Dis. 148:1-6.

25. Felix, A. 1956. Phage typing of Salmonella typhimurium: its place in epidemiological and epizootiological investigations. J. Gen. Microbiol. 14:208.

26. Fierer, Joshua and Winifred Fleming. 1983. Distinctive biochemical features of Salmonella dublin isolated in California. J. Clin. Microbiol. 17:552-554.

27. Gemski, P., J. R. Lazere, T. Casey, and J. A. Wohlheiter. 1980. Presence of a virulence-associated plasmid in Yersinia pseudotuberculosis. Infect. Immun. 28:1044-1047.

28. Gershman, M. 1977. Single phage typing set for differentiating Salmonella. J. Clin. Microbiol. 5:302-314.

29. Gershman, M. and G. Markowsky. 1983. Reduced set of phages for typing Salmonella. J. Clin. Microbiol. 17:240-244.

30. Hall, M. L. M. and J. Taylor. 1970. Salmonella dublin: the relation between a living calf vaccine strain and those isolated from human and other sources. Vet. Rec. 86:534-536.

31. Harris, J. R., I. K. Wachsmuth, B. R. Davis, and M. L. Cohen. 1982. High-molecular-weight plasmid correlates with Escherichia coli enteroinvasiveness. Infect. Immun. 37:1295-1298.

32. Helmuth, R. 1984. Serotype specific plasmids in Salmonella and their role for pathogenicity. Page 135 in H. Errebo Larsen, ed. Agriculture priority aspects of salmonellosis research. Commission of the European Communities, Brussels, Luxembourg.

33. Hickman, F. W. and J. J. Farmer III. 1978. Salmonella typhi: identification, antibiograms, serology, and bacteriophage typing. Am. J. Med. Technol. 44:1149-1159.

34. Hickman-Brenner, F. W., J. J. Farmer III, and regional centers for Salmonella typhi bacteriophage typing in the United States. 1983. Bacteriophage types of Salmonella typhi in the United States from 1974 through 1981. J. Clin. Microbiol. 17:172-174.

35. Hinton, M. 1977. The diagnosis of Salmonella abortion in cattle with particular reference to Salmonella dublin. A review. J. Hyg. Camb. 79:25-38.

36. Holmberg, Scott D. and Mitchell L. Cohen. 1985. Investigating the epidemiology of salmonellosis in the United States: new approaches to an old problem. Pages 16-19 in Proceedings of the International Symposium on Salmonella. American Association of Avian Pathologists, Inc., Kennett Square, PA.

37. Holmberg, Scott D., Michael T. Osterholm, Kenneth A. Senger, and Mitchell L. Cohen. 1984. Drug-resistant Salmonella from animals fed antimicrobials. N. Engl. J. Med. 311:617-622.

38. Holmberg, Scott D., I. Kaye Wachsmuth, Frances W. Hickman-Brenner, and Mitchell L. Cohen. 1984. Comparison of plasmid profile analysis, phage typing, and antimicrobial susceptibility testing in characterizing Salmonella typhimurium isolates from outbreaks. J. Clin. Microbiol. 19:100-104.

39. Houston, D. L. 1985. Science and the necessity of Salmonella control. Pages 1-6 in Proceedings of the International Symposium on Salmonella. American Association of Avian Pathologists, Inc., Kennett Square, PA.

40. Ikeda, Jack S. and Dwight C. Hirsh. 1985. Common plasmid encoding resistance to ampicillin, chloramphenicol, gentamicin, and trimethoprim-sulfadiazine in two serotypes of Salmonella isolated during an outbreak of equine salmonellosis. Am. J. Vet. Res. 46:769-773.

41. Ishiguro, Naotaka, Jun Goto, and Gihei Sato. 1980. Genetical relationship between R plasmids derived from Salmonella and Escherichia coli obtained from a pig farm and its epidemiological significance. J. Hyg. Camb. 84:365-379.

42. Ishiguro, N., G. Sato, K. Takeuchi, and A. Nakayama. 1979. A longitudinal epizootical study of Salmonella infection on a piggery: a study on the mode of contamination by biotyping of Salmonella typhimurium and by the antibiogram. Jpn. J. Vet. Sci. 41:261.

43. Jones, G. W., D. K. Rabert, D. M. Svinarich, and H. J. Whitfield. 1982. Association of adhesive, invasive, and virulent phenotypes of Salmonella typhimurium with autonomous 60-megadalton plasmids. Infect. Immun. 38:476-486.

44. Jones, P. W., P. Collins, and G. T. H. Brown. 1984. Some factors underlying the variation in virulence of strains of Salmonella dublin. Pages 103-118 in H. Errebo Larsen, ed. Agriculture

priority aspects of salmonellosis research. Commission of the European Communities, Belgium, Luxembourg.

45. Jorgensen, Sigrid Tue. 1983. Prevalence and molecular epidemiology of antibiotic-resistant Salmonella typhimurium and Salmonella dublin in Danish cattle. Acta Pathol. Microbiol. Immunol. Scand. 91:163-168.

46. Kado, C. I. and S. T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.

47. Le Minor, L. 1984. Genus III. Salmonella. Page 427 in Noel R. Krieg and John G. Holt, eds. Bergey's manual of systematic bacteriology. Vol. 1. Williams & Wilkins, Baltimore, Md.

48. Le Minor, L. 1981. The Genus Salmonella. Pages 1148-1159 in Mortimer P. Starr, Heinz Stolp, Hans G. Truper, Albert Balows, and Hans G. Schlegel, eds. The prokaryotes, a handbook on habitats, isolation, and identification of bacteria. Vol. II. Springer-Verlag, New York.

49. Le Minor, L., M. Veron, and M. Popoff. 1982. Taxonomie des Salmonella. Ann. Microbiol. (Inst. Pasteur) 133:223-243.

50. Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy, ed. 1985. Salmonella species. Page 271 in Manual of clinical microbiology. Fourth edition. American Society for Microbiology, Washington, D. C.

51. Levy, Stuart B. 1984. Playing antibiotic pool: time to tally the score. N. Engl. J. Med. 311:663-665.

52. Macrina, Francis L., D. J. Kopecko, K. R. Jones, D. J. Ayers, and S. M. McCowen. 1978. A multiple-plasmid-containing Escherichia coli strain: convenient source of size reference plasmid molecules. Plasmid 1:417-420.

53. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Alkaline lysis method. Pages 368-369 in Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

54. Martensson, L., T. Holmberg, B. Hurvell, L. Rutquist, K. Sandstedt, M. Wierup. 1984. Salmonella isolated from animals and feed stuffs in Sweden during 1978-1982. Nord. Vet. Med. 36:371-393.

55. McConnell, M. M., H. R. Smith, J. Leonardopoulos, and E. S. Anderson. 1979. The value of plasmid studies in the epidemiology of infections due to drug-resistant Salmonella wien. J. Infect. Dis. 139:178-190.

56. Maimone, Francesco, Bianca Colonna, Paolo Bazzicalupo, Brunello Oliva, Mauro Nicoletti, and Mariassunta Casalino. 1979. Plasmids and transposable elements in Salmonella wien. J. Bacteriol. 139:369-375.

57. Makino, S., N. Ishiguro, and G. Sato. 1981. Change of drug resistance patterns and genetic properties of R plasmids in Salmonella typhimurium of bovine origin isolated from 1970 to 1979 in northern Japan. J. Hyg. Camb. 87:257-269.

58. Martin, P. A. J. and Bradford P. Smith. 1985. Control of salmonellosis in dairy calves. Pages 194-197 in Proceedings of the International Symposium on Salmonella. American Association of Avian Pathologists, Inc., Kennett Square, PA.

59. Meyers, Jane Aldrich, David Sanchez, Lynn P. Elwell, and Stanley Falkow. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127:1529-1537.

60. Nakamura, Masayuki, Shizuo Sato, Tatsuo Ohya, Shoko Suzuki, and Sumio Ikeda. 1985. Possible relationship of a 36-megadalton Salmonella enteritidis plasmid to virulence in mice. Infect. Immun. 47:831-833.

61. Neel, R., K. Jorgensen, L. LeMinor, and A. Machoun. 1953. Contribution a l'etude de Salmonella dublin. Nouvelles varietis biochemeques. Classification. Ann. Inst. Pasteur 84:410-419.

62. Neu, H. C., C. E. Cherubin, E. D. Longo, B. Flouton, and J. Winter. 1975. Antimicrobial resistance and R-factor transfer among isolates of Salmonella in the northeastern United States: a comparison of human and animal isolates. J. Infect. Dis. 132:617-622.

63. O'Brien, Thomas F., John D. Hopkins, Elaine S. Gilleece, Antone A Medeiros, Ralph L. Kent, Billie O. Blackburn, Marion B. Holmes, Joseph P. Reardon, James M. Vergeront, Wendy L. Schell, Eleanor Christenson, Marjorie Bissett, and Erskine V. Morse. 1982. Molecular epidemiology of antibiotic resistance in Salmonella from animals and human beings in the United States. N. Engl. J. Med. 307:1-6.

64. Orskov, Frits and Ida Orskov. 1983. Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the Enterobacteriaceae and other bacteria. J. Infect. Dis. 148:346-357.

65. Osborne, A. D., H. Pearson, A. H. Linton, and C. Shimeld. 1977. Epidemiology of Salmonella infection in calves: the source of calfhood infection by Salmonella dublin. Vet. Rec. 101:513-516.

66. Parker, M. T. 1983. Salmonella. Pages 332-354 in M. T. Parker, G. S. Wilson, and A. A. Miles, eds. Topley and Wilson- principles of bacteriology, virology, and immunity. Vol. 2. 7th edition. Williams & Wilkins, Baltimore, MD.

67. Platt, D. J., J. S. Sommerville and J. Gribben. 1984. Sequential acquisition of R-plasmids in vivo by Salmonella typhimurium. J. Antimicrob. Chemother. 13:65-69.

68. Pocurull, D. W., S. A. Gaines, H. D. Mercer. 1971. Survey of infectious multiple drug resistance among Salmonella isolated from animals in the United States. Appl. Microbiol. 21:358-362.

69. Pohl, P. and G. Ghysels. 1984. Salmonellosis in Belgium. Pages 21-28 in H. Errebo Larsen, ed. Agriculture priority aspects of salmonellosis research. Commission of the European Communities, Brussels, Luxembourg.

70. Popoff, M. Y., I. Miras, C. Coynault, C. Lasselin, and P. Pardon. 1984. Molecular relationships between virulence plasmids of Salmonella serotypes typhimurium and dublin and large plasmids of other Salmonella serotypes. Ann. Microbiol. (Inst. Pasteur) 135:389-398.

71. Rangnekar, V. M., D. D. Banker, and H. I. Jhala. 1983. Antimicrobial resistance and incompatibility groups of R plasmids in Salmonella typhimurium isolated from human sources in Bombay from 1978 to 1980. Antimicrob. Agents Chemother. 23:54-58.

72. Riley, Lee W. and Mitchell L. Cohen. 1982. Plasmid profiles and Salmonella epidemiology. The Lancet 1:573.

73. Riley, Lee W., George T. DiFerdinando, Jr., Thomas M. DeMelfi, and Mitchell L. Cohen. 1983. Evaluation of isolated cases of salmonellosis by plasmid profile analysis: introduction and transmission of a bacterial clone by precooked roast beef. J. Infect. Dis. 148:12-17.

74. Robinson, R. A., B. O. Blackburn, C. D. Murphy, E. V. Morse, and M. E. Potter. 1985. Epidemiology of Salmonella dublin in the U.S.A. Pages 182-193 in Prodeedings of the International Symposium on Salmonella. American Association of Avian Pathologists, Inc., New Bolton Center, Kennett Square, PA.

75. Saxena, S. N., M. L. Mago, Neelam Kumari, and S. N. Rao Bhau. 1984. R-plasmid of some rarely isolated Salmonella serotypes. Indian J. Med. Res. 79:307-311.

76. Schaberg, Dennis R., Lucy S. Tompkins, and Stanley Falkow. 1981. Use of agarose gel electrophoresis of plasmid deoxyribonucleic

acid to fingerprint gram-negative bacilli. J. Clin. Microbiol. 13:1105-1108.

77. Small, R. G. and J. C. M. Sharp. 1979. A milk-borne outbeak due to Salmonella dublin. J. Hyg. Camb. 82:95-100.

78. Smith, H. W. 1951. The typing of Salmonella dublin by means of bacteriophage. J. Gen. Microbiol. 5:919-925.

79. Sojka, W. J. and E. B. Hudson. 1976. A survey of drug resistance in Salmonella isolated from animals in England and Wales during 1972. Br. Vet. J. 132:95-104.

80. Sojka, W. J., P. D. Thomson, and E. B. Hudson. 1974. Excretion of Salmonella dublin by adult bovine carriers. Br. Vet. J. 130:482-488.

81. Stanisich, V. A. 1984. Identification of plasmids at the genetic level. Page 9 in P. M. Bennett and J. Grinsted, eds. Methods in microbiology. Vol. 17. Academic Press, London.

82. Steele, James H. and Kenneth D. Quist. 1965. Chain of infection-animal to human. Pages 71-73 in Proceedings National Conference on Salmonellosis. U.S. Government Printing Office, Washington, D.C.

83. Sutch, Kathleen and C. D. Murphy. 1984. Salmonella and Arizona serotypes from animals and related sources reported during fiscal year 1983. Pages 525-540 in Proceedings of the 88th annual meeting of The United States Animal Health Association. Carter Printing Co., Richmond, VA.

84. Swanstrom, M. and M. H. Adams. 1951. Agar layer method for the production of high titer phage stocks. Proc. Soc. Exp. Biol. Med. 7:372-375.

85. Taylor, David N., Joseph M. Bied, J. Stuart Munro, and Roger A. Feldman. 1982. Salmonella dublin infections in the United States, 1979-1980. J. Infect. Dis. 146:322-327.

86. Taylor, David N., I. Kaye Wachsmuth, Yung-Hui Shangkuan, Enmett V. Schmidt, Timothy J. Barrett, Janice S. Schrader, Charlene S. Scherach, Harry B. McGee, Roger A. Feldman, and Don J. Brenner. 1982. Salmonellosis associated with marijuana. N. Engl. J. Med. 306:1249-1253.

87. Terakado, N., T. Ohya, H. Veda, And Y. Isayama. 1980. A survey on drug resistance and R plasmids in Salmonella isolated from domestic animals in Japan. Jpn. J. Vet. Sci. 42:543-550.

88. Terakado, N., T. Sekizaki, K. Hashimoto, and S. Naitoh. 1983. Correlation between the presence of a fifty-megadalton plasmid in Salmonella dublin and virulence for mice. Infect. Immun. 41:443-444.

89. Tietze, E. and H. Tschape. 1983. Plasmid pattern analysis of natural bacterial isolates and its epidemiological implication. J. Hyg. Camb. 90:475-488.

90. Tietze, E., H. Tschape, W. Rabsch, and H. Kuhn. 1983a. Plasmid patterns of Salmonella typhimurium strains of n.c.1/72/n.c. phage-type from the G.D.R. Zbl. Bakt. Hyg., I. Abt. Orig. 254:69-77.

91. Walton, J. R. 1972. Bacteriological, biochemical, and virulence studies on Salmonella dublin from abortion and enteric disease in cattle and sheep. Vet. Rec. 90:236-240.

92. Werner, S., G. L. Humphrey, and I. Kamei. 1979. Association between raw milk and human Salmonella dublin infections. Br. Med. J. 2:238-241.

93. Wray, C. 1985. Salmonella dublin infection of cattle in England and Wales: its epidemiology and control. Pages 173-181 in Proceedings of the International Symposium on Salmonella. American Association of Avian Pathologists, Inc., Kennett Square, PA.

94. Wray, C. and W. J. Sojka. 1977. Reviews of the progress of dairy science: bovine salmonellosis. J. Dairy Res. 44:383-425.

95. Zink, D. L., J. C. Feeley, J. G. Wells, C. Vanderzant, J. C. Vickery, W. D. Roof, and G. A. O'Donovan. 1980. Plasmid-mediated tissue invasiveness in Yersinia enterocolitica. Nature (London) 283:224-226.

APPENDIX: RESULTS OF ANTIBIOTIC RESISTANCE TESTING

Culture	Antibiotic											
Number	AN	AM	C	GM	K	N	P	S	SD	TE	TM	TMP
85-62	25	23	28	24	24	22	11	15	11	22	25	20
85-171	24	6	29	24	6	6	6	6	17	6	25	24
85-174	24	6	6	25	6	6	6	6	6	6	23	26
85-309	25	6	6	25	6	9	6	6	6	6	25	29
85-322	27	6	6	26	6	9	6	6	6	6	24	29
85-331	26	6	6	26	6	11	6	6	6	6	26	27
85-460	25	6	6	26	6	9	6	6	6	6	24	29
85-513	26	6	6	26	6	11	6	8	6	6	25	28
85-518	27	25	29	27	26	23	13	17	17	24	26	28
85-522	27	6	6	26	6	11	6	6	6	6	25	30
85-523	28	6	6	27	6	12	6	6	6	6	26	29
85-525	26	27	30	26	26	23	21	15	20	26	25	28
85-527	27	6	6	27	6	12	6	6	6	6	26	30
85-530	28	6	6	27	6	10	6	6	6	6	26	29
85-531	28	25	25	27	26	24	11	18	26	25	27	30
85-534	27	26	28	26	26	23	13	16	19	25	24	28
85-637	27	6	32	26	6	6	6	6	23	7	22	29
85-674	27	6	24	26	6	11	6	6	23	6	26	25
85-763	29	6	6	27	6	12	6	6	6	6	27	34
85-764	27	6	6	27	6	12	6	6	6	6	26	34
85-813	26	25	26	25	25	23	14	18	18	24	26	33
85-950	27	6	6	26	6	8	6	6	6	6	25	31
85-965	26	6	27	27	6	10	6	6	25	6	25	29
85-970	28	6	6	26	6	11	6	6	6	6	25	26
85-991	29	25	25	27	28	23	17	17	18	23	27	26
85-992	27	6	28	27	6	10	6	8	20	6	25	28
85-998	27	29	31	28	27	24	22	12	24	25	26	30
85-1020	28	6	6	27	6	10	6	6	6	6	25	30
85-1031	26	6	30	25	6	14	6	6	15	6	24	32

Culture	Antibiotic											
Number	AN	AM	C	GM	K	N	P	S	SD	TE	TM	TMP
85-1040	25	27	31	24	6	12	20	15	15	24	24	24
85-1046	28	26	28	26	26	22	20	18	20	24	26	28
85-1047	28	6	6	26	6	10	6	6	6	6	25	29
85-1099	26	6	30	26	6	11	6	6	14	6	26	30
85-1129	27	25	28	26	8	14	14	18	20	25	26	28
85-1140	25	28	32	25	25	21	22	15	19	25	24	29
85-1148	25	6	26	25	6	9	6	6	21	6	25	30
85-1264	26	6	33	27	6	10	6	6	29	6	26	34
85-1266	26	6	30	27	6	8	6	6	24	6	25	29
85-1271	26	6	28	26	6	10	6	6	21	24	26	29
85-1439	27	29	31	27	27	22	23	17	17	27	25	29
85-1520	26	9	32	25	6	6	6	8	18	18	23	26
85-1521	28	11	30	26	6	6	10	11	22	18	25	30
85-1522	28	6	30	27	28	25	6	6	22	6	26	29
85-1555	28	6	6	28	6	11	6	6	6	6	27	32
85-1614	25	6	31	25	6	8	6	6	18	7	23	25
85-1628	25	6	30	25	6	10	6	6	20	6	25	29
85-1671	25	6	32	27	6	6	6	6	22	6	26	30
85-1715	29	29	33	28	28	25	22	17	24	28	28	30
85-1743	27	6	32	28	6	8	6	6	18	7	27	27
85-1794	27	6	6	26	6	10	6	6	6	6	25	28
85-1816	27	6	34	26	6	9	6	6	25	6	25	29
85-1849	26	6	34	27	6	6	6	6	25	7	25	29
85-1879	28	25	29	27	27	23	10	16	19	25	26	29
85-1903	26	6	31	27	6	9	6	6	13	6	27	31
85-1981	25	6	29	25	6	9	6	6	12	6	25	30
85-1996	26	6	27	25	6	11	6	6	20	6	25	31
84-1042	30	28	6	29	6	10	15	6	6	6	28	27
84-1541	27	25	6	27	6	10	12	6	6	6	27	30

Culture	Antibiotic											
Number	AN	AM	C	GM	K	N	P	S	SD	TE	TM	TMP
84-2235	28	6	6	28	6	12	6	6	6	6	27	31
84-2338	26	6	30	24	24	23	6	6	17	25	24	28
84-2357	27	6	32	27	6	11	6	6	20	7	26	29
84-2608	28	6	6	27	6	11	6	6	6	6	26	31
84-2684	28	6	6	27	6	10	6	6	6	6	26	30
84-2818	28	6	29	25	6	6	6	6	22	6	21	28
84-2819	26	6	26	25	6	6	6	6	18	6	22	28
84-2821	28	6	32	27	6	10	6	6	26	27	26	30
84-2932	25	6	31	26	6	9	6	6	21	6	25	25
84-2956	28	6	6	27	6	12	6	6	6	6	27	30
84-3155	25	6	32	26	6	9	6	6	20	26	25	28
84-3211	29	6	6	28	6	10	6	6	6	6	27	29
84-3509	25	6	31	26	6	9	6	6	22	6	25	29
84-3698	27	6	6	26	6	10	6	6	6	6	25	29
84-3700	27	27	32	25	26	21	21	15	19	25	25	28
84-3729	28	6	6	27	6	12	6	6	6	6	26	30
84-3788	26	6	6	25	6	10	6	6	6	6	25	30
84-3947	25	27	31	25	25	21	19	12	17	26	23	26
84-3950	26	6	6	26	6	8	6	6	6	6	24	29
84-3952	26	6	34	27	6	9	6	6	21	8	25	26
84-4013	26	6	31	25	6	6	6	6	23	7	22	29
84-4266	23	6	29	24	6	8	6	6	12	6	24	27
84-4576	20	6	6	10	6	6	6	6	6	6	10	6
84-4617	27	6	6	26	6	11	6	6	6	6	25	28
84-4637	28	6	33	27	6	6	6	6	24	7	23	30
84-4638	28	6	30	26	6	6	6	6	22	27	22	28
84-4640	26	6	30	24	6	6	6	6	19	26	20	27
84-4641	28	6	34	27	6	6	6	6	25	7	23	30
84-4642	27	26	30	27	26	22	19	15	18	26	24	26

Culture	Antibiotic											
Number	AN	AM	C	GM	K	N	P	S	SD	TE	TM	TMP
84-4645	28	29	33	27	27	24	24	16	14	26	26	34
84-4651	27	25	30	26	27	22	12	14	20	23	25	29
84-4702	27	6	6	27	6	9	6	6	6	6	25	27
84-4777	28	6	6	26	6	10	6	6	6	6	25	28
84-4795	25	6	35	28	6	9	6	6	18	8	25	28
84-4964	25	6	6	25	6	7	6	6	6	6	24	27
83-1101	26	27	28	24	26	22	21	17	14	22	25	25
83-225	27	28	28	27	26	23	20	17	18	25	26	29
84-254	26	24	27	25	25	22	10	14	8	21	25	25
84-340	27	27	28	26	25	23	22	17	15	25	26	29
84-341	27	29	30	28	27	24	20	16	13	28	27	27
84-594	27	27	28	27	27	23	21	16	17	24	26	26
84-757	28	27	28	27	27	22	22	16	19	24	26	29

AM=ampicillin
C=chloramphenicol
GM=gentamicin
K=kanamycin
N=neomycin
P=penicillin
S=streptomycin
SD=sulfamides
TE=tetracycline
TM=tobramycin
TMP=trimethoprim

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Charles O. Thoen and Dr. Robert E. Andrews, my co-major professors, for their time and assistance in the preparation of this thesis. Also, my thanks to Dr. Billie O. Blackburn for his assistance and encouragement. I would like to thank Dr. Steven Moseley, Childrens Orthopedic Hospital, Seattle, WA, who provided the plasmid extraction procedure; and Jeni Bungert and Bob Carroll, National Veterinary Services Laboratories, Ames, IA, for their assistance in computer analysis of data.

Special thanks are extended to my husband, James Ferris, and children, Chris and Mark Sutch, for their patience, understanding, and encouragement. Also, special thanks to my parents, Paul and Stella Grimes for their constant support.